

Biochar: A promising soil amendment to mitigate heavy metals toxicity in plants

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Abstract

Heavy metals (HMs) toxicity is serious abiotic stress that is significantly reducing crop productivity and posing a serious threat to human health, soil and environmental quality. Therefore, it is urgently needed to find appropriate measures to mitigate the adverse impacts of HMs on soil, plants, humans and the environment. Biochar (BC) has emerged as an excellent soil amendment to minimize the adverse impacts of HMs and to improve soil fertility and environmental quality. Biochar application decreases HMs uptake and their translocation to plant parts by forming complexes and precipitation. Biochar also has improved soil pH, soil fertility and soil cation exchange capacity (CEC) and it also increases adsorption of HMs thus reduces their mobility and subsequent availability to plants. BC application also maintains membrane stability and improves uptake of nutrients, osmolytes accumulation, antioxidant activities, and gene expression, therefore, improves the plant performance under HMs stress. Biochar application also improves the photosynthetic performance by increasing the synthesis of photosynthetic pigments, stomata conductance and increasing the water uptake by plants. Besides this, BC also scavenges ROS by increasing the antioxidant activities, gene expression, and accumulation of proline in HMs contaminated soils. This review highlights the role of BC to mitigate the HMs toxicity in plants. We have discussed the role of BC in the modification of soil properties to induce tolerance against HMs toxicity. Moreover, we have discussed various mechanisms mediated by BC at the plant level to induce tolerance against HMs. Additionally, we also identified research gaps that must be fulfilled in future research studies.

Keywords: anti-oxidant; biochar; heavy metals; oxidative stress; photosynthesis; reactive oxygen species

Received: 01 Jul 2022. Received in revised form: 02 Aug 2022. Accepted: 04 Aug 2022. Published online: 25 Aug 2022.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

The rapid increase in urbanization and industrialization has increased heavy metals (HMs) pollution across the globe. HMs are serious concern for humans and animals and it is also negatively affecting soil health, crop productivity and quality (Han *et al.*, 2020; Hassan *et al.*, 2021). Though HMs are easily transportable and have difficult degrading in the environment, therefore they are easily concentrated by plants and enter into the food chain which imposes serious threats to human health (Shen *et al.*, 2018; Han *et al.*, 2020). In recent time, diverse pollutants are exerting negative impacts on soil, humans, plants and overall, the ecosystem (Rai *et al.*, 2019; Rahim *et al.*, 2021). Amid these pollutants, the potential toxic metals (PTM) have raised concern owing to their non-degradable nature, higher mobility and toxicity (Yu *et al.*, 2017). The PTM includes arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb), silver (Ag), manganese (Mn), mercury (Hg), molybdenum (Mo) and nickel (Ni) which are posing serious threats to agriculture productivity, food safety, food security and human health (Wang *et al.*, 2018; Sagbara *et al.*, 2020; Xu *et al.*, 2022).

The higher concentration of HMs imposes negative impacts on crops (Antonangelo and Zhang, 2019) owing to their higher mobility, bio-accumulation behaviour and toxicity (Seneviratne *et al.*, 2019). HMs impact various plant processes ranging from nutrient homeostasis, photosynthesis, gas exchange characteristics, osmolytes accumulation and antioxidant activities (Sanjosé *et al.*, 2021). Toxic metals also develop toxicity by binding the metal ions to sulfhydryl groups (-SH), in the amino acids and proteins which inhibit the plant's physiological activity and degrade structure of different enzymes (Rehman *et al.*, 2018). HMs also induces the production of reactive oxygen species (ROS) which is considered to be a major effect caused due to HMs (Zhang *et al.*, 2019). HMs induced ROS pose serious damage to cellular membranes, proteins, lipids, and various micro as well as macromolecules (Wang *et al.*, 2019). Besides this HMs also reduce the root and shoot growth, photosynthetic pigments, accumulation of proteins and free amino acids (FAA), and increase the accumulation of MDA and H₂O₂ which in turn reduce the overall productivity (Hassan *et al.*, 2019).

It is essential to develop the appropriate measures to reclaim the contaminated soils to ensure healthy soils and ecosystem. Various strategies including immobilization, precipitation, ion exchange, use of organic amendments, nano-materials, adsorption, and bioremediation are being used globally to remediate HMs contaminated soils (Yu *et al.*, 2013; Haider *et al.*, 2022). These conventional remediation strategies require sophisticated instruments and they are very costly to remediate the HMs contaminated soils (Rahim *et al.*, 2022). Thus, it is necessary to shift from conventional strategies to eco-friendly and nono-remediation strategies such as the use of carbon based nano-materials (Huang *et al.*, 2015). Biochar (BC) is a carbon rich material produced by pyrolysis of different feed-stocks in the absence of oxygen. It has gained considerable attention globally as a green, innovative and sustainable tool in agriculture, energy production and environment protection owing to its unique physio-chemical characteristics (Aamer *et al.*, 2020). BC has a high specific area and surface activity, and it also has a better porous structure, oxygen-containing functional groups and higher ion exchange capacity which make him an important amendment to be used in agriculture (Liu *et al.*, 2020; Rahim *et al.*, 2020).

In recent decades BC has got attention as an important tool to manage environmental pollution, and remediate contaminated soils with additional benefits of improved soil fertility, soil quality and carbon sequestration (Rahim *et al.*, 2019; Hu *et al.*, 2020; Azadi and Raiesi, 2021). Recently, BC has emerged as cost effective and environmentally friendly approach for minimizing the effects of HMs (Yuan *et al.*, 2019). BC application reduces the absorption of HMs and it also increases the antioxidant activities which provide protection to plants against HMs (Virk *et al.*, 2021). Besides BC also improves the photosynthetic efficiency, accumulation of osmolytes, soil water holding capacity (WHC), soil quality and reduces the HMs uptake and restricts their entry into the plant body resulting in substantial improvement in plant growth and development on contaminated soils (Virk *et al.*, 2021). Thus, it is imperative to highlight the role of BC in the remediation

of polluted soils. The present review article presents the recent progress on the role of BC to remediate HMs contaminated soils. We have presented information on how BC decreases the HMs toxicity by affecting the soil properties. Moreover, we also discussed the role of BC in mediating different responses of plants to alleviate HMs toxicity.

Plant responses to heavy metals

HMs enters into the food chain by plants and they pose serious threats to human and animals. HMs toxicity negatively affects plant biochemical, molecular and physiological processes (Figure 1) which in turn cause a significant reduction in growth and development (Rasheed *et al.*, 2020; Raza *et al.*, 2021). HMs have been recorded to reduce seed germination, plant growth and cause different morphological changes (Ghori *et al.*, 2019). For instance, Hg stress ($80 \mu\text{g ml}^{-1}$) significantly reduced the root and shoot growth and leaves production in *Jatropha curcas* (Marrugo-Negrete *et al.*, 2016). Likewise, in another study, it was noted that Ni toxicity reduced the germination owing to a reduction in protease and α -amylase activities (Ashraf *et al.*, 2011). Further, negative effects of Cd on leaf area were also observed in tomatoes and in another study authors also noted Cd induced chlorosis in *Phaseolus vulgaris* (Bahmani *et al.*, 2012).

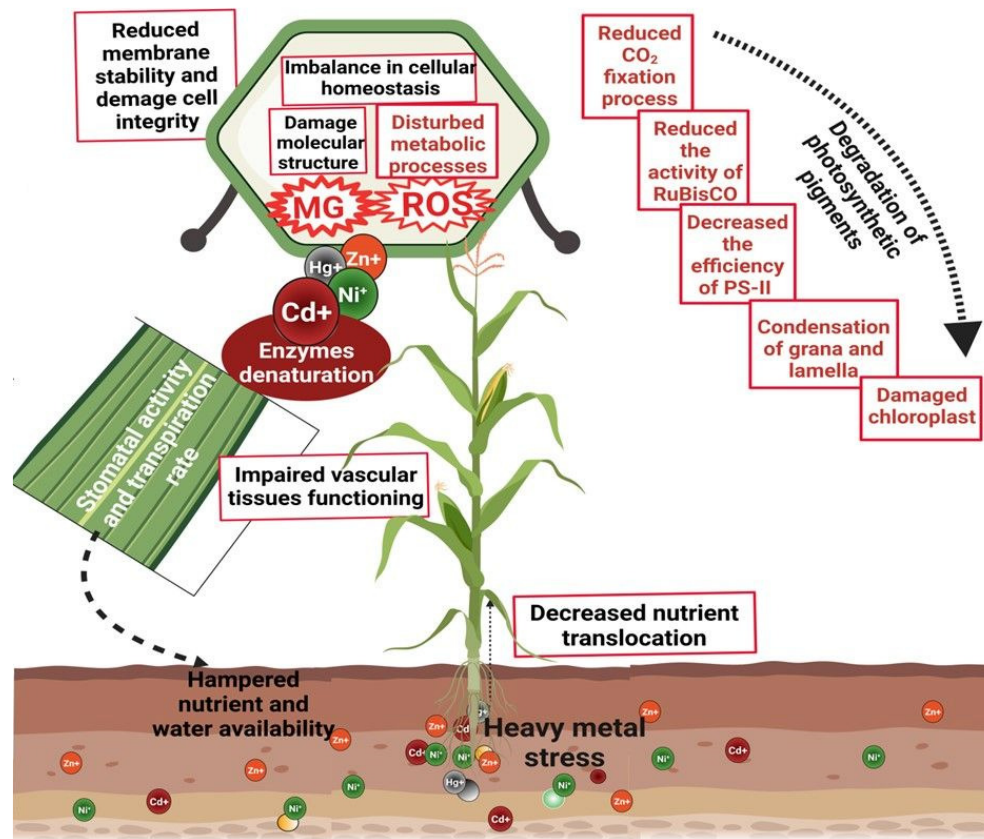


Figure 1. Plant response to HMs. The toxic metals decrease membrane stability, reduce water uptake, efficiency of PS-II, stomata conductance, photosynthetic and transpiration rates thereby resulting in substantial reduction in plant growth and development

Roots are the first plant organ that comes in contact with HMs and these HMs significantly reduced the root growth, root area, and increased root dieback which reduced the water and nutrient uptake resulting in a significant reduction in plant growth (Rucińska-Sobkowiak, 2016). The cell wall of roots also becomes hard

with exposure of toxic metals which also inhibit plant growth (Zhao *et al.*, 2010). Plant cell membranes prevent the entry of unwanted materials into plant organelles however; HMs disturbs membrane integrity and increase the loss of important solutes on exposure to HMs stress (Janicka-Russak *et al.*, 2012). HMs also disrupts uptake of nutrients and water which inhibit the synthesis of photosynthetic pigments which is one of the major reasons of growth reduction under HMs stress (Yang *et al.*, 2020). Toxic metals also damage the photosynthetic apparatus and reduce the photosynthesis rate by reducing the chlorophyll synthesis (Table 1) and thickness of mesophyll cells (Schmidt *et al.*, 2020). Toxic metals also reduce the rate of electron transport and reduce the efficiency of PS-II by decreasing the reaction site therefore, leading to a significant reduction in photosynthetic efficiency (Mathur *et al.*, 2016).

HMs also reduces the nutrient and water uptake and their translocation in the plant body (Raza *et al.*, 2021). The reduction in water uptake reduces transpiration, stomata activity, photosynthesis and water uptake (Hussain *et al.*, 2013). For instance, a substantial reduction in stomata activity was noted in *Pistacia vera* due to toxicity of Zn (Tavallali, 2017). Moreover, toxic metals also affect the functioning of the xylem and phloem and they also reduce the root xylem area (Vaculík *et al.*, 2012). HMs also competes with essential nutrients at the binding sites of different enzymes and inhibit their activity (Ghori *et al.*, 2019). Exposure to HMs (Cr) also reduces the chlorophyll contents, starch contents and protein contents (Rath *et al.*, 2019), conversely, HMs induced a significant increase in the accumulation of soluble sugars and proline (Song *et al.*, 2020). In another study conducted on kenaf; it was noted that Cu reduced the root and shoot growth by increasing the H₂O₂, MDA accumulation, and electrolyte leakage (EL), however, these negative effects were reduced by increasing the activities of antioxidant defense system (Saleem *et al.*, 2022). Cowpea plants explored to Fe toxicity also showed an increase in MDA contents and membrane damage (Ifle *et al.*, 2020). HMs also induced a substantial increase in MDA and H₂O₂ accumulation (Lokhande *et al.*, 2020), however, plants have an excellent defense system that search, neutralize and remove the ROS and protect the plants from HMs induced oxidative stress (Hasanuzzaman *et al.*, 2020).

Toxic metals also affect the CO₂ fixation and assimilation activities and it has been reported that toxic metals (Cd and Zn) severely inhibited the CO₂ fixation and assimilation in wheat plants (Paunov *et al.*, 2018). Rubisco plays a crucial role in photosynthetic processes however; HMs substantially reduced the Rubisco activity which in turn causes a significant reduction in photosynthetic performance (Son *et al.*, 2014). ROS produced owing to HMs damage DNA, proteins, cellular membranes which are also major reasons for reduction in plant performance under HMs stress (Hasanuzzaman *et al.*, 2020). Plants also produce diverse metal binding structures and chelating agents and ligands which plant an important role to cope with the toxic effects of HMs (Raza *et al.*, 2021). It has been reported that microRNA significantly improved the *Vaccinium myrtillus* tolerance against Cd and it regulated its responses to counter the effects of Cd stress (Casarrubia *et al.*, 2020; Ding *et al.*, 2020). MicroRNA expression also improves the accumulation of different secondary metabolites that appreciably improved the Al and Cd tolerance in transgenic tobacco plants (Sáenz-de la O *et al.*, 2020).

Biochar a potential amendment to improve plant performance under HMs toxicity

Biochar is a carbon rich material produced by pyrolysis (300-1000 °C) from different feedstock including crop residues, wood chips, animal and chicken manure, and municipal solid sludge under partial or anaerobic conditions (Diatta *et al.*, 2020). BC is a rich source of carbon besides this it also contains an appreciable amount of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), oxygen (O) and hydrogen (H) (Adnan *et al.*, 2020; Seleiman *et al.*, 2020). BC has got appreciable attention across the globe in the last two decades owing to its ability to improve carbon sequestration, soil fertility and remediate contaminated soils and waste-water (Alkharabsheh *et al.*, 2021). Biochar application improves nutrient retention, microbial

activities, soil structure, and nutrient absorption by plants which ultimately increased plant growth and yield (Bonanomi *et al.*, 2017). Biochar is considered to be recalcitrant C that slowly degrades in soil and takes thousands of years to full degrade (Pariyar *et al.*, 2020).

Table 1. Effect of HMs stress on growth, physiological and biochemical process of various crops

| Crop species | Heavy metal stress | Effects | References |
|--------------|-----------------------|---|-------------------------------------|
| Maize | Cu stress (160 mg/L) | Cu stress reduced germination, root and shoot growth and biomass yield increased SOD, POD and CAT activity | (Xin <i>et al.</i> , 2022) |
| Maize | As stress (100 mg/kg) | As stress decreased root and shoot growth, RWC, photosynthetic pigments and increase proline and MDA contents, EC and H ₂ O ₂ and activities of APX, CAT, POD and SOD | (Khan <i>et al.</i> , 2022) |
| Maize | Cd stress (500 µM) | Cd stress reduced the root and shoot growth, chlorophyll contents and increased MDA and H ₂ O ₂ accumulation and activities of APX, CAT and POD | (Saleem <i>et al.</i> , 2022) |
| Maize | Cd stress (100 mg/kg) | Cd stress reduced chlorophyll contents, photosynthetic and transpiration rates, growth and increased ROS production and activities of antioxidants | (Razzaq <i>et al.</i> , 2022) |
| Wheat | Cd stress (250 µg/mL) | Cr toxicity reduced germination percentage, root and shoot growth and increased ROS production activities of APX, CAT, POD and SOD | (Ilyas <i>et al.</i> , 2022) |
| Mash Bean | Cd stress (20 mg/kg) | Cd stress reduced the root, shoot growth, chlorophyll contents, RWC, antioxidant activities and increased the MDA, H ₂ O ₂ accumulation and EL | (Umer Chattha <i>et al.</i> , 2021) |
| Maize | Pb stress (7.5 mM) | Pb stress reduced the growth yield, photosynthetic pigments, N, P and K and increased MDA accumulation, EL and antioxidant activities | (Sofy <i>et al.</i> , 2020) |
| Oat | Ni stress (40 mg/kg) | Ni toxicity reduced germination, growth, biomass production, chlorophyll contents and increased SOD activity and proline accumulation | (Gupta <i>et al.</i> , 2017) |
| Maize | Pb stress (250 mg/kg) | Pb toxicity causes retarded growth and inhibits germination and reduced the chlorophyll and carotenoids | (Nawaz <i>et al.</i> , 2017) |
| Beans | Cu stress (75 µM) | Cu stress reduced biomass production, Zn, K and Fe uptake and increased GPX and CAT activity and accumulation of H ₂ O ₂ | (Bouazizi <i>et al.</i> , 2010) |

The field application of BC improves soil carbon, soil pH, soil porosity, bulk density, WHC, nutrient use efficiency, and nutrient availability (N and P) which in turn improved crop growth and yield (Wang *et al.*, 2020). Moreover, BC also reduces soil hardening and it also increases soil microbial activities and nutrient cycling (Figure 2) which favours a substantial increase in plant growth (Pariyar *et al.*, 2020). Biochar also helps to reclaim the HMs contaminated soils by increasing the adsorption of HMs and other contaminants (Inyang and Dickenson, 2015). Nonetheless, the effect of BC largely depends on the feedstock material, pyrolysis temperature, soil texture and particle size of BC (Gao and DeLuca, 2016). The use of BC also remediates soils,

reduces the wastage of resources. Moreover, BC also possesses excellent physiochemical characteristics including porous structure, hydroxyl and carbonyl groups which play an imperative role in the degradation of HMs (Zhu *et al.*, 2015). BC is considered to be an economical, environmentally friendly and superior quality amendment to remediate HMs contaminated soils (Liang *et al.*, 2021).

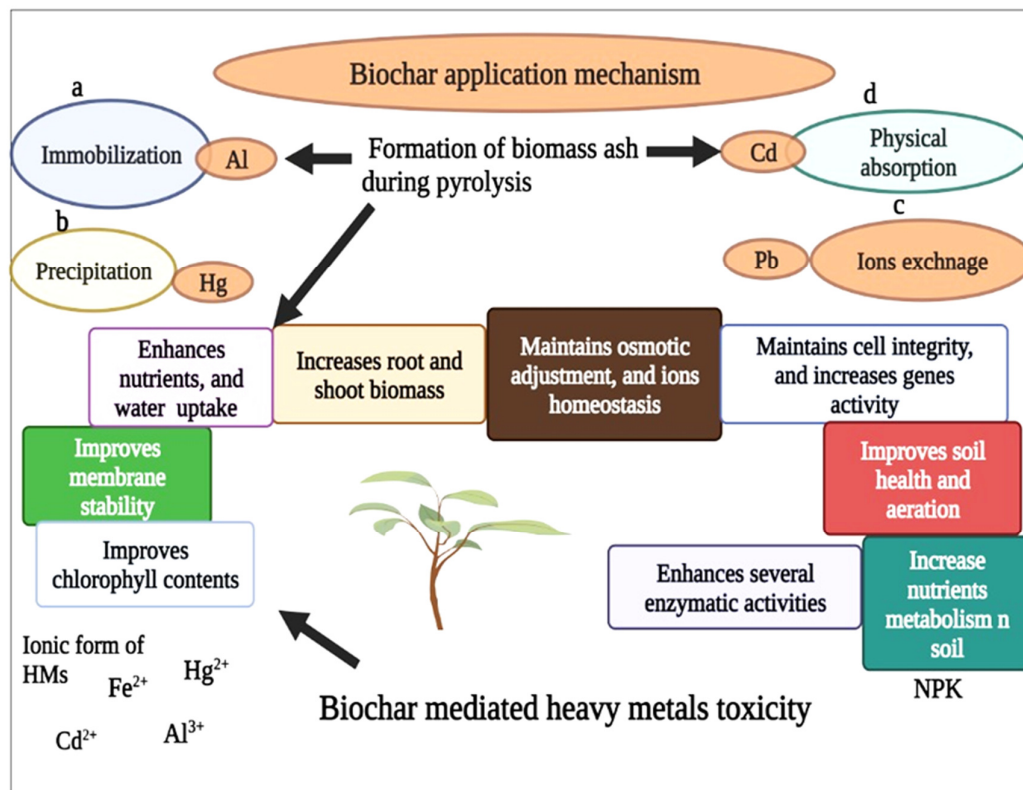


Figure 2. Role of BC in mitigating the HMs toxicity in plants. BC application cause immobilization, precipitation, and absorption of HMs and therefore, reduces toxicity to plants. Moreover, BC also improves chlorophyll contents, nutrient and water uptake, antioxidant activities, osmolyte accumulation and genes which in turn reduce the toxic effects of HMs on plants.

Biochar reduces HMs toxicity by forming complexes and precipitation

BC substantially mobilize the HMs thereby reduce the uptake and subsequent accumulation in plants (Meng *et al.*, 2018). Different functional groups such as carboxyl (COOH) and oxygen groups fix the HMs ions by ion exchange process and reduce their uptake by plants (Ho *et al.*, 2017). BC also releases Ca and Mg on its surfaces owing to higher CEC which fix the HMs ions and reduce their availability (Li *et al.*, 2015). Moreover, increased adsorption capacity of BC also attributed to its higher CEC (Tang *et al.*, 2013). In few studies it has been also reported that BC ensures electrostatic attraction of positively charged owing to its higher electro-negativity (Aamer *et al.*, 2020). The negatively charged surface groups affect electro-negativity which is increased at higher pH which in turn increases the adsorption of HMs (Tong *et al.*, 2011). BC fix the HMs by forming surface complexes which in turn reduce the HMs availability and their deleterious impacts on plants (Xu *et al.*, 2017). Similarly, surface functional groups of BC also immobilize the HMs by forming surface complexes (Xu *et al.*, 2017). The functional groups like COOH and OH in BC enable the binding site for HMs to complexes which increase the adsorption of HMs (Tan *et al.*, 2017). Moreover, different ions (Si, S

and Cl) in BC also combine with HMs and reduce the mobility in soils (Tan *et al.*, 2017). The mineral present in BC also form insoluble precipitates with fix the HMs and reduce their availability and mobility in soil (Cao and Harris, 2010). For instance, inorganic P present in BC induced the precipitation of Pb and reduced its mobility and availability (Liu *et al.*, 2016; Liang *et al.*, 2021).

Biochar mitigates HMs toxicity by modifying soil properties

Biochar improves the soil pH owing to its alkaline pH which in turn affects the HMs availability in soils (Yu *et al.*, 2019). The mobility of HMs in soils is governed by soil pH (Ding *et al.*, 2020). Similarly, HMs complexation is also significantly affected by soil pH and an increase in soil pH following BC application reduces the HMs desorption in soils (Jiang *et al.*, 2012). The increase soil pH by BC substantially reduced the toxicity of HMs. Moreover, BC application also significantly increased the soil CEC owing to the fact BC has very high CEC (Zhang *et al.*, 2017). Different authors noted that an increase in soil CEC due to BC significantly decreases the solubility as well as leach-ability of HMs (Bashir *et al.*, 2018). Increasing soil CEC owing to the application of BC improves the fixation of Cu and Pb (Ma *et al.*, 2010; Rees *et al.*, 2014). Similarly, it has been also reported that minerals present in BC increase the adsorption of HMs in soils (Bian *et al.*, 2014; Rees *et al.*, 2014). Similarly, organic carbon (OC) present in BC also reduces the availability, and mobility of HMs in soils (Zhu *et al.*, 2016). The labile forms of HMs are converted into less mobile forms by binding with OC present in BC which reduced HMs mobility in soil (Abdelhafez *et al.*, 2014). The carbon present in BC has a porous structure which forms complexes with HMs and reduces their mobility and subsequent availability to plants (Carter *et al.*, 2013). Biochar is considered as an effective for agriculture and the management of different environmental issues (Xu *et al.*, 2014). The application of BC modifies the polluted soils and reduced the availability of HMs and curtails the adsorption of HMs by plants (Namgay *et al.*, 2010). Because of the porous structure of BC, it increases the soil WUC, nutrient uptake and decreases the density of soil thereby improves soil aeration and consequently microbial activities (Mansoor *et al.*, 2021). The increase in microbial activity following the application of BC also plays a crucial role to manage the HMs (Liang *et al.*, 2014).

Biochar improves membrane stability and plant water relations under HMs

Toxic metals induce oxidative stress which damages the membrane integrity and increases the accumulation of MDA, H₂O₂ and electrolyte leakage (EL) (Abbas *et al.*, 2017; Farhangi-Abriz and Torabian, 2017). However, BC has excellent potential and it significantly reduces the HMs induced oxidative damage in plants. For instance, in spinach plants BC markedly reduced the MDA and EL and increased the membrane integrity under Cd stress (Younis *et al.*, 2016). The application of BC maintains membrane integrity by decreasing the accumulation of toxic metals in plant parts (Abbas *et al.*, 2017). The HMs induced oxidative stress induced ROS production which disturbs plant functioning and the integrity of cellular membranes (Hossain *et al.*, 2012).

Nonetheless, the application of BC significantly improved the accumulation of proline (Pro) which improved the antioxidant activities and protected the membranes from the toxic effects of HMs (Mazhar *et al.*, 2020). In another study, it was reported that rice residue bases BC reduced the MDA contents in rice plants by increasing CAT, POD and SOD activities under Cd stress (Zhang *et al.*, 2014). The application of BC substantially improved the activities of malic enzymes, glutamate dehydrogenase, iso-citrate dehydrogenase, and POD which boosted plant functioning and protected the cellular membrane of bean plants from the toxic effects of Zn stress (Hmid *et al.*, 2015). In another study, it was noted that BC application, enhanced photosynthetic pigments (Table 2), relative water contents (RWC), leaves/plant and improved anatomical features of plants (Hafez *et al.*, 2020). BC is carbon-rich product and it has a porous structure which increases

the soil WHC thereby improve the water uptake and maintains higher RWC in plants growing under stress conditions (Amonette and Joseph, 2009; Verheijen *et al.*, 2010; Fiaz *et al.*, 2014). The HMs toxicity increased the EL by increasing the formation of ROS, nonetheless, application of BC decreased the EL and increased the RWC which was linked with improved nutrient availability and soil WHC (Beesley *et al.*, 2014; Naveed *et al.*, 2020a). The increased RWC and decrease in EL following BC application helps the plants to sustain better under HMs toxicity (Bashir *et al.*, 2020).

Table 2. Effect of biochar on growth, and physiological attributes under HMs stress

| Crop | Heavy metal stress | Biochar application | Effects | References |
|-----------|------------------------|---------------------|---|-----------------------------------|
| Wheat | Cd stress (20 mg/kg) | 50 kg/ha | BC application improved the root and shoot growth, biomass production, chlorophyll contents, and accumulation of TSS, TSP FAA and phenolics | (Hussain <i>et al.</i> , 2022) |
| Barberry | Cd stress (30 mg/kg) | 125 g/kg | BC application increased plant height, plant biomass, chlorophyll contents, RWC, total phenolics, total flavonoids and antioxidants | (Khosropour <i>et al.</i> , 2022) |
| Wheat | Ni (1.5 mg/kg) | 50% (W/W) | BC improved the root and shoot growth, photosynthetic parameters and grain yield | (Shahbaz <i>et al.</i> , 2019) |
| Sunflower | Ni stress (77 mg/kg) | 50% (w/w) | BC reduced the Ni accumulation and increased the biomass production and grain yield | (Shahbaz <i>et al.</i> , 2018) |
| Wheat | Cr stress 50 mg/kg) | 5% (W/W) | Biochar improves growth, germination, chlorophyll and carbohydrate contents | (Arshad <i>et al.</i> , 2017) |
| Wheat | Cd stress (2.86 mg/kg) | 5% (w/w) | BC increased plant-height, root dry mass, photosynthetic pigments and gas exchange parameters | (Abbas <i>et al.</i> , 2017) |

Biochar improves uptake of nutrients under HMs stress

A balance between organic as well as inorganic nutrients is considered to be essential for getting maximum plant growth and yield. Toxic metals disturb nutrient uptake, therefore, cause a huge reduction in plant growth. BC application appreciably improved the nutrient uptake and resulted in significant improvement in plant performance under stress conditions. The application of BC increases the concentration of N, P, K, and S which in turn improved the plant growth and yield under stress conditions (Puga *et al.*, 2015). In another study, it was noted that sewage sludge BC markedly improved the N and P uptake and decreased the concentration of K in rice plants grown in metal contaminated soils (Khan *et al.*, 2014). The application of BC appreciably improved the concentration of P, K, Ca, and Mg in rapeseed shoots grown under metal contaminated soils as compared to un-amended soil (Houben *et al.*, 2014). Similarly, Younis *et al.* (2015) also reported a reduction in P and K in spinach plants due to BC application.

Biochar supply also reduced N, P and Ca availability in Cd and Zn polluted soils (Rees *et al.*, 2015; Rees *et al.*, 2014). Whereas, Yang *et al.* (2016) noted that BC application increased the available P in the soil in dose dependent manner. Moreover, Wagner and Kaupenjohann (2015) concluded that BC application had no impact on N and Mg concentrations however, BC application significantly increased the concentration of K while reduced the concentration of Ca concentration in plants. In another study, the application of maize residue-based BC in Cr polluted soil significantly improved the concentration of N, P and K in lettuce plants (Nigussie *et al.*, 2012). Similarly, BC application also increased the concentration of Si owing to the presence of phytoliths in the BC which in turn improved the growth of rice plants (Houben *et al.*, 2014; Dad *et al.*,

2020). BC also induced an increase in Si availability by increasing the soil pH (Houben *et al.*, 2014; Dad *et al.*, 2020).

It has been documented that an increase in soil pH following BC application solubilises Si in soil which in turn increases its uptake by plants (Rizwan *et al.*, 2012). Many authors also noted that BC application could increase Zn and Mn in plant parts growing in Cd contaminated soils (Abbas *et al.*, 2017; Rahi *et al.*, 2022). The uptake of N was appreciably increased in rice crop following the application of farm yard manure and BC (Majeed *et al.*, 2022). The supply of BC increases the supply of protons to reduce the Cr toxicity (Mansoor *et al.*, 2021). Many researchers also noted a substantial increase in nutrient uptake following BC application under HMs contaminated soils (Naveed *et al.*, 2020a; Naveed *et al.*, 2020b).

Biochar improves plant photosynthetic performance under HMs

Photosynthesis is an imperative physiological process that plays a crucial role in the growth and development of plants (Hassan *et al.*, 2022). HMs toxicity significantly decreased the synthesis of photosynthetic pigments and the number of stomata which cause a significant reduction in photosynthetic and transpiration rate, stomata conductance and inter-cellular CO₂ (Akhtar *et al.*, 2017; Bertel *et al.*, 2017; Li *et al.*, 2018). BC application significantly improved the synthesis of photosynthetic pigments, and carotenoids which in turn improved the photosynthetic performance under Cr stress (Zhu *et al.*, 2020; Bashir *et al.*, 2021). BC application substantially improved the stability of HMs in soil and alleviates the Cd induced toxic effects on chlorophyll synthesis and photosynthesis (Zoghi *et al.*, 2019). BC application also improved the gas exchange characteristics, and chlorophyll contents by immobilization of HMs (Mehdizadeh *et al.*, 2019; Kang *et al.*, 2022). The beneficial effect of BC can be seen in terms of improved physiological and biochemical traits. The application of BC substantially increased the synthesis of photosynthetic pigments which in turn improved the plant photosynthetic efficiency in Cr contaminated soils (Bashir *et al.*, 2020). The increase in chlorophyll contents, photosynthetic and transpiration rates following the addition of BC has been well documented under HMs stress (Akhtar *et al.*, 2015; Younis *et al.*, 2015; Younis *et al.*, 2016). The increase in photosynthesis following BC addition could be attributed to the improved ultra-structure of chloroplast (Abbasi *et al.*, 2015).

Biochar strengthens anti-oxidant defense system under HMs toxicity

Plants produce ROS in a controlled amount under normal conditions, however, under stress conditions; the production of ROS is increased many times which causes oxidative damage to DNA, proteins and lipids and also leads to cell death (Kohli *et al.*, 2019). Plants also have excellent defense system to cope with ROS (Niamat *et al.*, 2019). It has been noted that the application of organic amendments improved the plant's tolerance against abiotic stresses by increasing the antioxidant activities (Niamat *et al.*, 2019). The application of BC significantly improved the antioxidant activities by reducing the bioavailability of Cr (Naveed *et al.*, 2021). In another study conducted on maize and *Brassica rapa* it was reported that BC addition significantly improved the activity of CAT (Arshad *et al.*, 2017; Seneviratne *et al.*, 2017; Ali *et al.*, 2018; Bashir *et al.*, 2020). Moreover, Sabir *et al.* (2020) also noted that Cr stress reduced the antioxidant activities (CAT, GSH, GR, GP, GPX and GST) *Brassica* leaves, however, BC significantly increased this antioxidant activity.

Under HMs toxicity burst of ROS is considered to responsible for lipid per oxidation which ultimately reduced the cell membrane permeability (CMP) (Tanveer and Ahmed, 2020; Tanveer and Wang, 2019). The application of BC improved the antioxidant activities which leads to a significantly increase CMP under HMs (Dad *et al.*, 2020). BC also reduced the oxidative stress by reducing ROS production and increasing the antioxidant activities (Khan *et al.*, 2015). The increase in antioxidant activities following BC application

prevents the plants from oxidative damages (Mehdizadeh *et al.*, 2019). BC application appreciably increased the APX activities however; BC had no impact on activity of CAT (Dad *et al.*, 2020). Similarly, in another study, BC application significantly improved the CAT, POD and SOD activities (Table 3) in flag leaf of rice (Zhang *et al.*, 2014). Likewise, BC application significantly improved CAT and POD (Table 3) which in turn decreased the MDA and H₂O₂ accumulation and EL (Farhangi-Abriz and Torabian, 2017). The application of BC and bio-fertilizers increases the activities of antioxidant enzymes which in turn increase the plant response to Cd stress (Nookongbut *et al.*, 2019; Novak *et al.*, 2019). Likewise, Zhu *et al.* (2020) recorded that BC application improved the CAT and SOD activity and alleviated the Cd induced oxidative stress in cotton (Zhu *et al.*, 2020). Besides this BC also increases the activities of CuZn-SOD, Mn-SOD, and Fe-SOD which decompose the H₂O₂ and protect the plants from HMs induced oxidative stress (Zhu *et al.*, 2020). The improved antioxidant activities due to BC application are linked with improved plant health (Bashir *et al.*, 2021; Naveed *et al.*, 2021).

Table 3. Effect of biochar on oxidative stress markers and antioxidant activities under HMs

| Crop | Heavy metal stress | Biochar application | Effects | References |
|-----------|------------------------------------|---------------------|--|--------------------------------|
| Chili | Ni stress (50 mg/kg) | 150 g/kg | BC reduced the H ₂ O ₂ and MDA accumulation and increased CAT, POD, APX and SOD activities | (Turan, 2022) |
| Mustard | Pb stress (250 mg/kg) | (12.8 mg/g) | BC application decreased the MDA contents and increased activity of antioxidant enzymes (SOD, POD and CAT) | (Rathika <i>et al.</i> , 2021) |
| Quinoa | As stress (30 mg/kg) | 2% (W/W) | BC reduced the oxidative stress and increased the CAT, POD and SOD activities | (Shabbir <i>et al.</i> , 2021) |
| Cotton | Cd stress (4 mg/kg) | 6.8 t/ha | BC application reduced MDA accumulation and EL and increased the SOD, CAT and POD activities | (Zhu <i>et al.</i> , 2020) |
| Cotton | Cd stress (4 mg kg ⁻¹) | 3% (W/W) | BC reduced MDA and EC while increased SOD, POD and CAT activities | (Zhu <i>et al.</i> , 2020) |
| Corn mint | Pb stress (32 mg/kg) | 4% (w/w) | BC improved the antioxidant (SOD and POD) activity to encounter the Pb induced oxidative damage | (Nigam <i>et al.</i> , 2019) |

Biochar improves osmolyte accumulation under HMs toxicity

Stress related metabolites such as proline, soluble sugars, free amino acids (FAA) and total soluble proteins (TSP) induce resistance against stress conditions by avoiding cell damage from ROS (Sinay and Karuwal, 2014). Proline plays diverse functions in plants ranging from enzyme protection to osmotic adjustment, detoxification of ROS and maintenance of protein synthesis (Aly and Mohamed, 2012). BC application significantly improved the Pro accumulation which in turn increased the antioxidant activities and protect the plants from oxidative stress (Mazhar *et al.*, 2020). The BC induced increase in Pro improves osmotic adjustment, stabilizes enzymes and proteins and protects the plants from ROS (Szabados and Savouré, 2010). Phenolic compounds play a crucial role in plants and they prevent the plants from various stresses due to their higher antioxidant activities (Rohani *et al.*, 2019). Nonetheless, BC application improved the accumulation of phenolic compounds which in turn improved the antioxidant activities and protect the plants from Cd induced toxic effects (Dad *et al.*, 2020).

Similarly, radish plants showed a reduction of 13% in shoot phenolic contents however, BC application markedly improved the phenolic concentration in both roots and shoots of radish plants (Dad *et al.*, 2020). The exposure of radish plants to Cd stress also reduced the root and shoot protein contents however, BC application markedly improved the concentration of protein in roots and shoots of radish plants grown under Cd stress (Dad *et al.*, 2020). In addition, BC application also significantly increase Pro, TSP, FAA and crude fat contents which in turn improved the plant tolerance against Cd stress (Borchard *et al.*, 2014). Similarly, Chen *et al.* (2020) also noted that BC application improved the Pro, soluble sugars and fat concentration by 95.65%, 81.25% and 27.37%, respectively (Chen *et al.*, 2020). However, some authors also noted that BC application decreased the TSP owing to fact BC decreased organic N of soil by decreasing the production of proteins and their use by plants (Prommer *et al.*, 2014). Recently, it has been also documented that BC increased the abiotic stress resistance by affecting the signalling pathways of jasmonic acid and H₂O₂ hydrogen oxide accumulation (Mehari *et al.*, 2015).

Biochar improves genes expression and microbial activities to confer HMs toxicity

Toxic metals reduced the activities of antioxidant which in turn induce significant reduction in plant growth. BC application alleviated the biological activities of toxic metals by causing their inactivation. In another study, it was noted that BC application increased the expression of antioxidant genes (SOD, POD, and *CAT*) and metal-tolerant conferring gene (*OsFSD1*) thereby improved the tolerance against vanadium (Mehmood *et al.*, 2021). Likewise, Mehmood *et al.* (2020) also noted that BC application significantly boosted the expression of four genes (*CAT*, *APX*, *POD* and *SOD*) encoding antioxidant enzymes and significantly improved the growth and development of soybean plants (Mehmood *et al.*, 2020). Conversely, Kang *et al.* (2022) found that BC down-regulated the expression of *MnSOD*, *CAT*, and *GR* due to a reduction in ROS production and phyto-toxicity of Cd stress (Kang *et al.*, 2022).

The changes in composition and diversity of microbes following BC application have got considerable attention across the globe (Gul *et al.*, 2015). The application of BC considerably increased the abundance and richness of fungi and bacteria (Yao *et al.*, 2017). The addition of BC also increased the relative abundance of dominant soil bacterial phyla which in turn reduces the mobility of HMs in soils (Wang *et al.*, 2021). The application of BC also increased the abundance of *proteobacteria* which reduced the toxicity of HMs owing to their unique metabolic and ecological ability to adapt to HMs contaminated soils (Ghori *et al.*, 2019; Kang *et al.*, 2022).

Biochar improves plant growth, yield and quality under HMs stress

Toxic metals caused a substantial reduction in the growth of plants; however, BC exerts a positive influence on plant growth by decreasing the toxicity of HMs (Chattha *et al.*, 2021). The application of BC significantly improved the growth and biomass yield in HMs contaminated soils by improving the soil physicochemical and biological properties (Chen *et al.*, 2020). In another study, conducted on Brassica it was noted that BC application significantly improved root and shoot growth and biomass production in Cr contaminated soil (Naveed *et al.*, 2021). Likewise, Novak *et al.* (2019) also observed a marked improvement in plant growth under Cd stress after the application of BC. Moreover, Abbas *et al.* (2020) also noted that BC improved the length of shoots (93%), root length (222%), shoot dry biomass (60%) and root dry biomass (164%) of maize plants grown in Cr stress.

Arshad *et al.* (2017) documented that BC made from pine wood appreciable improved the root and shoot growth, biomass production and root surface area of maize plants. Likewise, different authors also noted that BC application caused an appreciable increase plant growth under Cr stress (Agegnehu *et al.*, 2017; Arshad

et al., 2017). BC increases SOC that improves soil fertility and overall plant growth (Rizwan *et al.*, 2016; Ali *et al.*, 2018; Cheng *et al.*, 2020). Moreover, BC also improves soil pH, CEC, SOM and availability of nutrients which ultimately increased plant growth and soil fertility (Agegnehu *et al.*, 2017). BC application decreased the availability of HMs (Cr) which enhanced the nutrient uptake, therefore, improved the plant growth (Rizwan *et al.*, 2016) addition also enhanced the mineralization of N and absorbs NH_4^+ more than NO_3^- on its surface, thereby ensuring better availability of N and better plant (Dad *et al.*, 2020).

Biochar application to mustard plants increases their growth and biomass production by improving antioxidant activities and reducing the production of ROS (Al-Wabel *et al.*, 2015; Rodríguez-Vila *et al.*, 2015). Khan *et al.* (2015) noted that BC made from sewage sludge markedly increase growth of turnip. Likewise, BC application also increased the growth and biomass productivity of ryegrass grown under As stress (Gregory *et al.*, 2014). Biochar application improved the plant growth in HMs contaminated soils by improving soil properties, nutrient uptake, antioxidant activities, photosynthetic performance and decreasing the availability of HMs (Rees *et al.*, 2015; Naveed *et al.*, 2021). BC application also improved the SOM, WHC and nutrient availability which in turn improves nutrient uptake in HMs contaminated soils (Chan *et al.*, 2008; Mendez *et al.*, 2012).

Conclusion

The concentration of HMs has been significantly increased due to human activities which are posing a serious threat to crop growth, soil quality and human health. HMs has devastating impacts on humans, agriculture and environment therefore, it urgently needed to develop the appropriate measures to counter the deleterious impacts of HMs. In this context, BC has emerged as an excellent tool to mitigate the adverse impacts of HMs. BC application improves plant performance ranging from growth to yield and providing resistance against HMs. BC application maintains plant water relations and improves the membrane stability which prevents the loss of important osmolytes therefore, improve the plant performance under HMs. BC application also improves nutrient and water uptake, synthesis of photosynthetic pigments and protects the plants from HMs induced toxic effects by increasing antioxidant activities, gene expression and accumulation of osmolytes. Moreover, BC also form complexes and ensures the precipitation adsorption of HMs therefore, reduce the HMs mobility and their uptake by plants. Additionally, BC also increases soil pH and soil CEC which also play a significant role in the immobilization of HMs.

Despite this recent progress the role of BC in plants growing under HMs is not fully explored and there are many un-answered questions. The role of BC in germination mechanism is not explored yet, thus is needed to explore the role of BC in germination mechanism in plants growing under HMs toxicity. The role of BC on nutrient uptake is well studied however, it is needed to explore the role of BC on nutrient channels and ion transporter under HMs toxicity. The effect of BC on photosynthetic enzymes, electron transport, and efficiency of PS-I and PS-II is not studied. Thus, is urgent to conduct research on these aspects to make him an important soil amendment to mitigate adverse impacts of HMs. The role of biochar on reproductive characteristics and crop quality is also not explored, thus it would be fascinating to explore its role in these aspects.

The role of BC on osmolytes and hormone accumulation is not explored yet. In literature, only information is available about the role of BC on proline and soluble sugar accumulation. Moreover, there is no study available about the role of BC on hormone accumulation under HMs. Therefore, it is also needed to explore the role of BC on osmolytes (glycine-betaine) and hormones (abscisic acid, auxin, cytokinin, ethylene and gibberellins) accumulation under HMs. The role of BC on antioxidant activities is also poorly studied and it needs to explore the effect of BC on enzymatic and non-enzymatic antioxidants and genes expression under HMs stress. Moreover, the effect of BC on the accumulation of secondary metabolites, and phenolic

compounds must be also explored in depth studies for increasing the tolerance of plants against HMs. The molecular mechanism mediating by BC to induce HMs is not explored in the future therefore, it is needed to conduct in-depth studies to explore the role of BC in mediating molecular mechanism for ensuring tolerance against HMs. In literature most of the studies are conducted in controlled conditions, thus it is mandatory to conduct long-term field studies in HMs contaminated soils to explore the role of BC. Moreover, it is also directly needed to optimize the rate of BC application for different field crops under a wide range of climate and soil conditions.

Authors' Contributions

Conceptualization; HT and GH; Writing - original draft: HT and SW; Writing - review and editing: YL, MUH, YS, GH, MH, SA and YSM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported and funded by the National Key Research and Development Program of China (2016YFD0300208), National Natural Science Foundation of China (41661070), Consulting research project of Chinese Academy of Engineering (2017-XY-28), the Project of the youth fund of Hunan University of Humanities, Science and Technology (2010QN23), Key Research and Development Project of Hunan (2021NK2030), Natural Science Foundation of Hunan Province (2021JJ30375). The authors also extend their appreciation to the Deanship of Scientific Research, King Khalid University for supporting this work through research groups program under grant number R.G.P. 1/97/43.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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