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Impacts of Bamboo Biochar Amendment on Growth, Morphological Traits, and Biomass Allocation of *Bambusa balcooa* under Copper-Contaminated Soil Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The accumulation of heavy metals in water streams and soil is considered a grave environmental threat that impacts plants and animals. Biochar has recently been widely used to overcome the effects of heavy metal contamination in plants and remediate the soil. A pot-trial study assessed the morphological traits of *Bambusa balcooa* under copper contamination. Each pot (twenty-four earthen pots) was filled with 7.0 kg of soil and spiked with copper sulfate of 0, 300, 600, and 1200 mg kg^{-1}. Of the total, twelve pots were amended with 7% (w/w of soil) bamboo biochar. Plant samples were harvested after one year (365 days) of treatment for biomass estimation. Data was

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recorded for different growth and morphological traits such as the number of clums, nodes, leaves, internode length, plant height, leaf area, root length, and dry biomass of root, shoot, and leaf to evaluate the impact of copper with or without bamboo biochar on *Bambusa balcooa*. The results indicated that the higher concentration of copper suppressed growth parameters such as shoot length, internode length, number of leaves, and leaf area; therefore, growth increment was significantly reduced at) mg Kg^{-1} copper-added soil. Biochar diminishes the impact of Cu stress on plants to some extent as at higher concentrations (600 and 1200 mg kg^{-1}) was enhanced root dry biomass (51 and 148%), shoot dry biomass (42 and 57%), and leaf dry biomass (38 and 48%). Thus, results confirm that biochar amendment under *Bambusa balcooa* reduces the impact of copper contamination on the plant and increases plant growth by improving soil health, suggesting that bamboo biochar application was effective in metal stabilization, thereby, reducing the bioavailability and phytotoxicity of Cu and can help to restore copper-contaminated soil.

Keywords: Bamboo; biochar; soil; Bambusa balcooa; growth.

1. INTRODUCTION

Soil is the top nominal layer of the earth's crust shaped by mineral particles, organic matter, living organisms, air and water. It is an essential natural resource on which the microorganisms proliferate and depend for their nutrient requirement. Faster urbanization and industrialization have prompted the contamination of soil and environmental pollution. The rapidly growing population also increases ecosystem contamination and soil toxicity complications. The major component responsible for soil contamination is heavy metals [1]. Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni) and lead (Pb) are common heavy metals classified as pollutants [2]. Some of them are non-essential and act as pollutants even at low concentrations.

In contrast, others are considered essential for some metabolic functions at low concentrations but very harmful at high concentrations. Among the notable heavy metal pollutants, Cu is a necessary element for plants engaged in various metabolic mechanisms such as respiration, photosynthesis and N_2 fixation. The major natural and anthropogenic sources of Cu are mining, production of Cu-related compounds, combustion of fossil fuels and wastes, waste dumps, volcanoes, sea spray, domestic wastewater, decaying vegetation, and phosphate fertilizer production [3]. Phosphate fertilizers usually have the highest share of Cu contamination in agricultural soils [4]. Cu at high concentrations can induce alteration in cell membrane integrity, photosynthesis apparatus, respiration and enzymatic activity. Extensive and continuous exposure to Cu can cause phytotoxicity by overproduction of reactive oxygen species (ROS) and damage to lipids, carbohydrates, proteins and DNA.

Bamboo is an evergreen, fast-growing, widely distributed multipurpose grass belonging to Poaceae and the sub-family bambusoideae [5]. It has various applications such as making houses, mats, baskets and mats, construction and scaffolding industries, food, used in pharmaceutical and nutraceuticals [6, 7, 8]. *B. balcooa* Roxb. is a drought-resistant, bamboo commercially cultivated in tropical countries such as India, Java, Africa, Bangladesh and Australia [9]. As one of the most economically important hardy bamboo species, it is well recognized for its solid culms, yield potential, rapid growth, drought resistance and high survival rate. Moreover, it can be used for carbon sequestration and remediation of heavy metal-contaminated soils.

Recently, there has been increasing interest in the role of biochar in the remediation of contamination and its impact on soil as a soil conditioner [1]. Biochar is a carbon-rich product of pyrolysis, a reducing agent, a carrier of slow-release fertilizer and a carbon storage agent. It also improves soil properties such as Cation exchange capacity, pH, organic carbon content, water-holding capacity and microbial activity [10]. Agricultural wastes such as rice straw; wheat straw; sugarcane bagasse; corm stalk; peanut shells; municipal wastes, and sewage sledges are some of the commonly used biomass for producing biochar [11]. Bamboo is fast-growing and inexpensive species widely used for furniture and scaffolding industries, the paper industry, house making, baskets, mats *etc*. that produces a large quantity of waste that can be employed to produce biochar efficiently and sustainably [12,13]. The characteristics of bamboo like

evergreen, accelerated growth rate, highly tensile, low cost, high biomass production and carbon sequestration, make bamboo suitable for biochar production. A high amount of biochar can be produced from bamboo i.e., 80% from *Dendrocalamus giganteus* [14]. The biochar can be yielded in only three to six years, making it desirable over other biomass sources. The excellent properties of bamboo-derived biochar, like high adsorption, large surface area and porous structure makes it efficient in adsorbing moisture, odors, organic and inorganic pollutants and carbon adsorbent and stabilize it for a long time. Several studies have been conducted on bamboo biochar for heavy metal immobilization and phytostabilization employed with plants [15, 16, 17]. Besides heavy metal immobilization in soil, biochar is utilized in water purification, carbon emission reduction and fixation [18]. With biochar, soil fertility is also enhanced by improving the soil structure and increasing the soil's organic carbon content, which is necessary for plant growth [1]. Although, several studies have been conducted on biochar amendments in the soil, the effects of biochar on *B. balcooa* morphology grown in Cu-contaminated soil have not been studied. Our analysis indicates that bamboo biochar, with its specific characteristics, may improve plant growth and helps mitigate the effects of Cu contamination on plant growth.

2. MATERIALS AND METHODS

2.1 Study Site and Experimental Design

The experiment was conducted at the Department of Botany, Panjab University, Chandigarh (latitude of 30^{o} 45' 38.2248" N and longitude of 76^{o}45'55.3968"E) in a controlled manner. Twenty four pots were arranged in a randomized block design with three replicates (n=3) for each treatment to reduce the heterogeneity in the results. The soil was spiked with Copper as copper sulfate in solution form at four concentrations of 0, 300, 600 and 1200 mg kg^{-1} dwt. basis. The duration of the experiment was one year. The morphological data were collected after every thirty days from the initiation of experiment.

2.2 Soil Collection and Analysis

The soil was collected from Botanical Garden, Sarangpur, Chandigarh, and initially tested for some selected physicochemical properties and copper concentration. The earthen pots (24 pots) were filled with 7 kg soil after putting polythene begs to avoid the leaching of Cu into the ground and 12 pots soil were amended with 7% bamboo biochar. The initial characterization of soil was done prior to the start of experiment (Table 1).

2.3 Test Plant

Five months old healthy plantlets of *B. balcooa* were collected from Forest Department, Rupnagar, Punjab, India and transplanted into the earthen pot for further experiment.

2.4 Bamboo Biochar Procurement and Characterization

Bamboo biochar was procured from Environment & Energy Management Group, Bhopal, Madhya Pradesh, India. The amount of biochar was chosen optimally to maximize the adsorption of Cu and reduce its impact on plant growth. Biochar was applied at the rate of 7% (w/w of soil) in four treatments (0, 300, 600 and 1200 Cu mg kg^{-1} dwt.).

The physicochemical properties of biochar were determined by the same methods used for soil analysis. The surface area was analyzed through Brumauer-Emmett-Tellet (BET) method and pore size distribution were measured by nitrogen adsorption- desorption isotherms measuring at-196.06^{0}C by Quantachrome Autosorb-1analyzer according to the method followed by Parthasarathy et al. [19]. The functional group and minerals were analyzed by FTIR and XRD, respectively at the SAIF facility of Panjab University, Chandigarh. The surface was studied by Field Emission-Scanning Electron Microscopy (FE-SEM) using biochar in pallet form.

2.5 Morphological Traits

Different morphological traits (number of leaves, culms, nodes, length of internode length, plant height and leaf area) were recorded after every 30 days to assess the impact of Cu on plants periodically. The leaf area was noted as a mean of 5 randomly selected leaves. Test plants were unpotted carefully after completion of the experiment and washed with tap water to make them soil free. The total, shoot, and root lengths were recorded from harvested test plants. Then some amounts of leaves, shoots, and roots were placed separately in polythene bags to transport the laboratory for further analysis. The dry weight of the samples (leaves, root and shoot) was recorded after oven drying at 70^{0} C for 24 hours.

Table 1. Initial characterization of soil used in experiment

Sharacters	Values for soil	Values for biochar	Method used for analysis
pH	7.56	9.9	Digital pH meter
EC (ds m^{-1})	0.34	3.21	Digital EC meter
CEC (Cmol kg^{-1})	7.56	20.81	Centrifuge method (29)
WHC (g g^{-1})	1.36	4.01	Cylinder method (30)
OC (%)	1%	62.61	Wet digestion Method (31)
Texture	Sandy- loam	-	International Pipette Method (32)
Silt	20%		
Sand	60%		
Clay	20%		
Ca+mg (meq/l)	1.8	-	EDTA method (33)
Nitrogen (meq/100 g)	0.046	-	Kjeldahl- Distillation method (34)
Cu (mg kg^{-1})	3.5	2.45	ICP-OES

2.6 Statistical Analysis

ANOVA (Analysis of variance) test was computed using the statistical software package SPSS. The difference between means was compared by the Duncan test at the $P<0.05$ confidence level. The difference between initial morphological traits value and at the end was compared to measure the plant's overall growth during the experiment in percentage terms.

3. RESULTS AND DISCUSSION

3.1 Biochar Characterization

The pH and EC of bamboo biochar is 9.9 and 3.21 ds m^{-1}, respectively. The possible reason behind high pH of biochar is destruction of acidic functional group on surface such as –OH and –COOH and existence of alkali and alkaline elements such as (Ca, Na, K, Mg) in biochar that increase basic functional group [20]. The CEC value was observed to be 20.81 cmol kg^{-1}. Wang et al. [21] reported that the value of bamboo biochar is higher than wood biochar and Chinese walnut shell biochar. The High CEC value is associated with improvement nutrient availability in soil that may improve crop yield. The surface area is an important factor that determines the ability of biochar to adsorb the chemical compounds. The surface area of biochar was 123. 614 m^2 g^{-1} with pore diameter of 3.363 nm and pore volume of 0.023 cc g^{-1} that reveals the potential of water holding capacity of biochar. The results are in coordination with Sahoo et al. [22].

The XRD analysis revealed that biochar is aromatic and crystalline structure and substantiated the presence of calcite, sylvite, quartz and silicates of Ca, Mg and Mn. The presence of various inorganic components such as carbonates, alicates and chlorides are responsible for alkaline nature of biochar. FTIR revealed the presence of different functional group on surface. As biochar is produced from biomass that contains C, H and O and the FTIR peaks were assigned with a limited number of bond vibrations. The strong peaks at 3414.13 cm^{-1} and 3238.52 cm^{-1} are ascribed to hydroxyl group stretching vibration for carboxyl and phenolic groups [23]. The FTIR analysis indicated the presence of functional group like carboxyl group (C=O), ester or ether and like compound, C-O-C, CH_4 C=C, aromatic and benzene ring skeleton. The presence of carboxyl groups on surface is known to contribute the negative charge on surface of biochar (Li et al., 2019). The degree of aromatization, volatilization, oxidation and carbonization depends on feedstock and pyrolization conditions [23]. FTIR analysis proclaimed that acidic and polar functional groups are removed during pyrolization [24].

Field Emission-Scanning Electron Microscopy (FE-SEM) was used to study the surface chemistry and morphology of biochar (Fig 1). The images revealed that the surface was relatively porous and have a honey comb like structure that shows the presence of well defined micropores and mesopores. The image shows the arrangement of pores. The porous structure of biochar was in accordance with high-performance adsorption capability of biochar and these findings are supported by Sahoo et al. [22]. The channel like formation of biochar and aromatic structure of biochar is due to the removal of heteroatoms and emission of large amount of volatile matters during pyrolization.

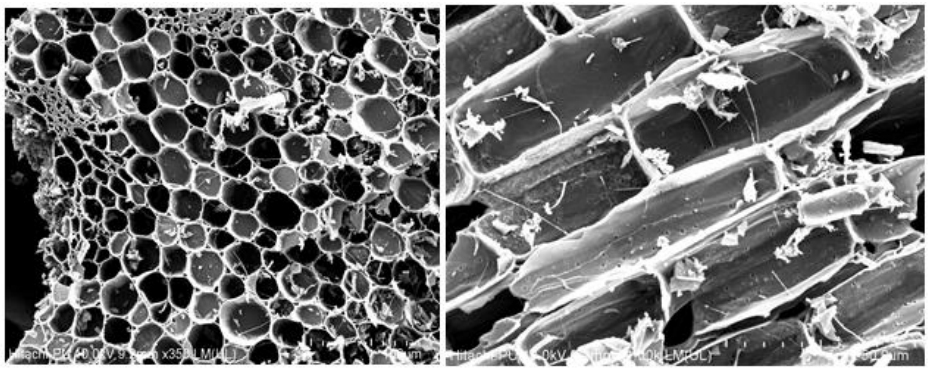

Fig. 1. FE-SEM images of biochar

Biochar is a carbon rich material found to have advantageous characteristics such as large specific area, unique pore structure, and active functional group on surface and stable chemical properties [25]. The removal efficiency of biochar for heavy metal removal depends on type, conditions during the process of pyrolization and type and concentration of heavy metal in soil. Biochar increase the pH. EC, CEC, specific surface area, water holding capacity and reduced the bulk density of soil. Zhang et al. [26] demonstrated that the liming effect of biochar precipitate heavy metal in pore water by increasing the pH of soil [27] Biochar immobilize the heavy metal in soil and reduce the bioavailability to the plants and reduce their toxic effect on growth of plant. The stabilization of heavy metal is carried out by different mechanism such as adsorption, precipitation, co-precipitation

3.2 Effect of Biochar on Plant Morphology under Cu Contaminated Soil

The results show that Cu is toxic to the growth and development of plants at higher concentrations. As the Cu concentration increased, the different morphological traits exhibited a decreasing trend. The leaf number and plant height in only Cu treated soil increased during the growing season of bamboo due to the seasonal progression of plant growth. Biochar enhance the soil pH due to its liming effect that would reduce bioavailability of heavy metal by promote adsorption and precipitation of heavy metals thus, reducing the effect on plant growth [28].

3.2.1 Number of culms, nodes, and internode length

The percentage of new emerging culms is highest in the treatment of biochar at 0 mg kg^{-1} concentration of Cu with 122% compared to 0 days of treatment (Table 2). The number of culms increased in treatments with biochar amendment, i.e., 300 mg kg^{-1}, 600 mg kg^{-1}, 1200 mg $kg^{-1,}$ by 64%, 44%, and 84%, respectively—the results indicating that biochar immobilize Cu in soil and reduce the impact of Cu on plant growth. Some culms died during the experiment at higher concentrations, possibly due to Cu's injurious effect and oxidative stress on plant culms. The same trend was seen in the impact on the number of nodes (Table 3). The number of nodes increased significantly except for 1200 mg kg^{-1} of Cu treatment. The increase was 1.80 and 1.20 fold higher than the control (zero days) in biochar and zero amendments of Cu concentration, respectively. The rise in the number of nodes in treatments of Cu with biochar is 78%, 52 %, and 44.4%, which was 16.7, 14.5 and 33.4 % higher when compared with the treatments without biochar of Cu 300 mg kg^{-1}, 600 mg kg^{-1}, 1200 mg kg^{-1}, respectively. Biochar application increases the porosity of soil that leads to improvement of water holding capacity of soil and thus, provides water and nutrient to the plant for a long time [29].

The internode length is adversely affected by the highest concentration of Cu (1200 mg kg^{-1}), as only a 25% increase was noted after 365 days of treatment compared to zero days of treatment. In contrast, the highest growth was in plants treated with biochar without Cu, which was 1.5 folds of the plantation time (Table 4). The plants grown in biochar with heavy metal treatments show more internode length when compared with only Cu-spiked soil at different concentrations. The internode length was increased by 25% only at higher concentration which was 69.75% less than control. The possible reason is that internode length at a higher concentration of Cu can reduce cell expansion [30].

Table 2. Effect of Copper and Copper+biochar (BC) on the number of culms. The number in the subscript of Cu represents the concentration of Cu in mg $kg^{-1,}$ and DAT represents days after treatment. Data represent the mean ± S.D. of three replicates (n =3). Superscript letters indicated a significant difference (p<0.05) concerning days of treatment

DAT (Days)	Cu_0	Cu_0+BC	Cu_{300}	Cu_{300}+BC	Cu_{600}	Cu_{600}+BC	Cu_{1200}	Cu_{1200}+BC
0	$3±0^{a}$	$3±0.00^{a}$	$4±0.00^{abc}$	$3±060^{a}$	$3±0.00^{a}$	$3±0.58^{a}$	$4±0.58^{b}$	$2±0.58^{a}$
30	$3±0^{a}$	$3±0.00^{a}$	$3±1.00^{ab}$	$3±0.58^{a}$	$3±0.00^{ab}$	$3±0.60^{a}$	$2±0.60^{a}$	$3±0.00^{ab}$
60	$3±0^{a}$	$3±0.00^{a}$	$3±1.00^{abc}$	$3±0.60^{a}$	$3±0.00^{a}$	$3±0.60^{ab}$	$2±0.60^{a}$	$3±0.00^{ab}$
90	$3±0.58^{a}$	$3±0.00^{a}$	$3±1.00^{ab}$	$3±0.58^{a}$	$3±0.00^{ab}$	$3±0.58^{ab}$	$2±0.58^{a}$	$3±0.00^{ab}$
120	$3±0^{a}$	$3±0.58^{a}$	$3±0.58^{abc}$	$3±0.58^{a}$	$3±0.58^{a}$	$3±1.53^{ab}$	$3±0.58^{a}$	$3±0.00^{ab}$
150	$4±0.58^{ab}$	$3±0.58^{a}$	$3±1.15^{a}$	$3±10.58^{a}$	$3±0.58^{a}$	$4±1.15^{ab}$	$3±0.58^{a}$	$3±0.00^{ab}$
180	$4±0.58^{bc}$	$5±0.00^{b}$	$4±1.00^{abcd}$	$4±0.00^{ab}$	$4±0.58^{a}$	$4±1.15^{abc}$	$4±0.58^{b}$	$3±0.58^{bc}$
210	$5±1^{cd}$	$6±1.00^{c}$	$5±1.53^{bcdef}$	$4±0.58^{abc}$	$5±0.58^{b}$	$4±1^{abc}$	$4±0.58^{b}$	$4±0.00^{cd}$
240	$6±0.58^{d}$	$6±0.58^{c}$	$5±1.53^{bcdef}$	$4±0.58^{bc}$	$5±0.58^{b}$	$5±1.15^{abc}$	$4±0.00^{b}$	$4±0.58^{c}$
270	$6±0.58^{d}$	$7±0.58^{c}$	$5±1.73^{cdef}$	$5±0.58^{bcd}$	$5±0.58^{b}$	$5±1.15^{bc}$	$4±0.00^{b}$	$4±0.58^{c}$
300	$6±0.58^{d}$	$7±0.58^{c}$	$5±0.58^{def}$	$5±0.58^{bcd}$	$4±0.58^{a}$	$5±1.15^{bc}$	$4±0.00^{b}$	$4±0.58^{c}$
330	$6±0.00^{de}$	$7±0.58^{c}$	$6±0.00^{ef}$	$6±0.58^{cd}$	$4±0.58^{a}$	$5±1.00^{c}$	$4±0.00^{b}$	$4±0.58^{c}$
365	$6±0.58^{de}$	$7±0.58^{c}$	$6±0.58^{ef}$	$6±0.00^{d}$	$4±0.58^{a}$	$5±1.00^{c}$	$4±0.00^{b}$	$4±0.58^{c}$
New emerging shoots(%)	100	122	33	64	9	50	44	86

Table 3. The number of nodes under Cu and Cu+biochar (BC) treatments. The number in the subscript of Cu represents the concentration of Cu in mg kg^{-1} and DAT represents days after treatment. Data represent the mean ± S.D. of three replicates (n=3). Superscript letters indicate a significant difference (p<0.05) for days of treatment

DAT (Days)	Cu_0	Cu_0+BC	Cu_{300}	Cu_{300}+BC	Cu_{600}	Cu_{600}+BC	Cu_{1200}	Cu_{1200}+BC
0	$5±0.6^{a}$	$5±0.6^{a}$	$9±1.2^{a}$	$8±1.5^{a}$	$8±1^{a}$	$8±0.6^{a}$	$7±1.0^{bcd}$	$6±1.00^{a}$
30	$6±1.73^{ab}$	$6±1^{b}$	$9±1^{ab}$	$8±1.53^{a}$	$8±1^{ab}$	$8±0.58^{a}$	$7±1.53^{bcd}$	$6±11.00^{a}$
60	$7±1^{bcd}$	$7±0.8^{bc}$	$9±0.6^{ab}$	$8±1.2^{ab}$	$8±0.6^{abc}$	$8±0.6^{a}$	$5±0.60^{a}$	$6±1.00^{a}$
90	$7±0.76^{bcd}$	$8±0.58^{cde}$	$10±0^{abc}$	$9±0.58^{ab}$	$8±0.58^{abc}$	$9±0^{ab}$	$5±0.58^{a}$	$6±1.00^{a}$
120	$7±0.58^{bcd}$	$8±0.76^{cde}$	$10±0.58^{bcd}$	$9±0.58^{ab}$	$9±0.58^{bcd}$	$9±0^{ab}$	$6±0.58^{ab}$	$6±1.00^{a}$
150	$7±1^{bcd}$	$9±1.44^{de}$	$11±0^{bcd}$	$9±0^{ab}$	$9±0.58^{cde}$	$9±0.58^{bc}$	$6±0.58^{ab}$	$6±1.00^{a}$
180	$8±0.58^{cdef}$	$9±0.58^{de}$	$12±0.58^{cde}$	$10±0.58^{bc}$	$9±0.00^{cde}$	$10±0^{c}$	$6±0.58^{ab}$	$7±0.58^{ab}$
210	$8±0^{cdef}$	$10±0.58^{ef}$	$12±0.58^{def}$	$11±0.58^{cd}$	$9±0.00^{cde}$	$11±0^{d}$	$6±0.58^{abc}$	$7±0.00^{ab}$
240	$8±0.58^{cdef}$	$11±0.58^{fg}$	$13±0^{gh}$	$11±0^{cd}$	$9±0.58^{def}$	$11±0^{d}$	$7±0.58^{abcd}$	$8±0.58^{bc}$
270	$9±0^{efg}$	$11±0.58^{fg}$	$13±0.58^{gh}$	$12±0.58^{de}$	$10±0.58^{ef}$	$12±0.58^{de}$	$7±1.15^{bcd}$	$8±0.58^{bc}$
300	$9±0.58^{efg}$	$12±0.58^{gh}$	$14±0^{h}$	$12±0.58^{de}$	$10±0^{fg}$	$12±0^{ef}$	$7±1.50^{bcd}$	$8±0.58^{bc}$
330	$10±0^{gh}$	$12±0.58^{gh}$	$14±0^{h}$	$13±1^{ef}$	$11±0.58^{gh}$	$12±0.58^{ef}$	$8±0.58^{cd}$	$8±0.58^{bc}$
365	$11±0^{h}$	$14±0.58^{i}$	$14±0^{h}$	$14±0.58^{f}$	$11±0.00^{h}$	13±0.58f	$8±1.00^{d}$	$9±0.58^{c}$

3.2.2 Plant height

Height is an important parameter to know the effect of heavy metal stress on plants. The present study showed that different concentrations of Cu significantly affected the plant height. The height of the plant decreased with elevation of Cu concentration. The increment in height in 300 mg kg^{-1}, 600 mg kg^{-1}, and 1200 mg kg^{-1} was less as compared to 0 mg kg^{-1} Cu treatment by 20.37%, 3.96%, and 73.17%, respectively (Table 5). Biochar amendment significantly overcame the effects of Cu stress on plant growth. The reduction in height in some treatments during the experiment was due to drying and browning of the top of the stem due to Cu stress. The slow growth rate in plant height at higher concentrations could be explained as inhibition of cell elongation and division due to heavy metal stress [31].

3.2.3 Leaves count and leaf area

The number of leaves increased in all the treatments over time, but the highest increase was noted in only biochar treatment which was 5.35 folds higher as compared to the control. The lowest growth was observed in the highest concentration, which is 96% compared to control. The number of leaves of plants grown in biochar-amended soil showed a significant increase of 36% in comparison to Cu contaminated soil. The leaf area increased in all the treatments significantly in comparison to the control. The decrease in the number of leaves in Cu stressed plants may be due to the reduction in cell division and uptake and transport of several minerals such as Ca, and Mg. P and K. [32]. Garcia et al.[33] pointed that the reduction in leaf area may be accredited to lignin accretion in xylem that ultimately induce thickening and hardening of cell wall posing fatalistic effects on cell division and leaf enlargement by declining its elasticity. Biochar improves the leaf count and leaf area in all treatments.

After 365 days of treatment, leaf area at different concentrations of 300, 600 and 1200 mg kg^{1} Cu in comparison of mg kg^{-1} was less by 11.28 cm, 17.85 cm and 17.24 cm, whereas in treatments with biochar was 0.84, 10.32 and 11.82 cm, respectively as compared zero mg kg^{-1} treatment of Cu. Biochar improves the leaf area growth even in Cu-stressed plants by mitigating the Cu effects (Table 7). The reduction in leaf area can be due to the decreased gaseous exchange related to stomata. The closer of stomata leads to water stress that lowers the photosynthetic rate and have injurious effects on mesophyll cells and oxidative stress [34]. Leaf area is a significant parameter for many remediation processes due to its relation to photosynthetic activity. Notably, the relationship between leaf area and aboveground biomass is essential for phytoremediation, as early prediction will be helpful for effective remediation [35].

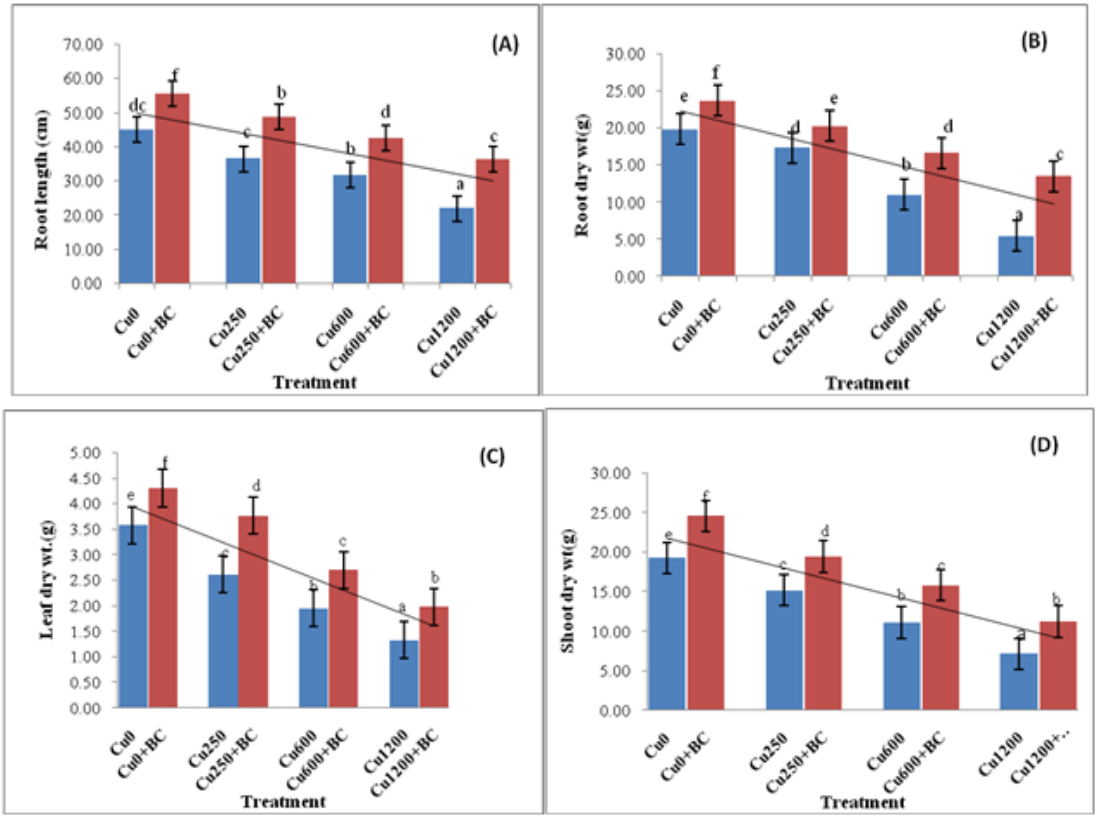

Fig. 2. Effect of biochar and Cu in different concentrations at root length (cm) (A), root dry weight(gm) (B), leaf dry weight(g) (C), and shoot dry weight(g) (D). The letter on the bars indicates the significant difference ($p<0.05$) regarding Copper treatment

Table 4. Internode length under Cu and Cu+biochar (BC) treatments. The number in the subscript of Cu represents the concentration of Cu in mg kg^{-1} and DAT represents days after treatment. Data represent the mean ± S.D. of three replicates (n=3). Superscript letters indicate the significant difference (p<0.05) for days after treatment

DAT (Days)	Cu_0	Cu_0+BC	Cu_{300}	Cu_{300}+BC	Cu_{600}	Cu_{600}+BC	Cu_{1200}	Cu_{1200}+BC
0	6.72±0.50^{a}	5.83±0.80^{a}	6.50±0.10^{a}	6.40±0.6^{a}	6.87±0.5^{a}	7.27±0.7^{a}	9.07±1^{a}	5.67±0.7^{a}
30	7.10±0.10^{ab}	6.90±0.20^{b}	7.30±0.26^{b}	6.70±0.7^{ab}	7.23±0.51^{a}	7.63±0.81^{ab}	9.33±0.91^{ab}	6.10±0.6^{ab}
60	7.97±0.40^{abc}	7.33±0.20^{bc}	7.77±0.20^{bc}	7.07±0.7^{abc}	7.50±0.5^{a}	7.97±0.9^{abc}	9.57±0.9^{abc}	6.37±0.6^{abc}
90	8.87±0.23^{abcd}	7.79±0.67^{c}	8.23±0.15^{cd}	7.40±0.44^{bcd}	7.73±0.58^{ab}	8.20±0.85^{abc}	9.77±0.9^{abc}	6.60±0.6^{bc}
120	9.42±0.40^{abcde}	8.83±0.20^{d}	8.53±0.25^{de}	7.77±0.42^{cde}	7.83±0.51^{ab}	8.57±0.8^{abc}	9.97±0.9^{abc}	7.13±0.5^{cd}
150	9.68±0.82^{bcde}	9.33±0.58^{d}	8.87±0.35^{ef}	8.10±0.36^{de}	8.10±0.53^{abc}	8.93±0.85^{bcd}	10.20±0.95^{abc}	7.83±0.31^{de}
180	10.64±1.01^{cde}	10.37±0.32^{e}	9.30±0.36^{fg}	8.40±0.44^{ef}	8.80±0.85^{bcd}	9.20±0.85^{bcd}	10.40±1^{abc}	8.33±0.25^{e}
210	11±1.91^{de}	12.03±0.06^{f}	9.73±0.40^{gh}	8.93±0.15^{f}	9.13±0.93^{cde}	10.13±0.87^{de}	10.6±0.9^{abc}	9.27±0.47^{f}
240	11.29±1.99^{de}	13.10±0.17^{g}	10.17±0.35^{hi}	9.67±0.15^{g}	9.43±0.76^{def}	10.57±0.76^{ef}	10.77±0.95^{abc}	9.83±0.35^{fg}
270	11.94±2.29^{e}	13.40±0.20^{g}	10.60±0.36^{ij}	10.28±0.20^{gh}	9.90±0.87^{def}	11.07±0.67^{efg}	10.97±0.91^{abc}	10.13±0.35^{g}
300	12.06±2.14^{e}	13.67±0.21^{g}	10.97±0.45^{jk}	10.83±0.15^{hi}	10±0.78^{def}	11.43±0.61^{efg}	11.03±0.96^{bc}	10.43±0.31^{g}
330	12.10±2.17^{e}	14.53±0.40^{h}	11.47±0.25^{kl}	11.43±0.47^{ij}	10.13±0.64^{ef}	11.93±0.59^{fg}	11.13±1^{bc}	10.50±0.3^{g}
365	12.19±2.15^{e}	14.69± 0.5^{h}	11.7±0.2^{l}	11.73±0.21^{j}	10.45±0.58^{f}	12.3±0.53^{g}	11.3±0.95^{c}	10.57±0.31^{g}

Table 5. Height under Copper and Copper+biochar (BC) treatments. The number in the subscript of Cu represents the concentration of Cu in mg kg^{-1} and DAT represents days after treatment. Data represent the mean ± S.D. of three replicates (n=3). Superscript letters indicate the significant difference (p<0.05) for days after treatment

DAT (Days)	Cu0	Cu0+BC	Cu300	Cu300+BC	Cu600	Cu600+BC	Cu1200	Cu1200+BC
0	39.54±4.00^{a}	32.94±1.30^{a}	54.61±4.4^{a}	43.26±2.80^{a}	57.23±5.50^{a}	44.53±2.80^{a}	56.9±2.70^{ab}	51.56±5.00^{a}
30	42.84±3.18^{ab}	35.98±4.72^{b}	60.2±4.84^{a}	51.73±2.11^{ab}	59.61±6.02^{ab}	49.19±2.29^{ab}	60.2±5.74^{b}	57.4±8.42^{ab}
60	46.23±4.40^{ab}	44.45±2.70^{b}	61.04±5.20^{a}	59.61±5.50^{bc}	61.55±5.50^{a}	53.51±2.70^{bc}	51.31±1.70^{a}	61.55±8.90^{ab}
90	50.04±3.6^{bc}	54.19±2.85^{c}	69.51±6.31^{b}	67.14±5.29^{cd}	66.72±5.21^{ab}	56.39±1.92^{cd}	52.41±1.69^{a}	65.62±8.45^{bc}
120	56.90±2.58^{c}	69.68±4.07^{d}	77.98±3.99^{c}	77.72±5.54^{de}	67.56±4.09^{bc}	61.55±2.54^{de}	62.06±4.23^{b}	69.6±8.49^{bc}
150	67.90±1.3^{d}	75.35±6.61^{d}	82.72±5.81^{cd}	86.87±5.47^{ef}	73.74±3.28^{cd}	66.97±2.36^{e}	72.05±6.15^{c}	76.45±7.65^{cd}
180	80.77±3.79^{e}	87.12±7.52^{e}	90.42±5.14^{de}	96.44±5.47^{fg}	79.25±5.21^{d}	74.59±0.89^{f}	81.53±5.82^{d}	83.23±7.92^{de}
210	92.37±5.34^{f}	96.94±5.14^{f}	99.31±3.6^{e}	101.85±11.07^{g}	87.8±6.35^{e}	81.28±0.92^{g}	87.04±2.64^{de}	85.26±5.6^{de}
240	103.72±7.87^{g}	112.69±3.99^{g}	106.09±3.88^{ef}	109.39±9.02^{gh}	94.15±4.8^{ef}	86.87±4.85^{gh}	88.65±5.86^{de}	90.34±6.67^{ef}
270	120.40±2.58^{h}	122.43±3.56^{h}	111.93±2.54^{fg}	117.86±10.68^{hi}	99.65±2.3^{fg}	90.68±5^{hi}	92.03±3.31^{ef}	95.33±5.65^{efg}
300	128.35±7.49^{h}	132.67±2.54^{i}	119.46±4.36^{gh}	122.94±11.82^{hi}	104.99±4.33^{gh}	95.42±5.57^{i}	93.90±2.56^{ef}	100.41±6.14^{fgh}
330	129.62±8.07^{h}	138.77±2.67^{ij}	122±4.95^{h}	128.02±10.82^{i}	108.8±3.52^{h}	101.68±5.04^{j}	98.3±5.51^{fg}	104.39±6.72^{gh}
365	129.88±7.96^{h}	142.07±1.94^{j}	126.24±5^{h}	131.83±9.27^{i}	112.69±1.63^{h}	106.34±4.95^{j}	101.77±4.83^{g}	108.71±6.76^{h}

Table 6. Number of leaves under Copper and Copper+biochar (BC) treatments. The number in the subscript of Cu represents the concentration of Cu in mg $kg^{-1,}$ and DAT represents days after treatment. Data represent the mean ± S.D. of three replicates (n=3). Superscript letters in a column indicate the significant difference (p<0.05) for days after treatment

DAT (Days)	Cu_0	Cu_0+BC	Cu_{300}	Cu_{300}+BC	Cu_{600}	Cu_{600}+BC	Cu_{1200}	Cu_{1200}+BC
0	21±3.80^{a}	25±7^{a}	36±6.66^{a}	30±6.56^{a}	48±6.24^{c}	35±7.02^{a}	47±16.77^{ab}	31±12.34^{bc}
30	25±7.00^{a}	26±8.08^{a}	26±9.02^{a}	33±5.29^{a}	31±7^{ab}	27±4.04^{a}	36±12.12^{a}	23±11.59^{ab}
60	31±3.80^{a}	33±5.5^{a}	23±9.71^{a}	34±4.16^{a}	25±6.11^{a}	17±4.36^{a}	30±12.66^{a}	12±8.74^{a}
90	53±3.06^{b}	60±9.07^{b}	54±8.02^{b}	45±23.52^{a}	36±2.65^{b}	31±17.62^{a}	53±12.66^{bc}	46±15^{cd}
120	70±9.87^{c}	84±16.62^{c}	62±8.74^{b}	86±12.06^{b}	61±7.51^{d}	72±14.53^{b}	70±4.73^{cd}	61±24.01^{d}
150	76±9.29^{c}	111±4.51^{d}	108±8.02^{cd}	91±9.71^{b}	70±6.56^{e}	87±5.03^{bc}	83±6.24^{de}	85±20.13^{e}
180	97±4.16^{d}	128±11.93^{de}	118±4.36^{def}	94±4.04^{bc}	80±1.53^{f}	96±6.51^{cd}	89±4.62^{e}	93±5.13^{ef}
210	111±5.03^{e}	141±11.14^{ef}	123±2.52^{ef}	102±6.51^{bcd}	89±5^{g}	107±12.5^{c}	95±4.58^{e}	99±2.08^{ef}
240	120±4.93^{ef}	145±11.59^{ef}	131±2^{f}	113±8.5^{cde}	98±5.69^{gh}	110±14.36^{c}	83±6.43^{de}	102±1.73^{ef}
270	123±11.02^{ef}	147±20.5^{ef}	109±10.41^{cd}	116±15.5^{de}	101±4.36^{h}	109±6.56^{c}	85±6.11^{de}	106±5.86^{ef}
300	128±9.07^{f}	155±18.33^{f}	101±9.17^{c}	126±9.54^{e}	102±3.79^{h}	108±8.14^{c}	84±12.22^{de}	103±5.69^{ef}
330	130±9.54^{f}	158±17.01^{f}	103±6.08^{c}	122±11.55^{de}	103±6.51^{h}	111±9.29^{c}	90±9.17^{e}	108±5.86^{f}
365	133±8.14^{f}	160±17.69^{f}	114±8.74^{cde}	123±15.14^{de}	105±4.51^{h}	113±11.06^{c}	92±9.07^{e}	103±4.93^{f}

Table 7. Leaf area (cm^2) under Copper and Copper+biochar (BC) treatments. The number in the subscript of Cu represents the concentration of Cu in mg $kg^{-1,}$ and DAT represents days after treatment. Data represent the mean ± S.D. of three replicates (n=3). Superscript letters in a column indicate the significant difference (p<0.05) concerning days after treatment

DAT	Cu_0	Cu_0+BC	Cu_{300}	Cu_{300}+BC	Cu_{600}	Cu_{600}+BC	Cu_{1200}	Cu_{1200}+BC
0	7.19±1.2^{a}	7.71±2.09^{a}	9.92±1.85^{a}	8.99±1.65^{a}	11.26±1.72^{a}	9.39±2.03^{a}	9.15±0.7^{a}	9.78±0.88^{a}
30	11.55±1.82^{b}	12.69±1.14^{b}	11.93±0.78^{a}	11.91±0.86^{ab}	13.41±1.64^{ab}	12.88±0.52^{ab}	11.69±1.46^{a}	12.86±0.42^{b}
60	17.42±1.65^{c}	19.11±1.74^{c}	16.59±0.95^{b}	13.78±1.92^{bc}	15.68±0.32^{b}	15.08±2.43^{b}	13.69±1.72^{ab}	14.08±1.01^{b}
90	21.29±1.37^{d}	22.75±1.87^{cd}	20.47±2.72^{bc}	16.31±0.63^{cd}	19.17±2.42^{c}	17.12±1.28^{b}	15.87±0.97^{cd}	16.54±1.01^{c}
120	22.82±3.31^{d}	25.64±1.25^{de}	22.12±1.7^{c}	19.02±1.52^{d}	19.7±2.14^{c}	17.02±0.77^{b}	16.53±0.1^{d}	19.01±1.8^{d}
150	25.14±1.97^{d}	27.91±0.68^{e}	25.19±1.64^{cd}	23.15±4.15^{e}	23.22±2.64^{d}	22.29±2.51^{c}	19.5±0.88^{e}	20.64±2.28^{d}
180	32.99±1.3^{e}	37.35±1.5^{f}	29.12±1.15^{de}	33.92±1.88^{f}	27.39±2.04^{e}	29.08±0.77^{d}	26.33±0.75^{f}	29.99±0.8^{e}
210	41.17±2.88^{f}	47.03±3.19^{g}	33.51±1.38^{e}	39.78±5.54^{f}	32.14±0.58^{f}	36.36±3.64^{e}	31.85±1.46^{g}	33.54±0.85^{f}
240	45.35±2.7^{g}	52.93±1.9^{h}	39.94±4.85^{e}	46.87±1.3^{g}	35.33±0.1^{g}	41.32±2.13^{f}	35.56±1.76^{h}	38.06±2.25^{g}
270	50.12±2.65^{h}	56.42±3.12^{hi}	41.69±3.4^{e}	49.51±0.68^{gh}	37.46±0.24^{gh}	42.98±3.12^{f}	36±1.99^{hi}	38.84±2.07^{g}
300	52±1.98^{h}	59.23±4.85^{i}	42.39±3.1^{e}	50.47±1.01^{ghi}	38.16±0.21^{h}	43.61±3.39^{f}	36.71±1.29^{hi}	39.78±1.73^{g}
330	52.62±2.02^{h}	59.92±4.75^{i}	43.55±3.68^{e}	51.68±0.9^{hi}	38.99±0.59^{h}	44.68±3.1^{f}	37.38±1.55^{hi}	42.73±1.05^{h}
365	53.43±2.37^{h}	60.99±4.65^{i}	44.88±3.75^{e}	54.39±1.88^{i}	39.65±0.97^{h}	45.3±3.26^{f}	38.14±1.48^{h}	44.2±1.16^{h}

3.3 Plant Biomass

The amendment with bamboo biochar effectively enhanced root, shoot and leaf biomass indicating that biochar has significant potential to remediate the soil polluted with heavy metals. The root biomass significantly decreased in all the parts in Cu-treated plants without biochar by 14.28%, 36.73% and 60.87% in 300, 600 and 1200 mg kg^{-1}, respectively, compared to the control (Fig. 2(B)). The biomass reduction could be due to a decrease in photosynthetic rate and disorganization of chloroplast, leading to a reduction in chlorophyll content and increasing malondialdehyde level [35]. Biochar improves the biomass quantity in all treatments compared to Cu treatments. A significant increase in leaf dry biomass in Cu plus biochar was noted by 43.91, 38.29 and 48.58 % in concentrations of 300, 600 and 1200 mg kg^{-1}, respectively (Fig. 2(D)). The shoot biomass was adversely affected in maximum concentration of Cu as it decreased by 62.19% compared to the control. At the same time, 70.92% decrease was noted regarding only biochar-amended soil (Fig.2(C)). The root length was also decreased with a higher concentration of Cu (Fig. 2(A)). A significant drop in growth indices is reported in different plant species such as *Indocalamus latifolius* [34], *Zea mays* L. [36], *Solanum lycopersicum* [37], *Fallopia japonica* and *Urtica dioica* [38] under Cu stress thereby supporting the present result. The trendline in all graphs shows that plants' biomass decreased with increased Cu concentration.

The results indicate that biochar addition reduces Cu's impact on plant growth by stabilizing them through surface adsorption and precipitation. Even under Cu contamination with biochar, the improvement of bamboo growth may be due to the improved physical properties of soil conferred by biochar, such as soil fertility, structure, and water-holding capacity. Biochar application decreases the availability of heavy metals to the plant and concomitantly improves soil fertility [39]. The bio-solubility of heavy metals reduces to some extent by adsorbing Cu ions on the surface [1]. Notably, the different functional groups such as oxygen carboxyl, phenolic and hydroxyl on the surface can effectively interact with soil pollutants [17]. A recent study has reported that organic amendments like biochar decreased the heavy metal availability and concentration in different parts of the plant, thus reducing the deleterious effects on the plant [36]. The growth of Cu-stressed plants might be due to oxidative damage caused in cells, alterations in cell organelles and DNA, and declined photosynthetic activity leading to reduced growth and necrosis in plants [40-46]. Biochar improves the soil physico-chemical and biological properties by supplying nutrients that could indirectly enhance the plant growth and biomass. The dry weight of leaves of moso bamboo has increased by 157% with the application of 5% rice straw biochar in heavy metal contaminated soil [21].

4. CONCLUSION

The present study revealed the toxic impacts of Copper on various morphological characteristics of plants grown in contaminated soil compared to contaminated soil amended with biochar and the tolerance ability of *B. balcooa* for heavy metal stress. Different morphological traits showed improvement with biochar amendment as the maximum increase of 109.13 cm and 53.28 cm^2 in height and leaf area was noted in biochar treatment. The plant biomass also improved in Copper with biochar at a maximum concentration of Copper by root (148%), shoot (57%), and leaf (48%) in comparison to Copper stressed plants. This study will provide theoretical references for trials on fields for future studies.

In conclusion, the application of biochar in restoring contaminated soil might be a sustainable option. Our study will potentially help to convey scientific knowledge of biochar uses and its potential benefits to policymakers and practitioners. The study will help farmers and environmentalists improve soil health and reduce the effects of heavy metal contamination on plant growth parameters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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