

Effect of biochar on phytoremediation of cadmium contaminated soil by using rice crop (*Oryza sativa* L.)

काशी हिन्दू
विश्वविद्यालय



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UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

Master of Science (Agriculture)
In
Soil Science and Agricultural Chemistry

Supervisor
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Submitted By
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Yours faithfully

(Pramod Kumar Sharma)
Chairman

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Head

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LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate
µg	:	Micro gram
BC	:	Biochar
Cd	:	Cadmium
cm	:	Centimetre
DAP	:	Diammonium phosphate
DAT	:	Days after transplanting
DTPA	:	Diethylene triamine pentaacetic acid
e.g.	:	(exempli gratia) for example
EC	:	Electrical Conductivity
<i>et al.</i>	:	et alia (and associates)
etc.	:	(et cetera) and the others
Fig.	:	Figure
g	:	Grams
h	:	Hours
i.e.	:	(ed est) that is
kg	:	Kilogram
kg ⁻¹	:	Per kilogram
KMnO ₄	:	Potassium permanganate
L	:	litre
m	:	Metre
Max	:	Maximum
mg	:	Milli gram
Mg	:	Mega gram
Min	:	Minimum
mL	:	Milliliter
N	:	Nitrogen
°C	:	Degree Celsius
pH	:	Potential of Hydrogen
RH	:	Relative Humidity
SOC	:	Soil organic carbon
q ha ⁻¹	:	Quintal per hectare
t/ha	:	ton per hectare
g pot ⁻¹	:	Grams per pot

CHAPTER - I

INTRODUCTION

Cadmium was discovered in 1817 by Friedrich Stromayer and Karl Samuel Leberecht hermen, it was derived from (Latin word *cadmia*, and Greek word *kadueia*) meaning calamine. It is a soft, silver-white metal usually not present in the environment as a pure metal, but is most often found in zinc, lead, and copper ores. Cadmium is a highly toxic heavy metal often found in nature combined with other elements such as oxygen, chlorine, or sulfur in the form of their oxides, sulfides, and carbonate complexes in zinc, lead and copper ores. Cadmium is regarded as non-essential to human and plant unlike other metals such as Zn, and Cu.

Cadmium is a highly toxic heavy metal having atomic no: (48), atomic weight (112.41gm); atomic radius $0.97\text{\AA}$; electronegativity- 1.69, density 8.65 Mgm^{-3} and having a hexagonal shape with an oxidation state of +2 belongs to group 2nd B of transition element, having melting point, 320.9°C ; and boiling point, 765°C ; of crystalline cadmium. Generally it exists in an oxidation state of +2, but it can also exist in the +1 state and it makes up about 0.1 ppm of earth's crust. Cadmium is generally found in association with Zn, in a ratio of about 1: 1000. This association is caused by the geochemical resemblance between zinc and cadmium which makes their geological separation unlikely.

Some of the inorganic cadmium compounds, are quite soluble in water e.g. cadmium acetate, chloride and cadmium sulphate where as cadmium oxide and sulphide are almost insoluble while cadmium carbonate might be soluble at gastric pH. The major natural sources of cadmium in soils include weathering of parent material and volcanic eruption along with soil erosion (Yoon *et al.*, 2007). However the anthropogenic sources are linked with the industrial activities such as metal finishing, paint pigment, battery manufacturing, leather tanning, mining activities, smelting and human activities too like urban composts, municipal sewage water, sludge deposition, and use of phosphatic fertilizers and pesticide (Omar *et al.* 2004).

The largest sources of cadmium in landfills are smelters, iron and steel plants, electroplating wastes, and battery production industries. Mine tailings generated from zinc mining ore have huge potential to transfer cadmium to the ambient environment. Cadmium is mainly used as an anti-corrosion, coating in electroplating as an alloying metal in solders and used as a stabilizer in plastics as a pigment, and as a component of nickel-cadmium batteries.

The average cadmium content in the geosphere is about 0.2 mg kg^{-1} (Lindsay, 1979) and in rocks, it ranges from 0.1 to 0.11 mg kg^{-1} (Alloway, 1990). Cadmium concentration in soil is less than 1 ppm but cadmium concentration ranges from 0.3 to $22.2 \text{ } \mu\text{g L}^{-1}$ depending on the geological sources of the soil. While in cultivable soil, cadmium concentration seldom exceeds 10 mg L^{-1} in the soil solution. Unlike the organic substances, heavy metals are basically non-bio-degradable and therefore accumulated in the soil.

Metal production, drying of zinc, concentrates as roasting, smelting, and refining of ores is the largest source of anthropogenic atmospheric cadmium contamination, followed by waste incineration and by other sources, including the production of nickel-cadmium batteries, fossil fuel combustion, and generation of dust by industrial processes such as cement manufacturing. Environmental pollution by means of heavy metals has become a severe problem in the world. The problem rises with the rising era of industrialization and disturbance of natural biogeochemical cycles.

Soil contamination is mainly due to accumulation of heavy metals and metalloids through emission of the growing industries, disposal of high metal wastes, application of phosphatic fertilizers, wastewater irrigation, animal manures, pesticides, sewage sludge, mine tailings, coal combustion residues, leaded gasoline, paints, and spillage of petrochemicals, along with atmospheric deposition that leads the accumulation of heavy metals in soil and water, poses a serious risk to the plant and human health.

The accumulation of heavy metal in soils and plants reaches an alarming zone and thus potentially toxic to plant and human health. The alteration by heavy metal in the food chain is one of the main pathways for the entry of these toxic pollutants in

human body next to these exposure to heavy metals by a number of ways, including at work in certain industries, from drinking water and eating contaminated foods. Garbage on an average accounts $12 \mu\text{g g}^{-1}$ cadmium which got burned in municipal waste incineration plant, i.e 30% of the total cadmium fumes elude from electrostatic precipitators, polluting to the environment.

Cadmium is more soluble and transportable than other heavy metals in soils (McBride, 1995) and is, therefore, the subject matter of concern in this experiment. Excessive accumulation of heavy metals in cultivated soils through wastewater irrigation, may not only end result of soil contamination, but it also leads to an elevated heavy metal uptake by crops and thus have an undesirable effect on food quality and safety. Soil is not a permanent sink for the contaminants and may be a source of pollutants, especially if get change in the local environment.

The mobility of heavy metals in soil is directly linked to their partitioning matter between soil and solution phase (Evans, 1989). Cadmium is easily taken up by plants and transported to above-ground tissues. Even if plants do not show symptoms of toxicity, it can potentially cause harmful effects in humans through the food chain (Davis, 1984). Cadmium causes many morphological, physiological, biochemical and structural changes in growing plants, resulting in growth inhibition, leaf chlorosis and even the plant death as well (Sanita` di Toppi and Gabbrielli, 1999).

Increasing evidence showd that cadmium toxicity in plants is due to H_2O_2 accumulation accelerating lipid peroxidation and affecting cell membrane fluidity and permeability due to an alteration in the composition of membrane lipids deposition (Shah *et al.* 2001 and Molina *et al.* 2008). Rice (*Oryza sativa* L.) is the primary food for over two billion people in Asia, Africa, and Latin America and has been used as a model plant in studies of cadmium toxicity and tolerance. Currently cadmium pollution in soils poses a serious threat to rice quality. Thus it is important to find ways to increase the rice tolerance to cadmium and limit their accumulation.

In rice seeds, cadmium, acts as a stress agent, increases auxin and increases gene products regulated by auxin (Hagen *et al.*, 1988). Auxin stimulates the biomass of rice crop. It opens the leaf pores in rice crop so that cadmium vapors can easily escape and hence preventing accumulation. An imbalance in the ratio of auxin and

abscisic acid results decrease in root growth and open pores making plants affected by cadmium exceptionally sensitive to drought. Because abscisic acid is responsible for cold resistance and auxin is associated with heat resistance cadmium exposed plants are also more susceptible to cold injury.

Processing food further depletes food of nutrients and may even increase cadmium content from the absorption of volatile fumes. Analysis of cadmium in food may be inaccurate using certain digestion procedures analyzed by (Cabanis *et al.*,1988) and concluded that cadmium accumulations may go unnoticed. Nutritional deficiencies play a vital role in cadmium toxicity (Webb, 1979). The vitamins, minerals, amino acids, essential fatty acids, and fibers in food are greatly modifying the toxic effect of cadmium exposure coming from the air.

Rice cropping systems cover almost more than 162.3 million ha of the land area throughout the world. Rice is a C_3 plant belong to the family *poaceae* having $2n=24$, characterized by a fibrous adventitious root system that can help the plant to pump water more proficiently from the soil, a significant characteristic for completing the primary need of metal accumulator plant. Fibrous adventitious roots of rice plants it not only supports to withstand crop but also helps to cope up with the nutrient deficiency. Extensive fibrous root systems adhere to soil particles thus it prevents soil from erosion.

Thus, the morphological characters of roots of rice plants facilitate to prevent the outflow of TEs from the contaminated soil through soil erosion. The rice introduction poses a promising phonological character of metal accumulation. Cultivation and adaptability to grow under varying environmental situations such as anoxia make rice plant suitable for phytoremediation. In addition to this, technology can be easily integrated into the rice cultivation. Rice cultivation also offers a huge amount of biomass that can be utilized for bioremediation purposes.

By poisoning plant roots, cadmium could harm plants during droughts when the rain finally comes, soil erosion and flooding occur. It causes flu-like symptoms associated with gastro-intestinal and respiratory viral illness. The maximum acceptable intake of cadmium as per FAO/WHO recommendation is 400-500 μg per week, or equal to 70 μg per day. Several technologies are present for the remediation

of heavy metal-contaminated soils. These methods are usually classified under physical, chemical and biological methods of remediation. Toxicity potential of heavy metals to the environment can be reduced by fixing them in the soil or thereby reducing the bioavailability and further mobility, by using a technology commonly called immobilization, and this technology frequently uses organic and inorganic amendment to accelerate the lessening of metal mobility and their toxicity in soils.

Remediation of heavy metal contaminated soils with immobilizing amendments represents a very cheap and feasible option (Knox *et al.* 2000). Amendments can offer a large surface area for immobilizing reactivity, such as biochar (Vacha *et al.* 2002) can change the soil pH, texture and soil strata. The use of economical and environmentally safe amendments for in situ immobilization of heavy metals has been found a promising way for the remediation of contaminated soils. Reduction of extractable metal concentration and phytotoxicity could be evaluated and verified by the feasibility of various amendments in fixing remediation. Addition of organic matter amendments, like biochar, compost and waste are common practice for immobilization of heavy metals or amelioration of calcium contaminated soils (Clemente *et al.* (2005).

Biochar application in cultivable soil has been recently attracted attention due to its potential agronomical, economically, and environmental feasibility. Biochar is a carbon rich material produced by pyrolyzing waste biomass between 250 and 900 °C under a constant flow of nitrogen or anoxic conditions. Number of waste biomass used for biochar include hard woods cuts, rice straw, rice husks, bamboo, wheat straw, peanut hulls, poultry litter, corn stover, bamboo, and animal manures. Amending the soil with biochar has been increasingly attracted and widespread attention for the given below two reasons.

First because of its chemical stability and suitability for carbon sequestration in soil. Secondly, it can be used as a soil conditioner because its application rapidly increases the soil fertility and plant growth by supplying and retaining the nutrients reserve and at the same time it also improves the physical, chemical and biological properties of the soil.

In addition to this, it also reduces the availability of toxic heavy metals, thus minimizes the bio-availability of organic contaminants to macro and micro organism, in plants, due to its high porosity, large surface area, and associated high sorption capacity. Biochar obtained mainly from plant waste characterized by a great variation in the content of biogenic elements such as nitrogen, phosphorus, and potassium. They contain huge amounts of carbon and other trace elements, which is the main part of organic substances. The quality and quantity of plant substrate for the production of biochar are influenced by the content of organic material and fertilizers along with the ratio of carbon to nitrogen.

The present study has the following objectives to extend and increase the knowledge about effect of biochar on phytoremediation of cadmium contaminated soil using rice crop.

Objectives

- To study the effect of biochar on growth, yield attributes and quality parameter of rice crop in cadmium contaminated soil.
- To study the effect of biochar application on N, P, K, content and their uptake by rice in cadmium contaminated soil.
- To study the Cd uptake ability of roots, shoots and grain of rice crop amended with biochar.
- To study the effect of biochar on soil fertility.

❧

CHAPTER - II

REVIEW AND LITERATURE

Cadmium is heavy metals, relatively water soluble and more mobile in soil. Heavy metals toxicity and their bioaccumulation in the food chain is one of the major environmental and health issues of our modern society. Basically heavy metals are carcinogenic in nature we are focusing here especially on cadmium. The ecosystem has been contaminated with certain toxic metals due to various natural and anthropogenic activities, at present, the entry and accumulation of this toxic metal in the soil are relatively very large quantities where formerly they were present only in traces.

Therefore everyone has to be very cautious about their use. Heavy metals get accumulated in the soil derives from a diverse group of sources including the burning of fossils fuels, mining, smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, sewage sludge, and use of pigment and batteries. Among all the non-essential heavy metals, cadmium is perhaps the metal which has attracted the wider attention in soil science and plant nutrition due to its potential toxicity to man, and their relative mobility in the soil-plant system.

Schroeder and Balassa (1963) were among the first researchers who highlighted the possible terror of cadmium accumulation in the food chain, through cadmium impurities in fertilizers and amendments which are applied to the soils. The interest of cadmium accumulation in soils and plants was further encouraged when industrial pollution of agricultural lands in Japan with cadmium-rich waste waters led to severe human health problems like ("Itai-Itai" disease).

Several scientists study the behavior of cadmium in the plants and soil to find ways to minimize health hazards including plant and animal. But the information pertaining to the effect of cadmium on the growth of cereals like rice plant, its physiological disorder and their behavior in relation to biochar is insufficient; therefore, it becomes necessary to include few other relevant findings related to this issues.

In this section work conducted by several scientists in India and abroad regarding the present studies has been reviewed and are presented as follows:

2.1 Natural and anthropogenic sources of cadmium in soils.

2.2 Effect of cadmium on growth, chlorophyll content and yield attributes, of rice crop.

2.3 Uptake of macronutrients and micronutrients.

2.4 Critical concentrations of cadmium in plants and soil.

2.5 Phytoremediation potential of rice in cadmium contaminated soils.

2.6 Effect of biochar on growth and yield attributes of the rice crop.

2.1 Sources of cadmium in soils

Cadmium is released to the biosphere from both natural and anthropogenic sources. A number of potential sources of cadmium contamination in the soil existing and these sources are listed here.

2.1.1 Natural sources of cadmium in soil.

Okada *et al.* (1997) reported that about 10% of total cadmium in the environment is derived from natural sources, whereas remaining 90% is derived from anthropogenic activity. Volcanic activity contributes about 62% of natural emissions and other natural sources include decaying of vegetation 25% airborne soil particles 12% and forest fire 2%. Although the degassing of crustal rocks has been suggested as an important source of metals accumulation in the atmosphere and the release of cadmium from such sources still remains to be quantified.

Bagul *et al.* (2015) reported the major natural sources of cadmium contamination in the earth's crust are volcanoes eruption and weathering of rocks that leach into the rivers, lakes, and oceans due to the action of water. The weathering of rocks releases cadmium contamination to lithosphere and hydrosphere. This process plays a significant role in the global cadmium cycle, within the biosphere and cadmium is translocated by different processes in plants. The major sources of air

contamination were reported due to the emission of volcanoes, airborne soil particles, sea spray, biogenic material, and forest fires.

Total emission to air from natural sources is estimated at about 150-2,600 tonnes and these figures may be compared to an estimated total global anthropogenic air emission of cadmium in 1995 of approximately 3,000 tones. Cadmium naturally exists in the earth's crust and got rank 7 of ASTDR's in top 20 toxic compound. The Percentage of cadmium in the upper soil has been reported dominant because it is found to come in the contact of insecticides, pesticide, fungicides, sewage, and sludge, along with the application of commercial fertilizers which are regularly used in agriculture. Dental alloys, electroplating, motor oil, and exhaust are other sources of cadmium pollution are prevalent in nature

2.1.2 Anthropogenic sources of cadmium

Kumar *et al.* (2007) reported anthropogenic sources of cadmium toxicity in biosphere like, smelting operations, use of phosphate fertilizers, pigment, cigarettes smokes, automobiles, combustion processes of coal and oil, from refuse incineration pigments in plastics, iron and steel industries, contributed to the entry of cadmium into human and animal via food chain. Cadmium is a non-corrosive and cumulative in nature that has made it very important due to its applications in electroplating or galvanizing. It is also used as color pigment for paints, plastics, and as a cathode material for nickel-cadmium batteries. More than 55 % of the total cadmium production is consumed by the manufacturing of cadmium-nickel batteries reported by Okada *et al* (1997).

Rizwan *et al.* (2018) and Wang *et al.* (2018) reported potential sources of cadmium in the lithosphere and concluded that with the development of industry and agriculture, anthropogenic activities such as mining, smelting, manufacturing, sewage disposing and wastewater irrigation are dominant sources of heavy metal accumulation in soil. The industrial production (IP) is the dominant source of anthropogenic cadmium emissions, which contributes to 62.1% of the total emissions of cadmium while other contributors include aquaculture, waste--water treatment, living consumption, crop farming and animal breeding, each accounted for less than 10% of the total emissions.

2.2 Effect of cadmium accumulation On plants growth and yield attributes

Pettersson (1977) reported three general groups of plants based on the degree of cadmium accumulation in plant. 1) Low cadmium accumulators i.e - Leguminosae. 2) Moderate accumulators i.e - Graminae, Liliaceae, Cucurbitaceae, Umbelliferae; and 3) High accumulators are - Chenopodiaceae, Cruciferae, Solanaceae, and Compositae. However, the pattern of accumulation of cadmium by different crop species may differ in solutions with high and low levels of cadmium present.

Stobart *et al.* (1985) and Barcelo *et al.* (1988) detailed on wheat crop under cadmium stress soil and reported that cadmium ions are readily taken up by roots and translocated into the leaves in many plant species, cadmium treatment damaged the structure of chloroplasts, as manifested by the disturbing shape and the dilation of the thylakoid membranes. These ultra structural changes suggested cadmium probably induced premature senescence. It inhibits germination of seeds and plant growth, nutrient distribution increases several enzymes activity, e.g., glucose-6- phosphate-dehydrogenase influenced since cadmium²⁺ ions accumulate at higher levels in leaves than in other parts of plants and leads phytotoxic effects of cadmium on the inhibition rate of photosynthesis.

Vazquez *et al.* (1989) reported the, influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Visual symptoms for heavy metal phytotoxicity are often indicated as leaf necrosis, chlorosis, reddish-brown discoloration of the leaf blade, browning of the root system especially in rice crop.

Padmaja *et al.* (1990) reported that total biomass reduction caused due to cadmium toxicity because it could have direct consequence on the inhibition of chlorophyll synthesis in plants. Long-term exposure of cadmium to plants may affect chlorophyll synthesis and thus have an important role in the chloroplast development in young leaves. Ultra structural study reported disorganization of grana, either by increasing in number or size of plastid globulin in chloroplasts that induced vesiculation in the cytoplasm.

Moya et al. (1993) reported that the presence of the excessive amount of cadmium in the soil causes many toxic symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism, therefore it might strongly reduce biomass production of the affected plant.

Das et al. (1997) reported that leaf chlorosis, leaf rolling and stunting are the main and easily visible symptoms of cadmium toxicity in plants. Cadmium also reduces the absorption of nitrate and its transport from root to shoot by inhibiting nitrate reductase activity reported in soya bean crop hence it reduces nitrogen fixation and primary assimilation of ammonia by decreasing root nodulation in cadmium contaminated soil.

Generally cadmium interfere with the uptake, transport and use of various other elements like Ca, Mg, P, K, and water uptake within soil and plant system.

Sandalio et al. (2001) and **Liu et al. (2014)** reported that heavy metal pollution is one of the great environmental threats in the present era which is growing with inevitable pace. Various industries such as metal refining discharge untreated xenobiotic pollutants containing different heavy metals into agro-ecosystem posing serious environmental problems. Cadmium is a widely spread toxic pollutant for humans, plants, and animals, which enters the environment mainly through various industrial emanations and anthropogenic activities, including herbicides, chemical fertilizers, and pesticides application, or when soil irrigated with contaminated groundwater, and is then transferred to the food chain causes a serious undesirable impact on human health.

Heckathorn et al. (2004) reported that emissions of cadmium residue in the atmosphere will affect the physiological activities of plants. Dust released from wear and tear of tires contains Cd^{2+} that is deposited on leaves leading to blockage of stomata and gas exchange, which culminates in the reduction of the photosynthetic activity of plants that reduces the yield and biomass of particular crop.

Hart et al. (2006) reported toxic relevance of cadmium among the major cereal crops, he went through a series of studies on durum wheat (*Triticum*

turgidum L.) during their experiment and characterized cadmium uptake, root-to-shoot, translocation, and grain accumulation. It is remarkable that the grain cadmium concentration was strongly associated with those of the xylem sap and vegetative tissues rather than that of the root. As with essential heavy metals, cadmium is thought to be transported into seeds or grains via the phloem in many plants.

Nagajyoti *et al.* (2010) reported that excess of cadmium accumulation by the plant not only have negative effects on the quality of foods grain, but it also reduces the growth, chlorophyll content and yield through a different mechanism such as the reduction in nutrient uptake, photosynthesis, and over production of reactive oxygen species which can alter the- physiology of plant and hence reported a drastic reduction of quality and quantity food grain production.

Meng *et al.* (2014) conducted an experiment by inoculating higher content of Ca and Mg in rice grain with *B. subtilis* and reported that these elements may relate to reducing cadmium accumulation in rice plants. Thus results indicated that microorganism can promote plant growth and uptake of high Ca and Mg, especially *B. subtilis* which can highly decrease cadmium uptake by rice plants. Considering that Mg and Ca contain divalent cations with a small molecular size and weight, due to high diffusion action for transportation into a plant these ion might competed with cadmium ion. Moreover, microorganisms have the ability to produce siderophores and other organic acids that might be suitable to solubilize Mg and Ca from the soil and increase Mg and Ca solubility in soil.

Song *et al.* (2015) reported that cadmium toxicity restricted rice growth as estimated in terms of root length, shoot length, leaf area, root area, and number of leaves and roots per plant.

Gao *et al.* (2016) and Rehman *et al.*, (2017) conducted an experiment in rice and reported that rice plant has the ability to accumulate cadmium which negatively affected rice growth and quality. Therefore, minimization of cadmium in edible parts of the crops is important mainly to reduce its negative impacts on human's health.

Muhammad *et al.* (2016) studied on cadmium toxic potential in paddy field and reported it as a serious threat to human health due to the accumulation of

cadmium in human through the food chain. Cadmium toxicity decreased rice seedling germination, growth, photosynthesis, yield, and quality of rice grain. Excessive cadmium caused oxidative stress by increasing ROS generation and causes genotoxicity in rice. Response of rice towards cadmium toxicity varies with cultivars, growth conditions, and duration of cadmium stress. Rice induced many tolerance mechanisms both at physiological and molecular levels to restrict the cadmium-induced undesirable effects and toxicity.

Adhikari et al. (2018) reported that cadmium contamination in agricultural soil has become an increasingly deteriorated environmental constraint which could pose a serious exposure to the productivity of crops.

Rizwan et al. (2019) reported the antagonistic behavior of cadmium in the soil-plant system and found that Zn application may possibly reduced cadmium accumulation in plants.

2.3 Effect of cadmium toxicity in the uptake of micro and macronutrient

Clemens et al. (2002) conducted an experiment on nutrient imbalance and reported that nutrient imbalance leads toxic mechanism of heavy metal in plants and it alters the uptake and distribution of macro and micronutrients such as Cu, Fe, Mn, and Zn affected due to cadmium stress. The cadmium accumulation in food crops is not only issues for food safety but also a prominent threat to agricultural productivity due to its severe biological toxicity which hinders plant growth and physiology by affecting nutrient homeostasis.

Akhtar et al. (2016) conducted a hydroponic experiment to investigate the various levels of cadmium on growth, physiology, mineral nutrients and cadmium accumulation in different tissues of rice and wheat crop. The plant's seedlings grown with four replicates and cadmium were applied after seven days of transplantation and harvested after twenty days of cadmium application. The cadmium uptake by roots of wheat and rice was increased up to 42 and 304 $\mu\text{g pot}^{-1}$ respectively in cadmium containing solution compare to control plot the 15 μM cadmium treatment decreased the copper (Cu), manganese (Mn) and zinc (Zn) contents by 6-61%, 6-41% and 6-17% in wheat and 18-39%, 6-29% and 4- 32% in rice, respectively. Interestingly he

found root iron contents in wheat were increased up to 28% at 10 μM cadmium level while in rice both root and shoot Fe contents were reduced to 10 and 15 μM under cadmium stress condition.

Kashem and Kawal, (2007) reported that the addition of Mg could probably reduced cadmium concentration within the soil and plants system.

Zhang et al. (2007) reported that heavy metal accumulation in the soil interrupts the normal functioning of soil and plant ecosystems. Plants absorb various kinds of heavy metals when available in the soil or added with irrigation water. Metals like manganese (Mn), magnesium (Mg), copper (Cu) and iron (Fe) is classified as essential plant metals. These metals are required in a specific amount and their deficiency elevated concentrations will result in toxic effects and reduces the plant productivity. For example, Mn is involved in splitting water molecules necessary for photosynthesis. Other metals like magnesium deficiency are responsible for chlorosis in plant leaves and also induce oxidative stress. Cadmium is phytotoxic in nature and it affects the plant's photosynthesis by reducing the chlorophyll content. This is because cadmium reduces the uptake of chlorophyll essential elements such as Mg and Fe, affecting chloroplast, changing essential enzymatic processes for photosynthesis and the closing of stomata lead to significant impacts on seedling dry mass, root length, shoot length, and weight by adversely affecting the process of respiration and metabolism of plants system.

Mallick et al. (2010) reported that uptake of both macronutrients (eg. N, P, K), as well as micronutrients, decreased with an increase in cadmium level on irrigation and its long term exposure decreased copper absorption in (*Zea mays* L.) roots, while leaves were not affected and also reported a decreased in uptake of the micronutrients such as Mn, Fe, Cu, and Zn in (*Viridis* L.) when exposed to cadmium contamination.

Sarwar et al. (2010) conducted an experiment on cereal crops and they observe the negative effect of cadmium stress in soybean, wheat, rice, barley, and maize because cadmium has an antagonistic effect with these essential nutrients, while decreased the Mn translocation to the shoots of wheat Similarly, in rice distribution of Cu, Fe and Zn was significantly minimized under the presence of

cadmium toxicity. The disturbance in nutrient uptake under cadmium stress is due to disturbed plasma membrane documented a synergistic relation among cadmium and zinc uptake in field cultivated corn and wheat crop. Cadmium alters the nutrient homeostasis and growth of globally top produced cereals and showed the effect of cadmium on availability, uptake, accumulation, and translocation of selected micronutrients from roots to aerial parts of wheat and rice crop.

Mukhtar *et al.* (2013) reported that increase in cadmium concentration decreased the net photosynthetic rate in plants, and gas exchange parameters such as carbon fixation, stomata conductance, and transpiration rate also influenced by cadmium toxicity. Cadmium can increase reactive oxygen species (ROS) production and causes oxidative stress to scavenger ROS production plants have developed enzymatic and non enzymatic antioxidant mechanisms depending upon plant species, age, and growth conditions.

Bolan *et al.* (2014) reported that immobilization of cadmium and other metals in soils through phosphorus-containing compounds. Precipitation of metals with phosphorus has been proved to be one of the main mechanisms for the immobilization of metals in soils. These compounds are stable and have extremely low solubility over a wide range of soil pH, thereby reducing metal bio-availability and plant uptake. Phosphorus application in soil increased the soil pH and caused the redistribution of cadmium to less mobile fractions.

Nagarajan (2014) reported that highly evident reductions were recorded in the uptake of nitrogen 23-82%, potassium 6-42% and phosphorous 4-37% content of cadmium- treated plant leaves. Cadmium accumulation was extremely higher in roots 35-50%, followed by shoots 23-58%, and then seeds 21-24% of treated plants compared with control.

2.4 Critical level of cadmium in soil and plant system

Bingham *et al.* (1975) reported a method that was employed to calculate the critical toxic concentration of cadmium in soil and plants ecosystem by relative percent of dry matter yields plotted against the extractable content of cadmium in soil

and plant tissues when it got exposed. Critical toxic limits of cadmium in soil and plants were determined for a 10% reduction in dry matter yields.

McLaughlin *et al.* (1994) set maximum permissible concentrations of cadmium in various foods, for example including 0.05 mg cadmium kg⁻¹ fresh weight for potatoes.

Alloway (1995) studied the mechanism of cadmium concentration in plants grown on normal soils then he reported a critical range of cadmium in a plant that ranges from 0.1 to 2.4 mg kg⁻¹.

Uraguchi *et al.* (2009) reported that in general cadmium accumulation in shoots and grains are potentially higher in Indian rice cultivars compared to Japonicum cultivars. The World Health Organization set the highest provisional and acceptable intake limit for an adult at 60 to 70 µg cadmium per day. World Health Organization 1972 and the Codex Alimentarius Commission of FAO/WHO set a limit of 0.1 mg kg⁻¹ for cereal grains and oilseeds traded on international markets.

2.5 Phytoremediation potential of rice in cadmium contaminated soil

Morishita *et al.* (1987) and Liu *et al.*, (2005) examined the exploitation of rice crop in cadmium contaminated soil and reported that rice could be an ideal crop which can absorb and translocate less cadmium to the grain which is a promising technology for reducing cadmium contamination in rice crop and concluded that grain cadmium concentration varies greatly among different rice cultivars.

Fodor *et al.* (1995) reported cadmium treatment reducing in ATPase activity of the plasma membrane in wheat and sunflower roots grown under cadmium contaminated soil.

Zhen *et al.* (2008) conducted an experiment on rice crop in heavy metal contaminated soil because of its huge growth area, high total yield, and wide distribution with rapid economic development. Because most part of cultivable soils was contaminated by heavy metals hence accumulation of cadmium in rice grain were found much more higher than the food hygiene standard.

Dinakar et al. (2009) reported that cadmium stress could increase soluble proteins in the plants. Some fragmented proteins, like Rubisco, and others are stress-related proteins and, antioxidants, are increased due to cadmium stress in the plant.

Payus et al. (2015) carried out an experiment on heavy metal contaminated paddy field and reported that rice roots accumulated relatively large amount of lead, cadmium, chromium, and copper, while the stem accumulated a large amount of zinc. Due to possibly an enrichment factor (EF) showed that lead, cadmium, and copper were concentrated in the root of *Oryza sativa*, while chromium and zinc metals were more concentrated in the shoot part. In addition to this, the translocation factor (TF) showed that *Oryza sativa* was able to transfer zinc and cadmium from root to the shoot.

Gao et al. (2016) and Rehman et al. (2017) observed the negative impact of heavy metal especially cadmium in rice crop and reported that rice plant has the ability to accumulate cadmium which negatively affects rice growth and quality in the number of cultivars.

2.6 Effect of biochar in phytoremediation of cadmium contaminated soil

Singh et al. (1995) reported cadmium behavior in the presence of lime and biochar and concluded that cadmium phytoavailability generally decreased with increasing soil pH while in acid soils, application of lime may reduced cadmium concentration, but the effect does not seem consistent in both the situation.

Nordberg et al. (1997) and Honda et al. (2010) reported that in some region of China and Thailand, production of rice in highly cadmium-polluted soil leads renal disfunction among the populations on a huge scale.

Hsu and Kao (2003) reported the synthesis of amino acids in plants is controlled through certain environmental factors. Cadmium stress increased the synthesis of the amino acid in tissues and these amino acids increased at various levels of cadmium application, but the effect was more visible at 3 % biochar application with cadmium stress condition. The increase in cadmium concentration activated the proteolytic enzymes and also increased the accumulation of amino acids that play a protective role against cadmium toxicity in plants.

Mishra *et al.* (2006) reported that the increase in proteins synthesis in a plant with the biochar application under cadmium stress condition due to an increase in the formation of metal-binding complexes.

Borchard *et al.* (2012) reported that application of biochar also improves C, N and P status within the soil strata.

Fellet *et al.* (2011) reported the advantage of biochar incorporation in cadmium contaminated soil might improves soil fertility parameters such as nutrient contents, pH and CEC and water retention capacity thereby lessening the need for fertilizer and irrigating water. Moreover, the agronomic benefits of biochar are last longer than those offered by any other form of organic matter e.g. manure or compost commonly applied to land due to its greater efficiency in retaining nutrients and keeping them available as well as its favorably long persistence in soil.

Thies and Rillig, (2009) reported general benefits of biochar application in soils increases water holding capacities as well.

Lee *et al.* (2010) and Witters *et al.* (2012) reported proper evidence of phytoremediation, using bio-energy crops including rapeseed, could positively abate atmospheric CO₂ while being economically efficient as well. Energy can be obtained from such crops using pyrolysis and when biochar incorporated in the soil found that it has the potential of reducing greenhouse gases emmision from the soil.

Major *et al.* (2010) reported the application of biochar in soil enhanced availability of Ca, Mg and Zn but reduction in the leaching of some macronutrients below soil solution as well. That might increased the soil fertility and plant growth by supplying and retaining nutrients while simultaneously improving the physical and biological properties of the soil even under cadmium stress.

Wang *et al.* (2014) studied the mechanisms of biochar and reported its role in controlling and removal from aqueous solutions including precipitation, complexation, ion exchange, chemisorption, and physical sorption. Biochars having the high surface area and pore volumes have a greater affinity for metals because metallic ions physically sorbed on biochar surface and retained within the pores. Biochar surfaces have negatively charged and can sorb metals having positively

charged through electrostatic attractions, ligands Specificity and various functional groups present on biochar can also interact with various heavy metals by forming complex and precipitates of their solid mineral phases compared with activated carbon, biochar appears to be a new potential low-cost and effective adsorbent.

Uchimiya *et al.* (2011) and Zheng *et al.* (2012) defined that biochar is a highly porous and carbon-rich material, has been applied for soil remediation due to its capability of minimizing the mobility and bioaccumulation of PTEs as well as other organic contaminants such as polycyclic aromatic hydrocarbons and pesticides. Although surface adsorption and complexation are known to play important roles in the interaction between biochar and contaminants the mechanisms of contaminant immobilization by biochar are generally complex and vary from soil properties and types of biochar.

Uzoma *et al.* (2011) reported that biochar is the result of biomass pyrolysis under minimal oxygen supply. Amending the soil with biochar has increasingly attracted widespread attention for two reasons first, because of its chemical stability and is ideally suited for sequestering carbon in soil Secondly biochar is often regarded as a soil conditioner because it's efficient and rapid action within soil system.

Rothenberg *et al.* (2014) reported biochar might enhance both pH and Eh of the rhizosphere, which is important for plant growth and metal solubility. Alternate wetting and drying cycles were used in his experiment which is widely employed in low land paddy soils with sufficient rainfall found effective in mitigating cadmium transfer from soil to rice crop in a continuous flooding situation.

Kim *et al.* (2013) reported that biochar application increases the exchangeable bases and inorganic compounds in soil which further increased the porosity and surface area that in turn, decreases toxic elements in the amended soil solution. Biochars having different pH with the increasing temperature that could change the soil pH differently and influenced the toxic elements mobility and their accumulation in the rice plants grown on biochar-amended soil. The negatively-charged sites on the surfaces depend on soil pH which is changed with biochar application that could also be affected by the sorption of selected toxic elements.

Ok et al. (2015) reported that biochar application may decrease the cadmium concentration in plants as compared to the same treatments without biochar application. The decrease in cadmium concentration might be due to biochar-mediated cadmium immobilization in the soil.

Rizwan et al. (2016) examined on the ability of biochars to reduce the bioavailable toxic forms of cadmium in soil and hence reported various immobilization mechanisms in soil including adsorption, precipitation, and complexation reactions which can significantly remove cadmium toxicity from soil and plant system.

Abbas et al. (2017) reported that phytoremediation through biochar is economically feasible tool to mitigate cadmium toxicity having the characteristics contains 22.5% ash contents, 24% volatile matter, 42.3% carbon contents, 2.4 dS/m electrical conductivity, and pH value of 10. Commercially available ZnO, NPs were obtained from *Alfa Aesar* and the specific characteristics of these NPs were the purity of 99%, size of 20–30 nm, and density 5.606 g/cm³.

Abbas et al. (2017) reported that biochar may possibly improve the growth of the plant by supplying essential micronutrient (i.e. Fe, Zn, and Mn) and at the same time decreasing toxic metal concentration from the plant.

CHAPTER - III

MATERIAL AND METHOD

A pot experiment was conducted to study the “**Effect of biochar on phytoremediation of cadmium contaminated soil by using rice crop (*Oryza sativa* L.)**” in net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The pot experiment was conducted in Completely Randomized Design (CRD) taking rice as a test crop in *kharif* season 2018.

3.1 Experimental site

The experiment was conducted in the net house of Department of Soil Science and Agril Chemistry in Varanasi and Varanasi is situated at an altitude of 78.71 meters above mean sea level and located in between 25 degree 18” North latitude and 80 degrees 36” East longitude.

3.2 Climate and weather

Varanasi falls in the belt of semi-arid to sub-humid climate receiving a mean annual rainfall of 1100 mm and potential evapotranspiration of about 1525 mm. Thus, there is a moisture deficit of 425 mm. The normal period for the onset of monsoon in this region is the third week of June and which lasts up to the end of September or sometimes extends to the first week of November. However, occasional showers are also common during winters. The winter months are cool whereas summers are hot and dry. Standard week wise data on meteorological parameters were obtained from the meteorological observatory, located at Agricultural Research Farm of Banaras Hindu University, Varanasi. A standard week-wise data on weather parameters are presented in Table 3.1 and depicted in Fig. 3.1.

Table 3.2: Mean standard week-wise meteorological parameters during *kharif* season 2018

Week No.	Month & Date	Rainfall (mm)	Temperature °C		R.H. %		Wind Speed (km/hr)	Sunshine (hours)	Evaporation (mm)
			MAX	MIN	Morn.	Even			
1	June 25-01	33.3	33.9	26.7	80	61	4.1	4.0	6.1
2	July 02-08	8.0	35.5	27.9	77	57	3.6	6.4	4.4
3	09-15	11.6	35.5	26.0	83	58	4.4	7.8	5.8
4	16-22	78.4	33.3	25.4	86	66	4.5	4.9	4.4
5	23-29	91.4	28.4	23.6	88	87	2.5	0.3	1.7
6	30-05	86.8	28.1	22.8	93	88	4.2	0.1	1.4
7	Aug 06-12	26.6	31.8	24.7	92	77	2.0	5.9	2.7
8	13-19	20.4	33.3	25.3	88	70	2.4	9.7	2.8
9	20-26	154.8	31.1	24.0	91	81	2.4	7.4	2.6
10	27-02	118.4	32.0	24.3	93	77	1.7	4.4	2.6
11	Sep 03-09	94.6	30.6	23.6	91	79	2.3	5.7	2.4
12	10-16	0.0	32.4	23.6	88	68	2.7	7.8	3.8
13	17-23	53.4	32.5	22.8	88	65	3.7	7.7	3.1
14	24-30	0.0	33.4	25.9	88	63	1.1	8.1	3.5
15	Oct 01-7	0.0	34.2	20.8	83	51	0.8	8.6	3.7
16	08-14	0.0	31.0	20.0	89	61	1.0	8.5	2.9
17	15-21	0.0	33.4	16.5	84	40	0.6	6.1	2.8
18	22-28	0.0	31.5	14.4	89	41	0.4	6.9	2.6
19	29-04	0.0	31.1	16.7	91	48	0.8	7.7	2.3
20	Nov 05-11	0.0	28.2	12.2	87	44	2.3	7.6	2.0
21	12-18	0.0	29.0	11.7	89	45	0.9	7.6	1.8
22	19-25	0.0	27.9	10.1	88	44	0.6	7.2	2.0
23	26-02	0.0	26.4	10.1	93	48	1.1	4.3	1.5

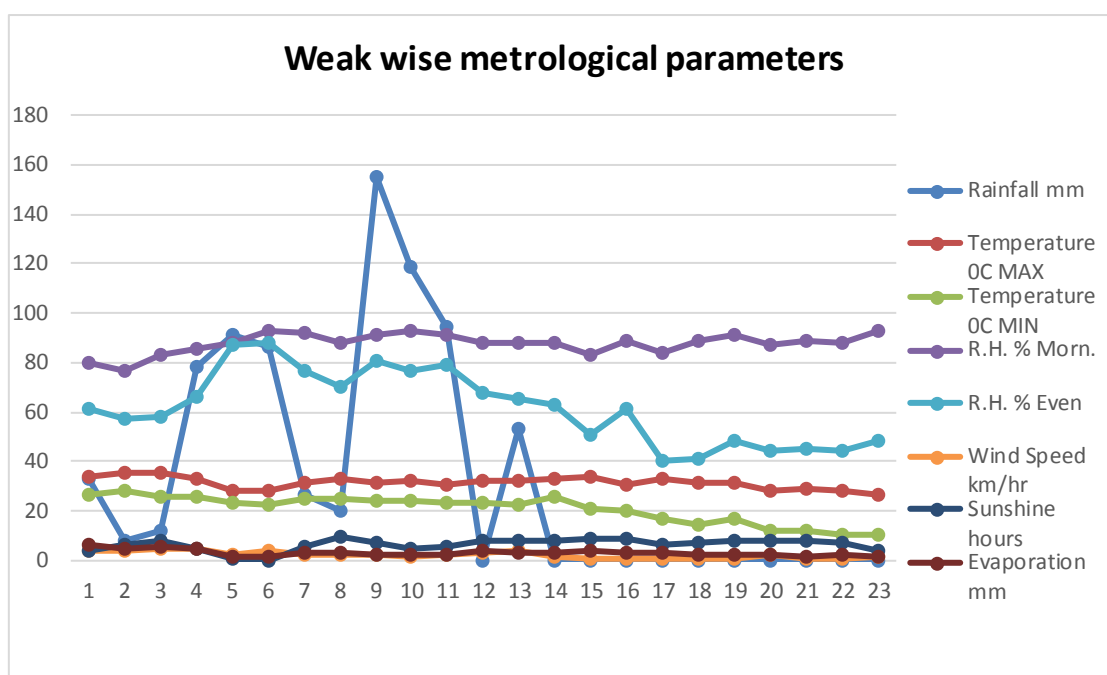


Fig 3.2: Mean standard week-wise meteorological parameters during *kharif* season 2018

3.3 Experimental soil

The alluvial soils of Indo-Gangetic plains are deep, flat, well drained with low available nitrogen and medium in available phosphorus and potassium. To carry out the pot experiment, upper surface (0-15 cm) soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The soil samples were analyzed for chemical properties and the results are presented in below. The data pertaining to physical and chemical properties of initial soil sample are depicted in Table 3.3. Experimental soil has pH -7.19, EC-0.15 dSm⁻¹ and organic carbon 0.25 %. The initial soil was low in available N content 191 kg ha⁻¹ while available P was recorded 17.89 kg ha⁻¹ and K 185.78.3 kg ha⁻¹ were in medium range respectively and available micronutrients were found very less in the initial soil sample.

Table 3.3: Chemical characteristics of the initial experimental soil

Properties	Values of initial soil	Method	Reference
Chemical analysis			
pH (1:2.5) soil: water suspension	7.19	Glass electrode digital pH meter	Jackson (1973)
Electrical conductivity (dSm ⁻¹) at 25°C.	0.15	Electrical conductivity meter	Jackson (1973)
Organic carbon (%)	0.25	Wet digestion	Walkley and Black's (1934)
Available N (kg ha ⁻¹)	195.70	Alkaline permanganate method	Subbiah and Asija (1956)
Available P ₂ O ₅ (kg ha ⁻¹)	17.89	0.5M NaHCO ₃ Extractable	(Olsen <i>et al.</i> , 1954) method
Available K ₂ O (kg ha ⁻¹)	185.78	Flame photometer	Hanway and Heidel (1952)
Available cadmium (ppm)	0.04	AAS	Lindsay and Norwell(1978)
Available Fe (ppm)	18.8	AAS	Lindsay and Norwell(1978)
Available Cu (ppm)	1.75	AAS	Lindsay and Norwell(1978)
Available Mn (ppm)	6.05	AAS	Lindsay and Norwell(1978)
Available Zn (ppm)	1.04	AAS	Lindsay and Norwell(1978)

3.4 Experimental details

Year of experimentation	<i>Kharif</i> , 2018
Date of soil treatment with CdCl ₂	27-06-2018
Date of biochar application	12-07-2018
Date of rice transplantation in pot	23-07-2018
Date of harvesting	15-11-2018
Recommended dose of fertilizer	N: P ₂ O ₅ : K ₂ O @ 150:60:60 kg ha ⁻¹
No. of treatments	18
No. of replication	3
Total no. of pots	54 (18 × 3)
Amount of soil	10 kg pot ⁻¹
Experimental design	C.R.D
Crop	Rice
Variety	HUR-105

3.5 Treatment details

Treatments	Abbreviations
T ₁ : Cadmium 0 ppm	Cd0
T ₂ : Cadmium 20ppm	Cd20
T ₃ : Cadmium 40ppm	Cd40
T ₄ : Cadmium 60 ppm	Cd60
T ₅ : Cadmium 80 ppm	Cd80
T ₆ : Cadmium 100ppm	Cd100
T ₇ : Cadmium 0 ppm + Biochar 20	Cd0Bc20
T ₈ : Cadmium 0 ppm + Biochar 40	Cd0Bc40
T ₉ : Cadmium 20 ppm + Biochar 20	Cd20Bc20
T ₁₀ : Cadmium 20 ppm + Biochar 40	Cd20Bc40
T ₁₁ : Cadmium 40ppm + Biochar 20	Cd40Bc20
T ₁₂ : Cadmium 40 ppm + Biochar 40	Cd40Bc40
T ₁₃ : Cadmium 60 ppm + Biochar 20	Cd60Bc20
T ₁₄ : cadmium 60ppm + Biochar 40	Cd60Bc40
T ₁₅ : Cadmium 80 ppm + Biochar 20	Cd80Bc20
T ₁₆ : Cadmium 80 ppm + Biochar 40	Cd80Bc40
T ₁₇ : Cadmium 100ppm + Biochar 20	Cd100Bc20
T ₁₈ : Cadmium 100 ppm + Biochar 40	Cd100Bc40

Where biochar is applied according to 20 and 40 ton ha⁻¹ respectively and N, P, K recommended dose was followed 150, 60, and 60 (kg ha⁻¹) to each pot as per recomonded.

3.6 Selection of variety

Malaviya Sugandh-105 (HUR-105)

(HUR-105) was used as a test crop and was developed at Banaras Hindu University, Varanasi and released in 2009. It is a semi-dwarf, medium maturity scented rice variety suitable for cultivation in irrigated areas of U.P. Its maturity duration is 130-135 days; average yield potential reported in this variety is 50 q ha⁻¹. The plant grows up to a height of 100 to 110 cm. It is tolerant to stem borer, leaf and neck blast and a brown spot under field condition.

3.7 Sources of nutrients

3.7.1 Inorganic sources

1. **Urea:** Urea is a most widely used N fertilizer in India which is produced by heating ammonia with CO₂ under high temperature (160-170 °C). Urea contains the highest percentage of nitrogen (46.0%) among solid fertilizers. It contains nitrogen in the amide form. This organic fertilizer is cheaper than any other solid nitrogenous fertilizer in India.
2. **DAP:** Di-ammonium phosphate is the most widely used phosphatic fertilizer in India and is produced by reacting calcium nitrate with mono-calcium phosphate and finally to Di ammonium phosphate which contains 18% N and 46% P₂O₅.
3. **MOP:** Muriate of potash or potassium chloride (KCl) is most common and cheapest fertilizer among the potassic fertilizers. It contains 60% K₂O.

3.7.2 Biochar

The biochar was collected from a private rice mill situated at Mirzapur district.

Biochar is collected and then it was applied according to 20 and 40 ton ha⁻¹ i.e. 89.2 g pot⁻¹ and 178.6 g pot⁻¹ respectively.

3.8 Procedure

Step1: Filling of pots

Processed 10 kg soil was filled in each polythene lined pots. The pots were irrigated up to field capacity and moisture level is maintained.

Step 2: Application of cadmium:

Ten kg of soil was treated with the required amount of cadmium through cadmium chloride solution (CdCl_2) with six different levels of concentration like (0, 20, 40, 60, 80, 100 ppm) 10kg^{-1} of soil in pots and were kept to maintain equilibrium.

Step 3: Application of biochar

After 15 days of cadmium contamination in soil, biochar was applied 89.2g and 178.6 g pot^{-1} respectively and then mix thoroughly by hand with wearing of hand gloves homogenously in each pot so that it can effectively mitigate the toxic behavior of cadmium from soil.

Step 4: Transplanting of rice seedlings into contaminated pots

Pots were incubated for next 15 days with biochar and watered at field capacity almost on a daily basis after a month of incubation (four-week old-seedlings) was transplanted with 5 seedlings pot^{-1} on 23rd of July 2018.

Step 5: Application of fertilizers

The sources of nitrogen, phosphorus, and potassium were UREA, DAP, and MOP, respectively. The recommended dose of N, P and K were 150, 60 and 60 kg ha^{-1} as per the standard set for cereal crop. One-third N and a full dose of P and K has been applied at the time of sowing and remaining dose of N has been applied in two equal splits after 30 and 60 days of transplanting in each pot. All the pots received a uniform dose of N, P, K and irrigation were supplied to maintain field capacity up to the maturity of rice crop.

Step 6: Harvesting and collection of samples

Plants were harvested and plant parts like grain, straw, and root samples were collected separately from each pot and washed successively with tap water and

thereafter with single and double distilled water, oven dried at 65°C and grinded in Willy mill to pass through 0.5 mm screen.

3.9 Biometric Observation

For recording biometric observations at a regular interval of 30th, 60th and 90th days after transplanting (DAT) and at harvest, five plants in each pot were taken into consideration. Yield attributes and yield were studied before and after harvesting as per investigation required.

3.9.1 Growth Attributes

3.9.1.1 Plant height (cm)

The height of plants was measured at 30, 60 and 90 days after transplanting and harvest from the base of the plant to the tip of the uppermost fully opened leaf. After panicle emergence, the height was measured up to the tip of the panicle.

3.9.1.2 Number of the tillers hill⁻¹

Tillers were counted from each pot at 30, 60, 90 days after transplanting and at harvest total tillers hill⁻¹ and productive tillers hill⁻¹ from each pot were counted.

3.9.1.3 Dry matter accumulation hill⁻¹ in (g)

The plants were cut from ground level and then sun-dried thereafter the collected samples were oven dried at 70°C for 48 hours to constant weight. The weight thus obtained was recorded an average dry weight(g) hill⁻¹ after dividing the total weight of five plants by the total number of plants.

3.9.2 Yield and Yield attributes

The following observations on yield attributes were recorded during the experimentation.

3.9.2.1 Panicle length (cm)

Five panicles were randomly selected from plants in each pot and the length was measured from the neck node to the tip of the uppermost spikelet and the average length was recorded.

3.9.2.2 Filled grains panicle⁻¹

Grains of 5 panicles of randomly selected from each pot were separated and counted and their mean value expressed as average grains per panicle.

3.9.2.3 Panicle weight (g)

Five panicles were sampled from the plants in each pot and their weight was measured. The mean panicle weight was computed and expressed in g.

3.9.2.4 1000 grains weight or test weight (g):

Grain samples were taken from the threshed and cleaned produce of each pot and 1000 grains were counted and weighed.

3.9.2.5 Grain yield (g pot⁻¹).

The mature harvested produce from the pot was sun-dried and threshed to obtain grain yield in (g pot⁻¹).

3.9.2.6 Straw yield (g pot⁻¹)

The straw yield was worked out by subtracting the grain yield from total biological yield and finally it was counted g pot⁻¹.

3.9.2.7 Harvest Index (%)

It is the economic yield expressed as a percentage of biological yield and calculated as follows. It is expressed in percentage.

Harvest index (%)

Biological yield= Grain yield + Straw yield.

Economical yield= Grain Yield.

H.I = Economical yield/ Biological yield ×100

3.9.3 Chemical analyses of Soil Samples

3.9.3.1 Soil pH

A soil-water suspension was prepared in the ratio of 1:2.5 like 10 g soil in 25 mL of distilled water and pH was measured with the help of pH meter of collected soil sample.

3.9.3.2 Electrical conductivity

The soil water suspension prepared for determination of pH was used to determine the electrical conductivity of the soil. The soil suspension was allowed to settle till supernatant become clear. Electrical conductivity was measured with the help of EC meter and expressed as dSm^{-1} .

3.9.3.3 Organic Carbon

Organic carbon in the soil was determined using wet digestion method (Walkley and Black, 1934). One gram of soil was taken in 500 mL of the conical flask. 10 mL of 1 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution was added and mixed. Then 20 mL of concentrated H_2SO_4 was added, the flask was swirled 2-3 times and allowed to stand for 30 minutes in dark. Then the suspension was diluted with 200 mL of distilled water. Then after 10 mL of 85% H_3PO_3 and 1 mL of diphenyl indicator were added and titrated against the solution of 0.5 N Ferrous Ammonium Sulphate till color changed from violet to bright green. A blank titration was also carried out simultaneously.

3.9.3.4 Available nitrogen

Available Nitrogen content in soil was determined using Kjeltac Semi-Auto Nitrogen Analyzer by alkaline Potassium permanganate method as proposed by Subbiah and Asija (1956). The method has been adopted widely in order to get a reliable index of Nitrogen availability in soil due to its rapidity and reproducibility. Five gram of soil sample was weighed and transferred in a distillation tube. Twenty-five mL 0.32% KMnO_4 was added to it and the distillation tube was set to the instrument. In a 250 mL conical flask, 20 mL of 2 % boric acid mixed indicator was taken and placed under the receiver tube. Twenty-five mL of 2.5% NaOH was sucked and added to the distillation tube. Then it was put on distillation for 9 min. During this process, the N released in the form of ammonia is trapped in the boric acid, which develops green color. The flask containing the distillate was removed. The distillate was then titrated against 0.02 N H_2SO_4 until pink color developed.

3.9.3.5 Available phosphorous

Available phosphorus content in soil was determined by Olsen's method (Olsen *et al.*, 1954). Firstly reagent A was prepared by using ammonium molybdate, antimony potassium tartarate, and H_2SO_4 . Then reagent B was prepared with the help of reagent A. Now 2.5 gram of soil sample was taken in a 100 mL conical flask, then a pinch of Darco G-60 and 50 mL of Olsen's reagent 0.5 M NaHCO_3 was added to it. It was then shaken for 30 minute on the mechanical shaker and the suspension was filtered through Whatman No. 1 filter paper. Five mL of filtrate was transferred in a 25 mL volumetric flask and was acidified with 2.5 M H_2SO_4 to pH 5.0 and 20 mL distilled water was added followed by 4 mL of reagent B. After waiting for 10 min the intensity of blue color was measured on a spectrophotometer at 660 nm. Simultaneously a blank was also run. First standard reading was taken followed by sample reading.

3.9.3.6 Available Potassium

Flame Photometer is used to determine available potassium in soil by using 1 N ammonium acetate extract by using Honway and Heidel (1952). Five gram soil was transferred in a 100 mL conical flask and 25 mL of 1 N ammonium acetate solution was added and it was shaken for 5 min. The suspension was then filtered through Whatman No. 1 filter paper and- potassium concentration in the filtrate was measured using flame photometer. First standard reading was taken followed by sample reading.

3.9.3.7 DTPA extractable micronutrient.

Available Zn, Mn, and Fe in soil samples were determined by DTPA extraction Lindsay and Norvell, (1978). In this method, 10 g of soil was extracted with 20 mL DTPA extracting solution by shaking for 2 hours on a shaker. The suspension was then filtered and trace elements were determined by atomic absorption spectrophotometer (UNICAM969) using respective cathode lamps.

3.9.3.8 Di-acid digestion for cadmium extraction.

Plant sample of about 0.5g taken in conical flask and then pre-digested by adding with 10 ml of HNO_3 and left it over night in dark place and then added mixture of 20 ml Di-acid in the ratio of $\text{HNO}_3:\text{HClO}_4$ (3:1) on next day and allowed it

for heating on hot plate about 150°C temperature for initial one hour and then increase the temperature around 250°C for next hour until white fumes are not coming from the flask and then finally remove it and allowed to cool down at room tem and then add 20ml distilled water then filtered with whatman no; 01 filter paper in 50ml of volumetric flask and finally maintain its volume up to the mark then go for analysis of cadmium in AAS using cadmium cathode lamp.

3.9.4 Nutrient content and uptake studies

At harvest, the plant and grain samples were collected from each pot for chemical estimation and the samples were dried in an oven at 70°C for 48 hours, the plant material thus obtained was ground with the help of grinder and passed through 40 mesh sieve and preserved separately for determination of N, P and K content calculated by following formulae.

$$\text{Nutrient uptake kg ha}^{-1} = \text{nutrient content in \%} \times \text{yield kg ha}^{-1}$$

3.9.4.1 Nitrogen content (%) and its uptake

Nitrogen content (%) was estimated both in grain and in straw by modified Kjeldahl method as described by Jackson (1973). Total nitrogen content in grain and straw was multiplied by the respective dry matter yield to get the total nitrogen uptake kg ha⁻¹ by plants.

3.9.4.2 Phosphorus and its uptake

Phosphorus content (%) in grain and straw was determined by Vandomolybdo phosphoric acid yellow color method as suggested by Jackson (1973). Total phosphorus content of grain and straw was multiplied with their respective dry matter yield to get the total phosphorus uptake by plants in kg ha⁻¹.

3.9.4.3 Potassium and its uptake

Potassium content (%) was estimated with the help of Flame photometer as described by Jackson (1973). Potassium percent in grain and straw were multiplied by their respective dry matter yield to obtain total potassium uptake kg ha⁻¹ by plants. The uptake of the entire major nutrient in kg ha⁻¹ was calculated by using the formula given by Black (1967).

3.9.4.4 Cadmium uptake in a different part of rice plant

Cadmium content (%) was estimated with the help of AAS as described by Lindsay and Norwell (1978). Cadmium percent in grain and straw were multiplied by their respective dry matter yield to obtain total cadmium uptake kg ha^{-1} by plants.

3.9.4.5 Protein Yield

The protein yield is estimated with the help of protein content in rice grain and protein content is based on the percentage of nitrogen uptake in rice grain and calculated as.

$$\text{Protein content} = \% \text{ of nitrogen uptake} \times 6.25$$

$$\text{Protein yield} = \text{protein content} \times \text{yield of rice grain (g) pot}^{-1}$$

Table 3.9.4 Chemical analyses of plant and grain:

Analysis	Method	Reference
Total N	Micro Kjeldahl method	Jackson (1973)
Total P	Vandomolybdo phosphoric acid yellow colour method	Jackson (1973)
Total K	Flame photometer method	Jackson (1973)
Total Cadmium	AAS	Lindsay and Norwell (1978)

3.10 Enrichment factor (EF) and transformation factor (TF)

Quantification of enrichment factor and transformation factor are shown in the equation (1) from Lorestani *et al.*, (2011). The value of enrichment factors indicated the mechanism of heavy metal absorption from soil to *Oryza sativa*; however, the translocation factor is to examine the transport or transfer of heavy metals from roots to shoots of *Oryza Sativa*. The EF and TF values that were examined in this study are in mg kg^{-1} .

Enrichment factor (EF) = C_p/C_s

Where C_p = heavy metal concentration in plant

C_s =heavy metal concentration in soil

Translocation factor (TF) = C_s/C_r Where

C_s =heavy metal concentration in plant's shoot

C_r =heavy metal concentration in the plant's root

3.11 Statistical analysis

The observations recorded during the course of investigation were tabulated and analyzed through CRD statistically to draw a valid conclusion. The data were analyzed as per the standard procedure for "Analysis of Variance" (ANOVA) as described by Gomez and Gomez (1984). The significance of treatments was tested by F test (Variance ratio). The standard error of mean was computed in all cases. The difference in the treatment the mean were tested by using Critical Difference (CD) at 5% level of probability where F test showed significant differences among means by the following formula.

$$CD = \frac{t \cdot SE}{\sqrt{r}}$$

CHAPTER - IV

RESULTS AND DISCUSSION

This experiment was conducted to study the “Effect of biochar, on phytoremediation of cadmium contaminated soil by using rice crop (*Oryza sativa* L.) in the net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh.

In carrying out this experiment data has been recorded concerning the effect of biochar on growth, yield and quality parameter along with the nutrient content and uptake of rice crop grown in cadmium contaminated soil and regarding the potential of rice to uptake cadmium. The data were recorded analyzed statistically then drawing the valid conclusions on the given issue. Degree of difference responses exhibit by varying experimental variables are discussed in this chapter as follows.

4.1 Effect of biochar on growth parameter of rice crops in cadmium contaminated soil

4.1.1 Growth (plant height)

4.1.2 Total tillers hill⁻¹ and productive tillers hill⁻¹

4.2 Effect of biochar on physiological characteristics of rice crop in cadmium contaminated soil

4.2.1 Chlorophyll content

4.3 Effect of biochar on yield attributes of rice plants in cadmium contaminated soil

4.3.1 Panicle length (cm)

4.3.2 Numbers of grains panicle⁻¹

4.3.3 Unfilled grain panicle⁻¹

4.3.4 1000 Grains weight or test weight(g)

4.3.5 Grain yield of rice crop (g pot⁻¹)

4.3.6 Straw yield of rice crop (g pot⁻¹)

4.3.7 Harvest index (H.I %)

4.3.8 Nutrient content and uptake in rice straw and grain (%)

4.3.9 Phosphorus content in grain and straw (%)

4.3.10 Potassium content in grain and straw (%)

4.4 Effect of biochar, on soil properties of cadmium contaminated soil

4.4.1 pH

4.4.2 EC

4.4.3 Organic carbon (%)

4.4.4 Available Nitrogen (kg ha^{-1})

4.4.5 Available Phosphorus (kg ha^{-1})

4.4.6 Available potassium (kg ha^{-1})

4.5 Effect of biochar on cadmium content in soil and different part of rice plant

4.5.1 Cadmium accumulation in soil (mg kg^{-1})

4.5.2 Accumulation of cadmium in roots of rice crop (mg kg^{-1})

4.5.3 Accumulation of cadmium in shoots of rice crop (mg kg^{-1})

4.5.4 Accumulation of cadmium in grain of rice crop (mg kg^{-1})

4.6 Effect of biochar on quality attributes of rice grain in cadmium contaminated soil

4.6.1 Protein content (%)

4.6.2 protein yield (g pot^{-1})

4.7 Translocation and enrichment factor

4.1 Effect of biochar on growth parameter of rice crops in cadmium contaminated soil

4.1.1 Plant height

The data of total plant height of rice was recorded at 30 DAT, 60 DAT, and 90 DAT. The height of the rice plant was observed in cadmium contaminated soil and found that plant height was influenced due to the toxic nature of cadmium in a treated pot which is presented in table 4.1.1 and depicted in fig 4.1.1.

Plant height was reported to increase up to 90DAT and thereafter decreased. Plant height was significantly affected by different levels of cadmium at all growth stages. With an increase in the concentration of cadmium (Cd 0, 20, 40, 60, 80, and 100 ppm) plant height decreased at all stage of rice crop. A similar type of result was reported by (Quadir *et al.* 2004).

Considerable increase in plant height was reported on 30 DAT on the addition of biochar as compared to control pots. The highest plant height 71.2 cm was recorded in treatment (Cd0+Bc40) followed by the treatment (Cd0+Bc20) recorded with 70.0cm. On the other hand, the lowest height was obtained from control pot with treatment Cd100 i.e. 46.0cm. The similar trend was noticed with plant height at 60 DAT and 90 DAT except a slight increase in plant height and then after reduction in plant height was observed.

The toxic effect of cadmium disrupted the physiological responses of rice crop by altering the physiology of rice crop. The toxic effect of cadmium not only reduces plant height but it can also reduce the number of leaves and surface area of leaves per plant hence due to easy accessibility biochar in Indian context it is found very promising tools in mitigating the undesirable effect of cadmium in soil.

Table 4.1.1: Effect of biochar on plant height of rice crop at 30, 60, and 90 DAT in cadmium contaminated soil

Plant height (cm)			
Treatments	30 DAT	60 DAT	90 DAT
Cd0	69.3	103	105
Cd20	65.4	98.7	101
Cd40	60.8	92.7	92.8
Cd60	54.0	89.0	91.2
Cd80	48.0	85.0	89.0
Cd100	46.0	80.0	82.0
Cd0Bc20	70.0	104	107
Cd0Bc40	71.2	105	109
Cd20Bc20	68.4	99.9	102
Cd20Bc40	69.1	101	102
Cd40Bc20	61.3	92.7	94.0
Cd40Bc40	64.9	94.0	96.9
Cd60Bc20	55.0	89.8	92.9
Cd60Bc40	58.6	90.0	94.8
Cd80Bc20	49.0	86.0	91.3
Cd80Bc40	51.0	88.3	93.3
Cd100Bc20	46.9	80.9	83.0
Cd100Bc40	49.6	83.6	85.0
CD (0.05)	2.52	1.64	1.70
SEm $\pm$	0.88	0.57	0.59
S/NS	S	S	S

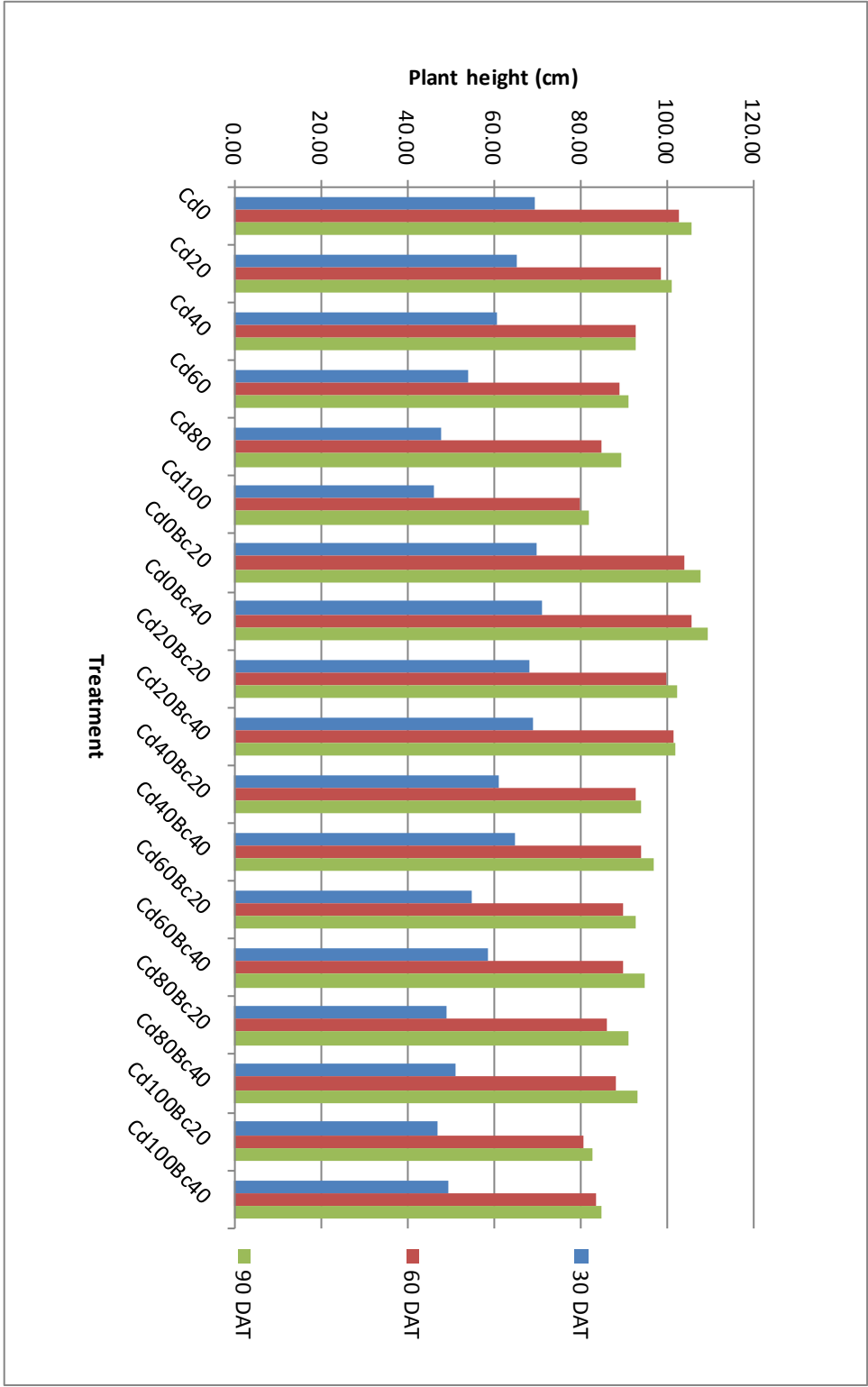


Fig. 4.1.1: Effect of biochar on plant height of rice crop at 30, 60, and 90 DAT in cadmium contaminated soil

4.1.2 Number of tillers hill⁻¹

The data of total tillers per hill and productive tillers per hill recorded at different observation days was presented in table 4.1.2 and depicted in fig no: 4.1.2. An inspection of data existing showed that the number of tillers per hill increased slowly from 30 to 60 DAT. Levels of cadmium usually affected the tiller number. As the cadmium concentration increased there was a decrease in the number of the total tillers and productive tiller per hill. Similar results had been reported by (Ahmad *et al.* 2011). Result showed that number of tillers per hill of rice and productive tillers per hill were significantly affected due to the application of different doses of cadmium.

The maximum total tillers count hill⁻¹ was recorded 13.0 and productive tillers hill⁻¹ was recorded 11.8 respectively and pot treated with biochar recorded maximum tiller count i.e 13 in treatment (Cd0+Bc4) followed by 12.8 in treatment (Cd0+Bc20) recorded respectively. while total minimum number of tiller count per hill was 6.20 and productive tillers count per hill was 4.80 both in treatment (Cd100). The effect of biochar was found significant on tiller count per hill because it increased the number of tillers per hill and productive tillers hill⁻¹ in rice crop and it also supplies of the better and adequate amount of macro and micronutrient by organic sources resulted in better toleration in rice crop. Biochar increased soil fertility, enhancing plant growth by decreasing phyto-available fractions of metals, while cadmium toxicity restricted rice growth as estimated in terms of root and shoot length, leaf area, root area, number of leaves and roots per plant (Song *et al.* 2015).

The variation in the above-mentioned parameters recorded might be attributed due to the availability of nutrients. Nutrient availability from organic sources is due to microbial action which is slow and steady and further through improved physical conditions of the soils (Mirza *et al.* 2010). Biochar showed the better result as compared to another organic amendment because of its chemical stability as it widens the possibility of sequestering carbon besides that it also increased soil fertility and acts as a soil conditioner, application of biochar could also reduced metal uptake in various plants species and increased available nutrient uptake for the plant (Houben and Sonnet 2015).

Table 4.1.2: Effect of biochar on total tillers and productive tillers count hill⁻¹ of rice crop in cadmium contaminated soil

Tillers count hill ⁻¹				
Treatments	30 DAT	60 DAT	Total tillers hill ⁻¹	Productive tillers hill ⁻¹
Cd0	7.20	8.60	9.60	7.50
Cd20	6.10	8.20	9.30	7.20
Cd40	5.20	7.10	8.10	6.50
Cd60	4.60	6.20	7.20	6.00
Cd80	4.20	5.80	6.80	5.10
Cd100	4.00	5.20	6.20	4.80
Cd0Bc20	7.40	9.80	12.8	11.2
Cd0Bc40	7.60	10.2	13.0	11.8
Cd20Bc20	6.40	8.60	12.2	11.0
Cd20Bc40	6.80	8.90	12.8	11.2
Cd40Bc20	5.60	8.20	11.2	9.50
Cd40Bc40	5.80	8.60	11.6	9.80
Cd60Bc20	4.80	7.20	10.2	8.20
Cd60Bc40	5.30	7.60	10.4	8.50
Cd80Bc20	4.60	6.46	8.20	7.10
Cd80Bc40	5.00	6.70	8.40	7.00
Cd100Bc20	4.40	5.80	8.00	6.60
Cd100Bc40	4.60	6.20	8.26	6.00
CD (0.05)	0.34	0.30	0.37	0.43
SEm±	0.11	0.10	0.13	0.15
S/NS	S	S	S	S

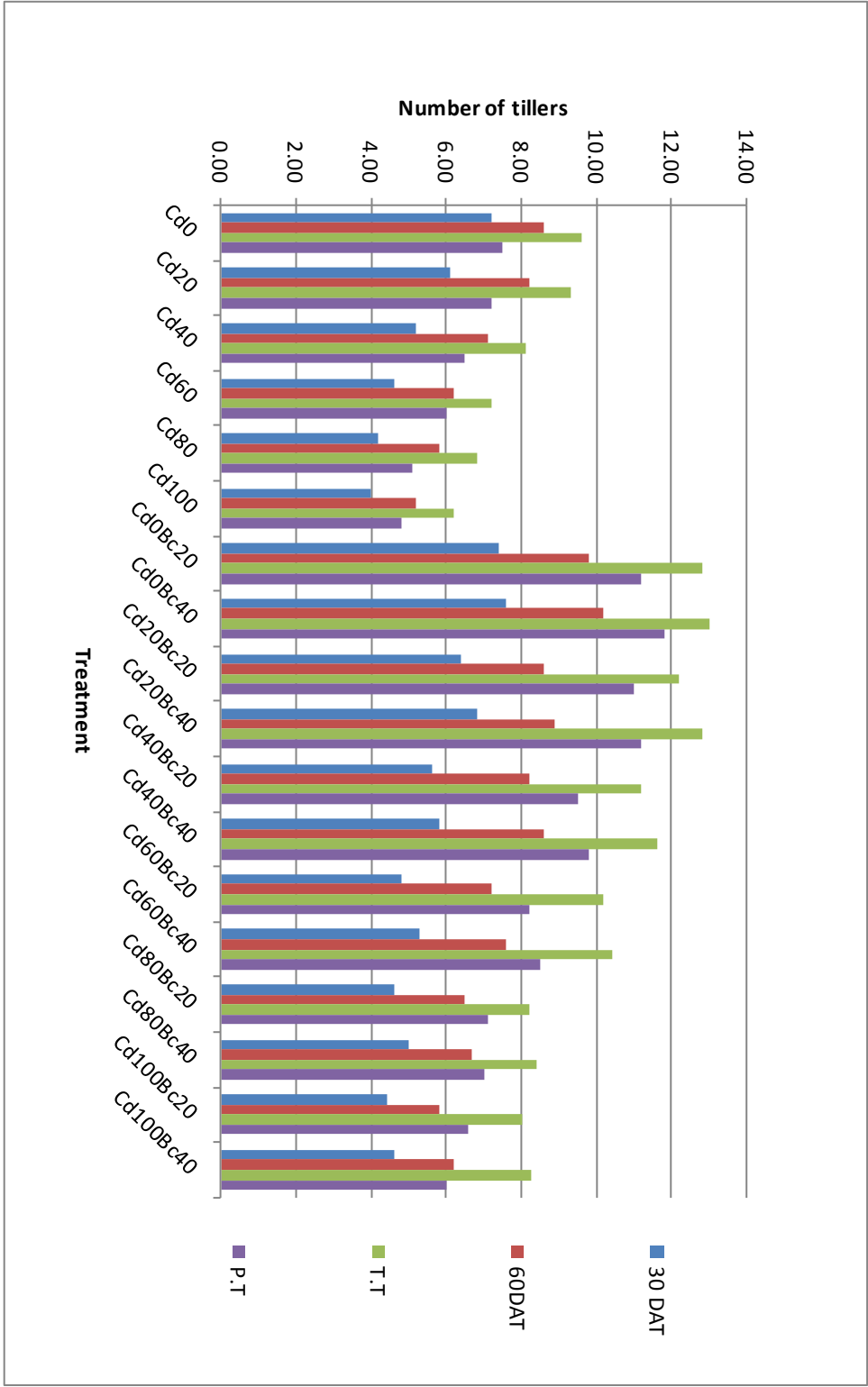


Fig. 4.1.2: Effect of biochar on total tillers and productive tillers count hill⁻¹ of rice crop in cadmium soil

4.2 Physiological Characteristics of Plants

4.2.1 Chlorophyll

The data of chlorophyll was recorded by SPAD meter at different observation days is presented in table no: 4.2.1 and depicted in fig no: 4.2.1. Data presented here for chlorophyll content increased slowly from 30 to 60 DAT and then after a gradual decline was observed on 90 DAT when plants move towards maturity. It is evident from the data that chlorophyll content was significantly affected by different levels of cadmium at different growth stages.

Data ensured that level of cadmium increased from 0 to 100 ppm the amount of chlorophyll content were decreased respectively because of oxidative stress induced by cadmium accumulation in the plant which resulted in lipid peroxidation in plants that cause severe damage to the cell membranes. The oxidative stress is induced by cadmium initiates the degradation of photosynthetic pigments which causes a decline in growth of the plant. High cadmium concentration affects photosynthesis in terms of CO₂ fixation, electron transport, photophosphorylation, and enzyme activities that can disturb the chloroplast ultra structure there by disturbing the photosynthetic process. Under cadmium stress situation photosynthesis, chlorophyll a, chlorophyll b, carotenoids, net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency was reported decreased in rice plant (Rascio *et al.* 2008).

The cadmium toxicity results in growth inhibition of rice crop more or less showing chlorosis while excessive cadmium exposure Cd 100 mg kg⁻¹ of soil causes leaf chlorosis and necrosis (Liu *et al.* 2003). High cadmium concentration inhibits photosynthesis and seriously inhibits the root growth (Dheeba *et al.* 2012). The results regarding chlorophyll content after 30 days of transplantation in rice crop indicated that chlorophyll content significantly enhanced due to the application of biochar. The highest chlorophyll content was recorded 42.10 in treatment (Cd0+Bc40) followed by treatment (Cd0ppm+ Bc20) i.e. 41.50 respectively. While plants grown on soil treated with Cd100ppm without application of biochar leads to an extreme yellowing of leaves due to lack of chlorophyll content. The application of biochar significantly increased chlorophyll content in rice crop.

Table 4.2.1: Effect of biochar on chlorophyll content at 30, 60, and 90 DAT of rice crop in cadmium contaminated soil

Chlorophyll content (SPAD reading)			
Treatments	30 DAT	60 DAT	90 DAT
Cd0	40.1	38.2	27.9
Cd20	37.5	36.1	25.4
Cd40	35.4	34.1	24.3
Cd60	33.1	32.1	22.3
Cd80	30.2	29.1	20.6
Cd100	28.3	27.0	18.6
Cd0Bc20	41.5	39.1	28.6
Cd0Bc40	42.1	40.2	29.5
Cd20Bc20	38.2	37.4	26.2
Cd20Bc40	39.0	38.3	27.2
Cd40Bc20	36.4	35.5	25.4
Cd40Bc40	37.2	36.1	26.1
Cd60Bc20	34.1	33.1	23.2
Cd60Bc40	35.2	34.1	24.3
Cd80Bc20	31.2	30.1	21.1
Cd80Bc40	33.4	32.1	22.0
Cd100Bc20	29.5	28.0	19.1
Cd100Bc40	30.5	29.0	20.2
CD (0.05)	1.88	1.62	1.78
SEm±	0.65	0.56	0.62
S/NS	S	S	S

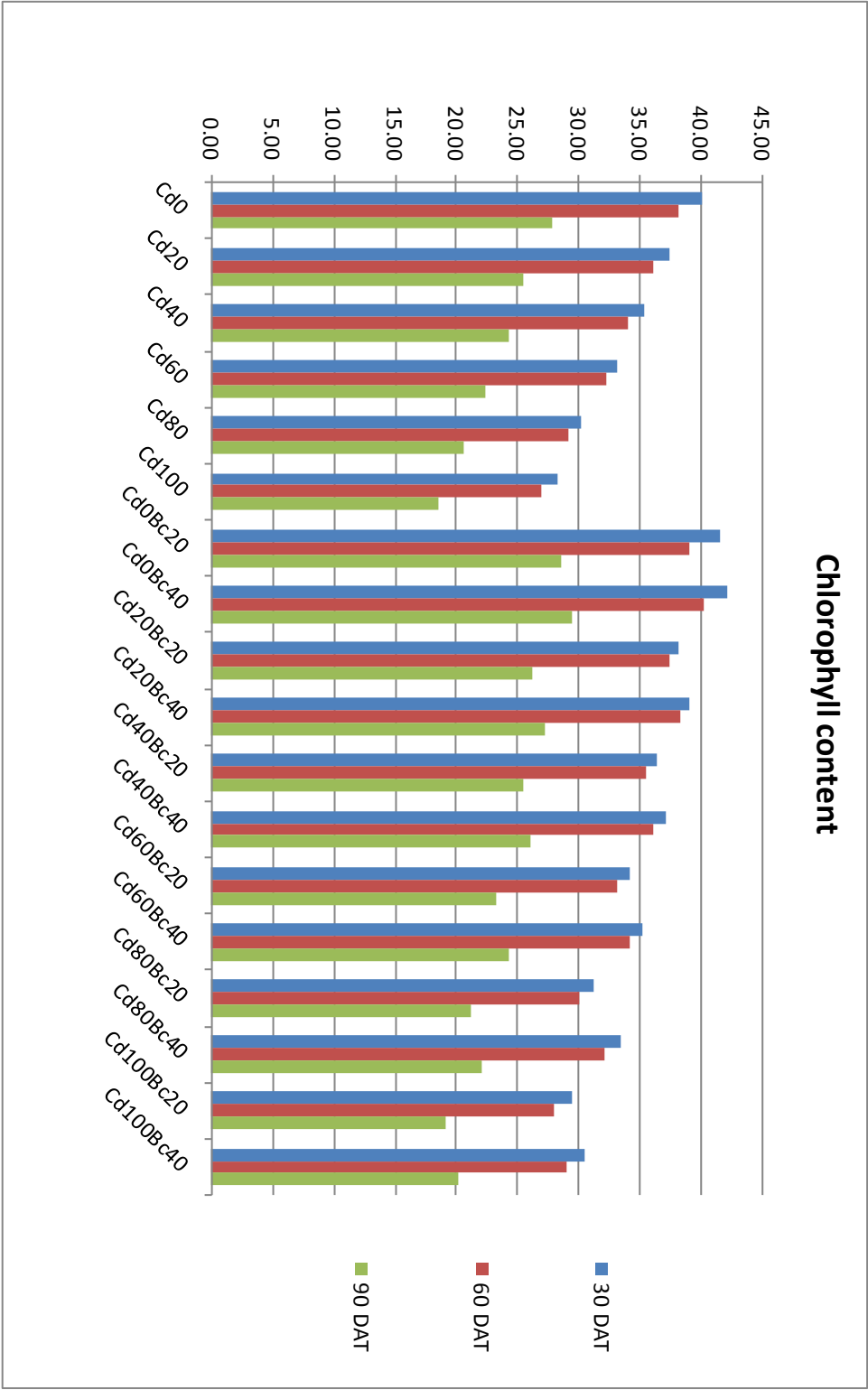


Fig. 4.2.1: Effect of biochar on chlorophyll content at 30, 60, and 90 DAT of rice crop in cadmium contaminated soil

4.3 Effect of biochar on yield attributes of rice crop in cadmium contaminated soil

4.3.1 Panicle length (cm)

The length of the panicle is basically influenced by cadmium concentration along with the addition of biochar. Data collected regarding the length of panicle which is presented in table 4.3. revealed that plant has considerable variations and can survive with an increased in the levels of cadmium but resulted in shorter panicle length over control treatment. It is evident from the results that panicle length varied from 23.10 to 16.2cm among different level of cadmium concentration. Maximum panicle length 24.9cm was found associated with the treatment (Cd0+Bc40) which was significantly higher due to the application of biochar followed by (Cd0+Bc20) i.e 23.7cm. Cadmium levels and biochar with respect to the length of panicle and weight of panicle was found significant at every stage of rice crop.

4.3.2 Number of grains panicle⁻¹ or (grain hill⁻¹)

There was a significant effect reflected on number of grains per panicle with different level of cadmium application presented in table no: 4.3 and depicted in fig no: 4.3. The number of grains per panicle varied from 95 to 132 grain hill⁻¹ in case of different levels of cadmium. The highest total number of grains panicle⁻¹ was observed in treatment (Cd0+ Bc40) i.e 142 followed by treatment (Cd0+Bc20) i.e. 136. Result revealed that there was a decreased in grains per panicle as the concentration of cadmium increased. It was reported that cadmium could transfer from root of rice crop to grains by the mechanism of translocation and decreases grain yield, quality, and nutrient uptake by rice crop (Rodda *et al.* 2011).

4.3.2 1000 grains weight (g) or test weight

Cadmium levels and application of biochar may influence weight of rice grain at reproductive stage that has been presented in table 4.3 and depicted in fig no: 4.3. Data revealed that 1000 grains weight varies from 25.0g to 20g in treatment (Cd100). Data reflected that, increased in cadmium content decreasesd test weight recorded in rice crop. Application of biochar can significantly influenced test weight of rice grain.

Maximum 1000 grains weight was recorded 26.6g in treatment (Cd0+Bc40) followed by treatment (Cd0+Bc20) i.e 25.2g respectively..

These yield attributes like panicle number, grains panicle⁻¹ test weight, increased with the addition of biochar compared to control treatment. Biochar significantly increased grain yield in case of rice and grain yield sustained over 3 years after using a single dose of soil amendment like wheat straw or biochar (Khan *et al* 2013). Improving growth coupled with the transport of photosynthates towards reproductive structure might have increased yield and yield attributes due to addition of biochar (Manikandan and Sriram Chandrasekharan, 2009). The results indicated that cadmium affected yield attributes in rice crop even though it was reported as a hyper accumulator and high tolerant crop for heavy metals.

Table 4.3: Effect of biochar on yield attributes of rice crop in cadmium contaminated soil

Yield attributes			
Treatments	(Total grain hill ⁻¹)	(Unfilled grain hill ⁻¹)	Panicle length (cm)
Cd0	132	9.00	23.1
Cd20	124	12.0	20.6
Cd40	117	13.0	18.3
Cd60	109	15.0	17.9
Cd80	105	17.0	17.0
Cd100	95.0	19.0	16.2
Cd0Bc20	136	8.00	23.7
Cd0Bc40	142	7.00	24.9
Cd20Bc20	126	11.0	20.9
Cd20Bc40	130	9.00	22.0
Cd40Bc20	125	12.0	20.4
Cd40Bc40	132	10.0	21.1
Cd60Bc20	115	14.0	18.6
Cd60Bc40	120	12.0	20.0
Cd80Bc20	108	16.0	17.8
Cd80Bc40	114	14.0	18.9
Cd100Bc20	98.0	18.0	17.4
Cd100Bc40	102	16.0	17.9
CD (0.05)	8.41	2.61	1.76
SEm±	2.93	0.91	0.61
S/NS	S	S	S

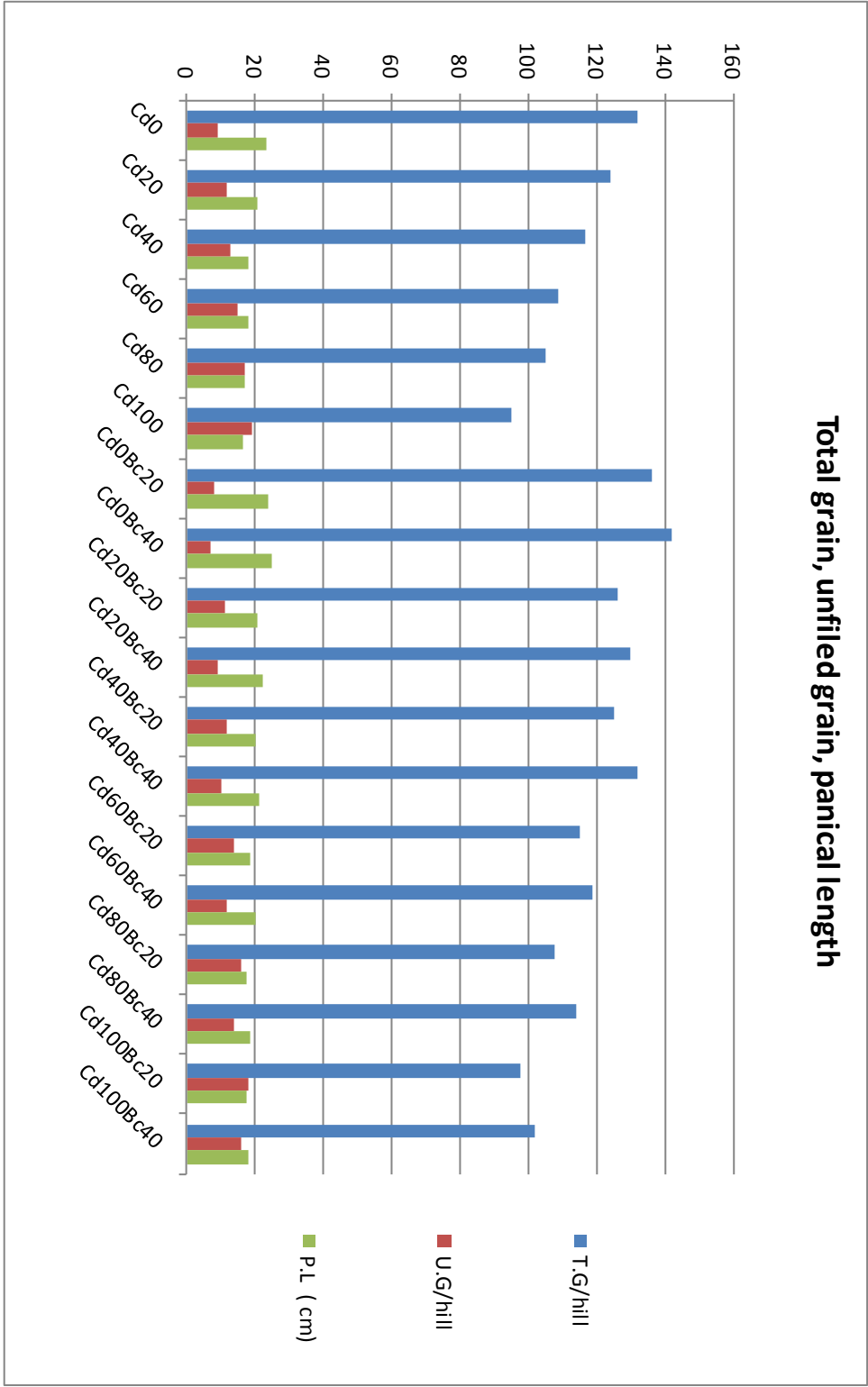


Fig. 4.3: Effect of biochar on yield attributes of rice crop in cadmium contaminated soil

4.3.5 Grain yield and straw yield

The yield of rice grain and straw has been depicted in table 4.3.4 and depicted in fig no: 4.3.4. Cadmium directly has an adverse effect on rice yield but it can be mitigated by using an adequate amount of biochar. The lowest value of rice grain yield was 29.2 g pot⁻¹ in the treatment (Cd100) with a reduction in the yield over 30% compared to the treatment of (Cd0+Bc40) was reported highest yield i.e 45.7g. Similar results were also reported in case of the straw yield of rice because it translocated in grain through the shoot. The highest reduction in straw yield due to the direct effect of cadmium concentration observed in treatment (Cd100) having yield 40.2g pot⁻¹.

The beneficial effect of cadmium was seen at treatment (Cd20ppm) in rice crop which could be attributed to increased absorption of nutrients like K, Ca and Mg, while at very higher cadmium concentration it might have adversely affected yield attributes due to phytotoxicity (Parmar and Patel 2015).

The straw yield of rice was enhanced due to the direct effect of biochar over control treatment which is shown in table 4.3.4. Highest straw yield was reported in treatment (Cd0+Bc40) i.e 50g followed by (Cd0+Bc20) i.e 48.1g respectively. The improvement in yield of rice could be attributed due to the addition of biochar to the soil, which increased the availability of nutrients due to enhancement in important soil properties (Ganal and Singh 1988). The adverse effect of cadmium on the yield of rice could be alleviated to some extent especially with biochar addition.

The role of biochar is to reduce the toxicity of cadmium by improving the efficacy of nutrient uptake which leads improvement in yield of rice crop that could be attributed due to the reduction in the bioavailability of cadmium and thus reduces toxic effects of Cadmium in the soil. Bioavailability of heavy metal in the soil is said to be the fraction of the total metal in the interstitial pure water that is available to the receptor organism. More specifically, it refers to the biologically available fraction or pool that can be taken up by an organism and can react with its metabolic machinery. By immobilization of this fraction of metals reduced its bioavailability.

This could be achieved by the addition of biochar which increases the immobilization of metal through adsorption reactions, and formation of organo-cadmium complexes. The grain and straw yield has been drastically reduced due to the toxic effect of cadmium on the roots and shoots of rice crop. The characteristics of rice crop vary under various treatments of cadmium. High organic matter, macro and micronutrients enhanced soil physical, chemical, and biological properties. The decrease in biomass or productivity of rice crop was due to a disruption in nitrogen metabolism of seedlings under cadmium stress condition (Chatterjee, and Chatterjee 2000).

Table 4.3.4: Effect of biochar on yield of rice crop in cadmium contaminated soil

Yield attributes			
Treatments	Test weight in (g)	Straw yield (g) pot ⁻¹	Grain yield (g) pot ⁻¹
Cd0	25.0	50.0	45.7
Cd20	24.0	48.1	42.1
Cd40	23.0	46.8	39.1
Cd60	22.0	44.5	36.1
Cd80	21.0	42.3	33.2
Cd100	20.0	40.2	29.2
Cd0Bc20	25.2	50.3	46.2
Cd0Bc40	26.6	50.4	47.0
Cd20Bc20	25.0	48.4	42.7
Cd20Bc40	25.8	48.9	43.3
Cd40Bc20	24.0	47.1	39.8
Cd40Bc40	25.2	47.4	40.3
Cd60Bc20	22.8	45.0	36.8
Cd60Bc40	23.2	45.4	37.2
Cd80Bc20	22.1	42.6	33.9
Cd80Bc40	22.3	42.8	34.1
Cd100Bc20	21.8	40.6	29.8
Cd100Bc40	21.9	40.9	30.2
CD (0.05)	2.13	1.18	0.43
SEm±	0.74	0.41	0.15
S/NS	S	S	S

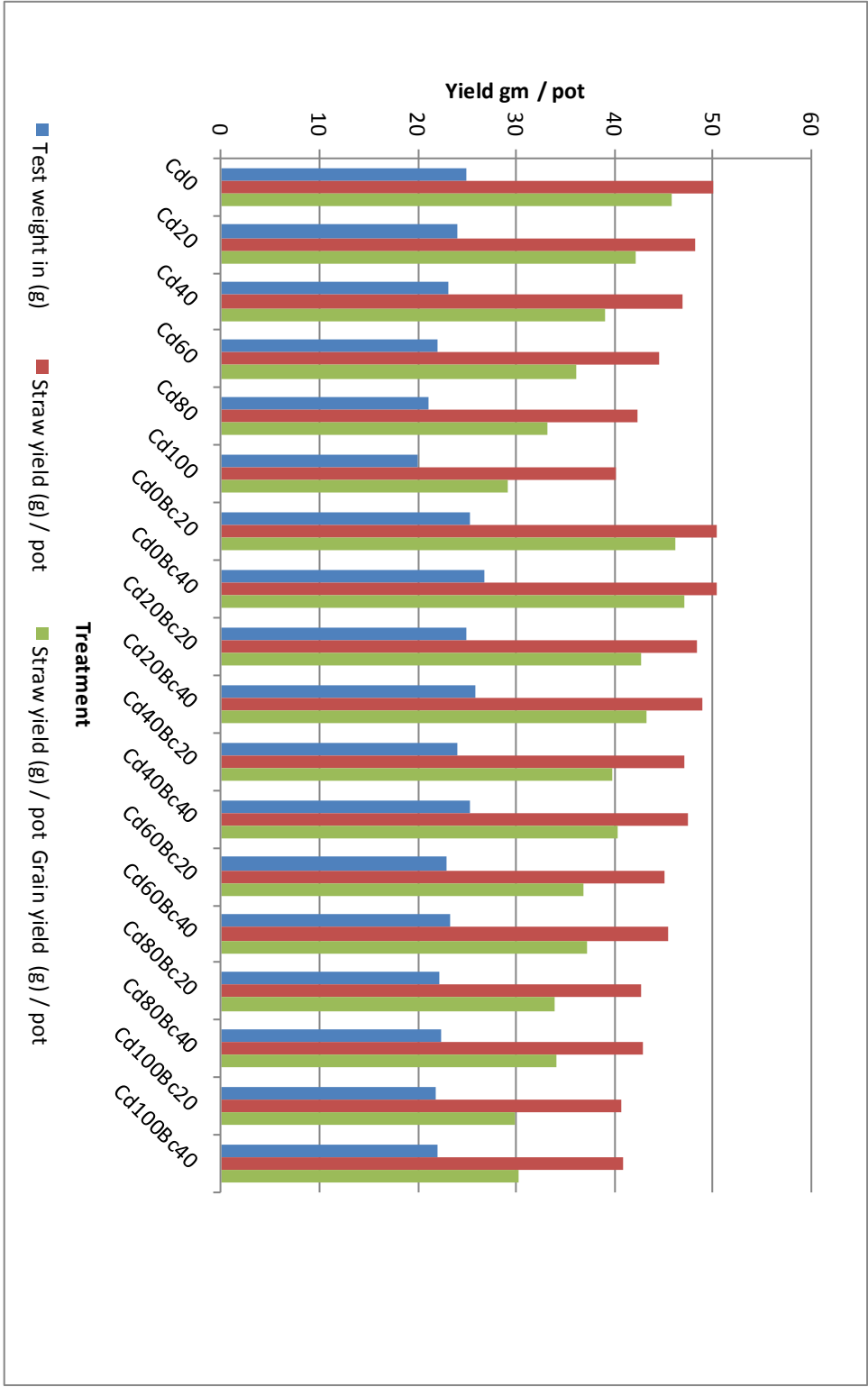


Fig. 4.3.4: Effect of biochar on yield attributes of rice crop in cadmium contaminated soil

4.3.7 Harvest index (H.I %)

Harvesting index of rice was affected by different concentration of cadmium and biochar applied in experimental soil that has been presented in table 4.3.7 and depicted in fig 4.3.7. Data regarding harvesting index revealed that (H.I) varied from 48.2 % to 42.01%. However, the highest harvest index recorded in treatment (Cd0+Bc40) i.e 48.2 followed by the treatment (Cd0+bc20) i.e 47.2 respectively and lowest harvesting index reported in treatment Cd100 i.e 42.01 percent. Biochar significantly influenced the yield of rice and hence interaction effect of biochar regarding to harvest index was found significant.

Table 4.3.7: Effect of biochar on harvesting index of rice crop in cadmium contaminated soil

Treatments	Harvesting index (H.I %)
Cd0	47.7
Cd20	46.6
Cd40	45.5
Cd60	44.7
Cd80	43.9
Cd100	42.0
Cd0Bc20	47.8
Cd0Bc40	48.2
Cd20Bc20	46.8
Cd20Bc40	46.9
Cd40Bc20	45.8
Cd40Bc40	45.9
Cd60Bc20	44.9
Cd60Bc40	45.0
Cd80Bc20	44.3
Cd80Bc40	44.3
Cd100Bc20	42.3
Cd100Bc40	42.4
CD (0.05)	1.73
SEm $\pm$	0.60
S/NS	S

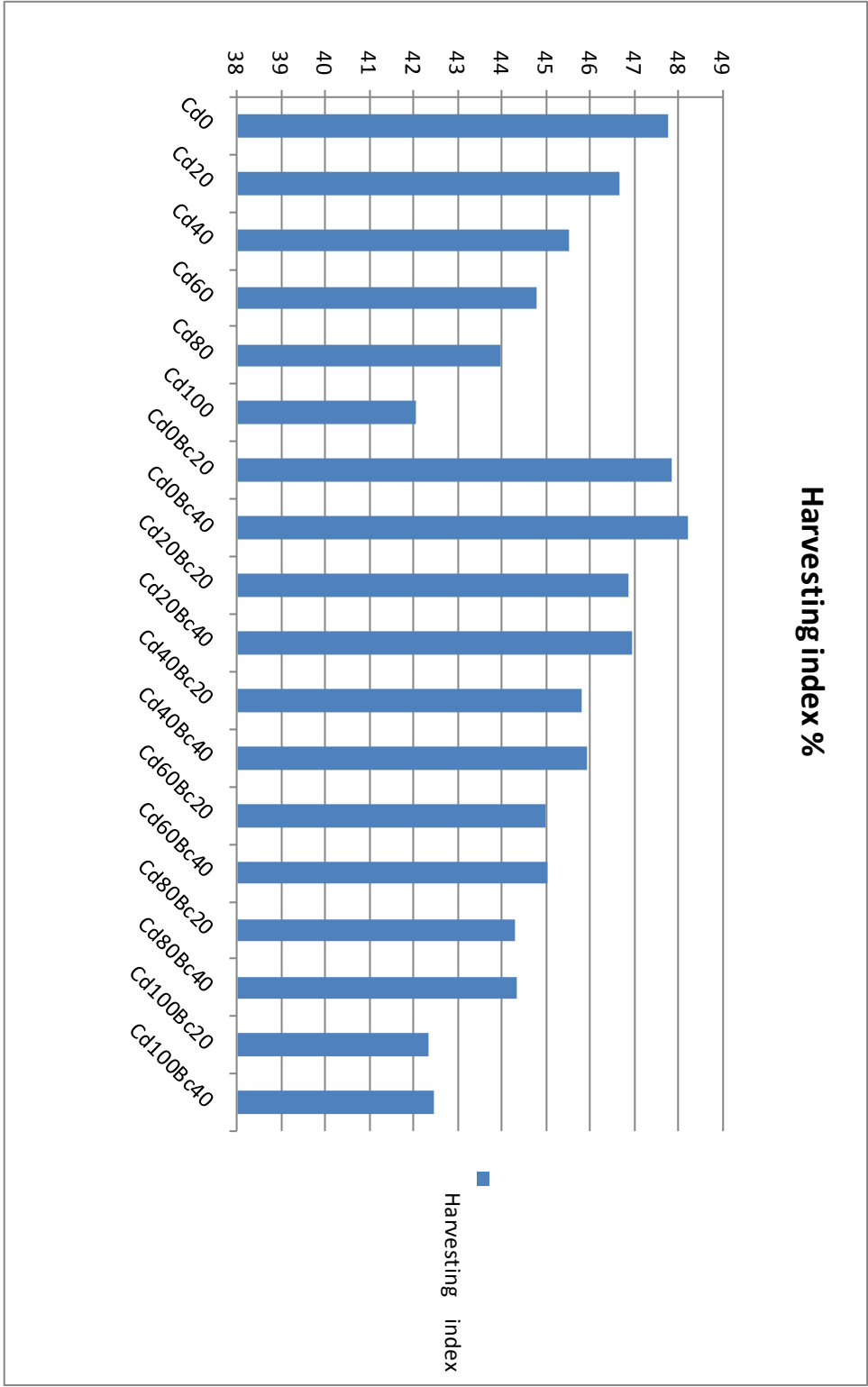


Fig. 4.3.7: Effect of biochar on harvesting index of rice in cadmium contaminated soil

Data pertaining to analyses of N, P and K contents in grain and straw and their uptake affected by cadmium levels and biochar have been presented in table no: 4.3.8, 4.3.9 and 4.3.10 below respectively.

4.3.8 Nitrogen content in grain and straw (%) and its total uptake

The data regarding nitrogen content in grain and straw has been shown in table 4.3.8 and depicted in fig 4.3.8. Data revealed that nitrogen content in grain was 1.40% compared to straw 0.61%. The lowest nitrogen uptake in grain was reported in treatment (Cd100) i.e. 0.98% while in straw it was reported 0.38%. Nitrogen content in grain ranged from 0.98% to 1.4% while N content in straw ranged from 0.38% to 0.61%. Application of cadmium decreased N content upto 100ppm biochar application increased the nitrogen content in cadmium contaminated soil and mitigated toxic effect of cadmium.

4.3.9 Phosphorus content in grain and straw (%) and its total uptake

Phosphorus content in grain and straw is affected by cadmium concentrations and biochar application that has been depicted in table no: 4.3.9 and depicted in fig 4.3.9. Data collected revealed that minimum phosphorus content was in grain and straw was found under treatment (Cd100). Generally phosphorus content found more in grain compared to straw. The reduction in the nutrient content may be due to the inhibition of enzymes involved in the synthetic process.

As the use of biochar along with the control pot i.e (Cd100+Bc40) recorded the highest phosphorus content in grain and straw, i.e 0.39% and 0.19% respectively and resulted highest P uptake in grain and straw through mass flow and diffusion proved significantly higher over rest of other treatment. However the lowest phosphorus content in both grain and straw and their uptake was recorded in treatment (Cd100ppm).

4.3.10 Potassium content in grain and straw (%) and its total uptake

Potassium content in grain and straw was also affected by different level of cadmium concentration as we observed that potassium uptake was more in straw than grain with respect to nitrogen, and phosphorus has been presented in table 4.3.10 and depicted in fig 4.3.10. Data revealed that maximum potassium uptake in grain was

0.55% and it was recorded 1.6% in straw under the treatment (Cd0+Bc40) followed by (cd0+Bc20) i.e 0.5% and 1.4% respectively over rest of other treatments.

Application of biochar in control pot recorded the highest potassium content in grain and straw & the highest amount of potassium uptake as compared to other treatments. Potassium content in grain ranged from 0.36% to 0.58% while in straw it varied from 1.16% to 1.63%. Application of cadmium lowered the K uptake in grain and straw but the application of biochar mitigated the ill effect of cadmium application in soil.

Table 4.3.8: Effect of biochar on nitrogen uptake by rice crop in cadmium contaminated soil

Treatments	N% uptake in rice grain	N% uptake in rice straw	N uptake in grain (g pot⁻¹)	N uptake in straw (g pot⁻¹)	Total N, uptake by grain + straw(g pot⁻¹)
Cd0	1.350	0.580	61.790	29.000	90.790
Cd20	1.320	0.563	55.571	27.099	82.670
Cd40	1.250	0.540	48.878	25.271	74.150
Cd60	1.170	0.490	42.239	21.808	64.049
Cd80	1.050	0.430	34.860	18.190	53.050
Cd100	0.980	0.380	28.619	21.945	50.561
Cd0Bc20	1.380	0.593	63.759	29.842	93.600
Cd0Bc40	1.400	0.617	65.800	31.110	96.910
Cd20Bc20	1.340	0.580	57.219	28.071	85.290
Cd20Bc40	1.360	0.600	58.889	29.340	88.230
Cd40Bc20	1.280	0.560	50.941	26.379	77.320
Cd40Bc40	1.300	0.580	52.390	27.491	79.880
Cd60Bc20	1.220	0.517	44.899	23.250	68.150
Cd60Bc40	1.253	0.543	46.621	24.669	71.290
Cd80Bc20	1.100	0.453	37.290	19.311	56.600
Cd80Bc40	1.127	0.470	38.420	20.119	58.539
Cd100Bc20	1.020	0.387	30.399	15.700	46.099
Cd100Bc40	1.080	0.410	32.619	16.770	49.389
CD (0.05)	0.088	0.043	1.706	2.657	1.866
SEm±	0.031	0.015	0.595	0.926	0.651
S/NS	S	S	S	S	S

Table 4.3.9: Effect of biochar on Phosphorus uptake in different part of rice crop in cadmium contaminated soil

Treatments	Phosphorus % uptake in rice grain	Phosphorus % uptake in rice straw	Phosphorus uptake in grain (g pot ⁻¹)	Phosphorus uptake in straw (g pot ⁻¹)	Total P ₂ O ₅ uptake by grain + straw (g pot ⁻¹)
Cd0	0.360	0.190	16.479	9.503	25.981
Cd20	0.320	0.180	13.471	8.659	22.130
Cd40	0.290	0.170	11.340	7.959	19.299
Cd60	0.270	0.140	9.749	6.230	15.980
Cd80	0.230	0.120	7.639	5.079	12.719
Cd100	0.190	0.110	5.549	4.421	9.970
Cd0Bc20	0.370	0.193	17.091	10.060	27.150
Cd0Bc40	0.390	0.201	18.330	10.592	28.921
Cd20Bc20	0.340	0.174	14.519	9.199	23.719
Cd20Bc40	0.360	0.176	15.589	9.780	25.370
Cd40Bc20	0.320	0.163	12.739	8.479	21.219
Cd40Bc40	0.340	0.168	13.701	9.009	22.710
Cd60Bc20	0.290	0.144	10.671	6.750	17.420
Cd60Bc40	0.310	0.149	11.531	7.261	18.791
Cd80Bc20	0.250	0.121	8.478	5.539	14.019
Cd80Bc40	0.280	0.127	9.549	5.657	15.209
Cd100Bc20	0.210	0.114	6.259	4.871	11.130
Cd100Bc40	0.230	0.119	6.949	5.319	12.269
CD (0.05)	0.057	0.021	1.024	0.616	1.380
SEm±	0.020	0.007	0.357	0.215	0.481
S/NS	S	S	S	S	S

Table 4.3.10: Effect of biochar on Potassium uptake in different part of rice crop in cadmium contaminated soil

Treatments	Potassium % uptake in rice grain	Potassium % uptake in rice straw	Potassium uptake in grain (g pot⁻¹)	Potassium uptake in straw (g pot⁻¹)	Total K₂O Uptake by grain + straw (g pot⁻¹)
Cd0	0.540	1.580	24.719	78.000	102.720
Cd20	0.507	1.430	21.330	68.781	90.110
Cd40	0.480	1.380	18.769	64.581	83.350
Cd60	0.450	1.290	16.248	57.398	73.649
Cd80	0.400	1.220	13.280	51.609	64.890
Cd100	0.360	1.160	10.511	46.631	57.140
Cd0Bc20	0.560	1.600	25.871	80.480	106.350
Cd0Bc40	0.580	1.630	27.260	82.565	109.822
Cd20Bc20	0.530	1.460	22.630	70.660	93.290
Cd20Bc40	0.550	1.480	23.818	72.371	96.190
Cd40Bc20	0.507	1.400	20.168	65.940	86.109
Cd40Bc40	0.530	1.440	21.360	68.259	89.619
Cd60Bc20	0.480	1.320	17.661	58.400	76.060
Cd60Bc40	0.520	1.350	19.341	61.290	80.630
Cd80Bc20	0.420	1.250	14.239	53.250	67.490
Cd80Bc40	0.450	1.280	15.348	54.781	70.130
Cd100Bc20	0.380	1.180	11.321	47.909	59.230
Cd100Bc40	0.400	1.210	12.080	49.490	61.570
CD (0.05)	0.062	0.056	1.197	1.837	1.585
SEm±	0.022	0.020	0.417	0.640	0.553
S/NS	S	S	S	S	S

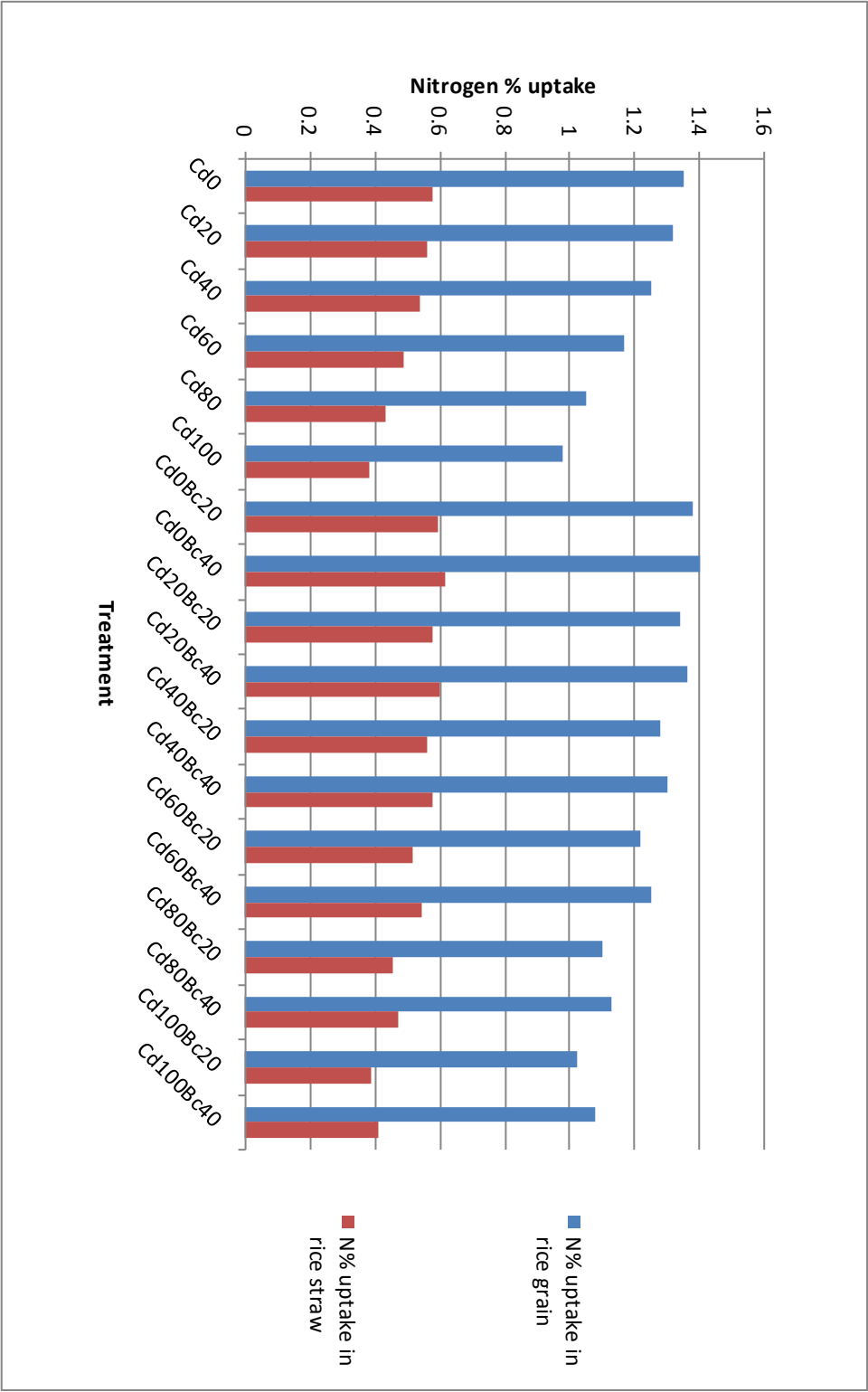


Fig. 4.3.8: Effect of biochar on nitrogen uptake by rice crop in cadmium contaminated soil

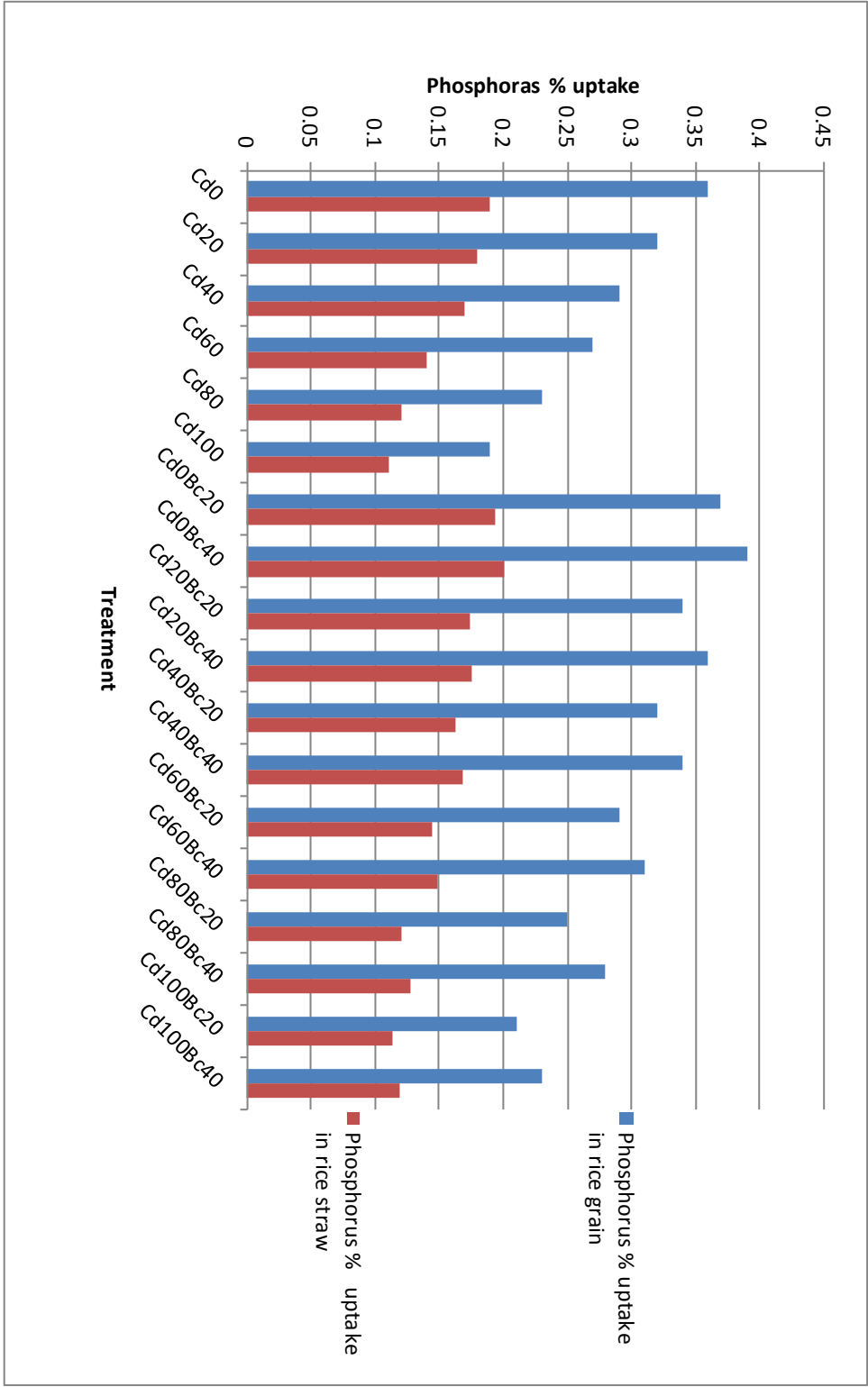


Fig. 4.3.9: Effect of biochar on Phosphorus uptake in different part of rice crop in cadmium contaminated soil

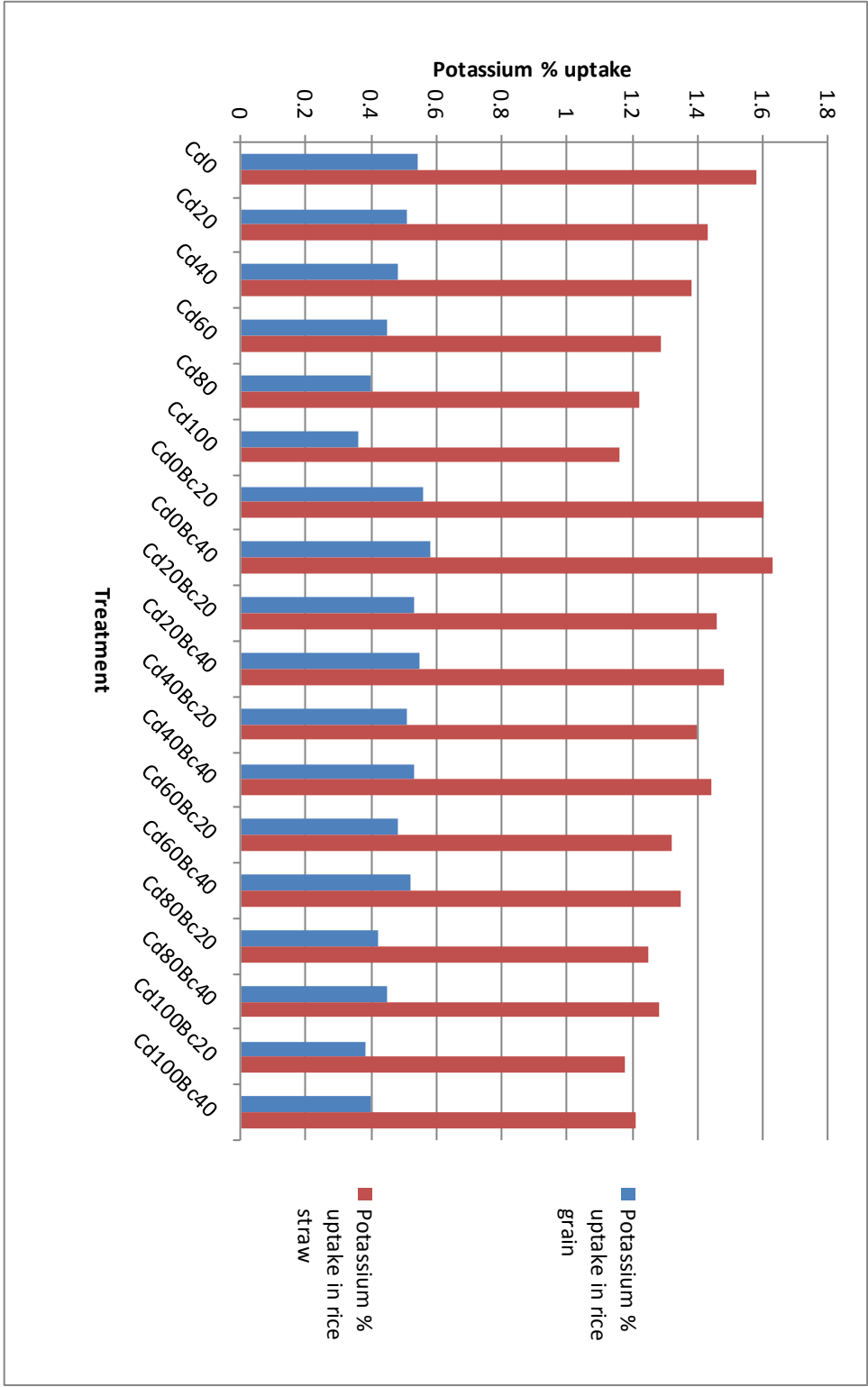


Fig. 4.3.10: Effect of biochar on Potassium uptake in different part of rice crop in cadmium contaminated soil

4.4 Effect of biochar on chemical properties of post-harvest soil

4.4.1 Soil pH

The results indicated that the initial pH of the soil was (7.1). The data pertaining to the pH of the post-harvest soil presented in table 4.4.1 showed that pH varied from 7.1 to 8.4. Maximum pH 8.4 was observed with treatment (Cd0+Bc40) while minimum pH 7.1 was observed in treatment (Cd0). Application of biochar in cadmium contaminated soil showed a slight increase in the pH value of the soil. Soil pH is the most important factor responsible for affecting metal availability. High pH under biochar soil increased adsorption sites by increasing negative charges on the soil surface and reducing competing for metal cations (Alamgir *et al.* 2011). Availability of all metals was significantly higher in non amended soil due to lower soil pH. With the increase in soil pH, the bioavailability of cadmium decreased (Kim *et al.* 2009).

The application of biochar resulted increase in pH of soil which determines the immobilization or mobilization of heavy metals. The pH levels were expected to rise with the addition of biochar due to wider C: N ratio, carbon sequestration and forming complexation by soil colloids. Initial soil samples did not show a significant difference in pH but at the time of harvest, pH was significantly higher due to the application of biochar in the soil as compared to the control treatment. Significant increase in pH of soil showed a significant relevance exist with the application of biochar in soil.

4.4.2 EC

Data regarding EC of soil has been presented in table 4.4.1. It is evident from table that EC of the soil ranged from 0.31 to 0.48 dsm⁻¹. Highest EC was recorded in biochar amended soil. The EC of the post harvest soil increased significantly with the application of biochar. The interaction effect of EC and biochar was found significant. Metal content in the soil is directly associated with plant uptake, which is indirectly associated with physiochemical characteristics of soil and plant species (Tariq and Rashid 2013). The EC of the soil increased when biochar levels in soil increased up to

a maximum value of 0.48 dS m^{-1} which was approximately 30% higher compared to the control treatment.

4.4.3 Organic Carbon

Data regarding organic carbon (%) of post harvest soil presented in table 4.4.1 and depicted in fig 4.4.1. The highest OC% recorded with the treatment (Cd0+Bc40) i.e (0.48%) followed by (Cd+bc20) i.e (0.45%) At the time of harvest, organic carbon increased about 25% in biochar amended soil as compared to the control treatment (Cd0). With the increased in cadmium concentration organic carbon decreased. The minimum organic carbon found in treatment (Cd100). Upon addition of biochar soil biological activity consequently increases the organic carbon content in the soil. The application of biochar resulted in (Cd) reduction either from an enhancement of the microbial population or due to microbial phytoremediation mechanism.

Table 4.4.1: Effect of biochar on soil pH, EC and OC% in cadmium contaminated soil

Soil parameters			
Treatments	pH	EC(dS m ⁻¹)	OC%
Cd0	7.100	0.310	0.380
Cd20	7.300	0.340	0.360
Cd40	7.600	0.370	0.350
Cd60	7.620	0.400	0.320
Cd80	7.830	0.420	0.293
Cd100	7.900	0.450	0.240
Cd0Bc20	7.810	0.330	0.450
Cd0Bc40	8.430	0.350	0.480
Cd20Bc20	8.000	0.350	0.410
Cd20Bc40	8.140	0.370	0.450
Cd40Bc20	8.000	0.390	0.390
Cd40Bc40	8.130	0.400	0.440
Cd60Bc20	8.020	0.410	0.380
Cd60Bc40	8.100	0.430	0.430
Cd80Bc20	8.010	0.437	0.350
Cd80Bc40	8.060	0.450	0.370
Cd100Bc20	8.000	0.470	0.300
Cd100Bc40	8.070	0.480	0.330
CD (0.05)	0.343	0.041	0.035
SEm±	0.119	0.014	0.012
S/NS	S	S	S

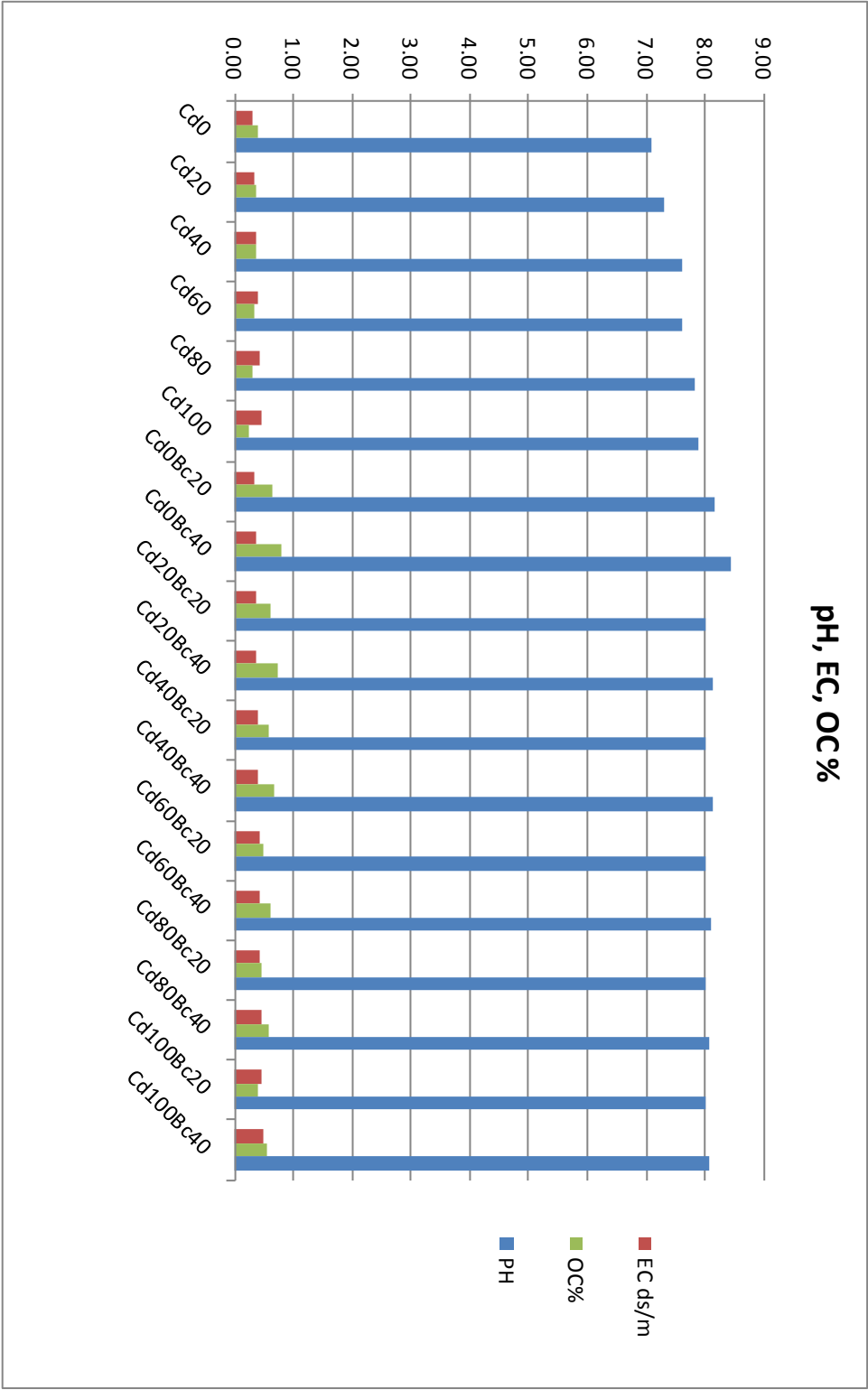


Fig. 4.4.1: Effect of biochar on soil pH, EC and OC% in cadmium contaminated soil

4.4.4 Available N (kg ha^{-1})

The data regarding available N in soil was given in table 4.4.4. The available N were recorded highest with the treatment (Cd20+Bc40) i.e $206.33 \text{ kg ha}^{-1}$ followed by (Cd20+Bc20) i.e $206.13 \text{ kg ha}^{-1}$ respectively while minimum amount of available nitrogen recorded in treatment (Cd100) i.e 194 kg ha^{-1} . At the time of harvest, available N increased due to the application of biochar. The interaction of biochar and cadmium were found significant with respect to available nitrogen in soil. The available nitrogen in the soil reported lower than normal range. Upon increasing cadmium concentration at first N content increases upto certain concentration but then upon the higher concentration of cadmium addition, the nitrogen content further decreased. The addition of biochar not only ensured plant growth by supplying adequate amount of nutrients but also mitigated the undesirable toxic effect of cadmium upto a certain limits.

4.4.5 Available P (kg ha^{-1})

The data of total available phosphorus presented in table 4.4.4 and depicted in fig no: 4.4.4. Data regarding available (P) in post harvest soil was found in the medium range. A significant increased was recorded in available phosphorus with the application of biochar and it ranged from 18.7 to 24.1 kg ha^{-1} .

4.4.6 Available K (kg ha^{-1})

The data regarding available K content in soil was given in table 4.4.4. Highest available K in soil was recorded with the treatment (Cd0+bc40) i.e 222.3 kg ha^{-1} . Interaction effect between biochar and available potassium were found significant. Post harvest soil had medium range of available K_2O . Upon addition of biochar, there was an increase in soil available (K) content. But with an increase in cadmium concentration, the available (K) decreased. Application of biochar increased the available K content in all treatment.

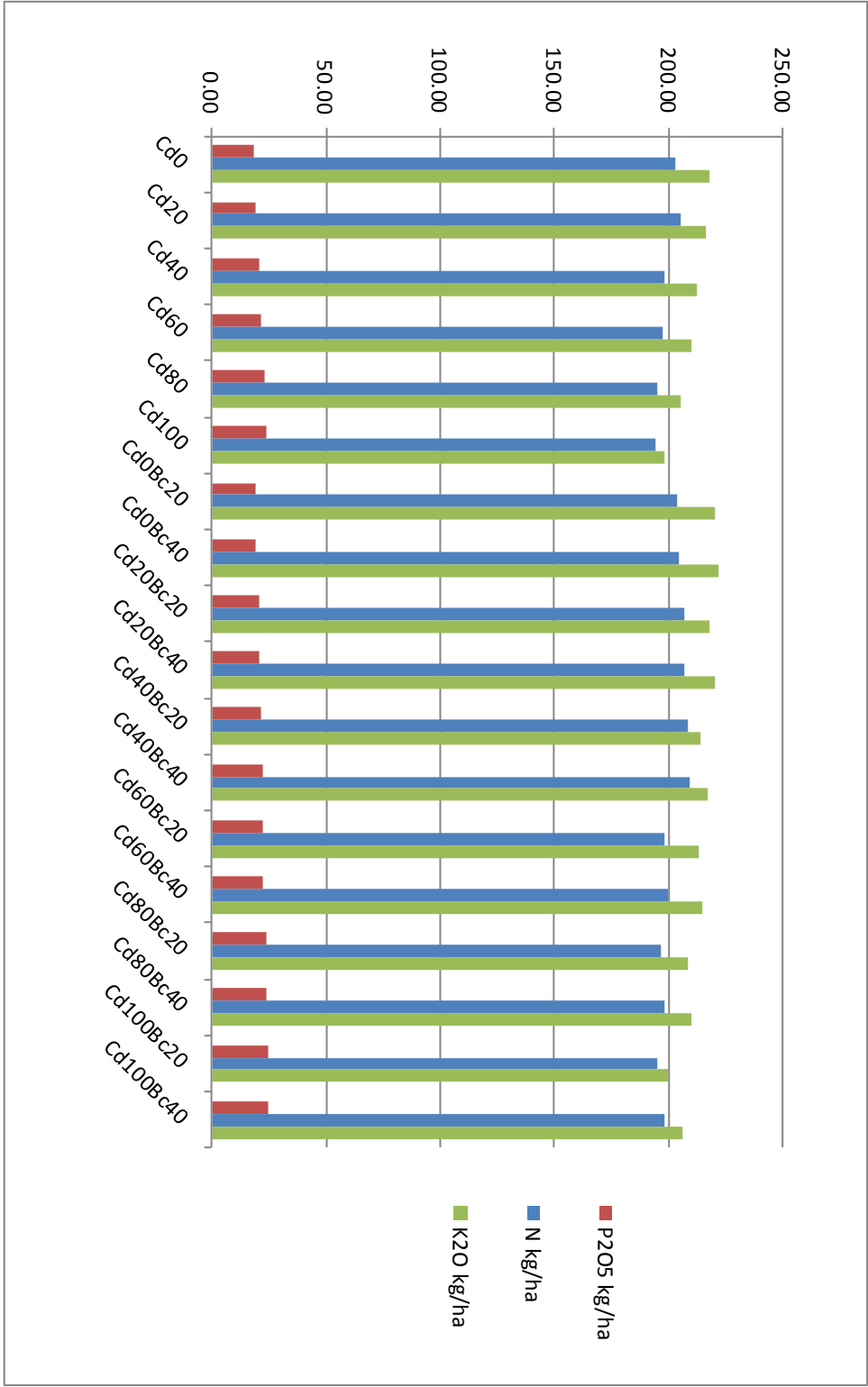


Fig. 4.4.4: Effect of biochar on available N, P₂O₅, and K₂O in cadmium stress soil

Table 4.4.4: Effect of biochar on available N, P₂O₅, and K₂O in cadmium stress soil

Soil parameters			
Treatments	N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)
Cd0	202	18.7	218
Cd20	205	19.2	216
Cd40	197	20.9	212
Cd60	197	21.9	210
Cd80	195	23.1	205
Cd100	194	24.1	198
Cd0Bc20	203	19.0	220
Cd0Bc40	204	19.4	222
Cd20Bc20	206	20.6	218
Cd20Bc40	206	20.7	220
Cd40Bc20	19	21.9	214
Cd40Bc40	199	22.2	217
Cd60Bc20	197	22.3	213
Cd60Bc40	199	22.4	215
Cd80Bc20	196	23.6	208
Cd80Bc40	198	23.9	210
Cd100Bc20	195	24.5	200
Cd100Bc40	198	24.5	206
CD (0.05)	12.3	1.90	8.76
SEm±	4.31	0.66	3.05
S/NS	S	S	S

4.5 Effect of biochar on cadmium content

4.5.1 Cadmium content in grain and straw of rice crop

Data regarding cadmium content in rice grain and straw is given in table 4.5 and depicted in fig no: 4.5. Cadmium content in rice grain and straw increased significantly with the increase in Cd concentrations level in soil i.e 0,20,40,60,80 and 100 ppm. The maximum Cd content in straw and grain of rice crop was recorded in the treatment (Cd100) i.e $8.9 \mu\text{g g}^{-1}$ and $3.8 \mu\text{g g}^{-1}$ respectively. In general, rice takes up Cd in the form of Cd^{2+} from the soils. Cadmium uptake in rice plants varies with soil pH and organic matter content present in the soils reported by (Zeng *et al.* 2011). The main reason is due to the higher addition of Cd in the soil, which in turn increased in grain and straw via translocation.

The application of biochar significantly reduced the adverse effect and toxicity of cadmium content in different parts of rice crop. Thus the effect of biochar significantly decreased cadmium content in grain and straw of rice crop. Biochar is found to be more effective in decreasing cadmium concentration in grain & straw as compared to other organic amendments might be due to the action of plant root exudates and organic matter which contained various organic acids like fulvic acid and humic acid, which can mitigate the undesirable effect of cadmium from the plant.

These organic acids will reduce the immobilization of an cadmium thus helps in reduction of cadmium concentration from the soil. Cadmium concentration significantly found more in those soil which is not amended with the biochar. The application of biochar in cadmium contaminated soils, result less translocation of cadmium in grain and straw with the corresponding level of root concentration in rice crop. Similarly, the use of biochar could reduce the desorption of metals in soil due to the formation of various insoluble complexes. Several studies revealed that cadmium could transfer to rice grains and decreased grain yield, quality, and nutrient uptake as well noticed by (Arao and Ae 2003).

4.5.2 Effect of biochar on cadmium content in post-harvest soil

Data regarding cadmium accumulation in post harvest soil is given in table 4.5 and depicted in fig 4.5. Result indicated that DTPA-extractable Cd increased with an increase in Cd concentrations in soil. The highest Cd content was recorded in treatment (Cd100ppm) i.e. $40 \mu\text{g g}^{-1}$. This may indicate that the applied Cd may remain in soil for many years and impacted negatively on rice crop. The results reported that DTPA extractable Cd content will change in the soil after the rice harvest. There is a significant decrease in DTPA extractable Cd this is due to the desorption of heavy metals in soil or due to the formation of various insoluble compounds. DTPA-extractable Cd was decreased with the increasing rate of biochar application, similar results reported by (Narwal *et al.* 1992).

The interaction effect between Cd and biochar was found significant data revealed that of maximum concentration of Cd recorded in treatment (Cd100) i.e. $40.2 \mu\text{g g}^{-1}$ while lowest Cd was recorded in control pot (Cd0) i.e. $0.04 \mu\text{g g}^{-1}$. Results showed that the application of Cd alone caused a higher value of DTPA-extractable Cd in soil over other treatment. The addition of biochar with Cd decreased DTPA-extractable Cd as compared to Cd application alone. Root exudates containing organic acids form complexes with Cd making them unavailable for plant uptake.

4.5.3 Accumulation of Cd in rice root

The accumulations of Cd in rice root varied with different level of Cd concentration i.e. 0,25,40,60,80, and 100 ppm. The concentration of Cd was reported highest in treatment (Cd100) in root i.e. $20.9 \mu\text{g g}^{-1}$ as compared to straw $8.9 \mu\text{g g}^{-1}$ respectively shown in table 4.5. The results showed that Cd accumulation was higher in root tissue than in shoot and grain because of the high CEC of roots that can absorb more Cd when come in direct contact with contaminated soil.

Table 4.5: Effect of biochar in cadmium uptake by different part of rice crop in cadmium contaminated soil

Cadmium concentration in soil and plant tissue (ppm)				
Treatments	Cd in soil	Cd in root	Cd in shoot	Cd in grain
Cd0	0.04	0.04	0.02	0.01
Cd20	8.20	3.95	1.80	0.77
Cd40	16.0	7.50	3.20	1.50
Cd60	24.3	11.9	5.10	2.20
Cd80	32.5	15.6	7.12	3.02
Cd100	40.2	20.2	8.90	3.80
Cd0Bc20	0.02	0.02	0.02	0.01
Cd0Bc40	0.01	0.01	0.01	0.01
Cd20Bc20	7.10	3.50	1.49	0.64
Cd20Bc40	6.40	3.00	1.00	0.56
Cd40Bc20	14.9	6.90	2.64	1.30
Cd40Bc40	13.0	5.96	2.13	1.05
Cd60Bc20	23.2	11.0	4.40	1.95
Cd60Bc40	21.4	9.44	3.60	1.66
Cd80Bc20	30.5	14.0	6.02	2.80
Cd80Bc40	28.1	12.2	5.60	2.41
Cd100Bc20	38.7	18.5	7.10	3.20
Cd100Bc40	35.4	17.1	6.32	2.60
CD (0.05)	0.90	0.89	0.46	0.12
SEm±	0.31	0.31	0.16	0.04
S/NS	S	S	S	S

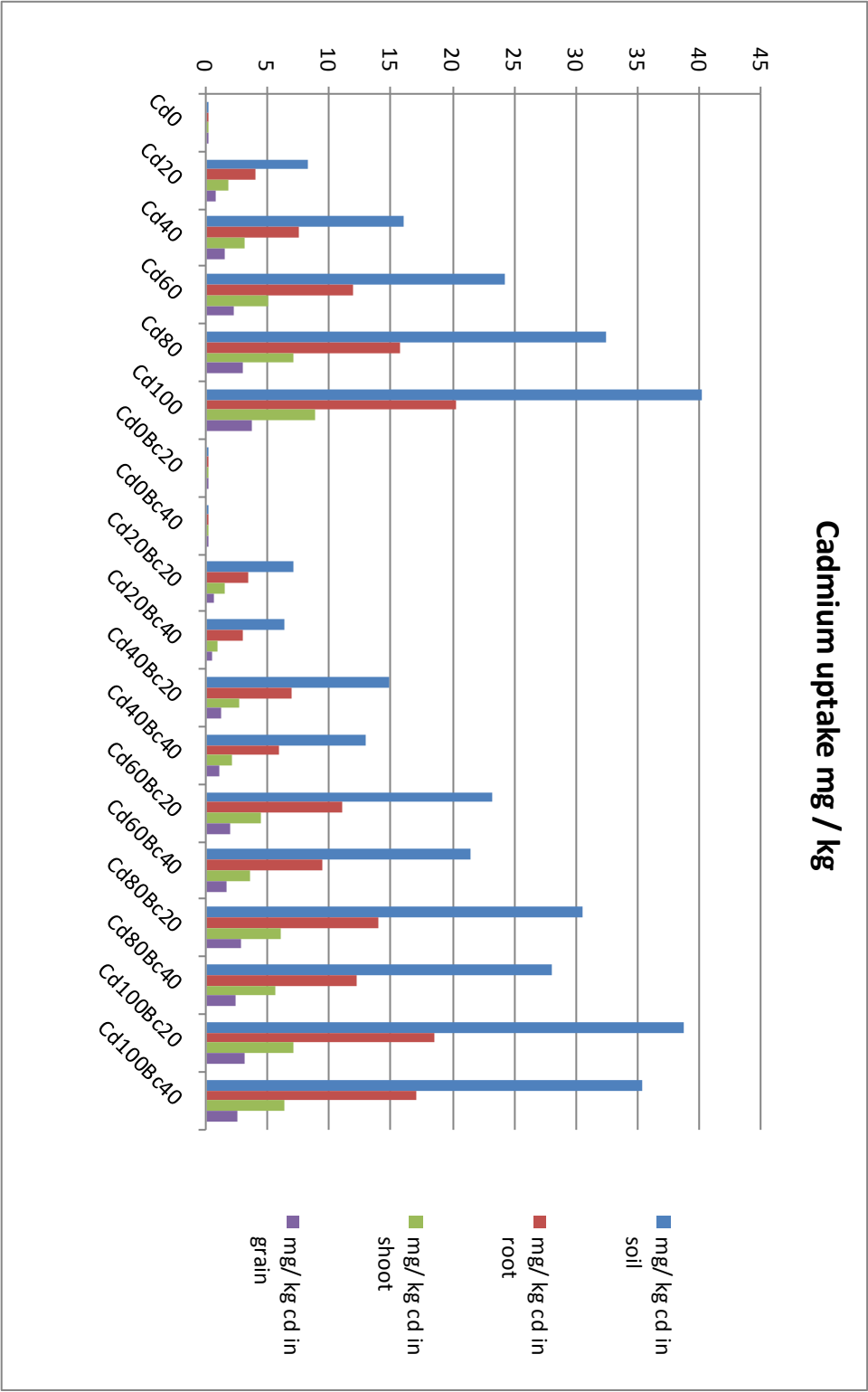


Fig. 4.5: Effect of biochar in cadmium uptake by different part of rice crop in cadmium contaminated soil

4.6 Effect of biochar on protein content in rice grain

Data regarding protein content and yield is shown in table no 4.6 and depicted in fig no 4.6. The highest protein content recorded in treatment (Cd0+Bc40) i.e 8.75 g pot⁻¹ followed by 8.62 g pot⁻¹ in treatment (Cd0+Bc20) respectively, while the lowest protein content was recorded in 6.12 g pot⁻¹ in treatment (Cd100). The interaction between biochar and protein yield found significant in rice crop. As cadmium level increased in soil then protein yield decreased in all pots while biochar application increased the protein yield in rice crop and reduced the toxic effect of cadmium.

$$\text{Protein content} = \% \text{ of nitrogen uptake on rice grain} \times 6.25$$

$$\text{Protein yield} = \text{protein content} \times \text{grain yield}$$

Table 4.6: Effect of biochar on protein content of rice grain in Cd contaminated soil

Quality attributes in rice grain (g pot⁻¹)		
Treatments	Protein content (%)	Protein yield (g pot⁻¹)
Cd0	8.43	386
Cd20	8.25	347
Cd40	7.81	305
Cd60	7.31	263
Cd80	6.56	217
Cd100	6.12	178
Cd0Bc20	8.62	398
Cd0Bc40	8.75	411
Cd20Bc20	8.37	357
Cd20Bc40	8.50	368
Cd40Bc20	8.00	318
Cd40Bc40	8.12	327
Cd60Bc20	7.62	280
Cd60Bc40	7.83	291
Cd80Bc20	6.87	233
Cd80Bc40	7.04	240
Cd100Bc20	6.37	189
Cd100Bc40	6.75	203
CD (0.05)	0.09	4.40
SEm±	0.03	1.53
S/NS	S	S

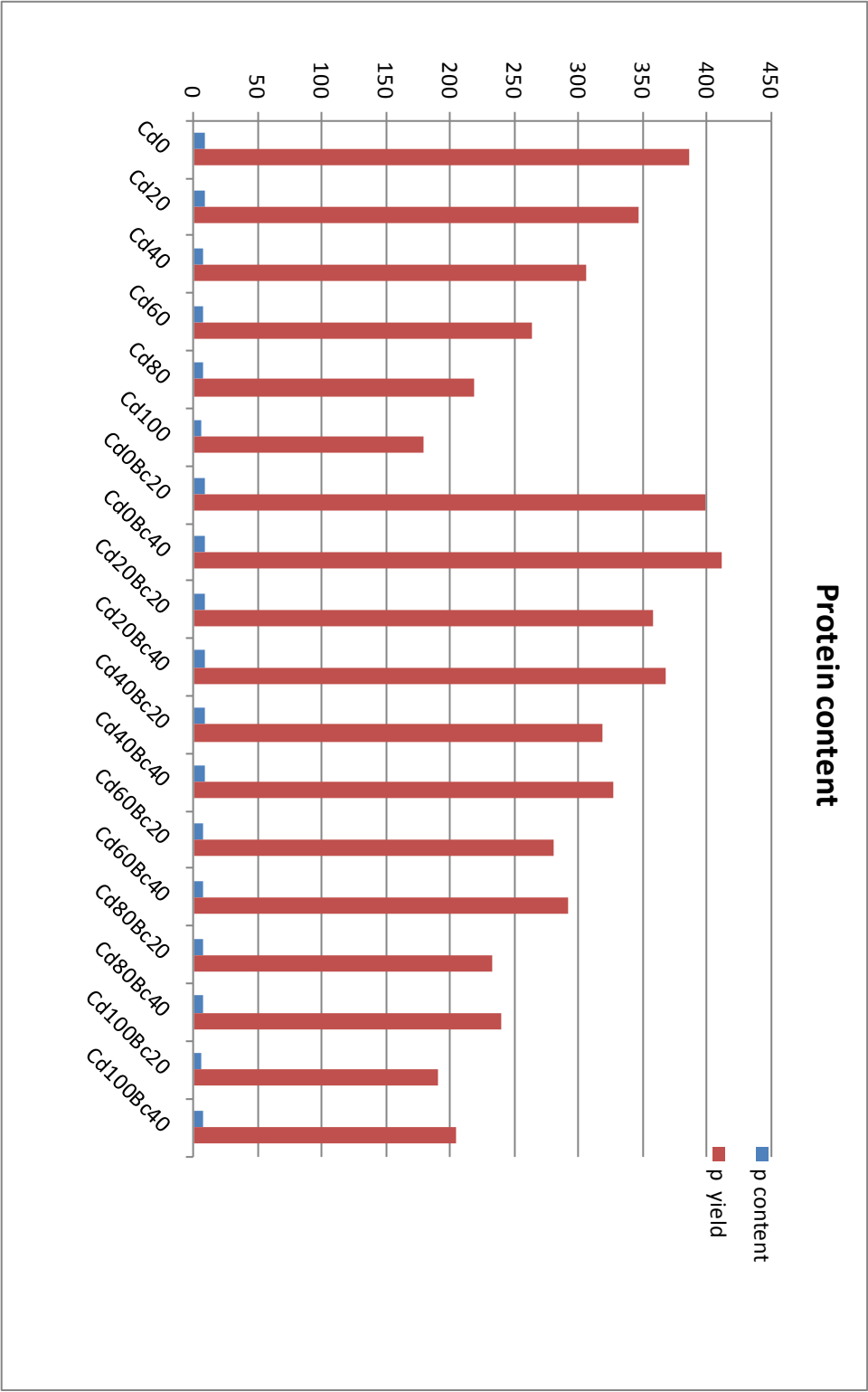


Fig. 4.6: Effect of biochar on protein yield of rice plant in Cd contaminated soil

4.7 Enrichment factor and translocation factor

The mechanism of absorption of heavy metal i.e cadmium by *Oryza sativa* has been analyzed by Enrichment factor and translocation factor analysis. The data regarding enrichment factor from soil to other parts of *Oryza sativa* shown in the table 4.7. Data revealed that enrichment factor was recorded greater than 1 which indicates that rice is a hyper accumulator with high potential to absorb metals from soil reported by (Lorestani *et al.*, 2011) .The values of translocation factor also given in table 4.7 found almost equal to 1 which indicates that *Oryza Sativa* is a hyper-accumulator from roots to shoot portion thus interaction between biochar and E.F and T.F of rice crop was found signifant.

Table 4.7: Effect of biochar on enrichment factor and translocation factor of rice crop in Cd contaminated soil

Translocation and enrichment factor in rice crop		
Treatments	Enrichment factor	Translocation factor
Cd0	1.750	0.500
Cd20	0.798	0.459
Cd40	0.761	0.429
Cd60	0.790	0.430
Cd80	0.792	0.451
Cd100	0.820	0.440
Cd0Bc20	2.500	1.000
Cd0Bc40	3.000	1.000
Cd20Bc20	0.791	0.429
Cd20Bc40	0.711	0.331
Cd40Bc20	0.729	0.381
Cd40Bc40	0.701	0.349
Cd60Bc20	0.750	0.399
Cd60Bc40	0.689	0.380
Cd80Bc20	0.749	0.430
Cd80Bc40	0.720	0.460
Cd100Bc20	0.748	0.381
Cd100Bc40	0.732	0.370
CD (0.05)	0.049	0.035
SEm±	0.017	0.012
S/NS	S	S

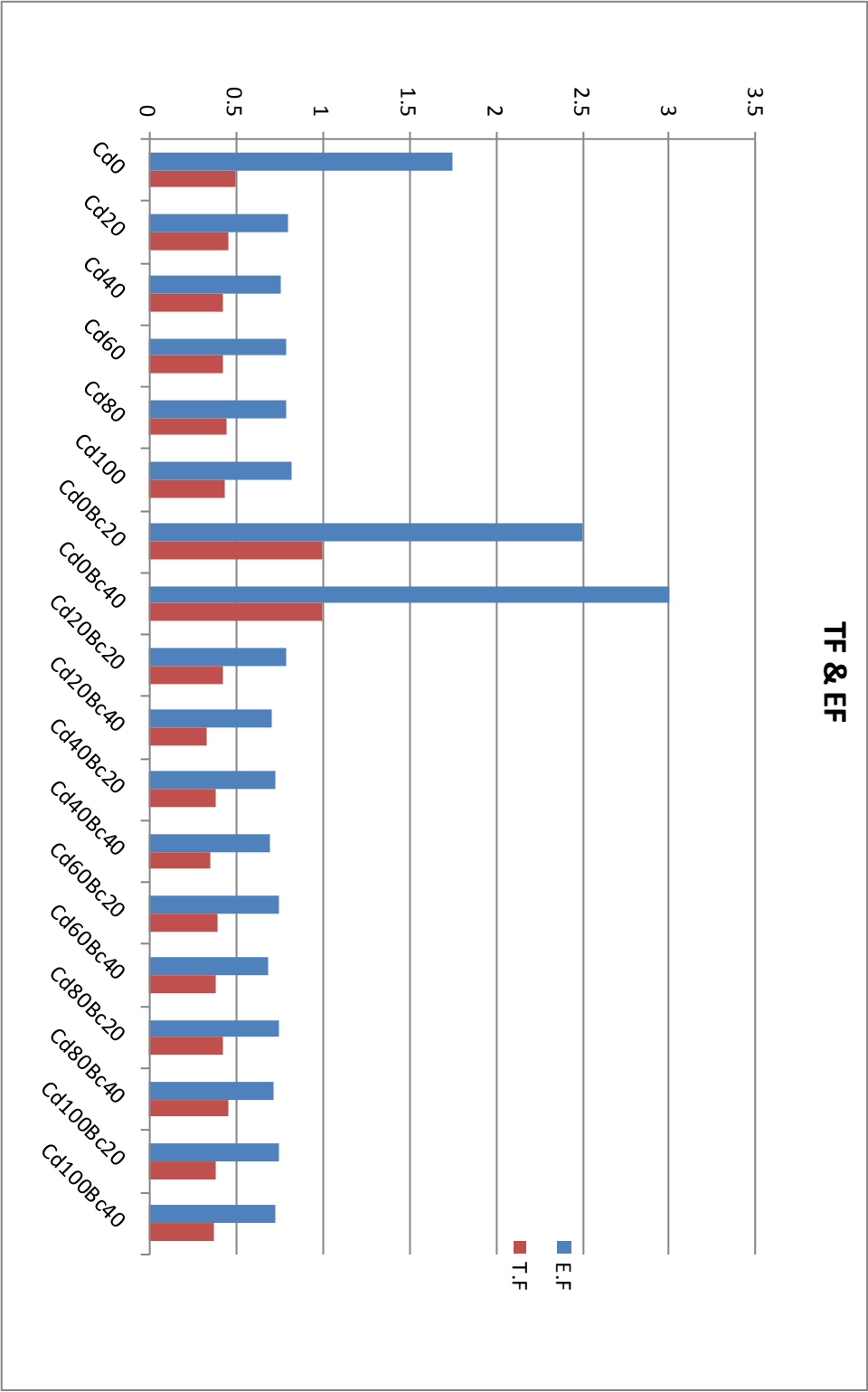


Fig 4.7: Effect of biochar on Enrichment factor and translocation factor of rice in Cd contaminated soil

CHAPTER - V

SUMMARY AND CONCLUSION

The heavy metal especially cadmium contamination in soil recognized as a serious global threat to human health via food chain. The accumulation of heavy metals in soil leads an adverse effect on sustaining soil ecology, crop production and human health. The application of biochar is found effective, low cost feasible tool in remediation of heavy metals from soil and thus minimizes its accumulation in plants and soil through the formation of organo-cadmium complex, phytodegradation and phytostabilization.

The present research work entitled “Effect of biochar, on phytoremediation of cadmium contaminated soil by using rice (*Oryza sativa* L.) as a test crop carried out in net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during *Kharif* season, 2018.

Malaviya Sugandha (HUR-105) is a test variety chosen for the experiment. The trail of 54 pots was conducted containing 18 treatments of different combination (Cd0), (Cd20), (Cd40), (Cd60), (Cd80), (Cd100), (Cd0+Bc20), (Cd0+Bc40), (Cd20+Bc20), (Cd20+Bc40), (Cd40+Bc20), (Cd40+Bc40), (Cd60+Bc20), (Cd60+Bc40), (Cd80+Bc20), (Cd80+Bc40), (Cd100+Bc20), and (Cd100+Bc40) with three replications to study the effect of biochar in association with different concentrations of cadmium in rice crop and data regarding different parameters of rice crop like plant height, chlorophyll content, straw and grain yield etc were recorded at different stages of crop growth.

After harvesting of rice grain, straw and root samples were analyzed for different macro and micronutrient along with cadmium content using the standard procedure. Soil samples were collected after harvesting of crop, processed and stored in polythene bags for subsequent laboratory analysis. Data recorded in various aspects statistically analyzed employing CRD technique. The facts observed during experimentation and then finally summarized and concluded in this chapter.

- Plant physical parameters like plant height, number of tillers hill⁻¹, productive tillers hill⁻¹ and chlorophyll content decreased with increasing doses of cadmium in soil i.e., (0, 20, 40, 60, 80, 100ppm). The addition of biochar, significantly increased the growth parameters in rice crop.
- Results indicated that cadmium had negative impact on the yield attributes like total tillers hill⁻¹, productive tillers hill⁻¹, grains panicle⁻¹ and panicle length, of rice crop. Upon increasing concentration of cadmium, the yield attributes decreased respectively. The application of biochar in control pot i.e. (Cd0+Bc40) was found more effective and recorded highest values of all parameter followed by the treatment (Cd0+bc20) respectively in cadmium contaminated soils. The application of biochar reduced cadmium toxicity to some extent but not completely.
- Biomass of rice crop grain and straw yield decreased with increasing doses of cadmium. The addition of biochar had significantly increased the biomass of rice and their interaction found significant. Among all these treatments with biochar, the highest biomass was recorded in the treatment (Cd0+BC40) followed by (Cd0+bc20) respectively. The results showed that biomass of rice plant decreased in both amended with biochar and non-amended soils upon an increase in cadmium concentration.
- Harvesting index varied from 48.2 % in (Cd0+bc40) to 42.0% in (Cd100) respectively. However harvesting index was decreased with increasing of cadmium concentration in soil. The application of biochar mitigated the toxic effect of cadmium up to certain limit but not completely. Harvesting index even decreased when soil amended with biochar with increasing concentration of cadmium in soil. Biochar significantly influenced harvesting index of rice.
- NPK content in straw and grain decreased with increasing levels of cadmium concentration. Lowest N, P, K content was observed in treatment (Cd100). Increasing cadmium levels resulted in significant increase in N and P content of the grain and straw up to certain limits (Cd20) tested in the experiment and then reduced slightly with an increase in cadmium concentration. N, P, K content in grain and straw was found significantly higher when soil amended with biochar.

The highest N content found in treatment (Cd0+Bc40) i.e. 206.3 kg ha⁻¹. N, P, K uptake by straw and rice grain was recorded minimum with treatment (Cd100). Data revealed nitrogen and phosphorus uptake was more in grain whereas potassium uptake was more reported in straw of rice crop.

- The pH of initial soil was recorded as 7.10. The data regarding to pH of post harvest soil varied from 7.10 to 8.42. Maximum pH 8.4 was recorded with treatment (Cd0+Bc40). Addition of biochar slightly increased the pH of post-harvest soil as compared to control. Basically pH of soil increases with increased level of biochar.
- A slight increase of EC the soil was reported is in case of biochar addition. This is evident that the EC of the soil ranges between 0.31 to 0.48 dSm⁻¹. The minimum EC was found in treatment (Cd0) i.e. 0.31dSm⁻¹. Normally with the increase in cadmium concentration the EC of soil also increases and hence the interaction between cadmium and EC of soil was counted significant.
- Organic Carbon was recorded highest with the treatment (Cd0+Bc40) i.e. 0.78% followed by treatment (Cd0+Bc20) i.e. 0.65 % respectively, while lowest OC% was recorded in treatment (Cd100). With the addition of biochar organic carbon percentage of soil increases because it is a rich organic source. While with the increase in cadmium concentration, the total biomass gets reduced due to decreased organic carbon percentage of post harvest soil.
- Available N in soil was recorded highest with the treatment (Cd20+Bc40) i.e. 206.32 kg ha⁻¹ followed by (Cd20+Bc20) i.e. 206.10 kg ha⁻¹ respectively while lowest amount of N reported in treatment (Cd100). Interaction between biochar and available nitrogen was recorded significant.
- Available phosphorus content increased in soil with an increase in cadmium concentrations. The treatment (Cd100+Bc40) recorded highest available phosphorus i.e. (24.5 kg ha⁻¹) compared to the rest of all treatments.
- Available K was recorded highest with the treatment (Cd0+Bc40) i.e. 222.3 kg ha⁻¹ and application of biochar generally increased the amount of available potassium.

- Cadmium content in rice grain and straw increased significantly with the increase in cadmium concentrations in soil. The highest cadmium content of rice grain and straw was observed with the treatment (Cd100) i.e. 3.8 and 8.9 ppm respectively which was much higher than control treatment. The use of biochar significantly decreased the toxic effect of cadmium content in different parts of rice crop. The direct application of biochar can decreased the cadmium toxicity more than 30% in grain and straw of rice crop respectively.
- The concentration of cadmium reported highest in root then in shoots and finally least in grain. Upon application of biochar the cadmium accumulation in roots was further decreased hence biochar reduced the Phytoavailability of cadmium to the plant.
- Enrichment factor was greater than 1 which indicates that *Oryza Sativa* was a hyper accumulator with high potential to absorb metals from soil. The mean values of translocation factor were also recorded greater than 1 which indicates that rice can hyperaccumulate cadmium from roots to shoots portion. Upon application of biochar there was more accumulation of cadmium observed in the roots and hence uptake of cadmium further decreased into grains.
- The present study ensured that rice is capable of accumulating cadmium in root and shoots; roots accumulation of cadmium is much more than the shoots accumulation.
- Exposure of rice to cadmium reduced overall growth, yield, nutrient content and uptake. However, application of biochar restored the damage caused by cadmium contamination and showed increased nutrient content and uptake as well. Biochar enhanced growth and yield parameters and accumulation of metal. So it may be concluded that rice can be used as ideal, effective, and hyper accumulator plant for phytoremediation cadmium in contaminated soil and application of biochar may be used as a source of nutrient (K, Ca, Mg) in rice crop and may be used as potential soil amendments for remediation of cadmium contaminated soil.

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