ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical &
Health Science Review

# REMEDIATION OF CADMIUM-CONTAMINATED SOIL USING BIOCHAR AND ITS IMPACT ON WHEAT GERMINATION AND GROWTH

**Iqra Nawaz[1], Attqa Afshan[2], Syeda Mahnoor Bukhari[3], Muniba Niaz[4], Sumbal Kamran[5], Faran Durrani[5], Ashique Ali Chohan[6], Zeshana Sikandri[5], Riffat Yasmin[1]**

[1]Department of Medical Laboratory and Technology, Riphah International University, Faisalabad, Pakistan

[2]Department of Botany, University of the Punjab, Pakistan

[3]Department of Chemistry, The Women University Multan, kutchery campus, Multan, Punjab, Pakistan

[4]Faculty of Science and Technology, University of central Punjab, Lahore, Pakistan

[5]Department of Botany, University of Science and Technology Bannu,Khyber Pakhtunkhwa Pakistan

[6]Department of Energy and Environment, Faculty of Agricultural Engineering and Technology, Sindh Agriculture University TandoJam, Sindh, Pakistan

| ARTICLE INFO | ABSTRACT |
|---|---|
| Keywords<br>Sustainability, Wheat Cultivation, Cotton Stick Biochar, Soil Toxicity, Germination, Growth, Physiological Responses<br>**Corresponding Author: Muniba Niaz,** Faculty of Science and Technology, University of central Punjab, Lahore, Pakistan, Email:Khanmuniba225@gmail.com | Heavy metal contamination, particularly cadmium (Cd), poses a severe threat to crop productivity and food safety due to its toxicity and non-biodegradable nature. This study aimed to assess the role of cotton stick biochar (CSB) in mitigating cadmium toxicity in soil and its impact on the germination, growth, and physiological responses of wheat (Triticum aestivum L.). A controlled pot experiment was conducted using soil artificially spiked with cadmium at 0, 4, and 8 mg $kg^{-1}$ and amended with CSB at 0%, 1%, and 2% (w/w). Results revealed that cadmium stress significantly reduced wheat germination percentage, shoot and root biomass, photosynthetic rate, stomatal conductance, and increased cadmium accumulation in plant tissues. However, the application of 2% CSB markedly improved germination (up to 88%), shoot and root growth, physiological parameters, and reduced cadmium uptake by up to 80% in shoots and 71% in roots. Furthermore, CSB application significantly enhanced soil organic carbon and cation exchange capacity while decreasing available soil cadmium levels. The study demonstrates that CSB is a cost-effective and eco-friendly soil amendment for reducing cadmium bioavailability, improving soil health, and supporting sustainable wheat cultivation under metal-stressed conditions. |

## 1. INTRODUCTION

Existence of heavy metals in soil environment is of great anxiety because these are environmental contaminants and can be toxic for the growth of crops, production and food status and even for human health (Khan et al., 2015a). Un-needed metals like cadmium and lead are present and dispersed all over the world. Through natural and anthropogenic sources they are discharged to the surrounding environment (Farahat and Linderholm, 2015; Khan et al., 2014). Different studies discovered that, heavy metals are toxic even at low concentration due to which serious diseases and health problems are caused in plants, animals and human due to their higher bio-accumulation rate, non-biodegradability and bio-toxicity (Di Salvatore et al., 2008). Due to the exposure poisonous metals like Cd can cause the severe health risks, because they are cyto-toxic (Monteiro et al., 2007) and in humans can cause the cancer and transformation. In current years, towards the heavy metals tolerance method by plants, the concern has been developed (Caporale et al., 2014). Many methods have been adopted to treat Cd-contaminated soil i.e. chemical, physical, and biological methods (e.g. phytoremediation and bioremediation approaches) (Akbal and Camci, 2011; Boudrahem et al., 2011; Malamis et al., 2011). However, most of these technologies can be related with high operational price, disposal problems and short term effect (Sud et al., 2008). For heavy metal contaminants these drawbacks have increased the requirement of alternatives, long term and low cost treatment technologies. Therefore, to satisfy this need bio-sorbents have been suggested to be a potential candidate, for remediation of toxic metals in contaminated soil. As an inexpensive bio-sorbent is the use of biochar to immobilize heavy metals in soil and is a rising and promising treatment technology (Uchimiya et al., 2010b; Park et al., 2011; Ahmad et al., 2014).

Biochar is a pyrogenic carbon (C) rich material, in a closed system it is derived from thermal decomposition of biomass with less or no oxygen (Lehmann et al., 2003). Biomass, particularly agricultural by-products, wood and manures are used for biochar production. The properties of biochar which make it more suitable for heavy metals immobilization in soil are high surface area ( > 400-1500 $m^2g^{-1}$), pH of biochar (wide range of pH value from 6 to 10), high CEC (> 40 cm mole $kg^{-1}$), long term stability (10-100 times of organic matter) and functional groups present on its surface especially oxygen containing functional groups (Uchimiya et al., 2010a; Park et al., 2011; Ahmad et al., 2014). These properties of biochar are helpful to immobilize the heavy metals for a long term basis. Biochar has 10 to 100 times more efficient as compared to other bio-sorbents for heavy metals immobilization in soil due to its high surface area. In addition, biochar may also provide some other benefits i.e. improved fertilizer use efficiency, increase crop yield and improve soil properties (Chan et al., 2007).

There are various mechanisms by which biochar decrease the bioavailability of heavy metals from soil i.e. sorption (Beesley and Marmiroli, 2011; Lu et al., 2012), precipitation (Park et al., 2013), altering soil pH (Wu et al., 2012). Sorption can be due to the binding of heavy metals with different functional groups present on the biochar surface especially oxygen containing functional groups (Uchimiya et al., 2011b) as well as by exchange of metals with different cations present on the biochar surface e.g. Ca, potassium (K) and magnesium (Mg) (Lu et al., 2012). Physical adsorption is simply electrostatic attraction between various functional groups present on heavy metals and surface of biochar (Lu et al., 2012). Different compounds (carbonates ($CO_3$), phosphates ($PO_4$) and sulphates ($SO_4$)) present on the biochar surface that precipitates the heavy metals by forming different compounds e.g. cadmium phosphate (Cao et al., 20009; Karami et al., 2011; Park et al., 2013). Similarly, most of the heavy metals are available at neutral pH when biochar (high pH) apply in the soil then it immobilizes the heavy metals by increasing the soil pH (Wu et al., 2012).

## 2. Materials and Methods

### 2.1. Experiment Location

A pot experiment was conducted in the wire house of Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad, Pakistan. The main purpose of this study was to evaluate the wheat germination and growth in Cd-contaminated soil amended with biochar. It was also evaluated that which rate of biochar show maximum wheat germination and growth in Cd-contaminated soil.

### 2.2. Biochar Production

Biochar was produced by using cotton sticks as feedstock at 400 ℃ according to the method described by Sanchez et al. (2009). The feedstock was placed in the quartz bottle. Then the filled quartz bottle was fitted in muffle furnace and pyrolysis was started. The heating ramp of 8-10 °C $min^{-1}$ was maintained. When desire maximum pyrolytic temperature of the furnace was achieved, the heating was stopped. This temperature was maintained for 20 min. Prepared biochar was analyzed for pH, electrical conductivity (EC) and total organic C (TOC) (Nelson and Sommer, 1982). Macronutrients (N, P and K) and Cd concentration in the biochar were analyzed with digestion method (Wolf, 1982).

### 2.3. Pot Experiment

A pot experiment was conducted after collecting the soil that was first air dried and then ground to pass through 2 mm sieve to make it homogenized. There were 27 pots each with 2 kg of soil. The experiment was conducted in wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad under 2-factorial CRD (completely randomized design) having three replications. The soil was spiked with different concentrations of Cd i.e. 0, 4 and 8 mg $kg^{-1}$ according to its permissible limit in soil by using cadmium nitrate ultra-pure salts. After that different rate of CSB i.e. 0, 1 and 2 % (w/w soil weight basis) was mixed in soil. Wheat was sown as test crop. All the recommended dose of nitrogen (N), phosphorus (P) and potassium (K) was applied with 1st irrigation after germination. The following treatment combinations were applied to the wheat sown in the pots.

**2.4. Treatment plan**

| Biochar rates / Cd concentration | 0 % (w/w) 0f soil ($B_0$) | 1 % (w/w) of soil ($B_1$) | 2 % (w/w) of soil ($B_2$) |
|---|---|---|---|
| $Cd_0$= 0 ppm application of Cd concentration) | $B_0$ $Cd_0$ | $B_1Cd_0$ | $B_2Cd_0$ |
| $Cd_4$= 4 ppm application of Cd | $B_0$ $Cd_4$ | $B_1$ $Cd_4$ | $B_2$ $Cd_4$ |
| $Cd_8$= 8 ppm application of Cd | $B_0$ $Cd_8$ | $B_1$ $Cd_8$ | $B_2$ $Cd_8$ |

**2.5. Harvesting & Data collection**

After 60 days of experiment, plants were harvested and analyzed for various parameters. Data regarding germination, physiological, agronomic, chemical and biochemical parameters were recorded as follows at different times during crop growth.

**2.6. Germination parameters**

1. Germination percentage
2. Mean emergence time

### 2.7. Growth and yield parameters

**2.7.1. Plant height (cm)**

Shoot length of highest plant from each pot was measured at harvesting with the help of meter scale from top to bottom. Then average of all the replications was obtained and recorded.

**2.7.2. Shoot fresh weight (g)**

After recording shoot length, these were cut into pieces and fresh weight of shoots was recorded with the help of electrical balance.

**2.7.3. Shoot dry weight (g)**

After recording shoot length, samples were cut into pieces, put into separate paper bags and allowed for sun drying. Then these bags were put into an oven at 65 $^{o}$C till the constant weight obtained and weights of the shoots were recorded.

**2.7.4. Root fresh weight (g)**

After recording root length, these were cut into pieces and fresh weight of roots was recorded.

**2.7.5. Root dry weight (g)**

After recording root length, these were cut into pieces, put into separate paper bags and allowed for sun drying. Then these bags were put into an oven at 65 $^{o}$C till the constant weight obtained and weights of the samples were recorded.

**2.7.5. Gaseous exchange measurement**

After 60 days the photosynthetic rate (A), transpiration rate (E), Stomatal conductance, Sub Stomatal conductance, Vapor pressure deficit three randomly selected plants from each treatment was determined using Cirus-3.

**2.7.6. Chlorophyll (SPAD)**

Leaf chlorophyll content was determined by using chlorophyll meter according to (Wellburn, 1994) (Minolta SPAD-502 Meter). Average (SPAD) reading was recorded from three measures (from the leaf tip to leaf blade).

**2.7.7. Relative Membrane permeability (RMP)**

For the RMP measurement, the leaves were cut into equal pieces and transferred to test tubes containing 20 ml of deionized distilled water. The test tubes were vortexed for 10s and the solution was assayed for initial electrical conductivity ($EC_o$). These tubes were kept at 4$^{o}$C for 24 h and then assayed for $EC_1$. The same samples were autoclaved at 121°C for 20 min to determine $EC_2$. Percent RMP was calculated as following the formula.

RMP (%) $=EC_1-EC0/EC_2-ECo\times 100$.

## 2.8. Chemical analysis

### 2.8.1. Digestion

The plant samples were digested as described by (Wolf, 1982). Ni concentration in the root/shoots, grain and soil were determined by drying all samples in an oven at 60 °C for 48h. Then, plant samples were grinded in rotary mill into the powder form. About 1 gram ground plant samples were taken in 100 mL Pyrex digestion flasks. 10 milliliters of concentrated nitric acid and 5 mm of per-chloric acid was added into the conical flask and left over night at room temperature in fume hood. Next day the flasks were put on the hot plate and heated up to the 350 °C until the sample appeared pale white. Samples removed from the hot plate and cooled them. When fumes were finished then distilled water was added in the flasks drop wise until the samples became colorless. Then filter it by using filter paper in conical flask. The volume of extract was made up to 50 mL by adding distilled water.

## 2.9. Biochemical analysis

### 2.9.1. Ash (%)

For ash determination one gram sample was taken in crucible. Then the crucible was placed in muffle furnace at 550°C and sample was heated, until the appearances of gray white ash. Gray white color indicates complete oxidation of all organic matter in the sample. Percent ash was calculated by following formula.

$$\%\ \text{ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

### 2.9.2. Soil texture

Hydrometer method was followed for particle size analysis (Bouyoucos, 1962). Dispersion solution was made by dissolving 10 g of sodium hexa-metaphosphate and 2.5 g of sodium carbonate in 250 mL of distilled water. 40 gram of air dried soil was taken in a 100 mL beaker and 60 mL of dispersing solution was added in it. Mixture was covered with watch glass and kept overnight. Next day, it was transferred to soil stirring cup and stirred at high speed for three minutes. Then material was quantitatively transferred into a graduated cylinder having 1 L volume. After obtaining homogenized suspension, plunger was taken out and first hydrometer reading ($HR_{sc}$) was recorded after 40 seconds. Again hydrometer was inserted to the graduated cylinder with minimum disturbance and second hydrometer reading ($HR_{c}$) was recorded after 2h. Since hydrometer is calibrated at temperature of 20 ºC, the readings were corrected for temperature variations, added 0.40 for each degree above temperature or subtracted 0.40 for each degree below temperature of 20 ºC and designated as $CHR_{sc}$ and $CHR_{c}$, respectively.

The quantity (%) of sand, silt and clay was determined as follows

% (Silt +Clay) = [($CHR_{sc}$) 100] / (Weight of soil)

% Clay = [($CHR_{c}$) 100] / (Weight of soil)

% Silt = % (Silt + Clay) - % Clay

% Sand = 100 – [% (Silt + Clay)]

Soil textural class was determined by plotting values of % Sand, % Silt and % Clay on USDA textural triangle.

### 2.9.3. Saturation percentage

A portion of saturated paste was transferred to a tarred china dish. It was weighed, dried to constant weight at 105 ºC and weighed again. Saturation percentage (SP) was calculated by using (U.S. Salinity Laboratory Staff, 1954; Method 27a) formula:

$$SP = \frac{\text{Loss in weight on oven drying (g)}}{\text{oven−dried soil weight}} \times 100$$

### 2.9.4. PH of the saturated soil paste (pHs)

About 250 g of soil was saturated with distilled water. Paste was prepared and allowed to stand for at least one hour and pH was recorded by pH meter (JENCO Model-671 P) with glass electrodes. Buffer of pH 4.1 and 9.2 were used to standardizing the instrument (U.S. Salinity Laboratory Staff, 1954; Method 21a).

### 2.9.5. Electrical conductivity of saturation extracts ($EC_e$)

Extract was obtained from saturated soil paste to determine $EC_e$ and soluble ions. It was obtained by applying positive pressure with the help of air pump. Sodium hexa-metaphosphate (2%) solution was added at the rate one drop per 25 mL extract to prevent precipitation of salts during storage. The electrical conductivity was noted with the help of conductivity meter

(WTW cond 315i). The conductivity meter was calibrated with 0.01N KCl solutions. Cell constant (k) was calculated by the formula:

$$k = \frac{1.4118 \text{ dS m}^{-1}}{\text{EC of 0.01 NKCl (dS m}^{-1})}$$

### 2.9.6. Cation exchange capacity (CEC)

Five grams of soil were saturated with 1N $CH_3COONa$ buffered at pH 8.2., washed thrice with ethanol and finally extracted with 1N $CH_3COONa$ thrice having pH 7.0 and volume was made 100 mL. Sodium ($Na^+$) in the extract was determined with the help of jenway PFP-7 flame photometer keeping Na filter in place. The CEC was calculated by the formula:

$$\text{CEC (cmol}_c\text{ kg}^{-}1) = \frac{\text{Na (mmol L}^{-}1) \times 100}{\text{Na (mmol}_c\text{ L}^{-}1) \times 100} \times 100$$

**Table 2.1. Selected physical and chemical properties of soil used for the experiment**

| Parameter | Unit | Value |
|---|---|---|
| $pH_s$ | --- | 7.95 |
| $EC_e$ | dS $m^{-1}$ | 1.30 |
| Textural class | --- | Sandy clay loam |
| Organic carbon | $gkg^{-1}$ | 4.3 |
| Total nitrogen | % | 0.062 |
| Available phosphorus | mg $kg^{-1}$ | 7.31 |
| Extractable potassium | mg $kg^{-1}$ | 109 |
| Cation exchange capacity | $cmol_c$ $kg^{-1}$ | 14.25 |
| Calcium carbonate | % | 3.38 |
| Total lead (Pb) | mg $kg^{-1}$ | 0.5 |

| | | |
|---|---|---|
| Total cadmium (Cd) | mg $kg^{-1}$ | 0.04 |
| Total chromium (Cr) | mg $kg^{-1}$ | 0.001 |

### 2.10. Statistical analysis

The data regarding all the parameters was statistically analyzed using software "Statistix 8.1" version. A general linear model was used to compare results of the study, and analysis of variance technique (ANOVA) was used to analyze data gathered from the experiment.

## 3. RESULTS

### 3.7. Germination parameters

#### 3.7.5. Germination percentage

Effect of different cotton stick biochar (CSB) rates on germination percentage of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.1.1. Wheat germination percentage was significantly decreased in cadmium contaminated soil and the minimum wheat germination percentage (57 %) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the wheat germination percentage in Cd contaminated soil and the maximum wheat germination percentage (88 %) was observed in the treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2 % CSB addition significantly increased the wheat germination in Cd spiked soil (8.0 mg $kg^{-1}$ w/w of soil) up to 35 % as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

#### 3.7.6. Mean emergence time

Effect of different cotton stick biochar (CSB) rates on mean emergence time (MET) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.1.2. Mean emergence time was significantly increased in Cd-contaminated soil and the maximum mean emergence time (4) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly decreased the mean emergence time in Cd contaminated soil and the minimum mean emergence time (3) was observed in the treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2 % CSB addition significantly decreased MET in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 8 as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

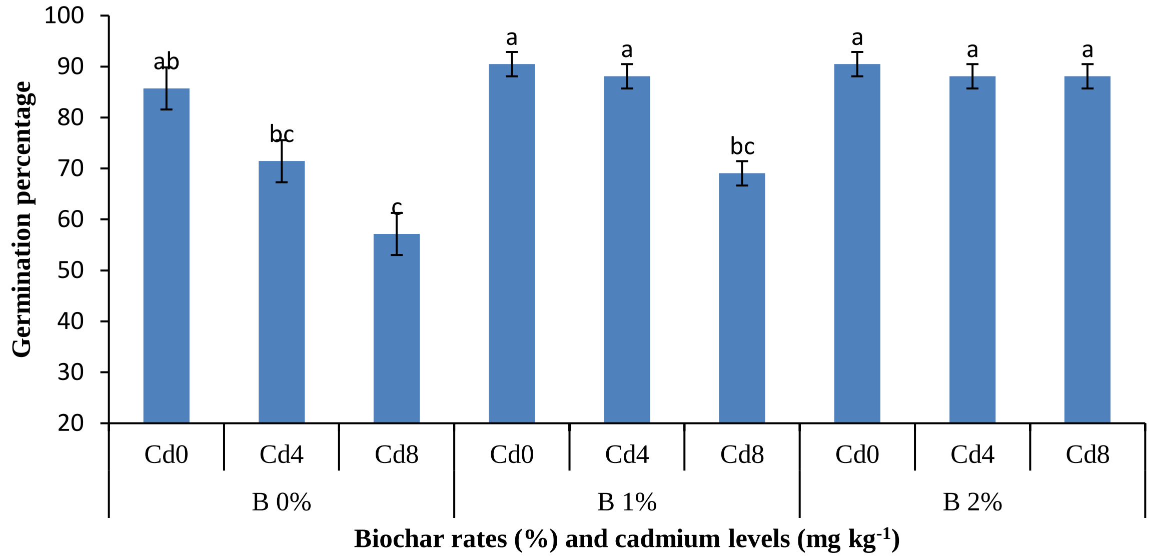



**Fig. 3.1.1: Effect of different biochar rates on germination percentage of wheat seed sown in soil contaminated with different levels of cadmium**

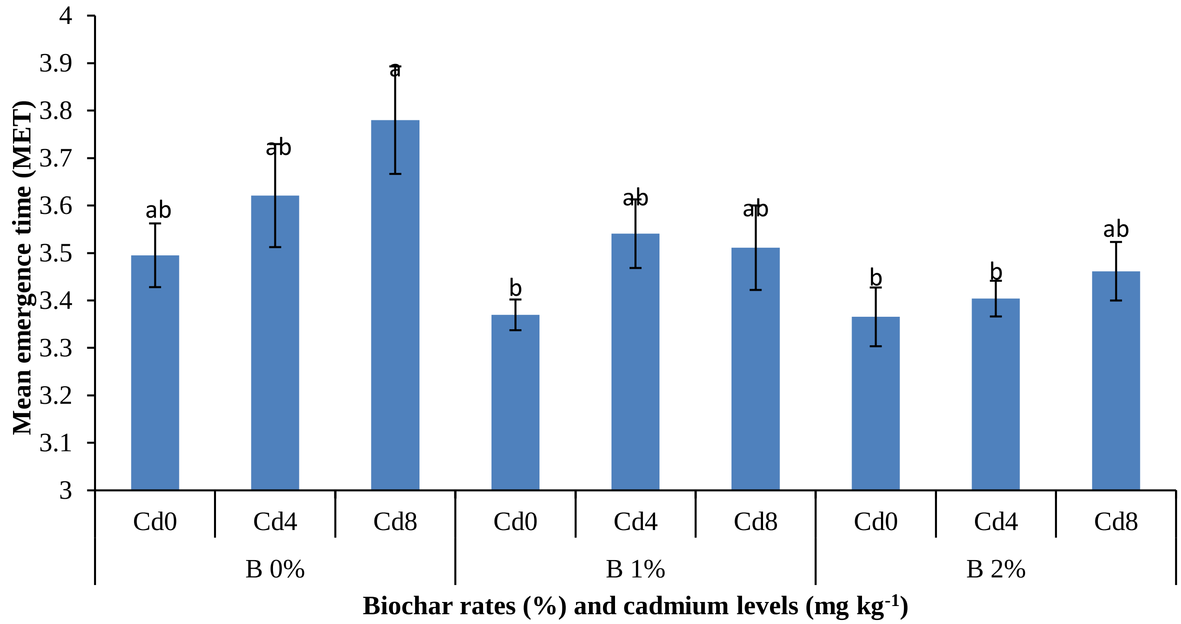



**Fig. 3.1.2: Effect of different biochar rates on mean emergence time (MET) of wheat seed sown in soil contaminated with different levels of cadmium**

## 3.2 Agronomic parameters

### 3.2.1 Shoot fresh biomass

Effect of different cotton stick biochar (CSB) rates on shoot fresh biomass (SFW) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.1.

Shoot fresh biomass was significantly decreased in Cd contaminated soil and minimum SFW (9 g) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the shoot fresh biomass in Cd contaminated soil and maximum SFW (14 g) was observed in treatment where 2.0 % CSB (w/w of soil) was added in the soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased the shoot fresh biomass of wheat in Cd spiked soil (8.0 mg $kg^{-1}$ w/w of soil) up to 36 g as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

**3.2.2 Shoot dry biomass**

Effect of different cotton stick biochar rates on shoot dry biomass (SDW) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.2. Shoot dry biomass was significantly decreased in Cd contaminated soil and minimum SDW (0.68 g) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the shoot dry biomass in Cd contaminated soil and maximum SDW (1 g) was observed in treatment where 2.0% CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0% CSB addition in soil significantly increased the shoot dry biomass of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 36 g as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

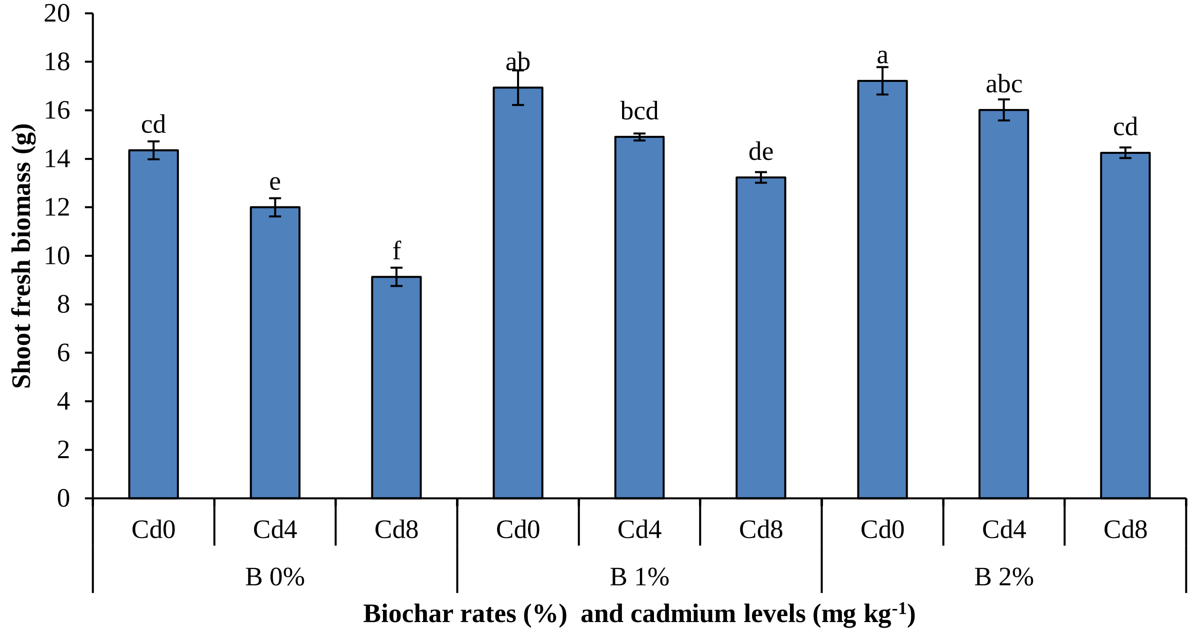



**Fig. 3.2.1: Effect of different biochar rates on wheat shoot fresh biomass sown in soil contaminated with different levels of cadmium**

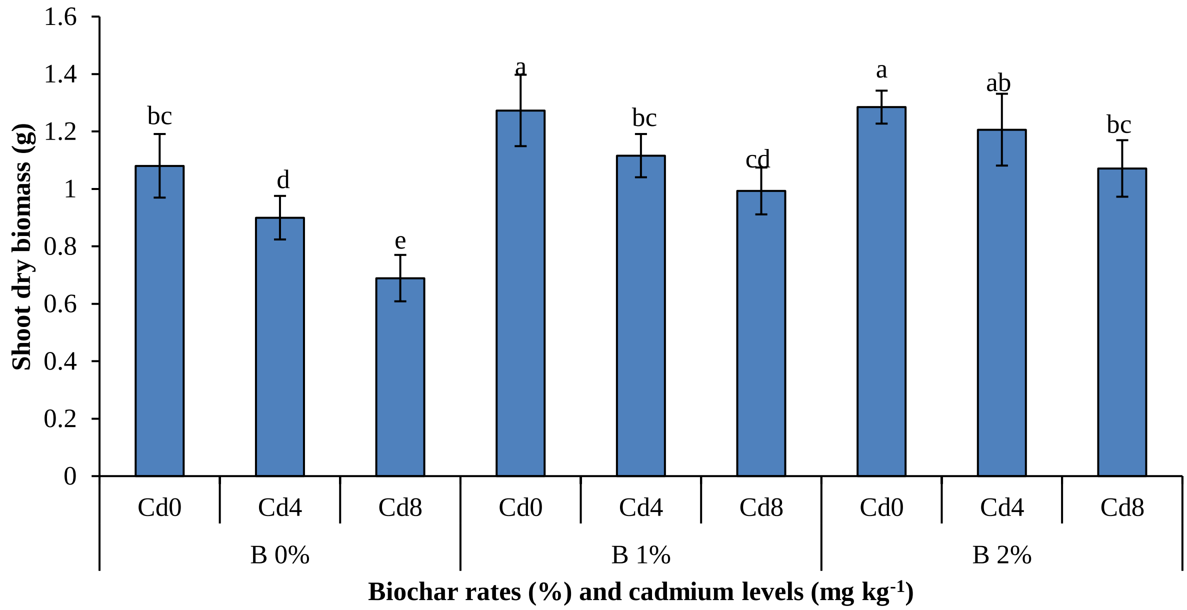



**Fig. 3.2.2: Effect of different biochar rates on wheat shoot dry biomass sown in soil contaminated with different levels of cadmium**

**3.2.3 Root fresh biomass**

Effect of different cotton stick biochar (CSB) rates on root fresh biomass (RFW) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.3. Root fresh biomass was significantly decreased in Cd contaminated soil and minimum RFW (2 g) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the root fresh biomass in Cd contaminated soil and maximum RFW (3 g) was observed in treatment where 2.0% CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0% CSB addition in soil significantly increased the root fresh biomass of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 40 g as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

### 3.2.4 Root dry biomass

Effect of different cotton stick biochar (CSB) rates on root dry biomass of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.4. Root dry biomass was significantly decreased in Cd contaminated soil and minimum RDW (0.78 g) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the root dry biomass in Cd contaminated soil and maximum RDW (1 g) was observed in treatment where 2.0% CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0% CSB addition in soil significantly increased root dry biomass of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 41 g as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

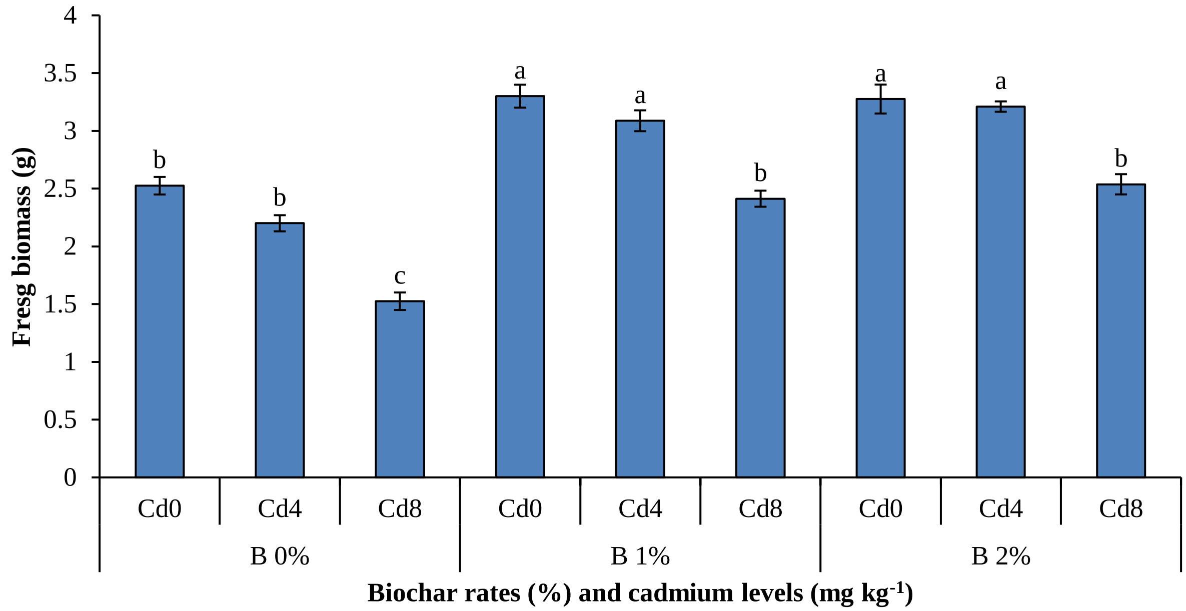



**Fig. 3.2.3: Effect of different biochar rates on wheat root fresh weight sown in soil contaminated with different levels of cadmium**

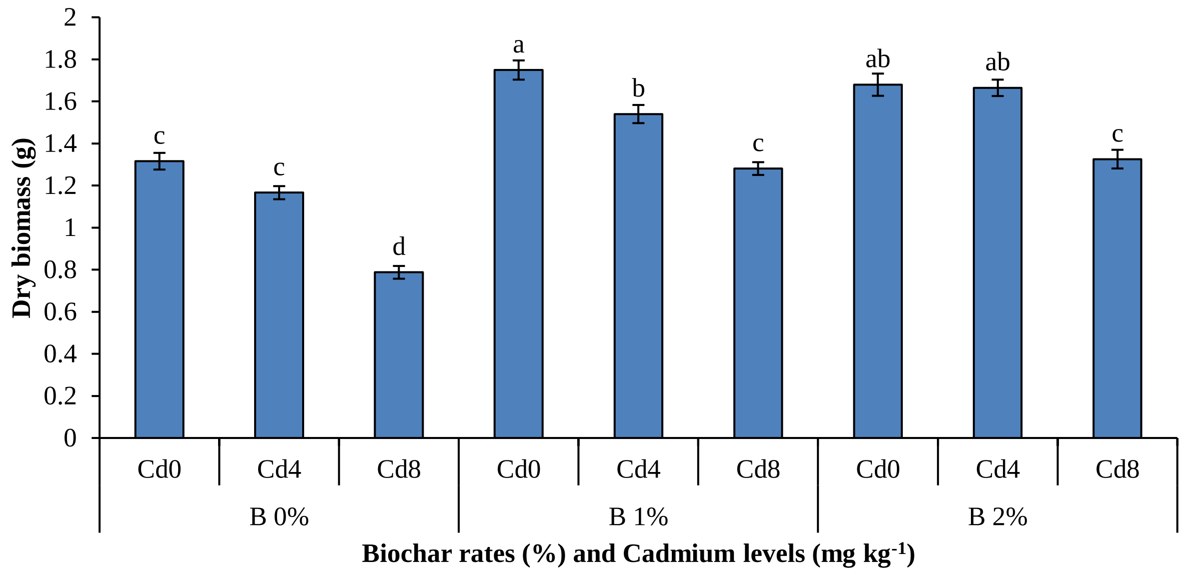



**Fig. 3.2.4: Effect of different biochar rates on wheat root dry biomass sown in soil contaminated with different levels of cadmium**

### 3.2.5 Shoot length

Effect of different cotton stick biochar (CSB) rates on shoot length (SL) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.5. Shoot length of wheat was significantly decreased in Cd contaminated soil and minimum SL (44 cm) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the shoot length in Cd contaminated soil and maximum SL (62 cm) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased the shoot length of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 28 cm as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

**3.2.6 Root length**

Effect of different cotton stick biochar (CSB) rates on root length (RL) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.6. Root length of wheat was significantly decreased in Cd contaminated soil and minimum RL (33 cm) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced root length in Cd contaminated soil and maximum RL (49 cm) was observed where the treatment 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased root length of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 32 cm as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

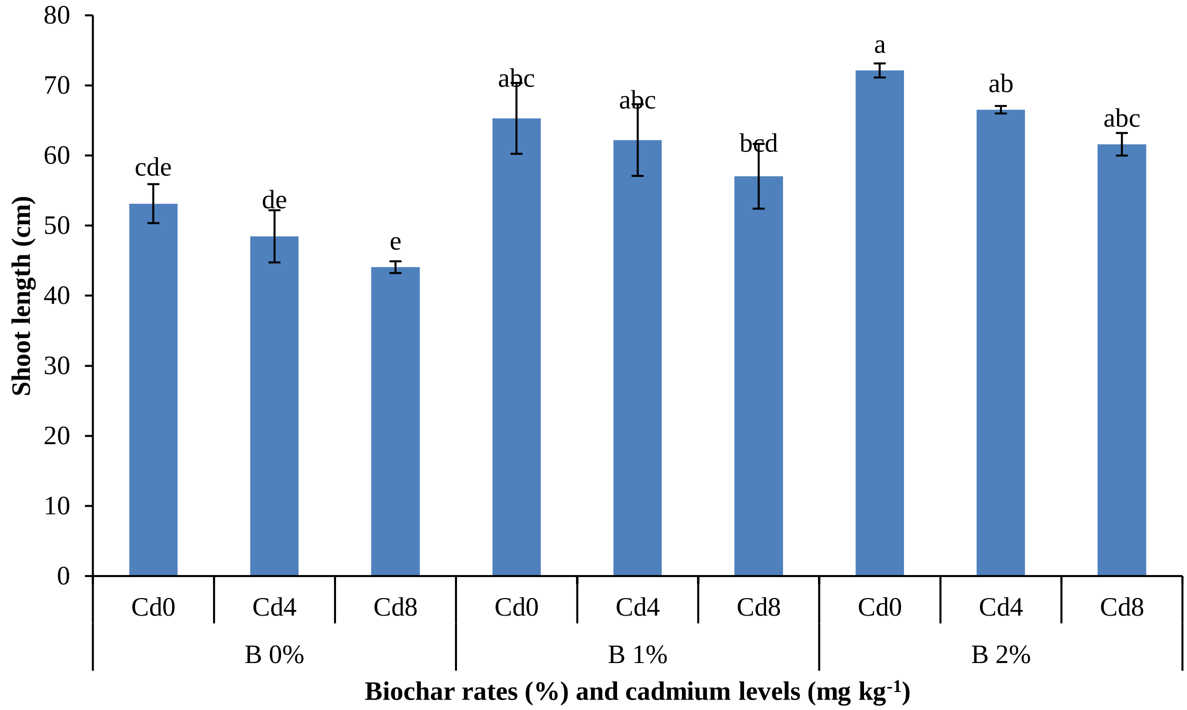



**Fig. 3.2.5: Effect of different biochar rates on wheat shoot length sown in soil contaminated with different levels of cadmium**

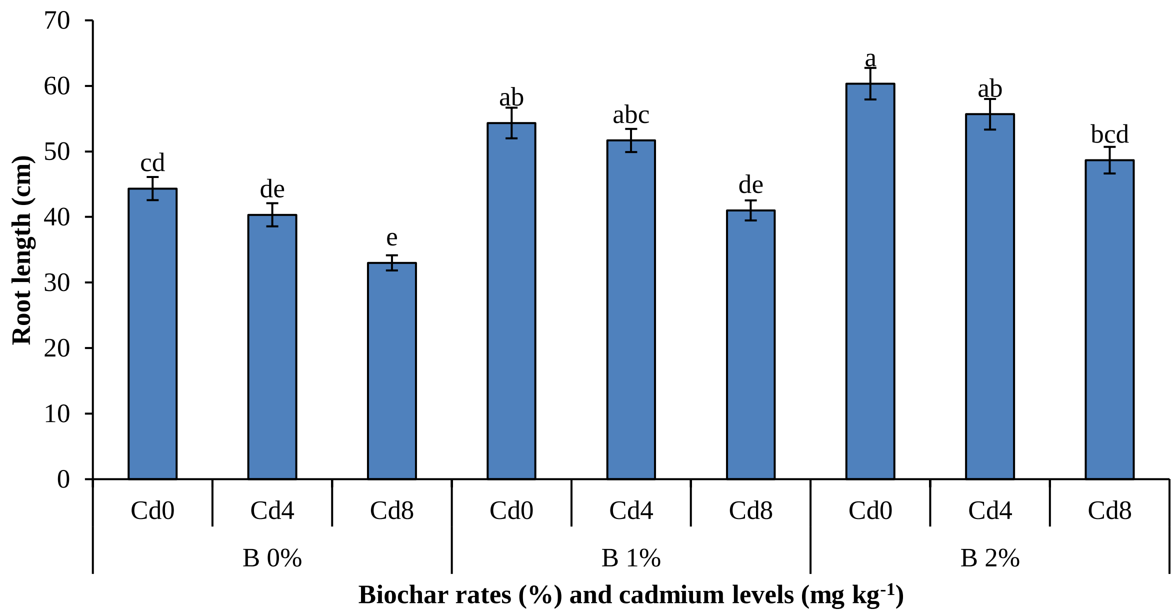



**Fig. 3.2.6: Effect of different biochar rates on wheat root length sown in soil contaminated with different levels of cadmium**

### 3.2.7 Root/shoot length

Effect of different cotton stick biochar (CSB) rates on root/shoot length of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.2.7. Addition of CSB (w/w of soil) showed non-significant effect on root/shoot length of wheat.

## 3.3 Physiological parameters

### 3.3.1 Photosynthetic rate

Effect of different cotton stick biochar (CSB) rates on photosynthetic rate of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.3.1. Photosynthetic rate was significantly decreased in Cd contaminated soil and minimum photosynthetic rate (11 µmol $m^{-2}$ $g^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly enhanced the photosynthetic rate in Cd contaminated soil and maximum photosynthetic rate (15 µmol $m^{-2}$ $g^{-1}$) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased the photosynthetic rate in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 29 µmol $m^{-2}$ $g^{-1}$ as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

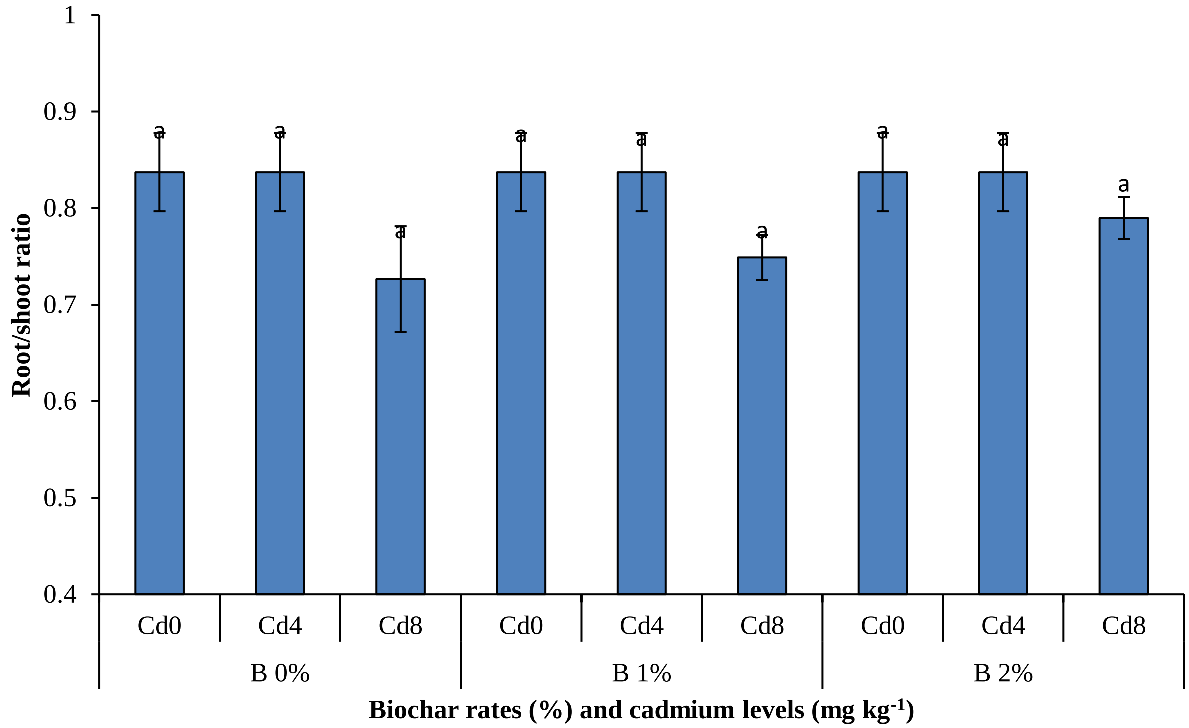



**Fig. 3.2.7: Effect of different biochar rates on wheat root/shoot length sown in soil contaminated with different levels of cadmium**

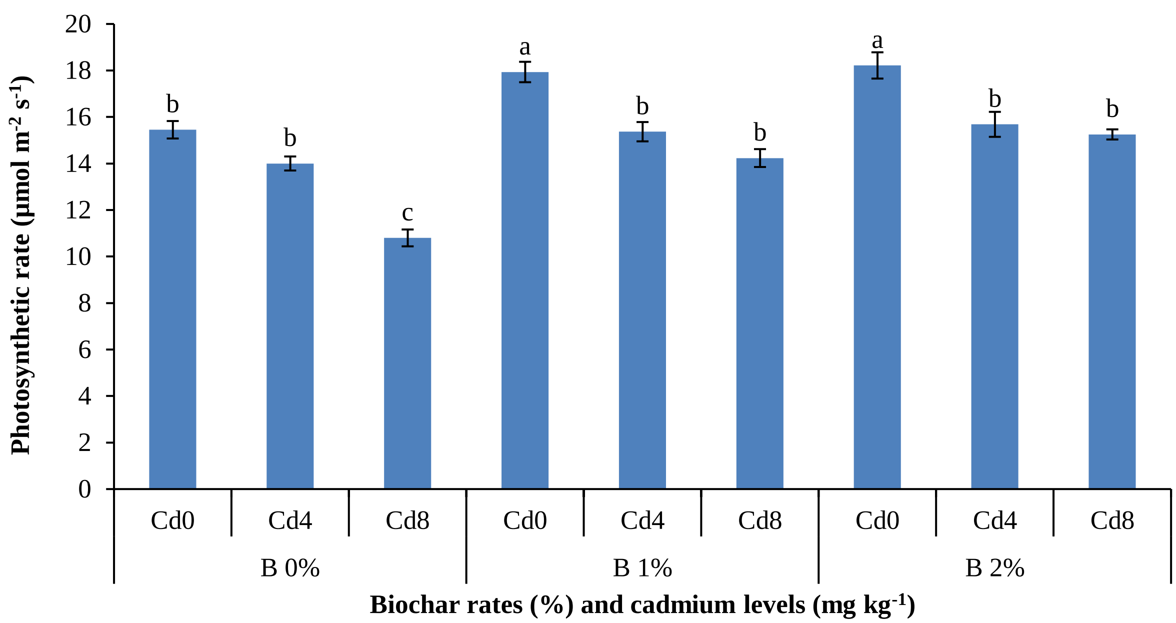



**Fig. 3.3.1: Effect of different biochar rates on wheat photosynthetic rate sown in soil contaminated with different levels of cadmium**

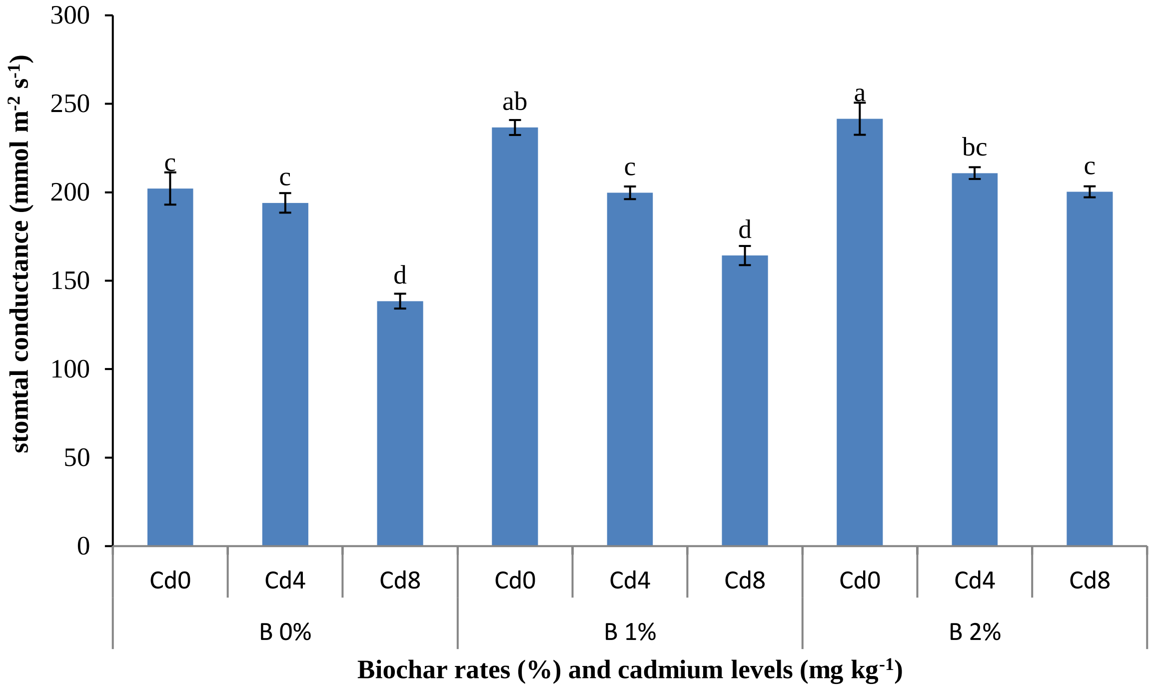
### 3.3.2 Stomatal conductance

Effect of different cotton stick biochar (CSB) on stomatal conductance of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.3.2. Stomatal conductance of wheat was significantly decreased in Cd contaminated soil and minimum stomatal conductance (138 mmol $m^{-2}$ $g^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly enhanced the stomatal conductance in Cd contaminated soil and maximum stomatal conductance (200 mmol $m^{-2}$ $g^{-1}$) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$. Thus, 2.0 % CSB addition significantly increased the stomatal conductance of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 31 mmol $m^{-2}$ $g^{-1}$ as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

### 3.3.3 Water use efficiency

Effect of different cotton stick biochar (CSB) rates on water use efficiency (WUE) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig.3.3.3. Water use efficiency was significantly decreased in Cd contaminated soil and minimum WUE (3 mmol $CO_2$ $mol^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly enhanced the water use efficiency in Cd contaminated soil and maximum WUE (4 mmol $CO_2$ $mol^{-1}$) was observed in treatment where 2.0 % CS (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased the WUE of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 28 mmol $CO_2$ $mol^{-1}$ as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

.




**3.3.2: Effect of different biochar rates on wheat stomatal conductance sown in soil contaminated with different levels of cadmium**

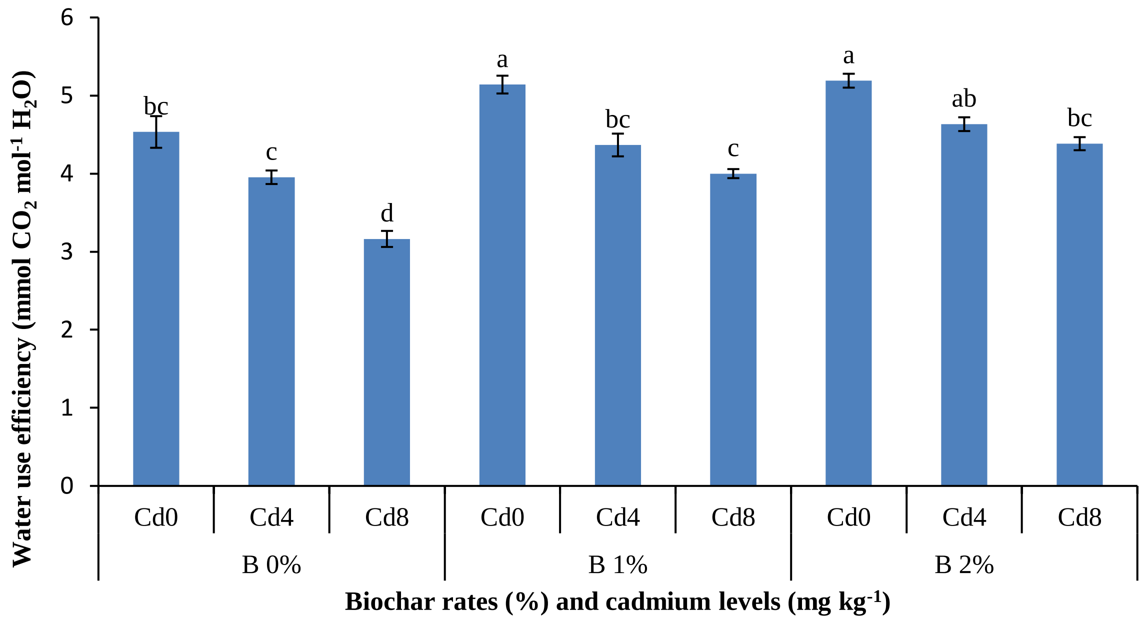



**Fig. 3.3.3: Effect of different biochar rates on water use efficiency of wheat sown in soil contaminated with different levels of cadmium**

### 3.3.4 Vapor pressure deficit

Effect of different cotton stick biochar (CSB) rates on vapor pressure deficit (VPD) of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.3.4. Vapor pressure deficit was significantly increased in Cd contaminated soil and maximum VPD (4 KPa) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly decreased the vapor pressure deficit and minimum VPD (3 KPa) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the VPD of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 19 Kpa as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

## 3.4 Plant analysis

### 3.4.1 Shoot cadmium concentration

Effect of different cotton stick biochar (CSB) rates on shoot cadmium (Cd) concentration of wheat seed sown in soil contaminated with different levels of Cd is presented in Fig. 3.4.1. Wheat shoot Cd concentration was significantly increased in Cd contaminated soil and maximum shoot Cd concentration (222 µg $kg^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly decreased the wheat shoot Cd concentration in cadmium contaminated soil and minimum shoot Cd concentration (44 µg $kg^{-1}$) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the shoot cadmium concentration of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 80 µg $kg^{-1}$ as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

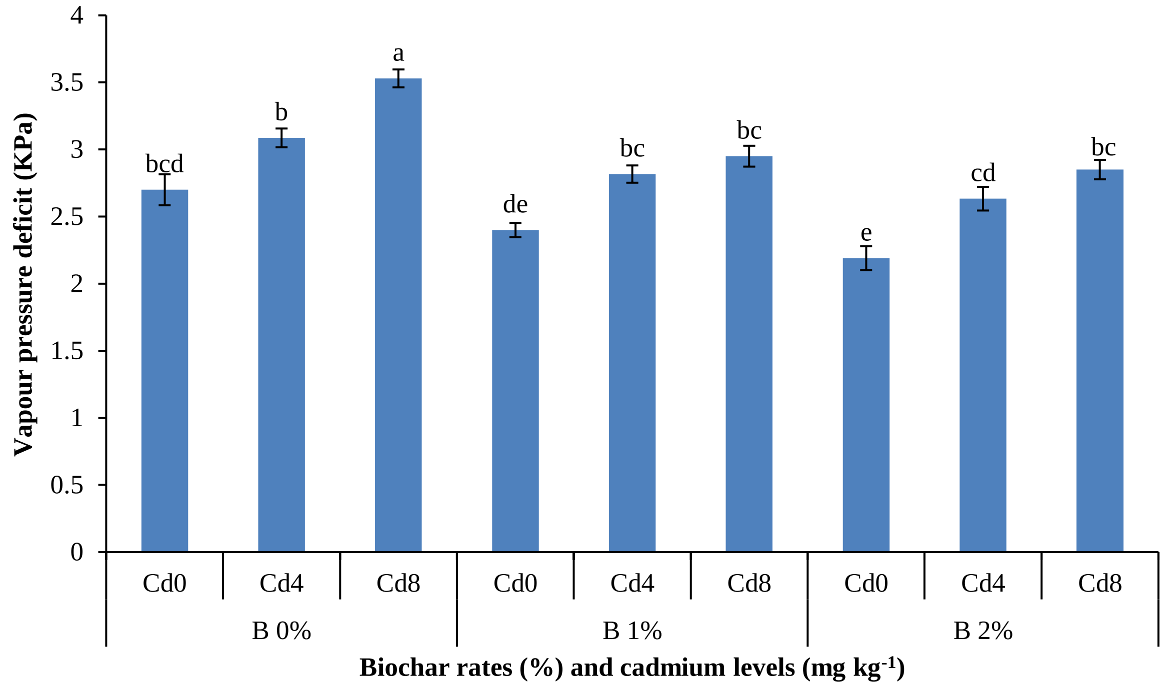



**Fig. 3.3.4: Effect of different biochar rates on vapor pressure deficit of wheat sown in soil contaminated with different levels of cadmium**

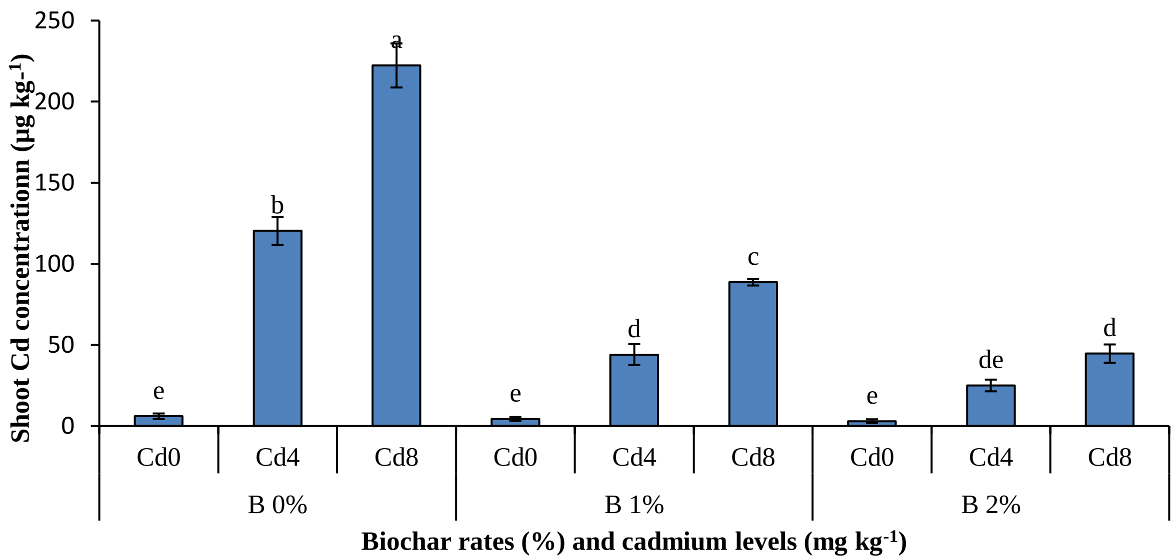



**Fig. 3.4.1: Effect of different biochar rates on wheat shoot Cd concentration sown in soil contaminated with different levels of cadmium**

### 3.4.2 Root cadmium concentration

Effect of different cotton stick biochar (CSB) rates on root cadmium (Cd) concentration of wheat seed sown in soil contaminated with different levels of Cd is presented in Fig. 3.4.2. Root cadmium concentration of wheat was significantly increased in Cd contaminated soil and maximum root cadmium (322 µg $kg^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly decreased the root cadmium concentration in Cd contaminated soil and minimum root Cd concentration (95 µg $kg^{-1}$) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the root cadmium concentration of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 71 µg $kg^{-1}$ as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

### 3.4.3 Translocation factor of cadmium from root to shoot

Effect of different cotton stick biochar (CSB) rates on translocation factor of cadmium (Cd) from wheat root to shoot sown in soil contaminated with different levels of Cd is presented in Fig. 3.4.3. Translocation factor of Cd from wheat root to shoot was significantly increased in Cd contaminated soil and maximum translocation factor of Cd from root to shoot (1) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly decreased the translocation factor of Cd from root to shoot and minimum translocation factor of Cd from root to shoot (0) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the translocation factor of Cd from root to shoot of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 31 as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

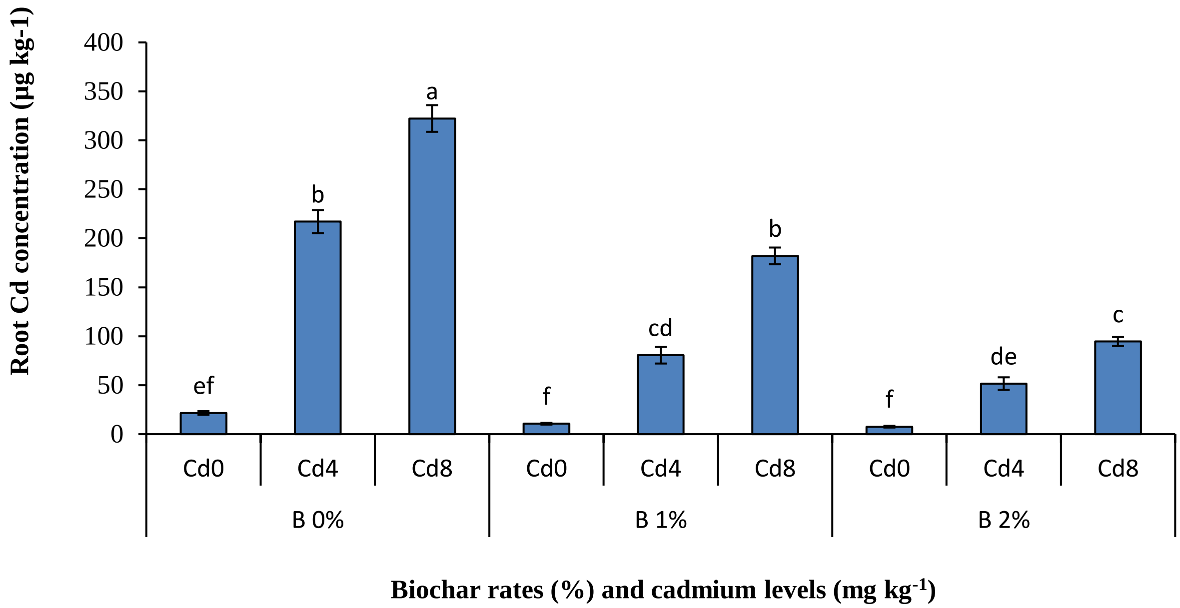



**Fig. 3.4.2: Effect of different biochar rates on wheat root Cd concentration sown in soil contaminated with different levels of cadmium**

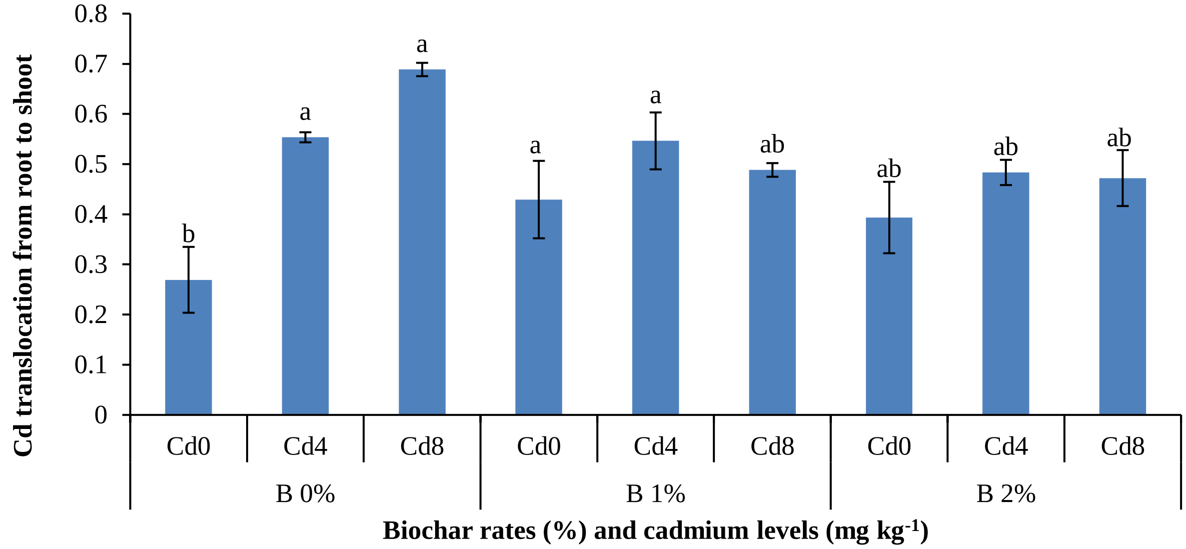



**Fig. 3.4.3: Effect of different biochar rates on translocation of cadmium from wheat root to shoot sown in soil contaminated with different levels of cadmium**

### 3.4.4 Lipid per oxidation

Effect of different cotton stick biochar (CSB) rates on Malondaldehyde (MDA) concentration of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.4.4. Malondaldehyde concentration of wheat was significantly increased in Cd contaminated soil and maximum MDA concentration (0.177 µmol MDA $g^{-1}$ FW) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB in soil significantly decreased the Malondaldehyde concentration and minimum MDA concentration (0.141 µmol MDA $g^{-1}$ FW) was observed in treatment where 2.0% CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0% CSB addition significantly decreased the MDA concentration of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 20 µmol MDA $g^{-1}$ FW as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

### 3.4.5 Total antioxidant activity

Effect of different cotton stick biochar (CSB) rates on total antioxidant activity (TAOA) in wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.4.5. Total antioxidant activity in wheat was significantly increased in Cd contaminated soil and maximum TAOA (9) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly decreased the total antioxidant activity of wheat in Cd contaminated soil and minimum TAOA (6) was observed in treatment where 2.0 % CSB (w/w of soil) was added in the soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the TAOA of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 35 as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

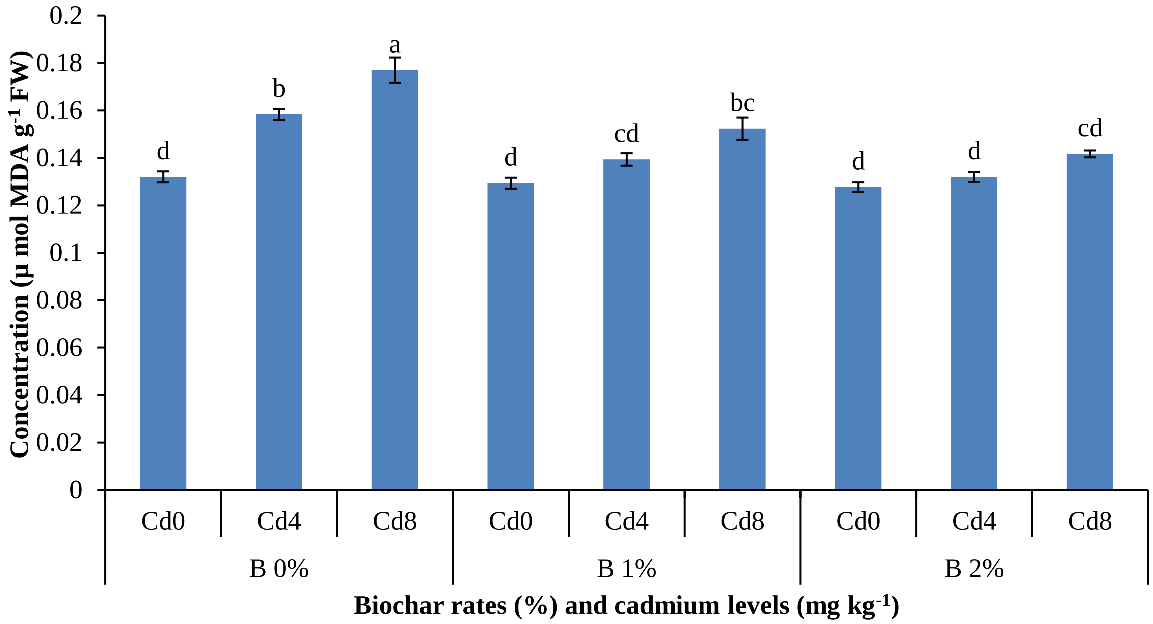



**Fig. 3.4.4: Effect of different biochar rates on wheat MDA concentration sown in soil contaminated with different levels of cadmium**

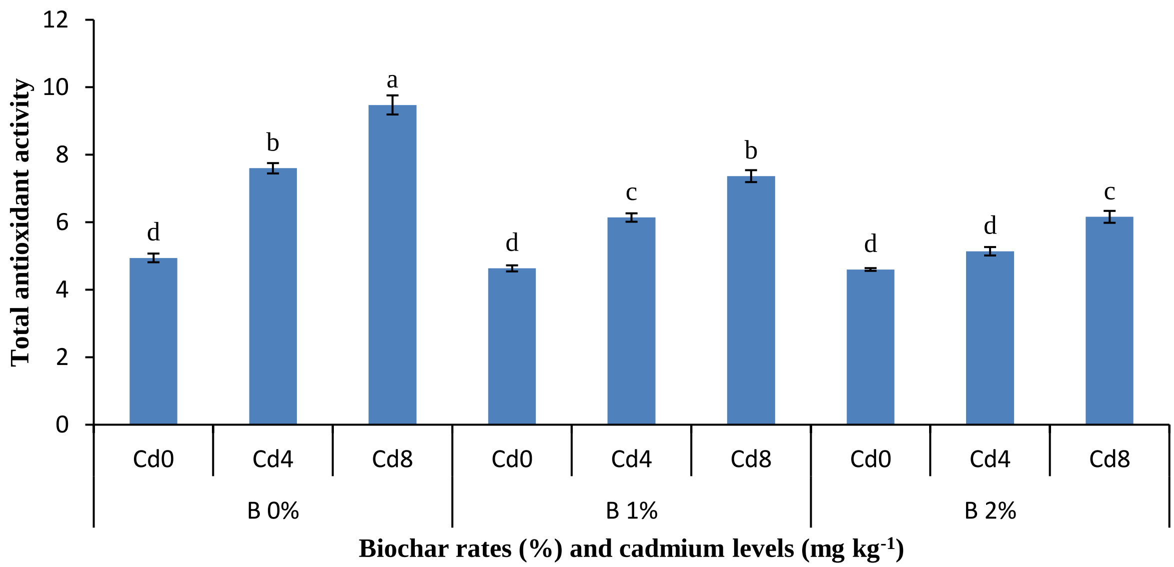



**Fig. 3.4.5: Effect of different biochar rates on total antioxidant activity in wheat sown in soil contaminated with different levels of cadmium**

### 3.4.6 Electrolyte leakage

Effect of different cotton stick biochar (CSB) rates on electrolyte leakage of wheat seed sown in soil contaminated with different levels of cadmium (Cd) is presented in Fig. 3.4.6. Electrolyte leakage of wheat was significantly increased in Cd contaminated soil and maximum electrolyte leakage (55 %) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly decreased the electrolyte leakage in Cd contaminated soil and minimum electrolyte leakage (35 %) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the electrolyte leakage of wheat in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 35 % as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

## 3.5 Soil analysis after harvesting

### 3.5.1 Total soil cadmium concentration

Effect of different cotton stick biochar (CSB) rates on total concentration of cadmium in soil contaminated with different levels of cadmium (Cd) after harvesting of wheat is presented in Fig. 3.5.1. Addition of CSB in soil showed non-significant effect on total soil cadmium concentration in soil contaminated with different levels of Cd.

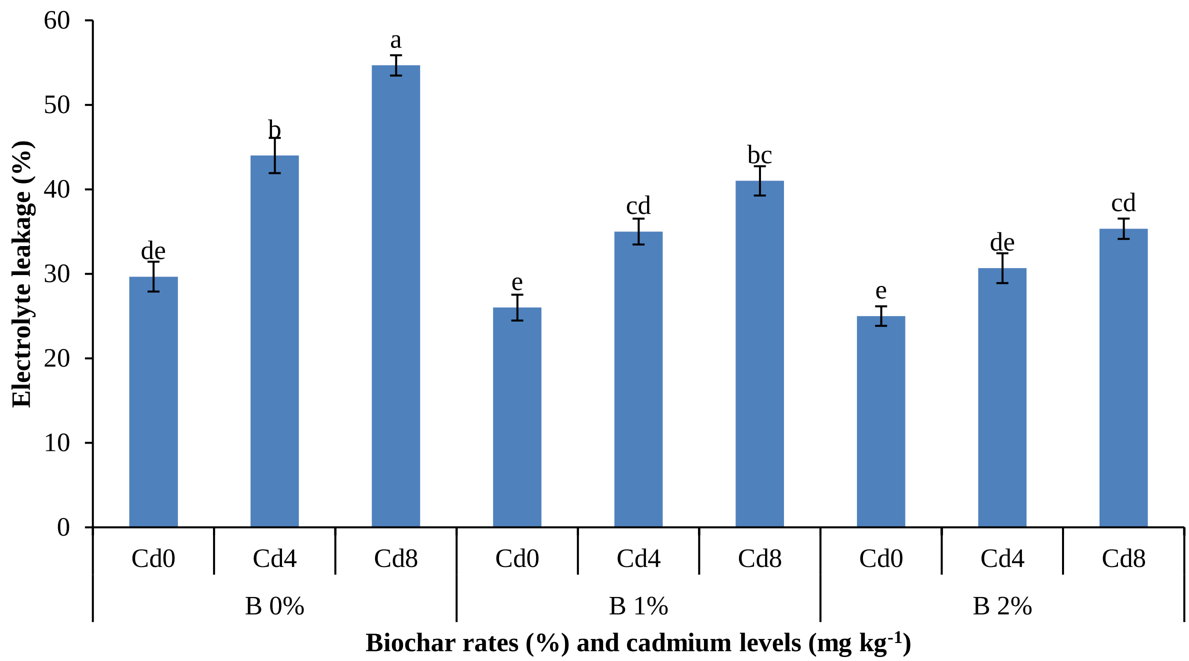



**Fig. 3.4.6: Effect of different biochar rates on electrolyte leakage of wheat sown in soil contaminated with different levels of cadmium**

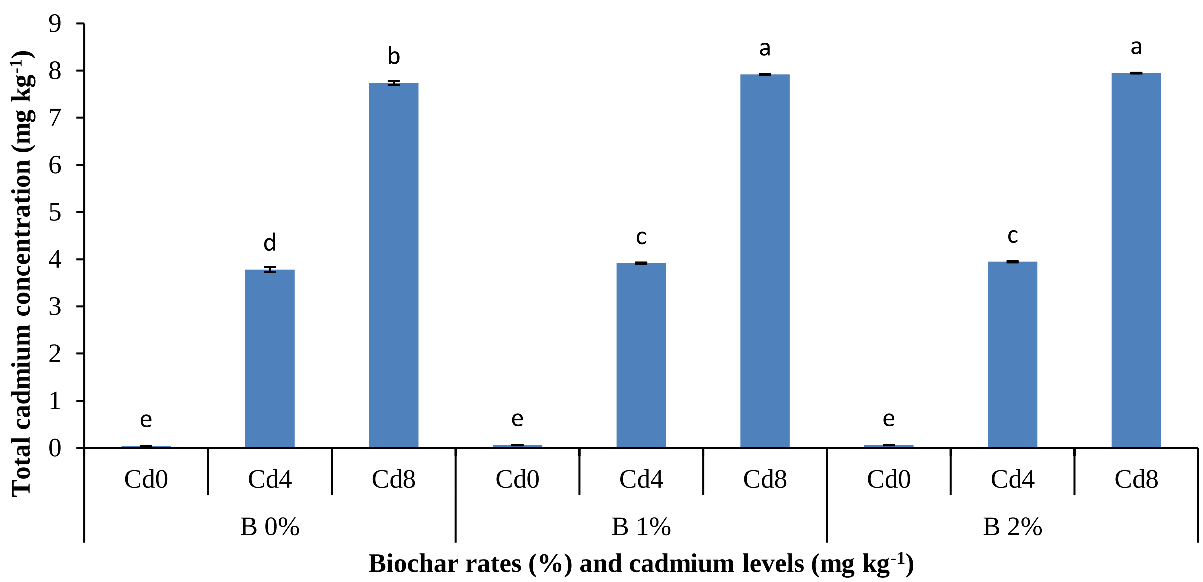



**Fig. 3.5.1: Effect of different biochar rates on total Cd concentration in soil contaminated with different levels of cadmium after wheat harvesting**

### 3.5.2 Available soil cadmium concentration

Effect of different cotton stick biochar (CSB) rates on available concentration of cadmium (Cd) in soil contaminated with different levels of cadmium after harvesting of wheat is presented in Fig. 3.5.2. Available soil cadmium concentration was significantly increased in Cd contaminated soil and maximum available soil cadmium concentration (6 mg $kg^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly decreased the available cadmium concentration in Cd contaminated soil and minimum available soil cadmium concentration (2 mg $kg^{-1}$) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the available cadmium concentration in soil contaminated with Cd (8.0 mg $kg^{-1}$ w/w of soil) up to 61 mg $kg^{-1}$ as compared with where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

**3.5.3 Translocation factor**

Effect of different cotton stick biochar (CSB) on translocation factor of cadmium (Cd) in soil contaminated with different levels of Cd after wheat harvesting is presented in Fig. 3.5.3. Translocation factor of cadmium was significantly increased in Cd contaminated soil and maximum translocation factor of Cd (0.02) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly decreased the translocation factor in Cd contaminated soil and minimum translocation factor of Cd in soil (0) was observed in treatment where 2.0% CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly decreased the translocation factor in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 80 as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

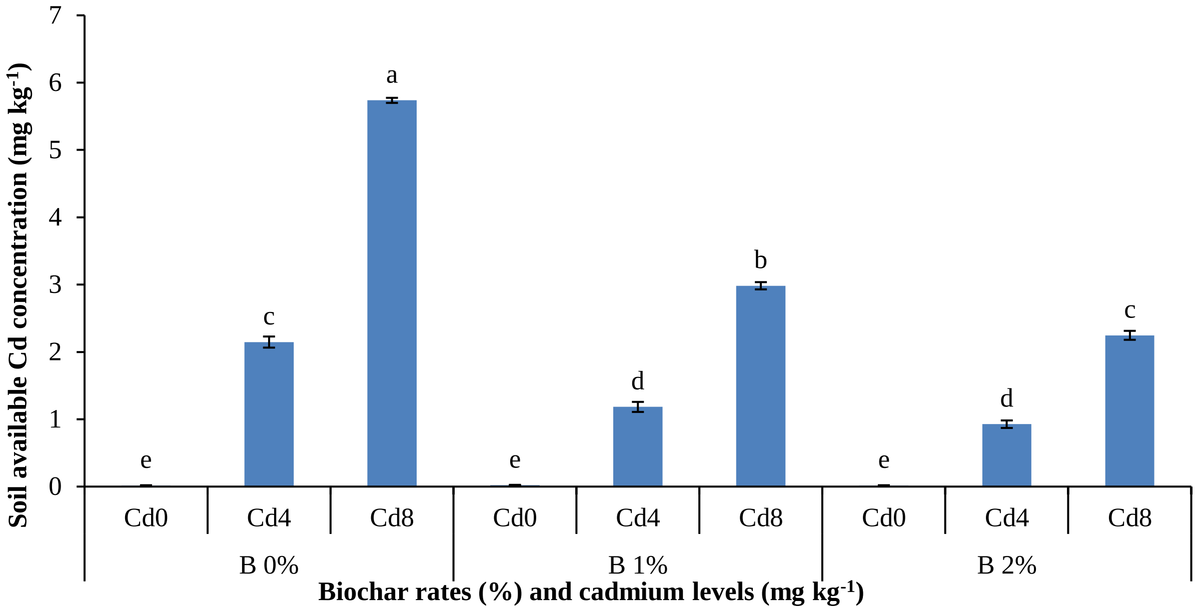



**Fig. 3.5.2: Effect of different biochar rates on available Cd concentration in soil contaminated with different levels of cadmium after wheat harvesting**

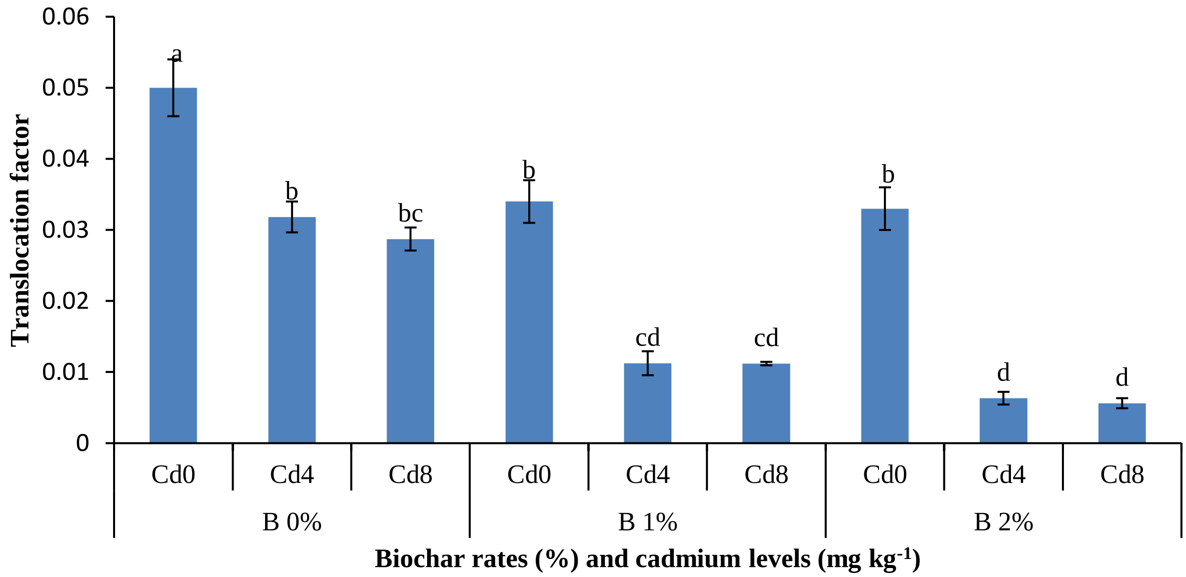



**Fig. 3.5.3: Effect of different biochar rates on translocation factor of Cd in wheat sown in soil contaminated with different levels of cadmium**

### 3.5.4 Soil pH

Effect of different cotton stick biochar (CSB) rates on soil pH contaminated with different levels of cadmium (Cd) after wheat harvesting is presented in Fig. 3.5.4. Addition of CSB (w/w of soil) showed non-significant effect on pH of soil contaminated with Cd.

### 3.5.5 Total organic carbon

Effect of different cotton stick biochar (CSB) rates on soil total organic carbon (TOC) contaminated with different levels of cadmium (Cd) after wheat harvesting is presented in Fig. 3.5.5. Total organic carbon in soil was significantly decreased in Cd contaminated soil and minimum TOC (4 g $kg^{-1}$) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly enhanced the total organic carbon in Cd contaminated soil and maximum TOC (7 g $kg^{-1}$) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased the TOC in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 34 g $kg^{-1}$ as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

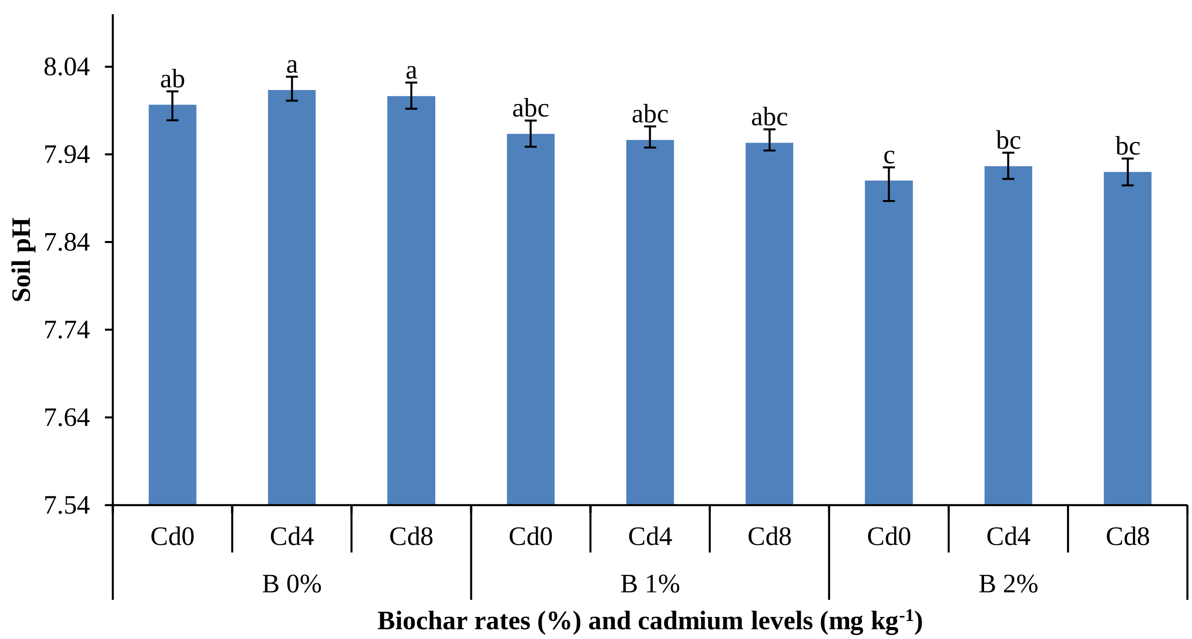


**Fig. 3.5.4: Effect of different biochar rates on soil pH contaminated with different levels of cadmium after wheat harvesting**

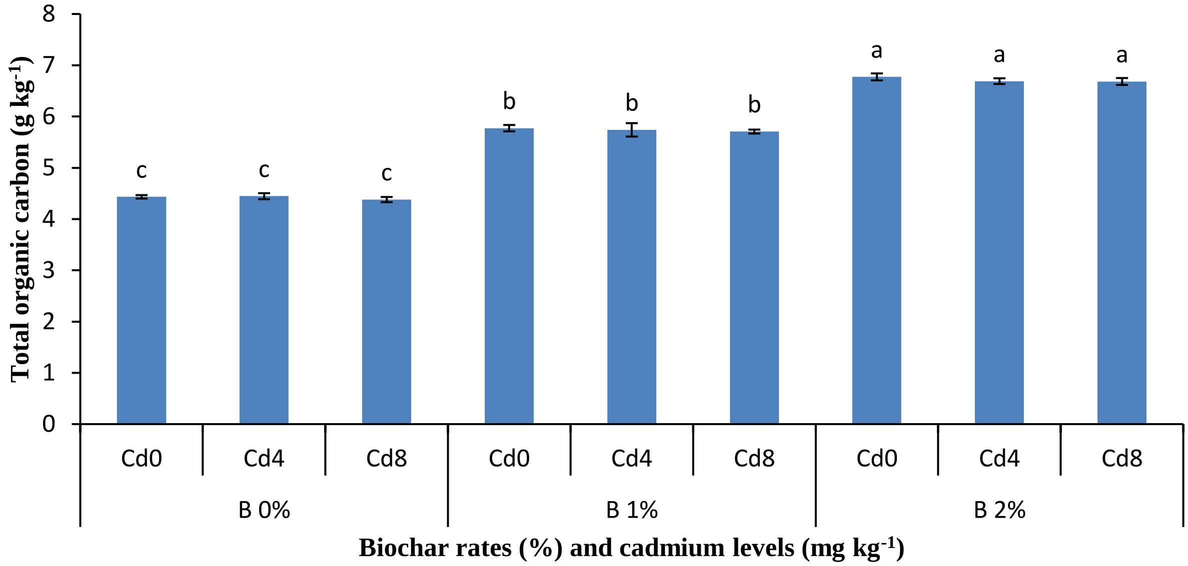



**Fig. 3.5.5: Effect of different biochar rates on soil TOC contaminated with different levels of cadmium after wheat harvesting**

### 3.5.6 Cation exchange capacity

Effect of different cotton stick biochar (CSB) rates on soil cation exchange capacity (CEC) contaminated with different levels of cadmium (Cd) after wheat harvesting is presented in Fig. 3.5.6. Soil cation exchange capacity was significantly decreased in Cd contaminated soil and minimum CEC (14) was observed where the soil was spiked with 8.0 mg $kg^{-1}$ Cd. Addition of CSB significantly enhanced the soil cation exchange capacity in Cd contaminated soil and maximum CEC (23) was observed in treatment where 2.0 % CSB (w/w of soil) was added in soil spiked with 8.0 mg $kg^{-1}$ Cd. Thus, 2.0 % CSB addition significantly increased the soil CEC in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil) up to 39 as compared with control where no CSB was added in Cd contaminated soil (8.0 mg $kg^{-1}$ w/w of soil).

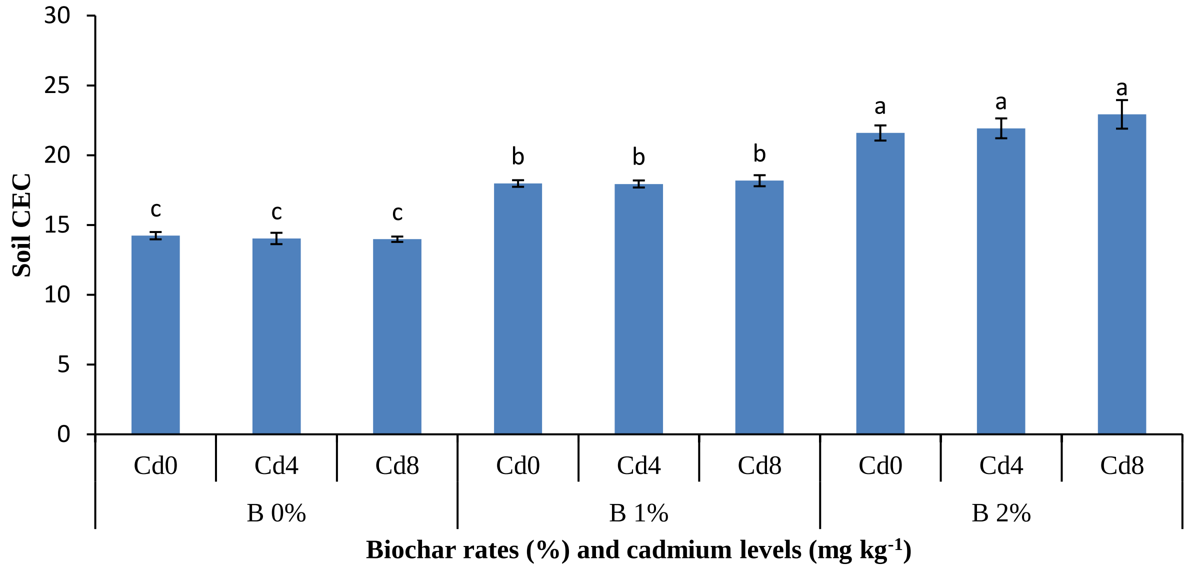



**Fig. 3.5.6: Effect of different biochar rates on soil CEC contaminated with different levels of cadmium after wheat harvesting**

## 4. Discussion

Heavy metal contamination is serious environmental problem in Pakistan and as well as in other developing countries. Cadmium (Cd) is un-needed heavy metal and very harmful to all living organisms including animals, plants and human beings. Although many techniques have been used for the remediation of metal polluted soils and these techniques include (1) chemical stabilization (2) replacement of polluted surface soils with un-contaminated soils (3) wrapping the polluted surface soils with normal contamination free soils (4) on site chemical leaching with acidifying agents (5) deep ploughing (6) phyto-remediation. But these techniques have high operational cost, sludge disposal problems and short-term effects. Therefore, this study low-cost bio-sorbent biochar was used to remediate and immobilize the Cd in soil that was spiked with cadmium. In acidic Ultisol through the formation of precipitates biochar enhanced immobilization of heavy metals and increased the specific adsorption of the metals (Jiang et al., 2012). In metal immobilization biochar application was effective, therefore phyto-toxicity and bioavailability of heavy metals reduced (Park et al., 2011).

For this purpose, a pot experiment was conducted to check the effect of biochar on wheat growth and germination in Cd-contaminated soil. Effect of cotton stick biochar (CSB) on physiological, agronomic, chemical and biochemical parameters of wheat in control and Cd-contaminated soil was also observed. Which rate of biochar (1 % or 2 % w/w of soil) shows the maximum wheat growth and germination in Cd contaminated soil was also observed.

However, in the present study effect of CSB amended soil is more efficient as compared to control in which no CSB was added in soil. Performance of CSB at the rate of (2.0 % w/w of soil) was best among all treatments. In the contaminated soils, biochar can stabilize the heavy metals, due to which quality of contaminated soil gets better (Ippolito et al., 2012) and significantly reduces the crop uptake of heavy metals. Therefore, for remediation of the heavy metals contaminated soils biochar application can potentially give a new solution

In the present study germination parameters like germination %age and mean emergence time of wheat was observed. Addition of CSB (2 % w/w of soil) significantly increased the germination % age of wheat in Cd-contaminated soil as compared to control where no CSB was added in soil. Similar results have been observed in sunflower using different types of biochar produced from five agricultural and forestry wastes (Alburquerque et al., 2013). As in our case CSB increased the wheat growth in Cd contaminated soil through immobilizing the cadmium and improving the soil properties. Biochar is being used for heavy metals immobilization in soil due to its high surface area (> 400-1500 $m^2$ $g^{-1}$), pH of biochar (wide range of pH value from 6 to 10), high CEC (> 40 cm $mole_c$ $kg^{-1}$), long term stability (10-100 times of organic matter) and functional groups present on its surface especially oxygen containing functional groups (Uchimiya et al., 2011a). Biochar application either alone or in combination with farmyard manure (FYM) or mineral nitrogen enhanced yield or yield components of wheat (Ali et al., 2015).

Different agronomic parameters like shoot length, root length, shoot fresh biomass; root fresh biomass, shoot dry biomass and root dry biomass were observed. In this present study addition of CSB significantly increased these parameters as compared to control treatments where no CSB was added in cadmium contaminated soil. Similar results were observed by Shen et al. (2016). Both heavy metal free biochar (FC) and heavy metal rich biochar (RC) improved plant growth. After the application of FC and RC in yellow brown soil (YBS) leaves, stem and root dry weights significantly decreased.

Different physiological parameters like photosynthetic rate, stomatal conductance, water use efficiency and vapor pressure deficit was observed. In this present study photosynthetic rate, stomatal conductance and water use efficiency significantly increased in treatments where 2 % CSB was added in Cd contaminated soil as compared to control where no CSB was added in soil spiked with cadmium. Similar results were observed by Akhtar et al. (2015), where biochar addition had significantly positive effect on photosynthetic rate and stomatal conductance. Anten (2005) explained that improvement in photosynthetic rate has a significant impact on crop growth and dry matter. Improvement in chlorophyll content indicates the improved nutrient availability and vigorous plant growth in citrus peel biochar (CPB) amended treatments.

In current study addition of 2 % CSB significantly decreased the root and shoot cadmium (Cd) concentration as compared to control where no CSB was added in soil. A soil contaminated with Cd and Zn was amended with a hardwood-derived biochar and the concentration of both metals in pore water reduced (Beesley et al., 2010). Choppala et al. (2012) proved that when chromate Cr (VI) contaminated soils amended with chicken manure derived biochar then it enhanced the reduction of mobile Cr (VI) to less mobile Cr (III), so leaching of Cr decreased. The decrease in the leaching of Cr (III) is attributed to the adsorption of Cr (III) on cation exchange sites and precipitated as $Cr\ (OH)_3$ resulting from the release of $OH^-$ ions during the Cr (VI) reduction process. Cd, Cu, and Pb uptake by Indian mustard significantly decreased by using the chicken manure and green waste-derived biochar. The study also concluded that by increasing the biochar application rates, the plant metal concentration increased except for Cu concentration (Park et al., 2011). Biochar treatments as compared to sewage sludge treatments reduced the plant availability of Cd, Ni, Zn and Pb (Méndez et al., 2012). As in our case, CSB significantly reduced the mobility of cadmium from soil to roots and shoots through adsorption.

It is also concluded that addition of CSB (2.0 %) significantly decreased the available soil cadmium concentration as compared to control where no CSB was added in Cd contaminated soil. Results are related with the result of Zhou et al. (2008) who stated that biochar derived from cotton stalk can decrease the bioavailability of soil Cd through adsorption or co-precipitation. There is also a decrease in soil pH by the addition of CSB (2.0 %) as compared to control without CSB. Because cotton stick biochar has high pH therefore its impact on soil pH was lower. Cheng et al. (2006) proved that soil pH reduces, with the application of biochar.

Carbon contents of biochar are present in highly stable form (Shenbagavalli and Mahimairaja, 2012). It is well known that soil organic matter plays important roles in both soil fertility and the global C balance. Increased soil organic matter can thus sequester C and dramatically enhances SOC content (Lal, 2004; Lehmann and Gaunt 2006; Kuzyakov et al., 2009).

In our case, addition of CSB (2.0 %) also significantly increased the TOC in soil as compared to control treatments where no CSB was added in Cd contaminated soil.

**References**


1. Ahmad, M., A.U. Rajapaksha, J.E. Lim, M. Zhang, N. Bolan, D. Mohan, M. Vithanage, S.S. Lee and Y.S. Ok. 2014. Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99: 19-33.
2. Akbal, F. and S. Camci. 2011. Copper, chromium and nickel removal from metal plating wastewater by electro coagulation. Desalination 269: 214–222.
3. Akhtar, S.S., M.N. Andersenb and F. Liu. 2015. Residual effects of biochar on improving growth, physiology and yield of wheat under salt stress. Agricultural Water Management 158: 61–68.
4. Alburquerque, J.A., J.M. Calero1, V. Barrón, J. Torrent, M.C. del Campillo, A. Gallardo and R. Villar. 2013. Effects of biochars produced from different feedstocks on soil properties and sunflower growth. J. Plant Nutr. Soil Sci. 000: 1-10.
5. Ali, K., M. Arif, M. T. Jan, T. Yaseen, M. Waqas and F. Munsif. 2015. Biochar: a novel tool to enhance wheat productivity and soil fertility on sustainable basis under wheat-maize-wheat cropping pattern. Pak. J. Bot. 47: 1023-1031.
6. Anten, N.P.R. 2005. Optimal photosynthetic characteristics of individual plants in vegetation stands and implications for species coexistence. Ann. Bot. 95: 495–506.
7. Beesley, L., E. Moreno-Jiménez and J.L. Gomez-Eyles. 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ Pollut. 158: 2282–2287.
8. Beesley, L., E. Moreno-Jiménez, J.L. Gomez-Eyles, E. Harris, B. Robinson and T. Sizmur. 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut. 159: 3269–3282.
9. Boudrahem, F., A. Soualah and F. Aissani-Benissad. 2011. Pb(II) and Cd(II) removal from aqueous solutions using activated C developed from coffee residue activated with phosphoric acid and zinc chloride. J. Chem. Eng. Data 56: 1946–1955.
10. Caporale, A.G., A. Sommella, M. Lorito, N. Lombardi, S.M. Azam, M. Pigna and M. Ruocco. 2014. Trichoderma spp. alleviate phytotoxicity in lettuce plants (Lactuca sativa L.) irrigated with arsenic-contaminated water. J. Plant Physiol. 171: 1378-1384.

11. Chan, K.Y., L.V. Zwieten, I. Meszaros, A. Downie and S. Joseph. 2007. Agronomic values of greenwaste biochar as a soil amendment. Aust. J. Soil Res. 45: 629–634.
12. Cheng, C.H., J. Lehmann, J.E. Thies, S.D. Burton and M.H. Engelhard. 2006. Oxidation of black carbon by biotic and abiotic processes. Organ Geochem. 37: 1477–1488.
13. Choppala, G.K., N.S. Bolan, M. Megharaj, Z. Chen and R. Naidu. 2012. The influence of biochar and black carbon on reduction and bioavailability of chromate in soils. J. Environ. Qual. 41: 1175–1184.
14. Di Salvatore, M., A.M. Carafa and G. Carratu. 2008. Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: a comparison of two growth substrates. Chemosphere 73: 1461-1464.
15. Farahat, E. and H.W. Linderholm. 2015. The effect of long-termwastewater irrigation on accumulation and transfer of heavy metals in Cupressus sempervirens leaves and adjacent soils. Sci. Total Environ. 512-513: 1-7.
16. Ippolito, J.A., D.A. Laird and W.J. Busscher. 2012. Environmental benefits of biochar. J. Environ. Qual. 41: 967–972.
17. Jiang, J., R. Xu, T. Jiang and Z. Li. 2012. Immobilization of Cu (II), Pb (II) and Cd (II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. J. Hazard. Mater. 229–230: 145–150
18. Khan, A., S. Khan, M.A. Khan, Z. Qamar and M. Waqas. 2015a. The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environ. Sci. Pollut. Res. 22: 13772-13799.
19. Khan, S., B.J. Reid, G. Li and Y.G. Zhu. 2014. Application of biochar to soil reduces cancer risk via rice consumption: A case study in Miaoqian village, Longyan, China. Environ. Int. 68: 154-161.
20. Kuzyakov, Y., I. Subbotina, H.Q. Chen, I. Bogomolova and X.L. Xu. 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by $^{14}C$ labeling. Soil Biol. Biochem.41: 210–219.
21. Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Sci. 304: 1623–1627.
22. Lehmann, J. and J. Gaunt. 2006. Bio-char sequestration in terrestrial ecosystems. Mitigation Adapt. Strateg. Glob. Chang. 11: 395–419.

23. Lehmann, J., J.P. da Silva Jr, C. Steiner, T. Nehls, W. Zech and B. Glaser. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249: 343–357.
24. Lu, H., Y.Y. Zhang, X. Huang, S. Wang and R. Qiu. 2012. Relative distribution of $Pb^{2+}$ sorption mechanisms by sludge-derived biochar. Water Res. 46: 854–862.
25. Malamis, S., E. Katsou and K.J. Haralambous. 2011. Study of Ni(II), Cu(II), Pb(II), and Zn(II) removal using sludge and minerals followed by MF/UF. Water, Air, Soil Pollut. 218: 81–92.
26. Méndez, A., A. Gómez, J. Paz-Ferreiro and G. Gascó. 2012. Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere 89: 1354–1359.
27. Monteiro, M., C. Santos, R.M. Mann, A.M.V.M. Soares and T. Lopes. 2007. Evaluation of cadmium genotoxicity in Lactuca sativa L. using nuclear microsatellites. Environ. Exp. Bot. 60: 421-427.
28. Nelson, D.W. and L.E. Sommers. 1982. Total C, organic C and organic matter. In: Methods of Soil Analysis, part 2: Chemical and Microbiological Properties. A. Klute (eds.). Am. Soc. Agron. Madison WI. pp. 570-571.
29. Park, J.H., G.H. Choppala, S.J. Lee, N. Bolan, J.W. Chung and M. Edraki. 2013. Comparative sorption of Pb and Cd by biochars and its implication for metal immobilization in soil. Water Air Soil Pollut. 224, 1711, doi:10.1007/s11270-013-1711-1.
30. Park, J.H., G.K. Choppala, N.S. Bolan, J.W. Chung and T. Chuasavathi. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348: 439–451.
31. Sanchez, M.E., E. Lindao, D. Margaleff, O. Martınez and A. Moran. 2009. Pyrolysis of agricultural residues from rape and sunflowers: Production and characterization of bio-fuels and biochar soil management. J. Anal. Appl. Pyrol. 85: 142–144.
32. Shenbagavalli, S. and S. Mahimairaja. 2012. Characterization and effect of biochar on nitrogen and carbon dynamics in soil. Int. J. Agri. Biol. Res. 2: 249–255.
33. Stiborova, M. 1988. $Cd^{2+}$ ions effect on the quaternary structure of ribulose-1,5- bisphosphate carboxylase from barley leaves. Biochemie und Physiologie der Pflanzen 183: 371–378.
34. Sud, D., G. Mahajan and M.P. Kaur. 2008. Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions – A review. Bioresour. Technol. 99: 6017-6027.

35. Uchimiya, M., I.M. Lima, K.T. Klasson and L.H. Wartelle. 2010b. Contaminant immobilization and nutrient release by biochar soil amendment: roles of natural organic matter. Chemosphere 80: 935–940.
36. Uchimiya, M., I.M. Lima, K.T. Klasson, S.C. Chang, L.H. Wartelle and J.E. Rodgers. 2010b. Immobilization of heavy metals ions (Cu, Cd, Ni, and Pb) by broiler litter-derived biochars in water and soil. J. Agric. Food Chem. 58: 5538-5544.
37. Uchimiya, M., L.H. Wartelle, K.T. Klasson, C.A. Fortier and I.M. Lima. 2011b. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. J. Agric. Food Chem. 59: 2501–2510.
38. Uchimiya, M., M. Isabel, K. Lima, T. Klasson and L.H. Wartelle. 2010a. Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. Chemosphere 80: 935–940.
39. Uchimiya, M., S.C. Changand and K.T. Klasson. 2011a. Screening biochars for heavy metal retention in soil: Role of oxygen functional groups. J. Hazard. Mater. 190: 432–444.
40. US Salinity Lab. Staff. 1954. Diagnosis and improvement of saline and alkali soils. USDA Handbook No. 60. Washington DC. USA.
41. Wolf, B. 1982. The comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plant Anal. 13: 1035–1059.
42. Wu, W., M. Yang, Q. Feng, K. Mcgrouther, H. Wang, H. Lu and Y. Chen. 2012. Chemical characterization of rice straw-derived biochar for soil amendment. Biomass Bioenergy 47: 268–276.
43. Zhou, J.B., C.J. Deng, J.L. Chen and Q.S. Zhang. 2008. Remediation effects of cotton stalk carbon on cadmium (Cd) contaminated soil. Ecol. Environ. 17: 1857–1860.