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EVALUATING THE EFFECTS OF CADMIUM UNDER SALINE CONDITIONS ON LEAFY VEGETABLES BY USING ACIDIFIED BIOCHAR

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Abstract

Crop development and yield are hampered by salinity and heavy metal (HM) stresses. Heavy metals enter the food chain due to crop plants' absorption when cultivated in areas where HM exceed their threshold levels. Among different heavy metals, cadmium (Cd) is a notorious one. Higher-water solubility made Cd a potential toxin for crops and consumers**.** On the other hand, salinity also deteriorates plant productivity by negatively affecting many morpho-physiological and genetic attributes. To address these issues, i.e., HM toxicity and salinity stress, acidified biochar can be a game changer. In recent years, the use of biochar has gained increasing attention. Due to the characteristic structure of the biochar, it absorbs Cd and releases critical micronutrients in the soil. Because of its many micropores and significant ion exchange properties, biochar is an appropriate amendment for improving soil properties and immobilizing Cd. Furthermore, improvement in soil microbial population can also play an imperative role in developing better rhizosphere ecology. That's why the current review addressed the detrimental effects of salinity and Cd, and the positive impact of biochar on crop productivity. This review also covers the knowledge gap regarding using acidified biochar in alkaline soils. The emphasis was placed on elaborating the beneficial impacts of acidified biochar on plant output and soil composition maintenance.

Key words: Activated carbon, Heavy metal, Microbial proliferation, Salt stress.

Introduction

Plant stress is an umbrella term for any force hindering a plant's growth and development (Foyer *et al.*, 2016). In recent years, crop yield worldwide has been highly influenced by different stressors, i.e., heat, cold, heavy metal (HM) toxicity, flooding, drought, and soil salinity (Gull *et al.*, 2019). Soil salinity is one of the most significant environmental stresses affecting the productivity of all annual crops worldwide (Kamran *et al.*, 2019; Demirkaya *et al.*, 2021). Abd El-Mageed *et al.,* (2021) stated that water shortage associated with high salinity negatively affects agricultural production in the world, especially in arid or semiarid regions. According to Kamran *et al.*, (2019), soil salinity is defined as the condition in which a sufficient concentration of salts is available in the rhizosphere, which results in impaired plant growth. Salt-impacted soils have osmotic stress of 0.2 MPa, an electrical conductivity (EC) of 4 dSm^{-1} or greater, and a replaceable sodium percentage (RSP) of 15% at 25°C (Kamran *et al.*, 2019).

Salts in the soil are found in the form of ions (Shrivastava *et al.*, 2015). The most frequent cations linked with salinity are ions like Na^+, Mg^{2+}, and Ca^{2+}, whereas the most frequent anions are HCO_3^-, SO_4^{2-}, and Cl^- (Safdar *et al.*, 2019). Plants need a small amount of salts in soil for their proper growth, but the elevated amount of salts in agricultural soils and irrigation water is a significant issue faced by crop plants (Kamran *et al.*, 2019).

High transpiration rates and inappropriate use of pesticides and fertilizers have become one of major reasons behind salinization, resulting in the conversion of agricultural land into barren land (El-Naggar *et al.*, 2019). Furthermore, the soil under salt stress grows by 10% yearly due to various primary and secondary causes. The primary and secondary causes of salinity may be natural and anthropogenic (Fig. 1) as entrance of salts into the soil by chemical weathering, geochemical activities and precipitation from ocean water involved in increasing salinity level of soil. Additional factors include the overuse of chemical fertilizers and the entry of industrial effluents into the soil (Bhise & Dandge, 2019; Haider *et al.*, 2022).

Besides salinity, HM stress has become a universal problem (Zwolak *et al.*, 2019),and is aggravating at an alarming rate (Jabeen *et al.*, 2022). Heavy metals are elements with high density compared to water. Considering the assumption that toxicity and heaviness are interlinked. Metalloids are also included in HMs, e.g. arsenic (As) or mercury (Hg), that, even on low-level exposure, have a toxic effect on other organisms (Tchounwou *et al.*, 2012). It has been established as true that the accumulation of any amalgam that exceeds the soil's limit is defined as a soil pollutant (Zafar-ul-Hye *et al.*, 2020).

Jabeen *et al.*, (2022) reported that soil is a natural source of HM origin. Heavy metals such as nickel (Ni), copper (Co), iron (Fe), and zinc (Zn) are crucial elements in plant development. Zinc is required by most plants for disease resistance and seed production, whereas Cu is essential in the metabolism of most plants. Nickel is an integral element of urease, even though it can cause risks to human health at excessive levels (Rai *et al.*, 2019). Briffa *et al.*, (2020) reported that the concentrations of HMs in soil are increasing due to geological and human activities, resulting in harmful effects on all organisms.

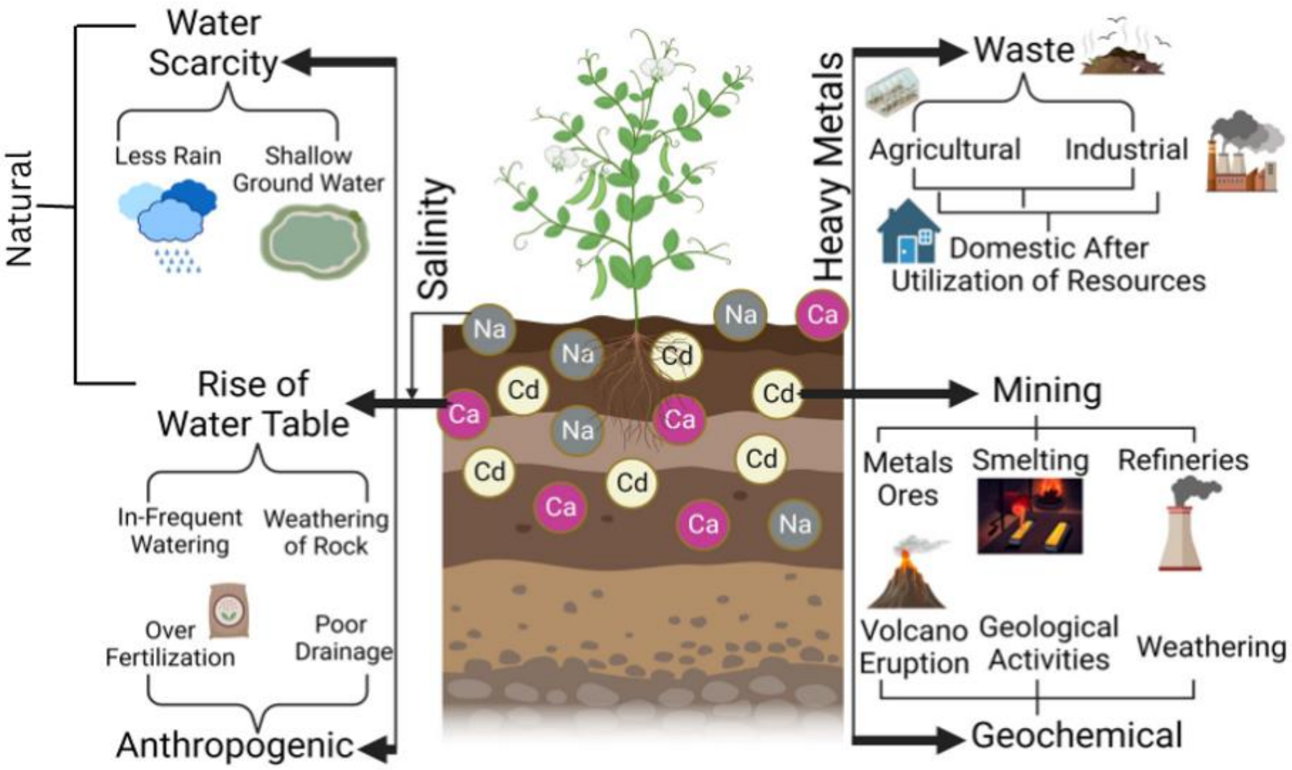

Fig. 1. Causes of cadmium and salinity stress development.

Cadmium (Cd) is also a non-essential HM**.** Cadmium pollution in soils has become a serious environmental issue, particularly in areas with naturally elevated Cd (Zhao *et al*., 2020). Cadmium is found naturally with Pb and Zn in sulfide ores. Sites around nonferrous mines and metal refineries comprise 73% of all anthropogenic sources of Cd (Zhao *et al*., 2020; Nordberg *et al*., 2022). Cadmium-Ni batteries, landfills, and municipal wastes are the principal contributors to Cd contamination worldwide (Khan *et al*., 2017). Elevated levels of soil Cd accumulation which mainly occur around the mining sites, are due to the extraction and transportation of products for refining, smelting, and improper disposal of tailing and wastewater (Zhao *et al*., 2020). In the EU, urban waste comprises 0.3-12 mg kg^{-1} of Cd, while landfill condensate includes 0.5-3.4 gL^{-1} of Cd (Haider *et al*., 2022).

The natural concentration of Cd for most soils is less than 1.0 mgkg^{-1}, but industrial effluents are applied continuously; their value may exceed the allowable limits (Jabeen *et al*., 2022). Because of its toxic effect, high solubility in water, and rapid mobility that easily carries it from soils to roots, Cd is recognized as a well-known harmful environmental contaminant (Rajamoorthy *et al*., 2015; Branca *et al*., 2020).

With time, Cd has accumulated in significant amounts in Pakistani soil due to improper agricultural practices that resulted in contamination of the food chain (Feleafel *et al*., 2012; Elgallal *et al*., 2016; El-Kady *et al*., 2018; Rai *et al*., 2019). It is estimated that 13,000 tons of Cd are released into our environment annually due to human activities (Bhatt *et al*., 2019). Unprocessed wastewater is the chief cause of Cd pollution, equally in plants and animals, particularly in the case of soil and vegetable crops (Rahi *et al*., 2022).

Effect of cadmium and salinity on plants at different growth stages: Heavy metals and salinity alter the morphology, physiology, viability, metabolism, and diversity of symbiotic and free-living soil microorganisms, resulting in affected plant development (Xie *et al*., 2016; Kamran *et al*., 2019). Heavy metals are renowned for causing abiotic stresses in plants; because they are highly accumulated in various plant components, these contaminants interfere with metabolic activities and restrict plant development (Jabeen *et al*., 2022). According to a crop's stage, various crops have varying tolerances to salinity and Cd toxicity; developing stages are much more vulnerable to Cd toxicity and salinity (Akhtar *et al*., 2015; Xie *et al*., 2016; Rahi *et al*., 2022). One of the most crucial steps throughout a plant's life is the germination of the seed, which is preceded by the breaking of the seed's inactive stage (Huybrechts *et al*., 2019; Ismael *et al*., 2019).

A trace amount of Cd may restrict seed germination, but when present in sufficient amounts, it also inhibits seed germination in the soil by modifying the concentrations of ABA, auxin, and gibberellic acid (GA), the key phytohormones that control seed germination (Huybrechts *et al*., 2019).Toxic Cd concentrations and salinity restrict seed germination, impede crop development, interfere with plantlet physiological processes, and decline crop production (Kaveh *et al*., 2011;Guilherme *et al*., 2015; Raza *et al*., 2020). It was observed that fivemgL^{-1} of Cd exposure decreases seed germination of lettuce, soybean, and sugar beet sprouts by 8.0, 18, and 19%, correspondingly (Li *et al*., 2013; Guilherme *et al*., 2015). Cadmium accumulation has been linked with a decrease in the activity of α-amylase, which reduces the starch release by cotyledons.

Furthermore, Cd and Ca ions competed for Ca-calmodulin binding sites in *Raphanus sativus* L. (radish) (Huybrechts *et al*., 2019). The link between calmodulin (CaM) and Cd is believed to be crucial in metabolism throughout the earlier stages of seed development (Raza *et al*., 2020). Citrus fruit sprouts were weakened, lifeless, and chlorotic after contact with $CdCl_2$ (Raza *et al*., 2020). Moreover, underneath the Cd effect, parsley saplings required much more Cd concentrations, even though there was no outward sign of stress on the plants. Consequently, poor germination causes a significant decrease in crop yield because of these stressors (Rahi *et al*., 2022).

Effect of cadmium and salinity on different physio-biochemical processes in plants: Cadmium in the topsoil is highly lethal for the growth attributes of significant plants. A surplus amount of Cd may often lead to several structural, functional, biochemical, and physiological disorders in plants (Ehsan *et al*., 2014). Cadmium poisoning and high salts in the soil cause a physical disturbance that can affect plant survival, reproduction, and mitigation (Naz *et al*., 2021; Haider *et al*., 2022). Plants cannot change their position actively to circumvent the polluted environment. Thus, their only hope for survival in hostile circumstances is to mobilize their defensive mechanisms and develop tolerance mechanisms and genotypes (Xie *et al*., 2016).

The significant tasks of plant roots are to absorb and uptake nutrients and water from the soil. They help anchor plants to the ground and play a role in asexual reproduction (Feleafel *et al*., 2012; Rucin'ska *et al*., 2016). If soils were Cd-enriched, the osmotic ability of the soil solution might be lower when compared with that of root cell sap (Haider *et al*., 2021). As a result, soil solution will significantly restrict plant roots from absorbing water by building reverse osmotic pressure (Rucin'ska *et al*., 2016).

If such conditions are followed by prolonged Cd exposure and salinity, the plant root becomes necrotic, decaying, and mucilaginous, restricting root and shoot growth and producing leaf roll and chlorosis (Abbas *et al*., 2017; Zafar-ul-Hye *et al*., 2020). Cadmium stress causes a rise in the mass of cortex and parenchyma cells, which enhances plant battle to solute and water transport, and induces changes in root thickness (Ismael *et al*., 2019). Exposure to Cd produced chromosomal anomalies in pea root tips, leading to mitotic disjunction and root elongation problems (Tran & Popova, 2013).

Salinity stress causes a buildup of Na^+ and Cl^- ions; it impacts the availability of other vital elements and can inhibit plant access to the absorption of minerals and essential nutrients, along with their distribution in plants, resulting in nutritional disparity and decreased physiological responses such as plant development (Bhise & Dandgev, 2019). Reduction in root size, rhizosphere, and root apex length was associated with high Cd toxicity. It also resulted in a poorer capacity for storing substances, such as food and water, in the Plant (Lu *et al*., 2013).A considerable reduction in total leaf area (TLA) and dry mass of numerous plant organs was also seen under salinity and Cd stress (Jinadasa *et al*., 2015).

Cadmium stress and salinity-induced plant growth stunting may be associated with reduced water and nutrient uptake, respiration, photosynthesis, phosphorous, carbon (C), nitrogen (N) acquisition, and antioxidant capacity (Li *et al*., 2013; Khan *et al*., 2021). Restricted absorption of Zn^{2+}, Fe^{2+} and Mg^{2+} also limit plant cells' functioning, resulting in reduced plant yield (Khan *et al*., 2021; Rahi *et al*., 2022). Cadmium poisoning affects the nitrogen, magnesium, and Phosphorous levels in alfalfa plant roots and shoots substantially (Zhang *et al*., 2019). In other studies, Cd exposure to alfalfa for 6-24 hours gives rise to rapid peroxide deposition and a reduction in homoglutathione (hGSH) and glutathione (GSH), which leads to a redox imbalance (Gutsch *et al*., 2019).

In plants, Cd, along with the high buildup of salts, initiate the synthesis of ROS, reduces gaseous exchange by affecting stomatal functioning, transportation and changes the photosynthetic machinery alleged to cause plants death (Rizwan *et al*., 2016; Bhise & Dandge, 2019). Decreased uptake of CO_2 due to elevated levels of Cd resulted in a disturbed photosynthesis rate in the plants (Li *et al*., 2013). Numerous symptoms (including chlorosis, dehydration, stunting, and cell death) have been seen in plant leaves as a result of Cd and salt stress; plants may develop hazardous symptoms if the Cd level in plant tissue out passes 3-30 mg kg^{-1} (Ismael *et al*., 2019; Demirkaya, 2021). In cereal, legume, and oilseed crops, a linear link is found amid transpiration and photosynthesis inhibition was observed, showing that Cd buildup in leaves reduces stomatal opening (Younis *et al*., 2016; Zhang *et al*., 2019). Salinity and Cd principal action areas include the photosynthetic machinery and its pigments, chlorophyll production, and carotenoid synthesis, as shown in Fig. 2 (Younis *et al*., 2016; Bhise & Dandge, 2019). They also limit the photoactivation of photosystem II (PSII) by inhibiting electron transport (Farooq *et al*., 2016; Bhise & Dandge, 2019). Cadmium reduced gas exchange (GE) properties, destroying photosynthetic pigments and chloroplast structure (Haider *et al*., 2022).

Cadmium and elevated salt levels often induce oxidative tension in plants, implicitly or explicitly, by the generation of ROS. Ahmad *et al*., (2011), Ehsan *et al*., (2014) and Haider *et al*., (2022) reported that plants show oxidative stress in response to salinity and Cd oxicity by enhancing electrolyte leakage stress generated oxidative responses in them by increasing H_2O_2 production, MDA (malondialdehyde) production in various parts of the plant. Peroxidation of lipids and proteins and DNA damage are common examples of ROS injury in plants in response to Cd toxicity and salinity (Younis *et al*., 2016; Bhise & Dandge, 2019; Haider *et al*., 2022), as shown in (Fig. 2).

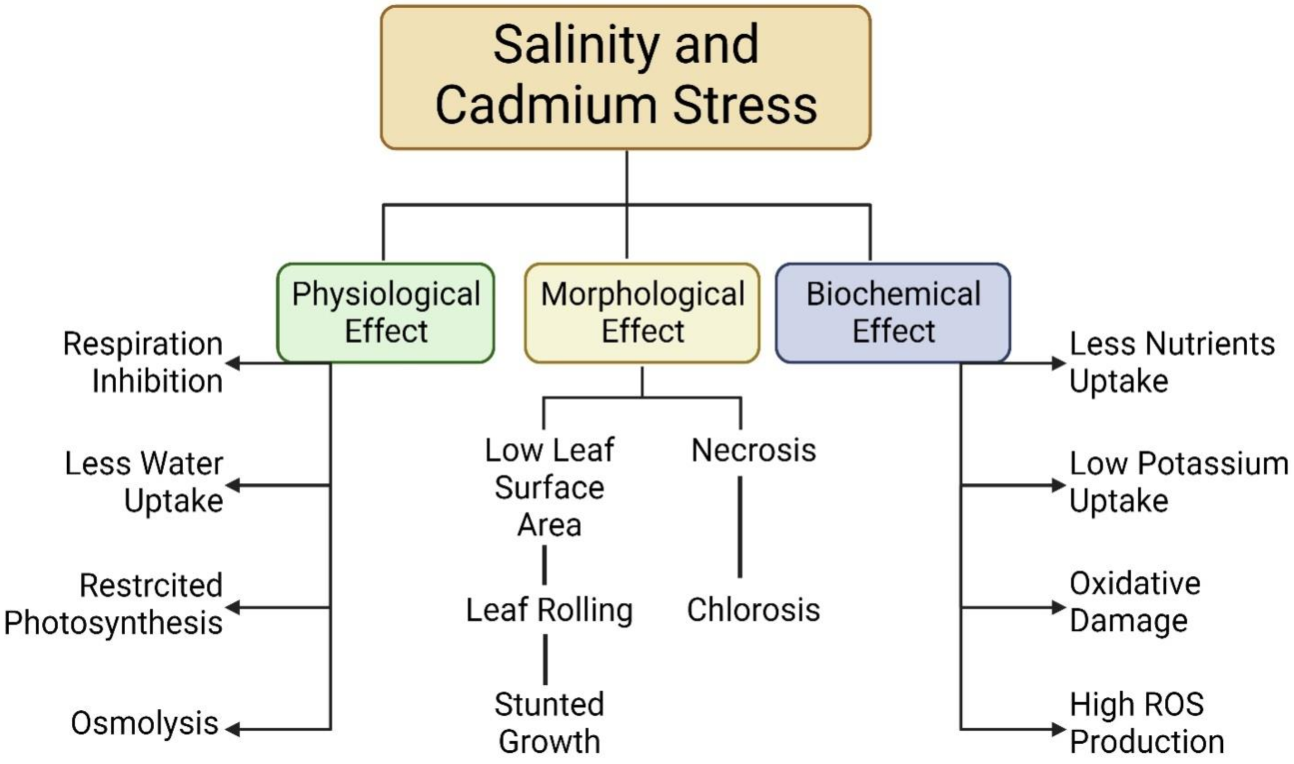

Fig. 2. Combined effects of salinity and cadmium toxicity on the plant.

Acidified biochar as remediation: For decades, many efforts have been made to minimize the HM stress and salinity in the loss of plants (Kamran *et al*., 2019). Strategies to incorporate naturally derived substances into sustainable agriculture have significantly amplified over the past decade. These bio-stimulants may boost plant productivity and resilience to various biotic and abiotic challenges (Akhtar *et al*., 2015; Semida *et al*., 2019). The most encouraging option now is using acidified biochar which can effectively boost soil fertility, encourage plant development, and boost plant adaptability to such hostile conditions (Guo *et al*., 2021; Jabborova *et al*., 2021).

Biochar is produced using organic wastes like crop plant residues, manures, etc. It is considered a cost-efficient strategy because it steadies the carbon at the lowest cost relative to organic compost and fertilizer (Younis *et al*., 2016). Physical properties, i.e., soil texture and pore structure, impact the aeration of the soil and water retention capacity. Besides, soil processing properties are directly influenced by biochar's applications in the soil as a modification strategy (Abd El-Mageed *et al*., 2021; Rahi *et al*., 2022). Adding biochar to the soil increases soil nutrient content and water-holding ability (WHA) of soil (Abd El-Mageed *et al*., 2021; Rahi *et al*., 2022). The amendment of soil with acidified biochar enhances soil nutrient content and water-retaining capacity (Ahmed *et al*., 2021), limiting the use of mineral fertilizers. Biochar helps stabilize the soil structure and remarkably decreases nutrient loss by leaching (El-Mageed *et al*., 2020; Abd El-Mageed *et al*., 2021). It also helps retain phosphorous due to the sorption process, which helps improve phosphorous availability and uptake by plant roots by enhancing soil anion exchange capacity (Ahmed *et al*., 2021). Biochar has a positive physical and biochemical influence on Soil microbiology and plant development. Besides, it has a positive and indirect role in conducting positive soil chemical reactions.

Demirkaya (2021) reported that using acidified biochar is quite helpful in lowering soil alkalinity. Biochar has an alkaline characteristic, so its addition to alkaline soil does not improve the soil. To fix this, the acid application is often used to acidify biochar. The creation of an acid functional group on the surface of organic char increased, thus helping play a regulating role in alkaline soils (El-Mageed *et al*., 2020;Ahmed *et al*., 2021; Demirkaya, 2021).

Acidified biochar regulates the nutrient content of soil (Abd El-Mageed *et al*., 2021). Sadegh-Zadeh *et al*., (2018) reported that when compared to untreated soil, acidified biochar amendment significantly increased the macronutrient content in *Faba bean* leaves (P, N, K^+, and Ca^{2+}). Chaganti *et al*., (2015) observed significant reductions in the electric conductivity of salt-affected soil by introducing biochar compared to the non-amended soil due to betterment in hydraulic conductivity of soil and leakage of excessive salt. Acidified biochar was effective at lowering soil EC levels. As, the acidified biochar can unconfined H^+ ions into the soil, where they react with HCO_3 and lead towards the formation of carbonic acid. (H_2CO_3), which then combined with calcium carbonate to form Ca^{2+} ions and HCO_3^- ions. On the surfaces of the soil colloids, the Ca^{2+}ions produced in such away were exchanged for Na^+ ions. The replaced Na^+ was released from the soil and plant rhizosphere by entering the soil solution. Furthermore, the biochar contained $Ca^{2+,}$ Mg^{2+}, and $K^{+,}$

which could replace Na^+ in soil colloids. Finally, Na^+ ions arrived in the soil solution and were easily expelled by the water used for irrigation purposes (Sadegh-Zadeh *et al*., 2018).

Numerous studies show that using biochar to treat soils contaminated with HM, like lead and Cd, can improve the situation for crops grown there Al-Wabel *et al*., (2015) and Ali *et al*., (2017) reported that toxic metal presence and uptake by maize plants could be reduced using soil modifications like biochar. They claimed that acidified biochar fundamentally reduced the amount of HMs that could be extracted from the soil, indicating metal immobilization, and increased the dry shoot biomass of maize. Additionally, biochar significantly decreased the amounts of Fe, Mn, Zn, Cu, and Cd in maize. Many functional groups are found on the surface of Activated char that function as binding sites for HMs (Sultan *et al*., 2020). When soil is amended with biochar, these active sites attract the heavy metals and bind them by chelating surface adsorption and precipitation, which significantly reduce their mobility and uptake by plants (Kloss *et al*., 2014; Rahi *et al*., 2022).

Houben *et al*., (2013) stated that a 10% application of acidified biochar helps improve plant growth and enhances carbon accumulation and nutrient uptake in *Brassica napus.* Bashir *et al*., (2018) reported that *Brassica oleracea* 3% application of biochar helps relieve the symptoms of Cd poisoning and improves plant biomass. In *Brassica chinensis,* a significant reduction is observed in the accumulation and translocation of Cd in the shoot and root due to the application of biochar in HM and salt-contaminated soil (Liu *et al*., 2018). In the case of *Brassica juncea,* the minimum amount of Cd translocation is observed in plant shoots, along with the decrease in HM uptake by plants roots because of biochar amendment. Younis *et al*., (2016) stated that a reduction in the level of reactive oxygen species (ROS) and an increased level of antioxidant enzymes were observed in the case of spinach (Table 1).

Table 1. Effect of acidified biochar on alleviating the stress of salinity and cadmium toxicity.

Plants	Acidified biochar dose (w/w; %)	Plant response	Target organ	Reference
Brassica napus	10	Improved plant growth, and enhanced nutrient uptake and carbon accumulation	Roots	(Houben *et al*., 2013)
Brassica oleracea	3	Cd poisoning reduced, and plant biomass improved	Shoots	(Bashir *et al*., 2018)
Brassica chinensis	2.5-5	Significant reduction in Cd accumulation rate, improved P and N uptake by plant roots	Shoots and Roots	(Liu *et al*., 2018)
Spinacia oleracea	5	Improved photosynthetic activity, and antioxidant enzyme synthesis, but reduced oxidative stress in plants	Shoots	(Younis *et al*., 2016)
Zea mays	0.45	Better P translocation to root, improved growth, lower oxidative stress	Roots, Stem	(Ahmed *et al*., 2021)
Brassica juncea	5	The lowest amount of heavy metal uptake and translocation in the shoot improved plant growth	Shoots	(Ali *et al*., 2017)

Conclusion

It is concluded that to alleviate salinity and HM pollution in soil and to provide a variety of advantages, biochar acts as an ecological solution to enhance the soil's nutrition cycle, cation exchange capacity, and humification. Studies reveal that addition of acidified biochar improves alkaline soil's physio-chemical (such as availability of nutrients, cation exchange capacity (CEC), soil pH, etc.) and living properties (such as microbial population). In the context of future prospective, more research should be done on acidified biochar regarding its impact on elevating salinity and Cd stress on plants, to achieve enhanced crop productivity.

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