Efficiency of different types of biochars to mitigate Cd stress and growth of sunflower (*Helianthus; L.*) in wastewater irrigated agricultural soil

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**Abstract**

Cadmium contamination in croplands is recognized one of the major threat, seriously affecting soil health and sustainable agriculture around the globe. Cd mobility in wastewater irrigated soils can be curtailed through eco-friendly and cost effective organic soil amendments (biochars) that eventually minimizes its translocation from soil to plant. This study explored the possible effects of various types of plants straw biochar as soil amendments on cadmium (Cd) phytoavailability in wastewater degraded soil and its subsequent accumulation in sunflower tissues. The studied biochars including rice straw (RS), wheat straw (WS), acacia (AC) and sugarcane bagasse (SB) to wastewater irrigated soil containing Cd. Sunflower plant was grown as a test plant and Cd accumulation was recorded in its tissues, antioxidant enzymatic activity, chlorophyll contents, plant biomass, yield and soil properties (pH, NPK, OM and Soluble Cd) were also examined. Results revealed that addition of biochar significantly minimized Cd mobility in soil by 53.4%, 44%, 41% and 36% when RS, WS, AC and SB were added at 2% over control. Comparing the control soil, biochar amended soil effectively reduced Cd uptake via plants shoots by 71.7%, 60.6%, 59% and 36.6% when RS, WS, AC and SB. Among all the biochar, rice husk induced biochar significantly reduced oxidative stress and reduced SOD, POD and CAT activity by 49%, 40.5% and 46.5% respectively over control. In addition, NPK were significantly increased among all the added biochars in soil–plant system as well as improved chlorophyll contents relative to non-bioachar amended soil. Thus, among all the amendments, rice husk and wheat straw biochar performed well and might be considered the suitable approach for sunflower growth in polluted soil.

**Keywords:**
- Cadmium
- Biochar
- Sunflower
- Antioxidant enzymes
- Wastewater irrigation

**1. Introduction**

The lack of good quality water and use of industrial effluents as the irrigation source is considered harmful for the productive land and food security. The farmlands contamination with PTEs is widely shared by the excessive mining of mineral resources, industrial products and the use of chemicals as agricultural inputs (Bashir et al., 2020a, 2020b, 2020c). Among the heavy metals, cad-
mium is mobile and neurotoxic pollutant which imposes serious threats to soil ecosystem and causes disorder in plants species. Cd accumulation in plants weakens the plants defence system resulting in disturbing the morphology, physiology such as antioxidant activity, photosynthesis and nutrients uptake (Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h). Unlike to other pollutants, Cd is more persistent and non-degradable in nature once introduced into the soil environment.

Sunflower (*Helianthus; L.*) is grown as a food and bioenergy product and belongs to Asteraceae family. Its dry biomass has a major contribution as an industrial product. It has important agronomic features such as extreme climate change adaptation and soils variations and is considered an important environmental clean-up crop (Rizwan et al., 2016). It is fast growing plant and has ability to remove contaminants from polluted soil and water. Accumulation and translocation of heavy metals in sunflower roots varies with soil properties, metals concentration and its cultivars (Cornu et al., 2016). Previous study observed by Junior et al. (2016) indicated that arsenic (As) and cadmium (Cd) effectively reduce the seed germination rate in four different cultivars of sunflower. It has great potential to translocate heavy metals form soil to edible parts via roots.

Therefore, several soil restoration strategies have been developed to ameliorate the PTEs polluted farmland including phytoremediation, soil washing, excavation and immobilization. It has been observed that the phytoavailable concentration is becoming a threat rather than total metal concentration in the soil. Hence, it is an important task to minimize the translocation of phytoavailable metal concentration to edible plant parts. In recent decades, biochar is widely recognized as a potential candidate to restore PTEs contaminated soils and useful option for soil fertility (Salam et al., 2019). It contains high carbon contents induced by thermal decomposition of organic biomass residues under limited hypoxia condition between (300–700 °C) (Bashir et al., 2019). Technically, it attains much attention because of the availability of excessive sources of organic residues, cost effective and eco-friendly nature on large-scale. Its efficiency has been proved both as an organic fertilizer and soil conditioner, it enhances soil pH, CEC and nutrients dynamics (Bashir et al., 2019), promote crop productivity (Ali et al., 2020) and reduced heavy metals mobility in contaminated soils (Rehman et al., 2019). Biochar is not only providing the solution of pollutants remediation, but also has ability to reuse the excessive quantity of agricultural wastes and has various application projections (Xu et al., 2020). Recent study reported by Cui et al. (2020) described that biochar pyrolyzed from wheat straw effectively enhances Cd immobilization and minimizes bioaccumulation because of its boundness with hydroxides, carbonates and organic substances of biochar. Another, recent study reported by Xu et al. (2020) suggested that the application of corn straw, peanut hull and kitchen waste derived biochar have potential to transform Cd and Pb into less available state and thereby declined their uptake by swamp cabbage.

While, various kinds of agricultural wastes and kitchen wastes induced biochar were applied for the stabilization of Cd, Pb and Ni in acidic polluted soils. However, there is no study conducted on the comparative efficiency of acacia, rice straw, wheat straw and sugarcane bagasse pyrolyzed biochar for wastewater irrigated Cd polluted alkaline soil and phytoavailability to sunflower. The prime objectives of the current study were, to examine the comparative role of studied biochar for Cd immobilization in soil and decreased its accumulation in sunflower tissues. In addition, to evaluate their efficiency for the defence mechanism of sunflower and its dry biomass as well as nutrients availability in sewage wastewater calcareous soil. Moreover, the surface functional groups of all used biochars were analysed using FTIR technique.

2. Materials and methods

Soil samples were taken from productive land in the suburb of Dera Ghazi Khan city, Pakistan that was irrigated with the wastewater of manka Canal. The polluted soil samples were collected from the upper layer at 0–30 cm depth in 2019. The soil was then shifted to the green house for air drying about three days. After drying, soil samples were ground and removed stones and pebbles then passed through 2-mm sieve for pot study. Some portion of prepared soil about 0.5 kg was further ground and passed through 0.25-mm sieve for the basic physico-chemical characteristics of the studied soil (Tab.1). Our previous study (Bashir et al., 2020a, 2020b, 2020c) explained that the studied soil of this area has more than 5 mg kg⁻¹Cd which is greater than the proposed value of Cd in Agricultural soils by the Chinese environmental and quality standard for soils (GB15618-1995). The selected physio-chemical properties of soil were given in the Table 1.

### 2.1. Biochar preparation and characterization

Several feedstock materials including rice straw, acacia, sugarcane bagasse and wheat straw were collected from Dera Ghazi Khan crop land areas. The biomass residues were washed with tap water to remove dust particles and impurities, and then chopped and ground into small pieces about 1 mm. The grinded residues were pyrolyzed at 400 ⁰C under limited oxygen supply for 2 hr according to our previous studies (Bashir et al., 2019a,b). The prepared biochar were meshed and passed from 0.05 mm sieve for pot study. The FTIR was used to analyze the surface properties of biochars amended soil. The selected chemical properties of all biochar were given in the Table 1.

### 2.2. Pot experiment

The sunflower (*Helianthus; L.*) growth experiment was performed to examine the influence of various kinds of biochar (rice straw, acacia, sugarcane bagasse and wheat straw) at 2% application rates on sunflower growth and Cd accumulation in its tissues. The pot study was arranged with the following treatments (1) Control (CK); (2) Rice straw biochar (RH); (3) Acacia (AC); (4) Sugarcane bagasse (SB); (5) Wheat straw (WS) and each treatment had three replicates. Each pot (16 cm diameter and 18 cm height) had 4 kg soil and homogeneously mixed with their respective biochar treatments. All the experimental units were irrigated with distilled water and kept their water contents at 70% water holding capacity and left them for 2 weeks for equilibrium. After 15 days, the sterilized 5 sunflower seeds were placed in each experimental unit. Each pot was fertilized with the basal dose of N (0.2 g kg⁻¹ soil), P (0.4 g kg⁻¹ soil) and K (0.3 g kg⁻¹ soil) in the form of urea, Ca(H₂PO₄)₂, and KCl respectively (Bashir et al., 2020a). After seed germination, 3 seedlings in each pot were maintained. After 4 months, the mature sunflower plants were harvested and the further plants and the soil analysis were performed.

### 2.3. Soil analysis

After the crop harvest, soil samples were taken from each pot and measured the soil pH and EC using pH and EC meter (Mettler Toledo Delta 320) and EC meter (DDS-307A), according to the described method in our previous study (Bashir et al., 2020a, 2020b, 2020c). Bioavailable Cd contents from each potted soil were estimated using the CaCl₂ extractable technique described by (Houben et al., 2013). After extraction and filtration, supernatant was analyzed for Cd concentration through atomic absorption spectroscopy (AAS) (AA-240FS Varian, USA). Soil organic matter
contests were calculated by using the wet oxidation method. Similarly, soil available nitrogen, phosphorous and potassium contents were estimated by (Lu, 2000).

2.4. Plant analysis

Fresh plant shoots samples were collected from each pot for antioxidant enzymatic activity including peroxidase (POD) as briefly described by Putter (1974) and superoxide dismutase (SOD) Beaucham and Fridovich (1971). While, chlorophyll contents were measured using the SPAD meter. However, the harvested portion of sunflower shoots and roots were oven dried and digested with di-acid HNO₃:HClO₄ mixture for the estimation of Cd contents and nutrients (NPK) as described in our previous study (Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h; 2019).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) followed by the LSD test were postulated at (P < 0.05) to analyze the data using Statistic 8.1 (Analytical software, USA). In addition, the means and standard deviations (SD) clearly indicated the statistically significant differences among all the treatments for each examined parameters.

3. Results and discussion

3.1. Effect of biochar on soil pH and Cd bioavailability

The incorporation of various kinds of biochars rice straw (RS), wheat straw (WS), acacia (AC) and sugarcane bagasse (SB) showed the significant alteration in soil pH and soil bioavailable Cd relative to without biochars amended soil (Fig. 1). The greater increase in soil pH was observed in rice straw derived biochar by 0.8 units relative to control. Similarly, the addition of WS, AC and SB induced biochar at 2% rate showed the increment in soil pH by 0.6, 0.4 and 0.3 units, relative to control soil.

While, the mixing of biochars in soil had the significant decline in soil bioavailable Cd contents against without biochar treated soil (Fig. 2). The maximum reduction was recorded in RS derived biochar by 53.4%, while the reduction was also observed when WS, AC and SB were added at 2% by 44%, 41% and 36% over without biochar treated soil, respectively.

3.2. Effect of biochar on soil organic matter and nutrients availability

The addition of biochar in polluted soil led to enhancing soil organic carbon contents over without biochar amended soil. The significant improvement in the soil carbon was recorded, when RS, WS, AC and SB biochars were incorporated at 2% rate by 35%, 25%, 19% and 14% relative to non-amended soil. Similarly, the bioavailable status of NPK nutrients efficiently improved in the biochars amended soil relative to non-amended soil (control) (Table 2). The prominent increase in the available-N was recorded by 36.6% when RS was added with polluted soil at 2% rate. Whereas, addition of WS, AC and SB at 2% dose level showed the prominent increment in the soil available-N by 25.4%, 20% and 14% respectively, over control soil. Available-K contents in biochar amended wastewater soil was prominently improved when RS, WS, AC and SB at 2% rate by 0.6%, 34.5%, 21.7% and 17.3% respectively, over control.

3.3. Effect of biochars on Cd uptake and antioxidant enzymatic activity

The results revealed that Cd accumulation in sunflower shoots and roots tissues was increased in wastewater polluted soil relative to biochars amended soil. However, addition of the various kinds of biochar has ability to reduce Cd accumulation in plant tissues (Fig. 3). The greater reduction in Cd accumulation in plant shoots was recorded by 71.7%, 60.6%, 59% and 36.6, when RS, AC and SB were added into wastewater polluted soil respectively, over control.
biochar incorporation also significantly reduced Cd accumulation in sunflower roots. The highest reduction was recorded by 67.2%, 53.5%, 44.9% and 31.6% after RS, WS, AC and SB were incorporated at 2% application level (Fig. 3).

The antioxidant enzymatic activity showed the different response among all the added biochars (RS, WS, AC and SB) in wastewater irrigated soil (Figs. 4 and 5). The enzymes SOD, POD and CAT showed the greater concentrations in the non-amended soil, while their contents were effectively declined in biochar amended soil–plant system. The greater reduction in enzymes activity was recorded in RS (49.2%, 40.5% and 46.5%), WS (42.80%, 33.5% and 40.4%), AC (37%, 33.2% and 32.19%) and SB (20%, 11.7% and 25.14%) for SOD, POD and CAT respectively over control (non-amended) soil.

3.4. Effect of biochars on chlorophyll contents and biomass

Results showed that the addition of various kinds of biochars in wastewater irrigated soil improved the soil health and plant growth. The chlorophyll contents was prominently improved in biochar amended soils relative to control soils (Table 3). The maximum increment in chlorophyll contents was recorded in RS, WS, AC and SB biochars amended soils by 33.9%, 28.6%, 11.11% and 18.84% at 2% application rate, respectively over control.

Sunflower fresh and dry shoot root biomass was also improved after the addition of RS, WS, AC and SB derived biochar at 2% dose level (Table 2). The maximum fresh shoot and root biomass was recorded by (35.7 g and 12.4 g) in RS, (33.3 and 9.4 g) in WS, (31.5 and 7.4 g) in AC and (26.7 g and 6.8 g) in SB amended soil relative to control (21.3 g and 4.8 g) respectively. While, the maximum dry biomass was also increased among all the biochar amended soils. Likewise, all other growth and yield parameters were improved among all the incorporated biochars (Table 3). Similarly, nutrients contents from sunflower shoots were improved when RS, WS, AC and SB biochars were added at 2% application rate. Among all the biochar, RS biochar showed the greater nutrients contents in plant shoot tissues relative to other biochars and control (Table 3). Similarly, sunflower achene, leaf area, flower diameter and 1000 achene weight was significantly increased among all the added biochar types (Table 4).

4. Discussion

Restoration of wastewater polluted soil and sewage wastewater treatment is an emerging concern for all around the globe and scientists to protect food chain. Recently, use of various organic soil amendments, especially biochar is a suitable approach for polluted soil restoration. Current investigation confirmed that the incorporation of various feedstock’s induced biochars have exhibited their potential to alleviate Cd mobility and phytoavailability to sunflower in wastewater irrigated farmlands (Bashir et al., 2019). As described in the Table 2, all biochars have sufficient amount of C, H, N and O. The greater amount of carbon content in all biochar attributed the presence of highest amount of lignin and cellulose.

| Treatments | Soil | Plants |
|------------|------|--------|
|            | N (mg/kg) | P (mg/kg) | K (mg/kg) | N (g/kg) | P (g/kg) | K (g/kg) |
| CK         | 20.9E     | 4.3D     | 92.3E     | 11.9D    | 1.97D    | 14.3C    |
| SB         | 24.3D     | 6.1C     | 102.0D    | 14.2C    | 3.57C    | 15.7C    |
| WS         | 29.0B     | 7.9B     | 115.0B    | 15.7B    | 4.97B    | 17.7B    |
| AC         | 26.2C     | 5.7C     | 108.3C    | 13.4C    | 3.60C    | 14.8C    |
| RS         | 33.4A     | 9.1A     | 123.3A    | 18.4A    | 6.27A    | 19.5A    |
Present study confirmed that the studied biochars have the maximum amount of carbon contents which could contribute to increasing the soil organic carbon contents. Similarly, it has been described that biochar incorporation in polluted soil could contribute the greater amount of carbon contents that ultimately enhance the organic matter status in the polluted soil (Abbas et al., 2020; Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h). In addition, the soil pH played a key role in the soil pH (Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h). Current findings are similar to our previous work, i.e. we established that the Cd adsorption was boosted after biochar incorporation with increasing their application rates and the solution pH (Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h). In addition, the soil pH played a key role in the soil nutrient status alteration, controlling the metals speciation, bioavailability and mobility in soil, as well as the metal accumulation in plants tissues and plants growth (Ahmad et al., 2017; He et al., 2019). It can be demonstrated that the prominent increase in soil pH could enhance the sorption cavities and bindings sites on the soil collides and increased the net electronegative charges on the soil constituents Kiran et al. (2017), which are thus responsible for the Cd immobilization through adsorption and precipitation (Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h). Several studies confirmed that the drought and metals toxicity pose severe stress on plants and cause the serious disorder in their physiological and morphological characters. Current results also revealed that Cd causes the stress on sunflower growth and yield relative to biochar amended soil (Fig. 3), this might be due to the excessive phytoavailabe pool of Cd. However, Cd accumulation in sunflower tissues significantly declined among all the biochar amended soils. This reduction may be attributed to the significant reduction in bioavailable Cd in soil, and because of the prominent increase in the soil available nutrient status after biochar amendments (Bashir et al., 2019). Our study suggested that biochar could enhance the organic matter status in the polluted soil (Abbas et al., 2020; Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h).

Table 3
Sunflower fresh and dry biomass, chlorophyll and plant height after treated with Sugarcane bagasse (SB), Wheat straw (WS), Acacia (AC) and Rice husk (RH) at 2% application level. Error bars are the SD of the means (n = 3) and different letters indicate that values are significantly different p < 0.05.

| Treatments | Fresh Biomass (g/plant) | Dry Biomass (g/plant) | Chlorophyll | Plant Height (cm) |
|------------|-------------------------|-----------------------|-------------|------------------|
|            | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| CK         | 21.3D | 5.7D | 6.8D | 1.7D | 21.0E | 49.3D |          |      |
| Su         | 26.7C | 6.8CD| 8.4CD| 2.4C | 25.0C | 59.3C |          |      |
| WS         | 31.5AB| 9.5B | 10.0BC| 2.8C | 28.5B | 63.0C |          |      |
| AC         | 33.3B | 7.4C | 12.1B| 3.5B | 22.9D | 75.3B |          |      |
| RS         | 35.7A | 12.4A| 14.8A| 4.3A | 30.7A | 83.0A |          |      |

Table 4
Sunflower yield, leaf area and flower diameter after treated with Sugarcane bagasse (SB), Wheat straw (WS), Acacia (AC) and Rice husk (RH) at 2% application level. Error bars are the SD of the means (n = 3) and different letters indicate that values are significantly different p < 0.05.

| Treatments | Achenes count | Leaf Area | 1000-achene | Flower diameter (cm) |
|------------|---------------|-----------|-------------|---------------------|
| CK         | 110.3E        | 735.7E    | 8.7D        | 9.3D                |
| Su         | 166.0D        | 815.0D    | 15.0C       | 11.4C               |
| WS         | 205.0C        | 846.0C    | 17.3C       | 12.9BC              |
| AC         | 227.3B        | 864.0B    | 21.7B       | 14.3B               |
| RS         | 244.7A        | 907.0A    | 26.3A       | 16.9A               |
neutralize the H ions in the polluted soil solution that ultimately enhances the negative surface sites for Cd complexation and thereby, declined the uptake of Cd by plant tissues (Bashir et al., 2019; Bashir et al., 2018a, 2018b, 2018c, 2018d, 2018e, 2018f, 2018g, 2018h). Several recent Khan et al. (2013b) and Rehman et al. (2020) studies assumed that the rice husk biochar efficiently reduced the health risk of Cd in rice and wheat crops.

The toxicological and physiological response of sunflower had been recognized by its biochemical, and intact plant behavior, such as antioxidant enzymes activity, chlorophyll contents, growth inhibition and yield reduction under Cd stress. These results are in line with the studies described by (Rizwan et al., 2016; Khan et al., 2013a). This study noticed that Cd stress declined the chlorophyll contents in leaves (Fig. 2) and enhanced the oxidative stress especially, (SOD, POD and CAT) activity in non-amended soil (Bashir et al. 2020c; Bashir et al. 2018h). Our study explored that, when sunflower is subjected to Cd stress then the balance system between the production and scavenging of reactive oxygen is disrupted, which ultimately affects the sunflower plants metabolism. SOD and POD are considered the protective enzymes that prevent the cell membrane disruption under Cd stress (Xu et al., 2020). As presented in the figures, the enzymatic (SOD and POD) activities of sunflower declined among all the biochars relative to the control soil. This study directed that all biochars have the potential to convert the phytoavailable Cd into more stable complexed form (residual form), which could reduce the stress-induced effect of Cd, and thus decrease the oxidative damage. Due to stress and accumulation of Cd in plant tissues, plants growth hindered to some extent, including the negative effect on plant biomass, height and yield parameters (Xu et al., 2019).

After biochar mixing in polluted soil, the biomass of sunflower was obviously improved than the without biochar treated soil. This improvement might be attributed to the nutritious significance of biochar that improves the soil productivity. Other possibility might be considered because of its potential to boost the organic matter mineralization and enhanced crop yield and growth (Rehman et al., 2017; Bashir et al., 2019). Particularly, biochar performs as a buffer and contains sufficient quantity of vital plant nutrients which pointedly rise crop yield (Ali et al. 2020). The establishment of these results might occurred due to the obvious reduction in the phytovailability of Cd in biochars amended soil, thus alleviating Cd uptake by sunflower, which might increase soil adsorption ability in biochar amended soil. It has been established that crop straw biochar has capability to improve soil acidity and exchange basic nutrients and metals from soil solution which might be able to tackle metals mobility in polluted soils and improved crop growth. Our previous study Bashir et al. (2018h) indicated that biochar addition in disturbed soils, efficiently overcome the nutrient loss through leaching and volatilization which is key factor to enhance soil fertility status and plant growth.

5. Conclusion

This study confirmed the comparative efficiency of rice straw (RS), wheat straw (WS), acacia (AC) and sugarcane bagasse (SB) biochar at 2% to sunflower growth and their incorporation into polluted soil can ameliorate the risk of the Cd spreading in the food chain. Results observed that all biochars have the ability to enhance soil pH, nutrients availability in the soil–plant system, reduce the mobility and uptake of Cd in sunflower plant as well as minimize its toxicological and physiological stress in the polluted soil. The present findings revealed plant biomass and chlorophyll contents in sunflower leaves were also increased, while SOD, POD and CAT activity was significantly decreased among all the biochars especially in rice husk. Biochar production from different feedstock’s could be systematically arranged on their comprehensive soil restoration performance in the following order RS > WS > AC > SB.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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