EFFECT OF BIOCHAR APPLICATION ON HEAVY METAL ACCUMULATION IN RED AMARANTH (*Amaranthus spp*.) **GROWN IN CONTAMINATED SOIL**

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CERTIFICATE

This is to certify that the thesis entitled **“Effect of Biochar application on heavy metal accumulation in red amaranth** (*Amaranthus spp*.) **grown in contaminated soil”** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE IN SOIL SCIENCE** in **SOIL SCIENCE,** embodies the result of a piece of bona fide research work carried out by **ABU BAKAR NUMAN**, Registration No.**17-08238** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

....

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ABSTRACT

A pot experiment was conducted at the net house of the research farm of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from January-June 2018. The experiment was conducted to observe the effect of biochar (BC) on cadmium (Cd) accumulation in red amaranth (*Amaranthus spp*.) and on soil health. In this experiment, the treatment consisted of four doses of BC viz. BC_0= no BC, BC_1=1% BC of soil wt., BC_2= 2% BC of soil wt., BC_4= 4% BC of soil wt. and Cd_0= no Cd, Cd_5 = 5 ppm Cd, Cd_{15} = 15 ppm Cd. The experiment was laid out in Complete Randomize Design (CRD) with five replications. The collected data were statistically analyzed for evaluation of the treatment effects. Results showed that significant variations were found among the treatments for the observed parameters such as Cd in stem, leaf and root, total and available Cd in bulk and rhizosphere soils, soil pH, bulk density and porosity. The lowest Cd accumulation in plant stem, plant leaf and plant root was found in the treatments with 4% BC of soil wt. addition. Higher amount of total Cd in bulk and rhizosphere soil was observed in the treatments of higher amount of BC .However, lower amount of bio-available Cd was found in soils with higher amount of BC indicating that stabilization of Cd is promoted by BC. Biochar application also reduced the bulk density and increased porosity and pH indicating improved soil health for better crop production in an acid soil. It was observed from this study that red amaranth accumulated significant amount of Cd even at low concentration (5ppm) which is alarming. As BC reduced the Cd accumulation significantly it can be concluded that metal stabilizer like BC may be used as soil amendment in reducing the heavy metal uptake by leafy vegetables grown in contaminated soil

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Chapter I

Introduction

Soil pollution with heavy metals is a major environmental hazard for developing nations like Bangladesh for rapid development of industrial activities over the decade. The excessive use of fertilizers and the addition of sewage waste in the soil have resulted in augment in soil Cadmium (Cd) concentrations. Generally, plants tolerate Cd better than human and animals. Cadmium toxicity in plants appears at higher than that of animals or human. In humans, excessive Cd intake can cause the "Itai-itai disease" and has also been related to some types of cancer or kidney damage. Heavy metals uptake by plants, accumulation in animals and humans (Dudka and Miller, 1999; Reeves and Chaney, 2008; Singh *et al*.,2010) showed that Cd penetrate into the food chain, consequently cause different health complexities, even cause cancer (Lee *et al*.,2005; Liu *et al*.,2005). Phyto extraction (Kumar *et al*., 1995), chemical stabilization (Kumpiene *et al*., 2008), and soil washing (Abumaizar and Smith, 1999) and various different ways are used to amend soil heavy metal concentration. Among the amendments, biochar (BC) adsorbs heavy metals and decrease potential bioavailability, and thus BC has been shown to be particularly benificial (Beesley *et al*., 2011; Houben *et al*., 2013).

Biochar is basically a stable, recalcitrant organic carbon compound produced by pyrolysis of biomass at temperatures between 300^0C and 1000°C under low oxygen or anaerobic conditions (Krull, 2011; Verheijen *et al*., 2010). Biochar is naturally high porous, thus its addition to soil is considered to develop a range of soil physical properties including soil porosity, pore size distribution, soil density, soil moisture content, water holding capacity or plant available water content (PAWC), and hydraulic conductivity (Atkinson *et al*., 2010; Major *et al*., 2009; Sohi *et al*., 2009, 2010; Zwieten *et al*., 2012).

Different materials can be used as feed stocks, including sludges, plant materials and manures. Generally, biochar has high CEC (cation exchange capacity) and alkaline in nature. Biochar has many potential benefits on soil properties physical, chemical and biological (Lehmann *et al*., 2011; Paz-Ferreiro *et al*., 2014), diminishing soil greenhouse gas emissions from agricultural sources and thus enhancing soil carbon sequestration due to its elevated content of recalcitrant forms of carbon (Gascó *et al*., 2012).

Furthermore, BC might have various effects on vegetative growth and crop yield or fruit quality. According to Vaccari *et al*. (2015) BC application had promising effect on soil fertility and stimulated growth of the plant (Hol WG. *et al*., 2017). Vegetables are essential edible crops and are an important part of the diet. They are rich in nutrients needed for human health, and are an essential source of carbohydrates, vitamins, minerals, and fibers (Hu *et al.*, 2013; Yang *et al*., 2009). Heavy metals can be easily taken up by vegetable roots, and can be accumulated at high levels in the edible parts of vegetables, even in lower level of heavy metal contamination in soil (Yang *et al.*, 2009; jolly *et al*., 2013).

In many countries and regions, vegetables are exposed to heavy metals by various means, thus vegetable consumption can cause adverse health effects. In Huludao City, China, the ranges of Pb and Cd concentrations in vegetables are 0.003–0.624 mg/kg and 0.003–0.195 mg/kg (fresh weight), respectively, and the maximum concentrations of Pb and Cd all exceed the recommended values (Hu *et al.* (2013) described that 16%, 26%, and 0.56% of market vegetables in Hong Kong were polluted by Pb, Cd, and Cr, respectively. Rahman *et al*. (2014) described that some Australian and Bangladeshi vegetables contained Cd concentrations higher than the Australian standard maximum limit (0.1 mg/kg). Therefore, vegetable consumption is considered to be one of the major sources of heavy metal intake for humans, and elevated levels of heavy metal in edible parts of vegetables can affect human.

Vegetable species vary widely in their capacity to take up and accumulate heavy metals, even among cultivars and varieties within the same species. Zhu *et al.* (2007); Säumel *et al*. (2012); Alexander *et al*. (2006) reported that Pb significantly accumulated in lettuce and onion, while Cd accumulated to the greatest degrees in spinach and lettuce. Yang *et al*. (2009) revealed that Chinese leek, pak choi, and carrot had higher Cd concentrations in their edible parts than radish, cucumber, and tomato. Säumel *et al*. (2012) stated that Zn concentrations in green beans, tomato, potato, kahlrabi, and carrots were significantly lower than the concentrations in leafy vegetables. Cd accumulation in vegetable species decreased in the order of leafy vegetables > solanaceous vegetables > root vegetables > allimus vegetables > melon vegetables > legumes vegetables (Yang *et al*., 2007).

In this study, BC is produced from rice husk intended to be examine regarding its ability to decrease the bioavailability of heavy metals in contaminated soils. The aim of this study was to investigate the effect of BC on heavy metal immobilization and subsequent heavy metal uptake by red amaranth plant in soil artificially contaminated with Cd. The objectives of the study were:

- To examine the effect of BC on Cd accumulation in red amaranth (*Amaranthus spp*.)
- To observe the changes in soil health as impacted by BC amendments

Chapter II

REVIEW OF LITERATURE

Many research works on heavy metal uptake and soil amendments by BC have been performed extensively in several countries especially in the South East Asian countries for its threat to environmental or human health. In Bangladesh, little attention has so far been given for the soil amendments through BC and ameliorate soil health. Currently Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) have started extensive research work on BC as amendments and its usefulness. Findings of various experiments related to the present study in home and abroad have been reviewed and discussed in this chapter.

2.1 Biochar

Biochar has been defined in similar ways by several authors. It is a 'black carbon produce by pyrolysis of biomass' (Lehmann *et al*., 2006); 'the high carbon materials obtained from the slow pyrolysis (heating in the absence of oxygen) of biomass' (Chan *et al*., 2007); and 'a fine-grained and porous substance, homogenous in its appearance to charcoal produced by natural burning or by the combustion of biomass under anaerobic (or limited oxygen) situation (Sohi *et al*., 2009). Even, it is a product of biomass obtained from heating in a suitable temperature regime in the absence of oxygen (the process of fast or slow pyrolysis) or from a gasification system.

2. 2 Effect of biochar on soil properties

Biochar is a steady form of carbon and can last for thousands of years in the soil. It is formed for the purpose of addition to soil as a means of sequestering carbon and developing soil quality. The conditions of pyrolysis and the materials used can considerably affect the properties of BC. The physical properties of BC contribute to its function as a tool for managing the environment. It has been stated that when BC is used as a soil amendment, it stimulates soil fertility and develops soil quality by increasing soil pH, increasing the ability to conserve moisture, appealing more useful fungi and other microbes, improving the ability of cation exchange, and stabilizing the nutrients in soil. Biochar decreases soil density and soil hardening, rises soil aeration and cation-exchange capacity, and changes the soil structure and consistency through the changes in physical and chemical properties. It also benefits to reclaim degraded soils. It has shown a better ability to adsorb cations per unit carbon as compared to other soil organic matters since of its greater surface area, negative surface charge, and charge density (Liang *et al*., 2006), thereby offering the probability of improving yields (Lehmann, 2006).

The physical characteristics of BC are directly and indirectly associated to how they affect soil systems. Soils have their own physical properties depending on the nature of mineral and organic matter, their relative amounts, and how minerals and organic matter are correlated. When BC is present in the soil combination, its influence to the physical nature of the system is substantial, affecting the depth, texture, structure, porosity, and consistency by altering the surface area, pore and particle-size distribution, density, and packing (Blanco and Canqui, 2006). The influence of BC on physical properties of soil directly affects the growth of plants, since the depth of penetration and accessibility of air and water in the root zone is determined mainly by the physical structure of the soil horizons. This affects the soil's response to water, its aggregation, and work ability in soil preparation, dynamics, and permeability when swells, as well as the ability to retain cations and response to changes at ambient temperature.

The smaller the pores on BC, the longer they can retain capillary soil water. The addition of BC can decrease the effects of drought on crop productivity in drought-affected areas due to its moisture-retention capacity. It has been shown that it eradicates soil restrictions that limit the growth of plants, and neutralizes acidic soil because of its basic nature (Hammes and Schmidt, 2009). Carbon dioxide and oxygen occupy air-filled spaces on the pores of BC or can be chemosorbed on the surface. As BC can encompass nutrients, microorganisms, and syngases, it can also retain nutrients in soil longer than other soils and prevent them from leaching.

As far as its chemical properties are concerned, BC diminishes soil acidity by increasing the pH (also called the liming effect) and aids the soil to keep nutrients and fertilizers, Lehmann *et al.* (2006). The application of BC increases soil fertility through two mechanisms: accumulation nutrients to the soil (such as K, to a limited extent P, and many micronutrients) or holding nutrients from other sources, including nutrients from the soil itself. However, the main benefit is to hold nutrients from other sources. In most circumstances, the addition of BC only has a net positive effect on the growth of crops if nutrients from other sources, such as inorganic or organic fertilizers, are used. BC rises the availability of C, N, Ca, Mg, K, and P to plants, because BC absorbs and slowly release fertilizers. It also aids to avert fertilizer drainage and leaching by allowing less fertilizer use and reducing agricultural pollution in the surrounding environment (Cao *et al.*, 2018). Biochar relieves the impact of hazardous pesticides and complex nitrogen fertilizers from the soil, thus dropping the influence on the local environment.

2.3 The Mechanism of interaction between biochar and heavy metals

Physical characteristic of BC is a function of several factors, including the type of feedstock, the particle size of the feedstock and temperature and conditions of pyrolysis. The wide range of physical characteristics that BC might possess makes some particular materials more suitable than others to remediate different heavy metals. Therefore, when selecting a BC for remediation purposes, scientists should be conscious not only on soil type and characteristics but also on BC properties. Moreover, it should also be considered that key BC properties such as surface area, pH, ash and carbon substances can be affected by post-treatments and thus improve BC's ability to immobilize heavy metals. Before studying the mechanisms implied in the interaction between BC and heavy metal it is essential to note that BC's act on the bioavailable fraction of soil heavy metals and that they can decrease also their leach ability. One of the characteristics of BC is holding large surface areas, which infers a high capacity for complex heavy metals on their surface. Surface sorption of heavy metals on BC has been proved on multiple occasions using scanning electron microscopy by Beesley and Marmiroli, 2011; Lu *et al*., 2012. This sorption can be due to complexion of the heavy metals with diverse functional groups present in the BC, due to the exchange of heavy metals with cations related with BC, such as Ca^{+2} and Mg^{+2} (Lu *et al*., 2012), K^{+}, Na^{+} and S (Uchimiya *et al*.,2011), or due to physical adsorption) (Lu *et al*., 2012). Also oxygen functional groups are known to stabilize heavy metals in the BC surface, principally (Uchimiya *et al*., 2011) for softer metals like Pb^{+2} and Cu^{+2} . In addition, Méndez *et al.* (2009) observed that Cu^{+2} sorption was associated to the elevated oxygenated surface groups and also with high average pore diameter, elevated superficial charge density and Ca^{+2} and Mg^{+2} exchange content of BC. Perhaps, sorption mechanisms are highly dependent on soil type and the cations present in both BC and soil. Some other combinations present in the ash, such as carbonates, phosphates or sulphates (Karimi *et al*., 2011; Park *et al*., 2013) can also help to stabilize heavy metals by precipitation of these complexes with the pollutants. Alkalinity of biochar can also be partially accountable for the lower concentrations of available heavy metals found in BC -amended soils. Higher pH values after BC application can result in heavy metal precipitation in soils. Due to application of BC, pH value rises with pyrolysis temperature (Wu *et al*., 2012), which has been related with a higher proportion of ash content. Biochar can also decrease the mobility of heavy metals, changing their redox state of those. As an example, BC addition could lead to the transformation of Cr^{+6} to the less mobile Cr^{+3}.

At present, researchers mostly focus on the remediation of heavy metals such as As, Pb, Cd, Zn, Cr, Cu and Hg. Unlike organic pollutants, heavy metals are hard to be biodegraded, when the new material, BC has wide sources, porous structure, larger surface area and plentiful surface functional groups, which can efficiently repair a certain quantity of heavy metal pollutants (Jin, 2011).

2.3.1. Ion exchange and adsorption of cationic π function

The exchange adsorption of BC surface is one of important reasons for the reduction of heavy metal activities as the bigger the amount of cation exchange, the stronger the retention of heavy metals (Lehmann, 2006; Reesa *et al.*, 2014). The nature of ion exchange is electrostatic relations between negative charge groups on the BC surface and positive charges in the soil. This type of reaction, with lower adsorption energy, belongs to nonspecific adsorption and has obvious reversibility. The cationic π function depends on the aromatization of BC. The more the π conjugate aromatic structure exists, the greater the negative charge in π orbital changes, thus the ability of losing electrons of function groups rises and the adsorption effect becomes more substantial (Li *et al.*, 2017).

2.3.2 Coprecipitation

Biochar can effectively diminish the activities of heavy metals by adsorption and dissolution- precipitation of mineral component. The addition of BC can rise the pH of soil (Reesa *et al.*, 2014), and the reaction of heavy metal ions with -OH, $PO4^{-3}$, $CO3^{-2}$can form hydroxide, carbonate or phosphate precipitation, which efficiently solids the heavy metal pollutants.

2.3.3. Complexation

This complexation is substantial for the fixation of heavy metal ions with strong kinship. A large number of studies have revealed that the reactions of heavy metal ions with oxygenic functional groups like hydroxyl group (-OH), carboxyl group (-COOH) and amino-group (-NH2) on BC surface create great contributions to the adsorption of heavy metal ions (Xu *et al*., 2012; Li *et al*., 2017).

2.3.4. Electrostatic absorption

The larger surface area and higher surface energy are cooperative for BC to strongly captivate the heavy metal pollutants and eliminate them from the soil (Li *et al*. 2017).

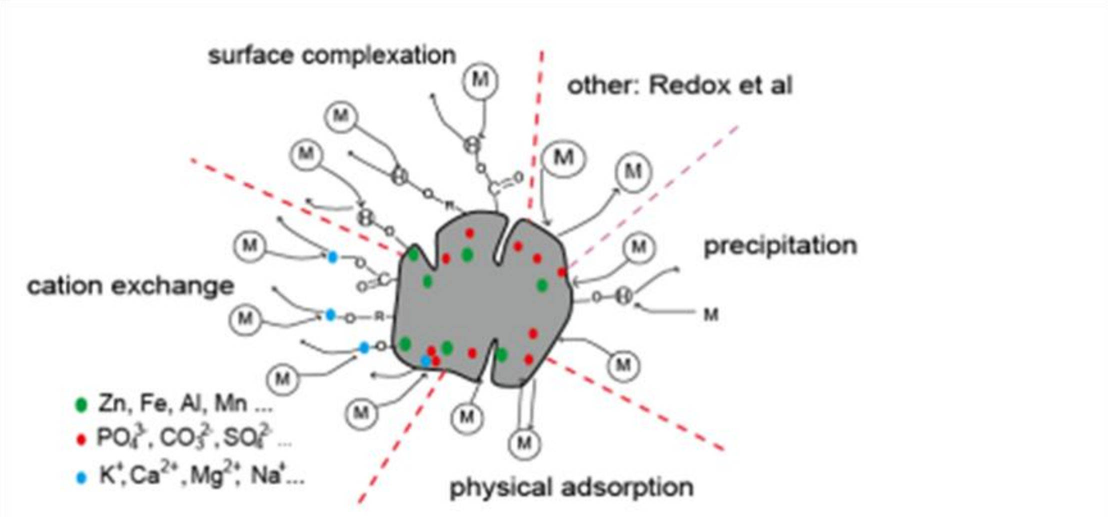

Fig-1: The removal mechanisms of heavy metals by biochar (Li *et al*. 2017).

The remediation mechanism of BC is different for different heavy metal pollutants. For the same heavy metal ion, the adsorption mechanism is dissimilar when the BC is unlike. The adsorption influence of BC on heavy metal ions is influenced by many factors such as raw materials of BC, pyrolysis temperature, pH of soil, physical and chemical properties of heavy metal ions and the amount of BC addition. Studies have shown that under the same state, although plant BC has the largest surface area, the adsorption effect of animal fecal BC on heavy metal ions is superior than sludge BC and plant BC. This is because P reserves in the animal waste BC can react with certain heavy metal ions through precipitation or co-precipitation, and make the greatest contributions during the repair procedure. Studies also showed that the adsorption effect of animal dung BC on $Pb^{+2}$is the best among the three types of BC.

2.4 Effect of biochar on crop growth and development

There are varied responses of crops to b BC. A study led by Van Zwieten *et al.* (2010) found that BC accumulation significantly increased biomass in wheat, soybean and radish in ferrosol soil but abridged wheat and radish biomass in calcaresol, amended with fertilizer in both soils. A significant reduction in dry matter content of radish was acquired when BC was applied at 10 ton ha^{-1} (Chan *et al*. 2008a). In a separate experiment, there was no significant effect of BC rates (0, 7 and 15 tons ha) on wheat yield (Brandstaka *et al*. 2010). Asai *et al.* (2009) displayed that BC amplified rice grain yields at sites with low P availability, which might be due to enhanced saturated hydraulic conductivity of the top soil, xylem sap flow of the plant and response to N and NP chemical fertilizer treatments.

Limiting soil N content by BC application in N deficient soils could be due to the high C/N ratio, hence it might diminish crop productivity temporarily (Lehmann et al. 2003). However, some BC enclose considerable amount of micronutrients. For example, pecan-shelled BC contained greater amount of Cu, Mg and Zn than the soil (Novak *et al.* 2009). In a separate experiment, concentrations of heavy metals including Cu and Zn amplified in sewage sludge BC but those of available heavy metals diminished (Liu *et al*., 2014). Furthermore, poultry litter BC was also rich with significant amounts of Zn, Cu and Mn (Inal *et al*., 2015). Thus, it is essential to compare its effect solely and in mixture with other nutrient sources. Some authors (Verheijen *et al*., 2009; Brandstaka *et al*., 2010) have accentuated the need for further research on potential benefits of BC as well as their economics. However, their interactions with other organic sources as well as microbes and release of nutrients from them are inadequately assessed.

Biochar at the rates of 20 and 40 t ha^{-1} without N fertilization in a carbon poor calcareous soil of China amplified maize yield by 15.8% and 7.3% while the rates with 300 kg ha^{-1} N fertilization improved the yield by 8.8% and 12.1% ,respectively (Zhang *et al*., 2012). In addition, BC application in a nutrient-poor, slightly acidic loamy sand soil had little effect on wheat yield in the absence of mineral fertilization but when applied with the highest rate of mineral fertilization, it produced yield 20–30 % more than mineral fertilizer alone (Alburquerque *et al*., 2014).

The yield of tomato fruit was significantly higher in beds with charcoal than without charcoal. Biochar application increased vegetable yields by 4.7- 25.5% as compared to farmers' practices (Vinh *et al.*, 2014). In another work, BC did not rise annual yield of winter wheat and summer maize but the collective yield over four growing season was significantly amplified in a calcareous soil (Liang *et al*., 2014). Biochar of maple was tested at different concentrations for root elongation of pea and wheat but no substantial difference was experiential (Borsari, 2011), possibly due to little consequence of BC in the short-term. The wood chip BC is produced at 290^{o}C and 700^{0}C had no effect on growth and yield of either rice or leaf sheet.

A rice-husk BC tested in lettuce-cabbage-lettuce cycle improved final biomass, root biomass, plant height and number of leaves in comparison to no BC treatments (Carter *et al*., 2013). An oak BC derivative from a slow pyrolysis process was verified for four years at 0 t ha^{-1}, 5 t ha^{-1} and 25 t ha $^{-1}$ with 100% and 50% of N fertilizer on a maize -soybean rotation in an alfisol soil, consequential in an overall positive trend in total above-ground biomass and grain yield (Hottle, 2013). A poultry-litter BC derived from slow pyrolysis experienced in cotton showed that a higher level (3000 kg ha $^{-1}$) with urea created better cotton growth than the lower rate (1500 kg ha^{-1}) which, in turn, did better than the control (Coomer *et al*., 2012).

Chapter III

MATERIALS AND METHODS

This study was conducted to find out the effect of BC on Cd uptake by red amaranth (*Amaranthus* spp.) plants. This chapter presents a short description about experimental period, site description, soil and climatic condition of the experimental area, experimental details, treatments, experimental design and layout, intercultural operations, data collection and statistical analysis. The details of experiments and methods are described below-

3.1 Experimental period

The experiment was conducted during the period from January 2018 to June 2018.

3.2 Description of the experimental site

The pot experiment was conducted in a net house of the research farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of the farm was $23^{0}77^{'}$N latitude and $90^{0}33^{'}$E longitude with an elevation of 8.2 meter from sea level.

3. 3 Soil characteristics

The soil belonged to The Modhupur Tract", AEZ — 28 (FAO, 1988). Top soil was silty clay in texture with common fine to medium distinct dark yellowish brown mottles. The collected soil was air-dried, grind and passed through 2 mm sieve and analyzed for both physical and chemical properties. Soil properties are p^{H} 6.20, OM 0.95, organic C (%) 0.55, N (%)-0.17, Available P(mg kg^{-1})- 8.4, bulk density (g cm^{-3})– 1.46.

3.4 Climatic condition

The geographical location of the experimental site was under the subtropical climate, categorized by three distinct seasons, winter season from November to February and the pre-monsoon period or hot season from March to April and monsoon period from May to October (Edris *et al*., 1979).

3.5 Treatments of the experiment

The treatments of experiment as follows:

BC_0CD_0 = No BC + No Cd

BC_0Cd_5 = No BC + Cd @ 5 ppm

BC_0Cd_{15} = No BC + Cd @ 15 ppm

BC_1CD_0 = BC @ 1% of soil wt + No Cd

BC_1Cd_5 = BC @ 1% of soil wt + Cd @ 5 ppm

BC_1Cd_{15} = BC @ 1% of soil wt + Cd @ 15 ppm

BC_2CD_0 = BC @ 2% of soil wt + No Cd

BC_2Cd_5 = BC @ 2% of soil wt + Cd @ 5 ppm

BC_2Cd_{15} = BC @ 2% of soil wt + Cd @ 15 ppm

BC_4CD_0 = BC @ 4% of soil wt + No Cd

BC_4Cd_5 = BC @ 4% of soil wt + Cd @ 5 ppm

BC_4Cd_{15} = BC @ 4% of soil wt + Cd @ 15 ppm

Seven kg soil/pot was taken for experiment. The volume of the pot was Approx. 0.3 cu. ft.

3.6 Experimental design

The two factors experiment was arranged out following Complete Randomized Design (CRD) with five replications.

3.7 Growing of crop

Lalshak (*Amaranthus spp.)*

3.7.1 Seed collection

The seeds of BARI LALSAK -1 used in this experiment were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur.

3.7.2 Biochar collection

Rice husk BC was collected from the Soil Science Division of BARI-Gazipur.

3.7.3 Preparation of the pots

Sufficient amount of soil was collected from a crop field of SAU farm in the last week of December, 2018. Collected soil was dried for two weeks in the air and pulverized. Dried soil was mixed with Cd at different rates and remained for eight weeks as barren for proper integration. After that different percentage of pulverized BC (rice husk burn) was mixed thoroughly with soil. Each pot contains seven kg soil. Pot was then kept for another four weeks until fertilizers were added. During the period of pot preparation, soil was kept moist by adding necessary amount water.

3.7.4 Application of fertilizers and manure

The fertilizers N, P, K and S in the form of Urea, TSP, MP and Gypsum, respectively were applied. The fertilizers application rate was 220, 180, 100 and 50 kg ha^{-1} respectively for urea, TSP, MoP and gypsum as per recommendation of BARI, (2006). The entire amount of TSP, MP, gypsum and 2/3 of urea were applied during the final preparation of pot soil. Rest of urea was top dressed after 15 days of sowing.

3.7.5 Seed sowing

Seeds were sown in the pot soil on 1st of April, 2019. It was confirmed that soil moisture was sufficient during seed sowing for seed germination.

3.8 Intercultural operations

After the emergence of seedlings, various intercultural operations such as watering, weeding, top dressing of fertilizer and plant protection measure were consummate for better growth of red amaranth seedlings as per the recommendation of BARI (2006).

3.8.1 Weeding and thinning

Weeding was done to keep the pots free from weeds which ultimately ensured better growth and development of red amaranth seedlings. The newly arisen weeds were uprooted carefully and thinning was done after 15 days of emergence of seeds. And again a thinning was done at mature stage.

3.8.2 Plant protection

The crop was confronted by different kinds of insects during the growing period. Sumithion-40 m1/20 liter of water was sprayed by hand sprayer on leaves as protection measure.

3.9 Harvesting, Drying and cleaning

The crop was harvested depending upon the maturity of plant manually from each pot from the Third week of May, 2019. The harvested crop of each pot was bundled separately and tagged properly. Fresh weight and plant height was taken immediately after harvesting. After enough sun dry whole plant was put in an oven at 27 degree Celsius. After three days of drying, dry weight was taken, and separated into root, leaves and stem. Entire amount then then pulverized in a grinding machine for Cd analysis.

3.10 Rhizosphere soil

Rhizosphere soil was collected from plant root zone after gentle shaking of pulled out root. It was then dried, pulverized and prepared for Cd analysis for measuring total and bio-available Cd.

3.11 Bulk soil

Bulk soil was collected from the pots and it was dried, pulverized and prepared for Cd analysis for measuring total and available Cd.

3.12 Bulk density, Particle density and porosity of soil

Bulk density of soil samples were measured by core sampler method. The bulk density is obtained by adding a known mass of powder to a graduated cylinder. The density is calculated as mass/volume.

1. Particle density was determined by volumetric flask method.
   Particle density= weight soil solid/ volume of soil solid.

2. Porosity =100(1- bulk density/particle density)

3.13 Cadmium analysis

Soil and plant samples were analyzed for total Cd using an atomic absorption spectrophotometer (AAS) method. Samples were accurately weighed 0.5g each and placed in a 100 ml PTFE beaker, then the samples were subjected to digestion with concentrated HNO_3 acid. After dilution the digested solutions samples were directly injected into flame AAS. DTPA extractable Cd was treated as bio-available Cd in this study. 0.5 M DTPA solution was used to extract Cd from the soil samples. In brief, 10 g of soil was taken in a 250 ml conical flask containing 50 ml of DTPA solution. The soil suspension was then shaken on a horizontal shaker for 15 hr. at 170 cycles per min. The suspension was then centrifuged at 2,000 rpm for 5 min and the supernatant was filtered through a suitable filter paper. Cd in the extract was then determined by an (AAS).

3.14 Statistical Analysis

Data recorded for Cd uptake against application of BC percentage was compiled and tabulated in proper form for statistical analyses. Analysis of variance was done with the help of MSTAT-C computer package program. The mean differences among the treatments were evaluated with DMRT test (Gomez and Gomez, 1984).

Chapter IV

RESULTS & DISCUSSION

The results obtained from the conducted study regarding the effect of BC application on Cd uptake by BARI Lalsak-1(*Amaranthus* spp.) and the properties of post-harvest soils have been presented and discussed in this chapter.

4.1 Effect of biochar on cadmium accumulation in plant leaf

Accumulation of Cd in plant leaf was significantly varied with the different doses of BC application. It was found that the highest amount of Cd accumulation occurred in BC_0Cd_{15} treatments (65.16ppm) (Table 1), and lowest accumulation was found in BC_4Cd_{15} treatment. Therefore, the results found that the higher doses of BC help to reduce accumulation despite high doses of Cd in soil. Regression analysis of Cd accumulation against bio-char percentage of this study (Fig. 2 & Fig. 3) supports the statement that bio-char can significantly reduce Cd accumulation in plants leaf. It may be due to the stabilization or immobilization of Cd by BC. In soil stabilizing, interacting with heavy metal helps to reduce Cd uptake. From Table-1 it is observed that increasing amount of BC reduced Cd phytotoxity or bioavailability in treatments of BC_1Cd_{15}, BC_2Cd_{15} and $BC_4Cd_{15.}$ At treatments BC_1Cd_{15}, BC_2Cd_{15} and BC_4Cd_{15}, Cd uptake was reduced 8.85%, 30.14% and 34.85%, respectively compared to BC_0Cd_{15} treatment. Similar results were found in several studies by others such as Zhang *et al*., (2014) stated that increasing amount of BC reduced the bio-availability, or phytotoxicity of heavy metal. Zhou *et al*. (2008) and Namagay *et al*. (2010) also observed that Cd accumulation was relatively higher in the treatments which contained relatively higher amount of Cd.

Table 1: **Effect of biochar on cadmium accumulation in plant stem, leaf and root**

Treatments	**Leaf**	**Stem**	**Root**
BC_0Cd_0	1.43 h	1.183 h	1.17 h
BC_0Cd_5	55.2 c	25.5 cd	39.37 c
BC_0Cd_{15}	65.16 a	36.06 a	47 a
BC_1Cd_0	1.31 hi	0.997 h	1.01 h
BC_1Cd_5	42.7 e	24.9 de	31.24 d
BC_1Cd_{15}	59.39 b	33.42 b	40.32 b
BC_2Cd_0	0.86 hi	0.84 h	0.79 h
BC_2Cd_5	31.49 f	18.5 f	24.28 f
BC_2Cd_{15}	45.52 d	26.27cd	31.43 d
BC_4Cd_0	0.66 i	0.75 h	0.72 h
BC_4Cd_5	29.25 g	17.25 g	21.61 g
BC_4Cd_{15}	42.46 e	24.44 e	28.23 e
$LSD_{(0.05)}$	0.69	0.79	0.65
Cv %	1.35	2.72	1.78
Level of significance	**	**	**

*indicates 5% level of significance BC_0: Control no BC, BC_1: 1% BC of soil wt., BC_2: 2% BC of soil wt, BC_4: 4% BC of soil wt., Cd_0= Control no Cd, Cd_5 = 5 ppm Cd, Cd_{15} = 15 ppm Cd.

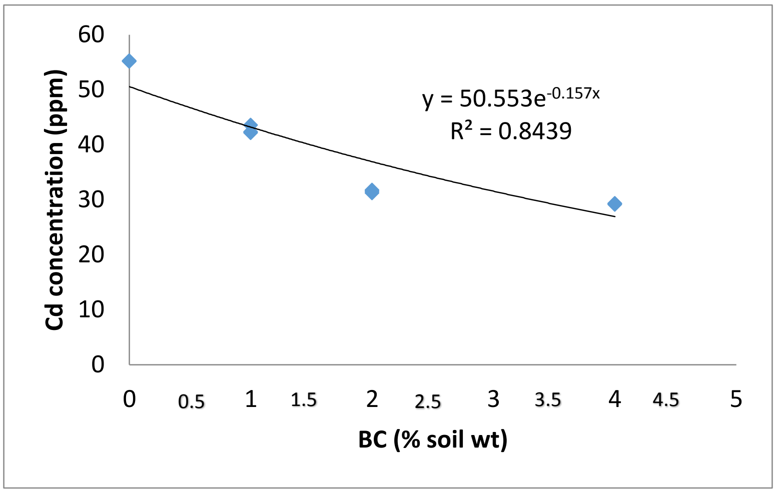

Fig. 2 Effect of biochar on cadmium accumulation by leaf (5ppm Cd)

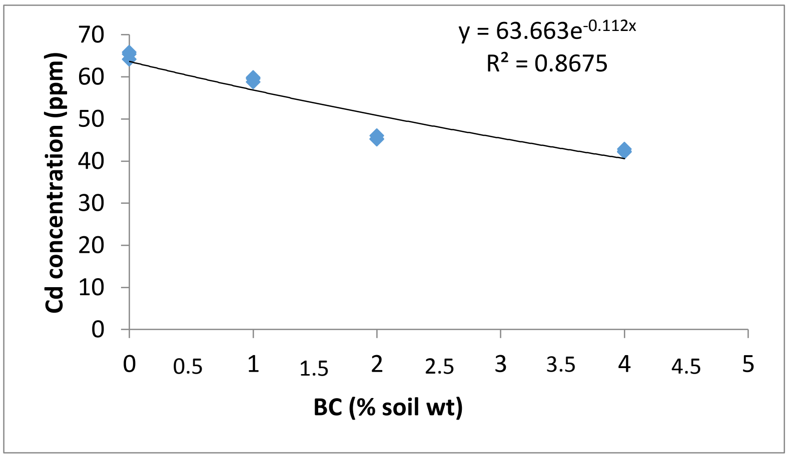

Fig. 3 Effect of biochar on cadmium accumulation by leaf (15 ppm Cd)

4.2. Effect of biochar on cadmium accumulation in red amaranth stem

Application of different levels of BC showed significant variation on Cd uptake by stem of red amaranth (Table 1). It indicated that the highest Cd concentration (36.06) was recorded in BC_0Cd_{15} treatment where BC was absent but Cd addition was the highest at 15ppm in this study. On the other hand, Cd accumulation was relatively low in the treatments where bio-char was present (Table 1). It was estimated that Cd accumulation was declined by 7.32%, 27.14% and 33.05% for the treatments of BC_1, BC_2 and BC_4, respectively. The probable reason for this low accumulation was due to the stabilization or immobilization of Cd by BC in soil. Jiang *et al*. (2012) observed that addition of bio-char was effective for decreasing Cd accumulation by 86% when the soil was treated with 3% of BC. Similarly, Břendová *et al*. (2015) demonstrated that BC was an effective soil amendment for stabilizing Cd and decreased the leach ability into the surrounding environment. Moreover, regression analysis of Cd accumulation and BC of this study (Fig. 4 & Fig. 5) supports the statement that BC can significantly reduce Cd uptake by plants. It was observed in this study that Cd accumulation was relatively higher in the treatments those contained relatively higher amount of Cd (Table 1). Therefore, apart from BC, the amount and availability of Cd in soil may be a factor by which Cd accumulation in plant is impacted.

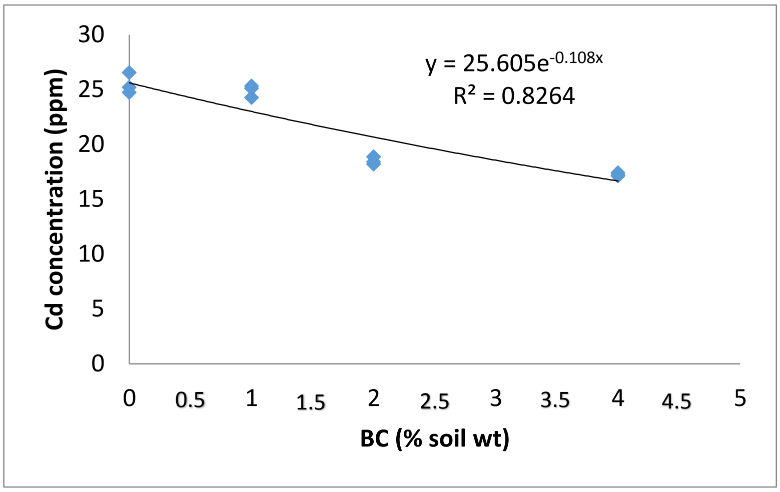

Fig. 4 Effect of biochar on cadmium accumulation by stem (5ppm Cd)

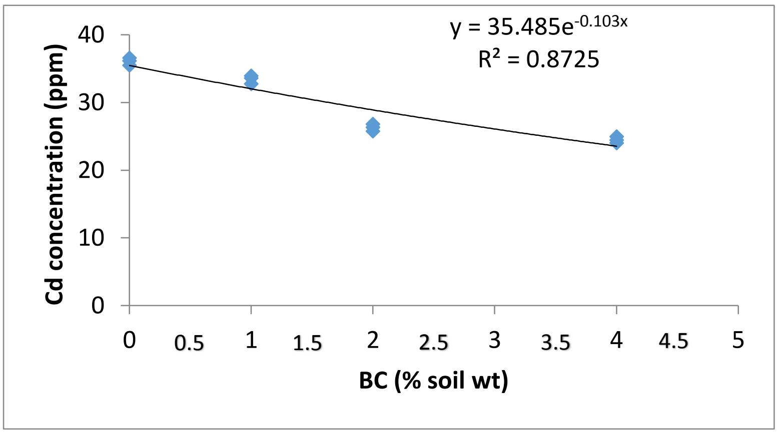

Fig. 5 Effect of biochar on cadmium accumulation by stem (15ppm Cd)

4.3 Effect of biochar on cadmium accumulation in plant root

Application of different level of BC also influenced Cd accumulation in root significantly. From the study the maximum amount of Cd (47 ppm) was accumulated in roots at BC_0Cd_{15}-treatment and the lowest accumulation was occurred in treatment of BC_4Cd_{15} (Table 1). Results reveled that Cd accumulation in plant root was reduced with the higher level of BC application in soil. From the regression analysis of Cd accumulation against biochar of this study (Fig. 6 & Fig. 7) supports the statement that BC has the ability to retard the metal accumulation in plants. In previous studies, it was reported that BC treatments were effective in immobilizing heavy metals, thereby reducing their bioavailability and phyto-toxicity, especially decreasing extractable heavy metals such as Cd, Cu and Pb (Mohamed *et al.*, 2015; Lu *et al.*, 2017). In this study, the reduction of Cd accumulation was found 14.21%, 33.12% and 39.93% respectively for BC_1Cd_{15}, BC_2Cd_{15} and BC_4Cd_{15} treatments compared to control. Mohamed *et al*. (2015) reported that the concentration of heavy metals in the shoots and roots of cabbage plants significantly decreased with the increasing application rate of bamboo BC. Yang *et al*., (2017) also reported that tobacco stalk and dead pig BC's were both effective in reducing the content of Cd and Zn in tobacco plants, and the content was significantly affected by the BC application rate. However, it was observed in this study that Cd accumulation was relatively higher in the treatments which contained relatively higher amount of Cd (Table 1). Therefore, again it can be postulated that amount and availability of Cd in soil would be a factor by which Cd accumulation in plant is affected.

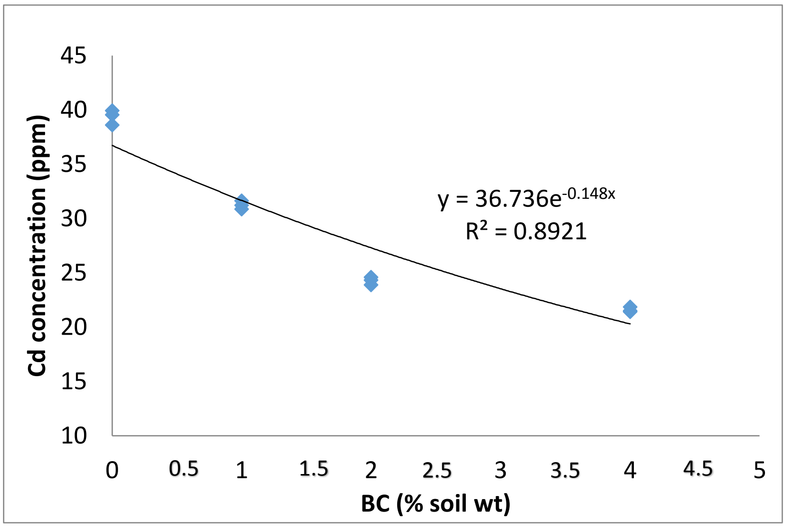

Fig. 6 Effect of biochar on cadmium accumulation by root (5ppm Cd)

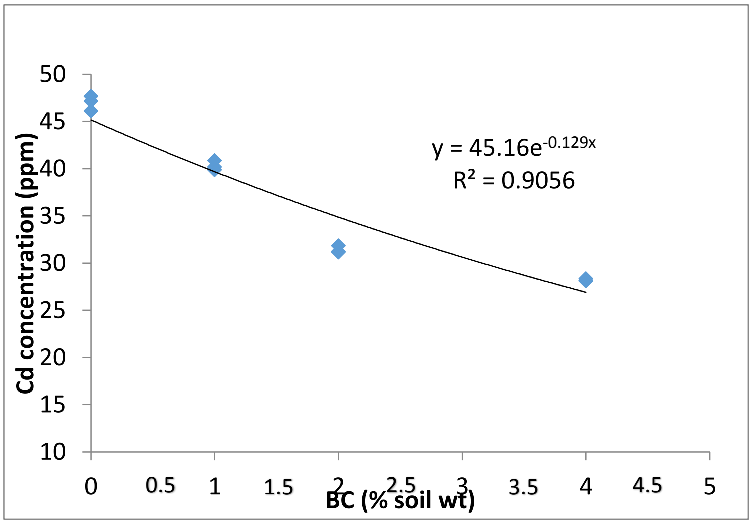

Fig. 7 Effect of biochar on cadmium accumulation by root (15ppm Cd)

4.4 Effect of biochar on cadmium bioavailability in bulk soil

Different doses of BC application in soil impacted on Cd availability in bulk soil. Cadmium bioavailability was significantly reduced with the increasing amount BC application (Table 2). Relatively higher amount of bioavailable Cd was observed in the treatments which contained greater amount of Cd. In this study, highest amount available Cd (4.06 ppm) was found in BC_0Cd_{15} treatment whereas lowest amount (0.22 ppm) was in BC_4Cd_0 treatment. However, higher amount of total Cd was found in the treatments which contained higher amount of BC in soil. Maximum amount of total Cd was observed in BC_4Cd_{15} treatments whereas BC_0Cd_0 treatment resulted minimum total Cd, which was similar to the values of BC_1Cd_0, BC_2Cd_0 and BC_4Cd_0 treatments.

4.5 Effect of biochar on cadmium bioavailability in rhizosphere soil

Different doses of BC application in soil impacted on Cd availability in rhizosphere soil. Cadmium bioavailability was significantly increased with the increasing amount Cd application (Table 2) irrespective of BC levels. Relatively higher amount of bioavailable Cd was observed in the treatments which contained greater amount of Cd. Highest amount available Cd (6.16 ppm) was found in BC_4Cd_{15} treatment whereas lowest amount (0.42 ppm) was in BC_0Cd_0 treatment. The results revealed that unlike bulk soil, bioavailability of Cd in rhizosphere soil increased with increasing BC rates. Increasing microbial activity in soil with biochar which produce different organic acids to dissolve Cd may be the reason to increase bioavailable Cd in BC treated soils (Abiven *et al.*, 2015). However, similar to bulk soil, higher amount of total Cd in rhizosphere soil was found in the treatments which contained higher amount of biochar and Cd. Maximum amount of total Cd was observed in BC_4Cd_{15} treatments whereas lowest amount of total Cd was found in BC_0Cd_0 treatment. In comparison rhizosphere total Cd percentage is lower than bulk soil but available Cd percentage is higher. In BC_0Cd_0 treatment total Cd 27.63% lower than bulk soil and available Cd is 10.52% higher than bulk soil. In BC_4Cd_{15} treatments rhizosphere total Cd 28.63% lower than bulk soil's total Cd and available Cd 158.82% higher than bulk soil.

Table 2: Effect of biochar on cadmium availability (ppm) in bulk soil and rhizosphere soil

Treatments	Bulk		Rhizosphere	
	Total Cd(ppm)	**Available Cd(ppm)**	**Total Cd (ppm)**	**Available Cd (ppm)**
BC_0Cd_0	0.76 g	0.38 f	0.55 f	0.42 e
BC_0Cd_5	2.84 f	1.41 d	1.15 e	0.81 d
BC_0Cd_{15}	7.70 c	4.06 a	6.54 b	5.83 a
BC_1Cd_0	0.98 g	0.35 f	0.75 f	0.62 d
BC_1Cd_5	3.28 e	1.32 d	2.75 d	1.79 c
BC_1Cd_{15}	9.48 b	3.54 b	7.78 a	5.73 a
BC_2Cd_0	0.91 g	0.27 f	0.85 f	0.68 de
BC_2Cd_5	4.73 d	1.42 d	3.14 c	2.41 b
BC_2Cd_{15}	11.52a	3.34 b	9.18 a	5.81 a
BC_4Cd_0	1.03 g	0.22 f	0.84 f	0.63 de
BC_4Cd_5	4.83 d	0.85 e	3.3 c	1.48 c
BC_4Cd_{15}	11.49 a	2.38 c	8.2 a	6.16 a
$LSD_{(0.05)}$	0.42	0.27	0.33	0.31
CV %	5.07	7.15	4.61	5.21
Level of significance	**	**	**	**

*indicates 5% level of significance BC_0: Control no BC, BC_1: 1% BC of soil wt., BC_2: 2% BC of soil wt, BC_4: 4% BC of soil wt., Cd_0= Control no Cd, Cd_5 = 5 ppm Cd, Cd_{15} = 15 ppm Cd

4.6 Effect of biochar on pH

In post-harvest soil the pH was significantly varied with different amount of BC treatments. From Table 4 it is shown that in control BCoCd0 treatment, pH was 6.25 which was significantly lower than the values of treatments containing 2% and 4% BC. It was also found that pH was increased gradually according to higher doses of BC application which may be due to the calcareous or alkalinity properties of BC. Similar results found by Lehmann et al. (2011); Khodadad et al. (2011), Lima et al. (2009) were reported.

4.7 Effect of biochar on Soil bulk density

Bulk density of soil was varied significantly due to BC application. It was found that bulk density was increased with decreasing rate of BC in soil (Table 3). It was found that the higher bulk densities were found in treatments where no BC was applied whereas lower values were found in treatments with higher levels of BC (Table 3). It was found that bulk density of soil in different treatments was reduced accordingly to higher doses of BC treatments (Table 3). Similar results of bulk density reduction from BC application were observed in the studies conducted by Mankasingh *et al*. (2011) and laird *et al*. (2010). Thus, the decrease in bulk density of BC amended soil could be one of the indicators of enhancement of soil structure or aggregation, and aeration.

4.8 Effect of biochar on Soil particle density

Different doses of BC treatment from the study showed that particle density was insignificantly varied with treatments (Table 3). In this short pot experiment, the results indicated that BC application may not have short term impact on soil particle density.

4.9 Effect of bio-char on Soil porosity

From the conducted study it was found that porosity was significantly varied with different treatments. Similar to bulk density, soil porosity was lower in treatments without BC application whereas it was found increased in treatments with BC (Table 3). Highest doses of BC application in this study resulted 30% more soil porosity than control. These results were expected because of decreasing soil bulk density with BC application results increase in soil porosity directly. The findings from a recent study conducted by Omondi *et al*. (2016) agree with the statement that that BC addition increased soil porosity. Addition of the porous material to soil can concomitantly increase soil porosity. The increased soil porosity caused by BC application can have positive implications for the movement of water, heat, and gases in the soil.

Table 3: Effect of biochar on pH, bulk density, particle density and porosity

Treatments	pH	BD	Pd	Porosity
$BCoCd_0$	6.25 c	1.46 a	2.11	30.10 c
$BC0Cd_5$	6.14 c	1.41a	2.12	33.45c
BC_0Cd_{15}	6.04 c	1.48a	2.14	30.84c
BC_1Cd_0	6.49 c	1.41 ab	2.12	33.49c
$BC1Cd_5$	6.24c	1.4 ab	2.10	33.33 c
BC_1Cd_{15}	6.54c	1.44 ab	2.05	29.75c
BC_2Cd_0	7.18b	1.35 b	2.13	36.62 b
BC_2Cd_5	7.11b	1.33 b	2.12	37.27 b
BC_2Cd_{15}	7.25b	1.34 b	2.11	36.49 b
BC_4Cd_0	7.85 a	1.10 c	2.16	49.07 a
BC_4Cd_5	7.65a	1.11 c	2.09	46.89 a
BC_4Cd_{15}	7.54a	1.08 c	2.16	48.08 a
CV %	5.07	3.15	4.61	5.21
Level of significance	**	**	NS	**

Chapter V

SUMMARY AND CONCLUSION

A pot experiment was conducted at the net house of the research farm of Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from January 2018 to June 2018. The experiment was conducted to observe the effect of BC on Cd accumulation in Lalshak (*Amaranthus* spp.) and on soil properties. In this experiment, the treatment consisted of four doses of BC viz. BC_0= no BC, BC_1= 1% BC of soil wt, BC_2= 2% BC of soil wt, BC_4= 4% BC of soil wt and Cd_0= no Cd, Cd_5 = 5 ppm Cd, Cd_{15} = 15 ppm Cd. The experiment was laid out in Complete Randomize Design (CRD) with five replications. The collected data were statistically analyzed for evaluation of the treatment effects. Results showed that significant variation among the treatments in majority of the observed parameters such as Cd in stem, leaf and root, total and bio-available Cd in bulk and rhizosphere soils, soil pH, bulk density and porosity. From the conducted study it was found that higher amount of Cd accumulation in leaf occurred in the treatments with no BC whereas lower accumulation found in treatments with BC_4Cd_{15}. It was found that the higher doses of BC helped to reduce Cd accumulation despite high doses of Cd was imposed. Effect of different levels of BC treatment on Cd accumulation in plant stem showed significant variations. It was found that the highest Cd content (36.06 ppm) by plant stem was recorded in BC_0Cd_{15} treatment whereas Cd accumulation was relatively low in the treatments where BC was present at BC_1Cd_{15}, BC_2Cd_{15}, BC_4Cd_{15} treatments. Compared to the control, it was estimated that Cd accumulation was declined by 7.32%, 27.14% and 33.05% for the treatments of BC1, BC2 and BC4, respectively. Similar results were observed in root Cd accumulation. In comparison with the control it showed that BC_1Cd_{15}, BC_2Cd_{15} and BC_4Cd_{15} treatments reduced Cd accumulation in roots by 14.21%, 33.12% and 39.93%, respectively. The probable reason for this low accumulation was due to the stabilization or immobilization of Cd by BC in soil. Bioavailability of Cd in both bulk and rhizosphere soil samples were impacted significantly by BC application. Relatively lower amount of bioavailable Cd was found in bulk soils with higher amount of BC application. In the other hand, higher amount of bioavailable Cd was observed in rhizosphere soils with higher BC levels, which was due to greater microbial activity producing organic acids in rhizosphere soils.

Soil properties including porosity and pH were impacted significantly by BC application in soil. Bulk density was decreased whereas porosity was increased with increasing BC rates. The increased soil porosity impacts on the movement of water, heat, and gases in the soil. Soil pH was increased with increasing BC rates, which was due to liming effect of BC.

Considering the findings of the present experiment, further studies in the following areas may be suggested:

1. The effect of BC on bioavailability of different heavy metals (Pb, Hg, Al) in industrially polluted soils of Bangladesh
2. Similar experiments may be carried out by using another porous materials like zeolite to examine its effect on accumulation of heavy metals in different crops.

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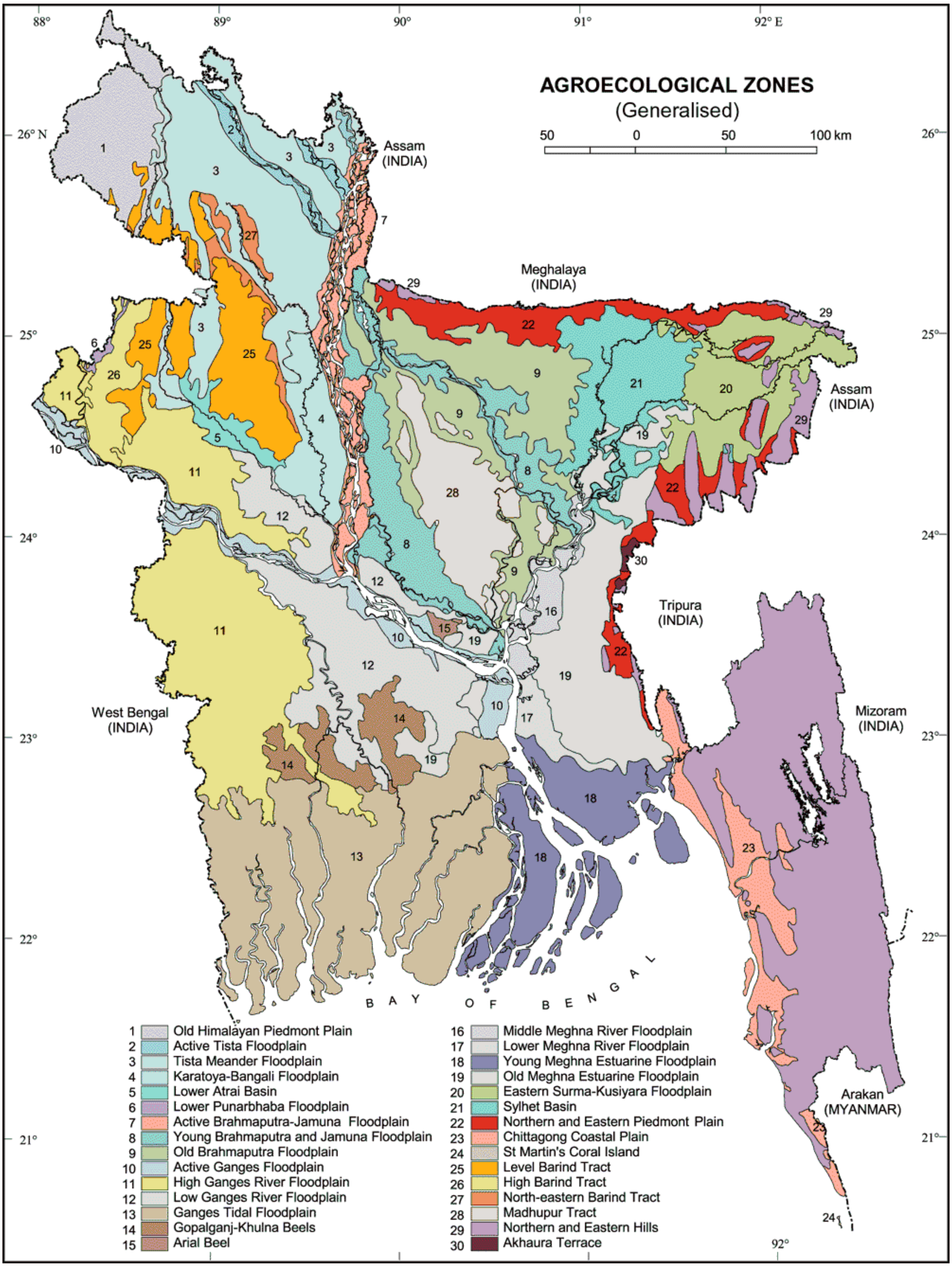
APPENDICES

Appendix I. The Map of the experimental site



Appendix. 2. Commonly used symbols and abbreviations

Abbreviations	**Full word**
%	Percent
@	At the rate
BC	Biochar
Cd	Cadmium
ANOVA	Analysis of variance
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BD	Bangladesh
BINA	Bangladesh Institute of Nuclear
Agriculture	
CEC	Cation Exchange Capacity
cm	Centi-meter
CV%	Percentage of coefficient of variation
df	Degrees of Freedom
LSD	Least Significant Difference
et al	and others
etc	Etcetera
FAO	Food and Agricultural Organization
g	Gram
H	Hours
J.	Journal
kg ha-1	Kilograms per hector
t ha-1	Ton per hectare
Kg	Kilogram
m	Meter

m2	square meter
MOA	Ministry of Agriculture
MSE	Mean square of the error
No.	Number
ppm	parts per million
RCBD	Randomized Complete Block Design
Sci.	Science
SE	Standard Error
var.	Variety