Production and application of nano-bonechar for immobilizing fluoride in naturally contaminated soil and it's potential towards climate smart agriculture

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APPROVAL CERTIFICATE

It is to certify that **Ms. Hina Imtiaz (Reg. No. 02312111007)** conducted the research work presented in this thesis, entitled **"Production and application of nano-bonechar for immobilizing fluoride in naturally contaminated soil and it's potential towards climate smart agriculture"** under the kind supervision of **Dr. Mahtab Ahmad**. Absolutely nothing from this thesis has been used in any other academic project for any other degree. This thesis is presented in the partial fulfillment of the requirements for the degree of **Master of Philosophy** in the field of **Environmental Science,** Department of Environmental Sciences, Quaid-i-Azam University Islamabad, Pakistan.

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NBC	Nano-bonechar
CSA	Climate smart agriculture
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
EDX	Energy dispersive X-ray
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
HAP	Hydroxyapatite
BC	Biochar
OM	Organic Matter
ANOVA	Analysis of variance
cm	Centimeter
dS/m	Deci siemens per meter
EC	Electrical conductivity
DTPA	Diethylene triamine Penta-acetic acid
DW	Distilled water
mg/l	Milligram per liter
mg/kg	Milligram per liter
ml	Milliliter
ºC	Degree Celsius
%	Percentage
WHC	Water holding capacity
IR	Irrigation rate

Abstract

Fluoride ion (F^-) is one of the major geogenic contaminants in water and soil. Excessive consumption of F^- poses serious health impacts on humans and plants. In this study, a novel carbonaceous material, nano-bonechar (NBC), was synthesized from cow bones and applied as a soil amendment to remediate and revitalize naturally F^--contaminated soil. The incubation experiments conducted revealed that NBC significantly reduced the mobility and bioavailability of F^- by 90% in the contaminated soil, and improved the soil quality by increasing the soil water holding capacity, soil organic matter, and the bioavailable contents of PO_4^{3-}, Ca^{2+}, and Na^+. Subsequently, the pot experiment results showed a significant reduction in the uptake of F^- by 93% in *Zea mays* plants. The F^--immobilization in soil was mainly due to the presence of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] mineral in the NBC. Ion exchange between OH^- (of NBC) and F^- (of soil), and the formation of insoluble fluorite (CaF_2) contributed to the attenuation of F^- mobility in the soil. To test the potential of NBC towards climate smart agriculture (CSA), a pot experiment was done under deficit irrigation, and the efflux of CO_2-C was measured using an incubation experiment. The experiment was conducted with three different irrigation rates (40%, 70%, and 100%), and 0 – 2% NBC application rates. Results revealed that NBC increases plant productivity and yield, and water retention capacity. The NBC application improved the plant's growth as indicated by the higher fresh and dry weights, root and shoot lengths, and total content of PO_4^{3-}, Ca^{2+}, and K^+ than those of un-amended soil. However, CO_2-C emissions remain higher in amended soil than in un-amended soil. This may be a result of high organic matter in the NBC that increased microbial activity in the soil. The findings indicate that NBC was not only beneficial for plant yield, but also for water conservation. Pot experiment showed the significant increment in fresh and dry weight, root an shoot length and total content of P, Ca and K in plants grown in NBC amended soil. The study concluded that the use of NBC is a natural waste management treatment for eliminating F^- from the soil, and a sustainable method for CSA. Moreover, due to its size and enrichment in hydroxyapatite, NBC could successfully be utilized for the rapid remediation and revitalization of F^--contaminated agricultural soil.

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Contamination of Fluoride in Soils

Fluoride pollution in soil is mostly neglected as compared to heavy metals and organic pollutants (Choudhary *et al.,* 2019). However, the endemic forms of fluorosis are a major issue across the globe. Fluoride is an electronegative form of fluorine, highly reactive and exists as an inorganic monoatomic anion (Sunkari *et al*., 2022). It is the 13th most abundant element and its occurrence reaches 0.06–0.09% of the earth's crust (Rusiniak *et al*., 2021).

Dry deposition, precipitation, and polluted water are all ways in which fluoride can enter into soil environment and be quickly absorbed by the cations already present there. Fluoride can also enter soil due to the application of phosphatic fertilizers and the unintentional release of chemicals or the emission of particulate matter into the atmosphere by volcanic eruptions (Khattak *et al*., 2022). It was reported that in Korea, the discharge of anhydrous hydrofluoric acid into the environment from a chemical industry led to an increase in the concentration of soil fluoride content within a radius of one kilometer from the location of the spillage (An *et al.,* 2015). Irrigation with fluoride-contaminated water and dissolved forms of fluoride from rock minerals had the potential to contaminate agriculture soil (Farooqi *et al*., 2009). Direct contact with polluted soil, breathing in vaporized soil toxins, eating polluted food, and groundwater contamination are all ways in which polluted soil can negatively impact human health (Cui *et al*., 2021). Soil is the only medium from which all life forms derive their sustenance. Therefore, remediation of fluoride-contaminated soil is extremely significant.

1.2 Sources of Fluoride

Both natural and anthropogenic activities affect fluoride's release into the environment and its deposition in soil (Makete *et al*., 2022). The most important natural sources of fluoride in soil are the eruptions of volcanoes and the spontaneous weathering of parent rocks (Ahmad *et al*., 2022). Fluoride exists in soil either as free fluoride ions or in the form of complexes with another element like iron (Fe), boron (B), calcium (Ca), sodium (Na), and aluminium (Al) and heavy metals (Fan et al., 2020; Xu *et al*., 2022). Fluoride concentration in soil is increased by weathering of rock-forming minerals that are rich in fluorine. Fluorine can be found in the soil in the form of fluoride

(F^-) or other complex anions, such as $(BF_4)^-$, $(AlF_6)^{3-}$, and $(SiF_6)^2$. As, fluoride bonds well with the aluminium contained by anion exchange, thus increasing aluminium solubility. It has been found that pollution of fluoride ultimately enhances the toxicity of aluminium as plants absorb more aluminium when fluoride levels in the soil are high. However, the induction of toxicity of aluminium depends upon plant species (He *et al*., 2021). Moreover, phosphorus-bearing minerals include apatite ($Ca_5(PO_4)_3F$) and other clay minerals like fluorite (CaF_2), topaz ($Al_2(SiO_4)F_2$), fluorspar (CaF_2), fluorapatite ($Ca_{10}F_2(PO_4)_6$), cryolite (Na_3AlF_6) are the major sources of the contribution of fluoride in soils (Rasool *et al*., 2018; Masood *et al*., 2022). Weathering and solubility of these minerals create an enrichment of fluoride in soils which is harmful to the entire food chain (Wang *et al*., 2022). The release of excessive fluoride from the weathering of mafic rocks like biotite, pyroxene, and amphibole may also contribute to polluting the soil (Zango *et al*., 2022). Anthropogenic activities include the utilization of phosphatic fertilizers, pesticides, disinfectants, wood picture tubes, glass, brick, and textile dyes all contribute to the release of fluorine into the environment (Yu *et al*., 2022; Khan *et al*., 2022).

1.3 Bioavailability of Fluoride

The bioavailability and mobility of fluoride in soils is a complex phenomenon. The bioavailability of fluoride in soils is determined by several different criteria, such as soil's pH, soil texture, the minerals present, organic matter, the concentration of calcium and phosphorus, and the amount of water that is lost from the soil (Roshni, 2022). Although, the water-soluble and exchangeable fluoride content is considered to be bioavailable for plants and animals, fluoride found in other forms is either less biologically active or not biologically active at all (Rizzu *et al*., 2020; Makete *et al*., 2020).

The bioavailability of fluorides in the soil is significantly influenced by pH because it has a significant role in fluoride solubility and the formation of stable aluminium and calcium complexes. In soil, fluoride exists in two forms at low pH: as complexes and as free F^- ions. Fluoride solubility in soils is highly varied but typically increases with pH values below 5 and above 6. Fluoride sorption is highest at pH levels between 5 and 6.5, where its solubility is also at its lowest (Yadav *et al*., 2018). The concentration of fluoride in soil solution rises as the pH of the soil increases because of the increased unfavorable electrostatic potential that causes the fluoride ion to be less likely to be retained by the soil (Choudhary *et al*., 2019). The greater solubility of F under acidic conditions was explained by the formation of AlF_x complexes, because fluoride has a low affinity for organic material, leading to leaching from the more acidic surface horizon and enhanced retention by clay fraction (Jha, 2015). When the pH of the soil is alkaline, the increased negative surface charge causes anionic F^- to be repulsed. The solubility of fluoride rises with an increase in soil pH because at pH 8-9, fluorides exist as free anions, and in the presence of hydroxyl ion (OH^-), all aluminium species form the aluminate, $[Al(OH)_4]$, complexes (Jha *et al*., 2011) .

Fluoride distribution typically enhances in the soil during the formation of texture and is mostly bound with colloid and clay fractions. Soils with a high clay content tend to have high retention fluoride levels, while those with sand content tend to have lower fluoride levels (Young et al., 2011). There is a negative correlation between fluoride adsorption and pH and cation exchange capacity of soil whereas, a positive correlation between soil clay and organic carbon concentration (Wang *et al*., 2022).

1.4 Status of Fluoride Contamination in Pakistan

The hazardous effects of fluoride have been documented throughout Pakistan, including Quetta (Balochistan province), Gujrat and Kalalan Wala (Punjab province), Dera Ismail Khan (Khyber Pakhtunkhwa province), Thar and Nagarparkar (Sindh province), and Thar (Balochistan province) (Ahmad and Qadir, 2011; Farooqi *et al*., 2007; Panezai *et al*., 2018; Rafique *et al*., 2009; Raza *et al*., 2016). The cities of Punjab province including Lahore and Kasur have been reported for incidences of bone abnormalities in 1.51 million residents, and approximately 17 localities (Farooqi *et al*., 2007). The consumption of F-contaminated water, vegetables and food is the primary factor in the development of these bone deformities in the majority of individuals (Kabir *et al*., 2020). Reviewing the fluoride data of 29 major cities in Pakistan reveals that 34 percent of those cities have fluoride levels with a mean value that is greater than 1.5 mg/L. Lahore, Quetta, and Tehsil Mailsi have the highest fluoride levels, with maximum values of 23.60, 24.48, and > 5.5 mg/L, respectively. Fluoride and arsenic contamination of groundwater has been documented recently in the cities of Lahore, Dargai, and Talab Sarai (Rasool *et al*., 2018; Rashid *et al*., 2020; Zulfiqar *et al*., 2020). In Talab Sarai, 80% of the children exhibited clear signs of thyroid hormonal imbalance, and four out of five of the children had four times the normal amount of fluoride in their urine serum, which may cause abnormalities in both the child's physical and mental development in later stages of development (Zulfiqar *et al*., 2020)

1.5 Effects of Fluoride

Fluoride is an important element that must be present in the body at levels below 1.5 mg L^{-1} for normal development and growth (Khan *et al*., 2022). Fluoride can be fatal to humans, animals, and plants if they are exposed to fluoride excessively and can cause adverse effects to all life forms. The effects of fluoride's toxicity on plants, animals, humans and are mentioned below:-

1.5.1 Effects on Plants

Fluoride (F) is known to cause phytotoxicity in most plant species by interfering with a number of metabolic processes. It is not an essential element for plant growth, it is an accumulative toxin that may be found in plant leaves, where its effects may be gradual over time (Devi *et al*., 2022). The symptoms that are visible in plants as a result of the toxic effects of fluoride are dependent on a wide variety of factors. These include the concentration of fluoride, time of exposure and the age of the plant (Kumar *et al*., 2022). Fluoride travels through plants through the stream of transpiration that originates from the roots or travels via xylem vessels to stomata, and it accumulates around the leaf edges (Rizzu *et al*., 2020). After fluoride has been absorbed by the plant in a dissolved state, it is carried to the leaf margins via the vascular tissue so that it accumulates there. When fluoride is absorbed by the plant and then transported to the shoots, it can cause physiological, biochemical, and structural damage to edges (Rizzu *et al*., 2020; Peng *et al*., 2021). Depending on the quantity of fluoride in the cell sap, it can even cause cell death (Khan *et al*., 2022). The uptake of fluoride can result in mild necrosis on the top edge of leaves, which can then spread down to the leaf root (Wang *et al*., 2022). By acting on the membranes and the stromal enzymes associated with carbon dioxide fixation, fluoride lowers the concentration of chlorophyll, which in turn harms the metabolism of plants. It exerts a powerful inhibitory effect on photosynthesis and other activities. According to the findings of histochemical research conducted on plants, fluoride first distorts the structure and function of spongy mesophyll cell and then inhibit the process of photosynthesis by disruption the chloroplast (Sharma & Kaur, 2018). It is also known to inhibit enzymes, particularly those enzymes that need cofactors such as Ca^{2+}, Mg^{2+}, and Mn^{2+} ions (Choudhary *et al*., 2019). Literature reported that seeds and seedlings are perhaps more vulnerable to the effects of fluorides than adult plants. The overabundance of fluorides that accumulate in plants is what causes obvious leaf harm, as well as damage to fruits and a reduction in output (Wang *et al*., 2022).

Sometime due to an imbalance in the absorption of nutrients by seedlings, fluoride toxicity causes a decrease in both the root length and the shoot length of the plants. Studies reported that fluoride contamination in the soil caused a reduction in the length of root and shoot development for *Prosopis juliflora* (Saini *et al*., 2013; Kumari & Khan, 2018). Fluoride toxicity causes a reduction in the development of both the shoot and the root, which ultimately led to a drop in the yield of plants (Gadi *et al*., 2021). Fresh weight, dry weight, and the percentage of seedlings all steadily reduced as the fluoride concentration increased. This was because the presence of fluoride caused a reduction in the metabolic activity of the plant (Rizzu *et al*., 2021).

1.5.2 Effects on Animals

Excessive fluoride ingestion by animals can induce either acute toxicity or a debilitating chronic disease. In the 1900s the earliest records of acute and chronic fluorosis in farm animals were found in Iceland, after volcanic eruptions (Kristinsson *et al*., 1997). Chronic fluorosis in grazing farm animals has been documented in several other regions of the world beginning as early as 1931 (Cronin *et al*., 2000). Fluoride contamination in animals depends on many factors like the quantity of fluoride that was consumed, solubility of the fluoride, time of exposure, and the specie and the age of the animal (Guth *et al*., 2020). The symptoms of chronic fluoride poisoning often appear after weeks or months, during which time excess fluoride is eliminated in urine and accumulated inside bones (Guth *et al*., 2020; Malik, 2021). When animals are consistently subjected to high levels of fluoride, accumulation in the bones can occur up to a saturation point, at which point the body's soft tissues are inundated with fluoride, leading to the breakdown of metabolic processes and eventual death (Wallis *et al*., 1996). Elevations in skeletal fluoride concentrations cause inhibition of various enzyme activity (Guth *et al*., 2020). It has the effect of slowing down the growth of their teeth, which can lead to mottling, erosion, excessive tooth wear, lameness, skeletal deformities, decreased feed and water intake, decreased weight gain, and decreased milk production are some of the other symptoms that can be seen (Guth *et al*., 2020).

1.5.3 Effects on Human

Fluoride can be taken up by food and feed crops through soil solution and it can enter the food chain and ultimately affect human health. In humans, fluoride can cause dental and skeletal fluorosis, retard biochemical and physiological processes, damage the reproductive system, renal failure, hypertension, arthritis, neurological impact, and carcinogenic risks (Kabir *et al*., 2020).

People who were exposed to fluoride for a long period had a comparatively high risk of developing dental fluorosis, osteoporosis, weight loss, fatigue, anemia, staining of teeth, and osteosclerosis (Kabir *et al*., 2020). In a research conducted in Germany on people who lived within the vicinity of two kilometers of an industrial area for twenty years, skeletal fluorosis and periosteal thickening were documented (Qiao *et al* ., 2021). Several hundred children living within one and a half kilometer of a fluoride mine that utilized outdated emission control technologies were found to have significant dental fluorosis (Kabir *et al*., 2020; Qiao *et al* ., 2021).

1.6 Soil Remediation Techniques

The entire globe is using different remediation techniques for the clean-up of contaminated soils, like in-situ, ex-situ, off-sight, on-sight, physical, chemical, and biological. In developing countries, reclamation and mitigation measures are not implemented potentially (Chen *et al.,* 2021). Contamination of agriculture soils has become a critical problem in Pakistan. Biological methods are used mostly for the remediation of contaminated soils as compared to physical and chemical methods in Pakistan (Ali *et al*., 2015).

Physical methods include soil washing, extraction, thermal desorption and soil excavation which are commonly used. Soil excavation is the process in which the replacement of contaminated soils with clean soils occurs and is mostly used in Pakistan. Physical methods increased the functionality of soil by dilution mechanism. Replacement of soils is also carried out by soil spading in which contaminated sites are dug deep down and contaminants spread into deep sites. New clean soils are imported to dilute the contaminants (Ayub *et al*., 2020). Soil washing is a process in which extractants and reagents were used including salts and chlorides which aid the process of extraction of pollutants from contaminated soils. In thermal desorption, contaminants turn into volatile form by applying heat (Chen *et al.,* 2021).

Chemical methods include vitrification, chemical leaching and fixation, and electrokinetics (Sui *et al*., 2021). Both physical and chemical methods are time-consuming, complicated, laborious, expensive and not viable economical options. Bioremediation provides non-invasive, environmentally friendly, cost-effective and permanent solution to rectify polluted soils (Ali *et al*., 2015).

Currently, in Pakistan the most accepted method for remedy of polluted soils is bioremediation. Concentration of pollutants can be reduced either by the degradation of pollutant by microorganisms or the dilution of agricultural soils by plant uptake and translocation of contaminants. The bioremediation techniques involve phytoremediation, mycoremediation and bacterial remediation. The use of different plant species to remove, dilute and immobilize contaminants in agriculture soil is called phytoremediation. Phytoremediation takes account on chelate assisted phytoremediation, phytoremediation with transgenic plants, phytovolatilization, phyto-stabilization and microbial-assisted phytoremediation (Chen *et al*., 2021). It is the most adapted successful and environmentally friendly in situ method, sometimes coupled with chelates like ethylenediamine tetra acetic acid (EDTA) (Ali *et al*., 2015). Pakistan has a diversity of flora which are used to eliminate contaminants from polluted soils (Kamran *et al*., 2014). Recent studies have reported the use of flora for phytoremediation from Pakistan. Indigenous flora of Pakistan has a high potential to remediate polluted soils. Due to the use of solar energy, the cost-benefit of this process is ¼ of a physical and chemical method for the reclamation of soils (Kamran *et al*., 2014). Myco-remediation is type of bioremediation which use fungi to decontaminate the soil environment. This method is effective, simple, environmentally sound, involves low cost and is applicable in Pakistan for the removal of contaminants from the soil environment (Shen *et al*., 2022).

1.7 Nano-bonechar as Immobilizing Agent for Soil Remediation

A wide range of adsorbents were used for the adsorption and immobilization of fluoride. For instance, activated alumina (Millar *et al*., 2017), activated carbon (Ravulapalli and Kunta, 2017), calcite (Cai *et al*., 2018b), activated sawdust (Ye *et al*., 2019), activated coconut shell carbon (Choong *et al*., 2020), and pinecone-derived biochar have already been reported in the literature for removing fluoride from soil and drinking water (Khan *et al*., 2022; Alkurdi *et al*., 2019; Wan *et al*., 2021). The use of bonechar for remediating fluoride-contaminated soil is one of the most promising solutions currently available (Zhou *et al*., 2019). If there is a significant amount of calcium carbonate present, fluoride in soils is fully fixed as calcium fluoride. Fluoride bonds to clay by displacing hydroxide off the surface of the clay, which is a necessary step in the binding process. When the soils have relatively high levels of Ca^{2+}, the electronegative F^- takes the place of the (OH^-) group, which was previously attached to the complex (Alkurdi *et al* ., 2020).

In recent years, nano remediation played a significant role in improving the environment by removing heavy metals, polyvinyl chlorides, poly aromatic hydrocarbon, anionic, and inorganic pollutants. Due to high surface area and increase surface energy, nano-sized particles got high potential for nutrient delivery**,** nutrient retention, soil microbial activity, pollutant adsorption and improving soil fertility. Therefore, nano biochars with the potential to exert more benefits get attention towards the remediation of the soil environment (El Refaey *et al*., 2022). According to certain reports, the fractions of biochar (BC) smaller than 0.45 microns have a larger amount of oxygen and polar functional groups, but they have lower aromaticity and less condensed aromatic clusters in comparison to the bulk BC (Qu *et al*., 2016). Because of their tiny sizes and negatively charged surfaces, these fine nano-bonechar particles have high colloidal stability (El Refaey *et al*., 2022). Nano-bonechar has the propensity to combine with minerals and organic matter in the soil to produce nano aggregates via organo-mineral interactions and is effective for immobilizing fluorides in contaminated soils due to the presence of calcium (Weng *et al*., 2017; Archanjo *et al*., 2017).

1.8 Climate Smart Agriculture

Climate-smart agriculture is defined as agricultural practices that sustainably improve agricultural production and incomes, adapt and contribute to system resilience, and at the same time reduce or remove greenhouse gases (Barasa *et al*., 2021). The Food and Agriculture Organization of the United Nations (FAO) has prompted a sustainable agricultural production system, also known as Climate-Smart Agriculture (CSA), as an alternative to conventional agriculture because of the social, environmental, and economic problems that are caused by climate change and conventional agriculture.

The three main goals of CSA are: (i) improved sustainable production, (ii) mitigation & reduced greenhouse gas emissions, and (iii) adaptation & systems resilience. A variety of technological advancements have been made in recent years to achieve the goals of climate-smart agriculture (CSA) (Wambi *et al*., 2021).

Due to a wide variety of obstacles that farmers confront in the production of crops and livestock, agricultural production has remained relatively unchanged over the previous three decades. Climate extremes and the unpredictability of the weather are two of the most significant obstacles that smallholder farmers face. These factors have contributed to an increase in the severity of the impact that abiotic factors such as soil deterioration, and scarcity of water which can result in infertile soils and biotic factors such as weeds, diseases, and pests limit the productivity (Jariwala *et al*., 2022). Moreover, the improvements in agricultural technology that have been made possible as a result of climate change are much more expensive and unaffordable (Barasa *et al*., 2021).

The application of CSA will play a significant role in agriculture of the future. The CSA have the potential to increase the size, efficiency, and production by promoting sustainable agricultural production methods. There will be a significant shift in the pattern of rainfall and temperature (Wambi *et al*., 2021). So, climate and changes to the climate will be major factors to be taken into consideration in the development, expansion, and acceptance of agricultural technology. Thus, there is a need to improve agricultural productivity and incomes in a sustainable manner by encouraging innovative, adaptable, and sustainable activities that could become essential input in the agricultural revolution (Wambi *et al*., 2021; Jariwala *et al*., 2022).

1.9 Pakistan and Climate Smart Agriculture

The agriculture sector in Pakistan is climate-sensitive and highly vulnerable to growing weather variability and climate change (Imran *et al*., 2022). Several studies indicated that the production of major crops in Pakistan could be significantly impacted due to receiving less rainfall and increasing temperature across the country. Pakistan is in 5^{th} place on the climate risk index in terms of vulnerability to climate risk (Kreft *et al*., 2013). The frequency of extreme climatic events is increasing, including droughts and floods with severe impact on climate-sensitive livelihoods. According to the literature, the entire amount of water used for irrigating crops in Pakistan is approximately 1531 km^3 every year. This represents more than 95% of the country's total water intake (Imran *et al*., 2018). The lack of available water in the country is getting worse, and the agricultural sector in particular is in danger as a result of the recurrent droughts that have been occurring. The silting up of major reservoirs, rapid population expansion, inefficient management of irrigation systems, the phenomenon of climate change, and a lack of consensus among the provinces about the construction of new dams are the primary factors contributing to water shortage in the country (Shahbaz *et al*., 2022). Several studies indicated that the production of major crops in Pakistan could be significantly impacted as a result of receiving less rainfall, an increase in temperature across the country by 0.5°C in the past three decades, and variations in the frequency and intensity of droughts and floods throughout 1995–2017 (Ullah *et al*., 2022). In a similar vein, forecasts suggest that the mean temperature in Pakistan will climb at a rate that is greater than the average increase predicted for the rest of the world, 1.4–3.7 °C. The vast majority of Pakistan's farmers continue to engage in the age-old practices of conventional agriculture. In addition to this, they are utilizing standard agricultural management procedures applying high doses of fertilizers, pesticides and herbicides etc. As a result of this, greater production costs as well as inefficient use of available resources.

Although Pakistan is one of the agricultural countries and largest producers of cotton, wheat and rice in the world, climate-sensitivity has endured multiple setbacks over the years as a result of conventional agricultural management practices, climate change, and market failures. The majority of the academics concluded that climate change and ineffective production management practices are having a detrimental impact on Pakistan's agricultural yield, which is a significant concern. Adopting CSA techniques and approaches and practices is a way to mitigate the negative impact that varying climatic conditions have on agricultural production, which can be done either individually or collectively (Imran *et al*., 2022). The implementation of CSA techniques has the potential to increase yield, improve resource use efficiency, and boost farm profitability, while mitigating the potentially detrimental effects of climate change on the production of crop yield (Imran *et al*., 2019).

1.10 Biochar and Climate Smart Agriculture

Biochar could sustainably play a major role to promote climate-smart agriculture (Nyambo *et al.*, 2020). Aa a soil amendment, biochar is a potentially useful approach for climate-smart agriculture (CSA) because it has the potential to increase crop output, strengthen agricultural resilience to climatic variability, and lower greenhouse gas emissions all at once (Huang *et al.*, 2023). However, feedstock material, pyrolysis settings, biochar amendment rates, and experiment duration all have significant impacts on crop yield, characteristics of soil, and greenhouse gas emissions. Biochar is made by pyrolyzing organic waste at temperatures typically between 300 and 1000 ºC and it is rich in carbon (C) that is resistant to decomposition. Biochar's unique stability makes it a useful soil additive for combating climate change by lowering atmospheric CO_2 and increasing soil C sequestration over the long term. Biochar can also increase crop yields by boosting soil quality and fertility (Herrera, 2022). Due to differences in biochar characteristics, studies have found a wide variety of reactions of physical, chemical, and biological soil parameters to biochar. Soil bulk density is decreased while soil pH, cation exchange capacity (CEC), water holding capacity, and nutrient retention are increased when there will be amendment of soil with biochar takes place. Soil microbial populations and their activity can be directly influenced by biochar addition (Huang *et al*., 2023). For instance, biochar addition raises microbial biomass C, N-acquisition enzyme activities, and symbiotic biological N_2 fixation. The addition of biochar to soils has been shown to improve agricultural output and poses a positive impact especially in nutrient-poor, acidic soils, whereas reports of no responses in nutrient-rich soils and negative yield responses for alkaline soils were also common. Responses to biochar amendments in soils that emit greenhouse gases i.e., carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are highly variable because of the complexity of the soil microbial processes that determine GHG fluxes and the interconnectedness of biotic and abiotic regulating factors (Das *et al* ., 2022). However, it has been shown that biochar amendment, especially when used in conjunction with fertilizer or manure, can lower N_2O emissions by anywhere from 12 to 49 percent. CO_2 emissions may be unaffected, increased, or decreased by biochar amendment. Biochar could have no impact on CH_4 emissions or could be used as a mitigating method (Das *et al* ., 2022; Huang *et al*., 2023).

1.11 Problem Statement

Climate changes severely affect agriculture practices in terms of yield, and water stress and continues to heat up challenged farmers' community worldwide and in Pakistan. Fluoride contamination and climate changes severely affect the soil environment and decline in yield of the crops. If fluoride is biomagnified through the food chain, it can cause toxicity in plants, animals and humans. Therefore, it is an urgent need to remediate the soils contaminated with fluorides and to cope with climate changes to promote safe and climate-smart agricultural practices.

1.12 Research Hypothesis

Nano-bonechar could be an effective immobilizer for immobilizing fluorides in contaminated soils due to the presence of calcium and phosphorus. Nano-bonechar can also capture carbon dioxide, strengthen plants against water stress conditions by increasing the water holding capacity of soils and give promising results in enhancing yield by increasing organic matter and nutrients.

1.13 Study Objectives

The following are the objectives of this study:

1. Synthesis and characterization of nano-bonechar from cow bones.
2. Immobilization of fluoride in naturally contaminated soil.
3. Mitigate and reduce the emission of carbon dioxide by adding nano-bonechar in soils.
4. Adaptation of plants to cope with water stress soil environment and improve sustainable production.

2. MATERIALS AND METHODS

2.1 Chemicals and Glassware

During the experimental work various chemical, glassware and instruments were used for the conduction of experimental work. Chemicals used for carrying out experiments mainly included pH buffer solution for calibration of the pH meter, ammonium bicarbonate (NH_4HCO_3), diethylene triamine penta acetic acid (DTPA), diphenyl-amine indicator and sodium hydroxide. Acids including sulphuric acid, hydrochloric acid, nitric acid, and perchloric acid were used for the analysis of organic matter for the digestion of plants. Barium chloride, hydrochloric acid, phenolphthalein, and sodium hydroxide for measurement of carbon dioxide. Other chemicals include orthophosphoric acid (H_3PO_4), ferrous sulphate solution ($FeSO_4.7H_2O$), ammonium heptamolybdate, ammonium molybdate, and lithium dichloride. In glassware, volumetric flasks, beakers, measuring cylinders and pipettes, falcon tubes, spatula, ceramics crucibles, cuvettes, burette, wash bottles, test tubes, stand, funnels and conical flask were used. Aluminium foil and distilled water were also used for covering, washing and preparation of standards and solutions, respectively. Polyethylene tubes were used for the storage of samples and filtrate.

2.2 Instrumentation

A drying oven (EQ-DHG-9140A) was used for drying the bones and for proximate analysis of nano-bonechar. Muffle furnace (Vulcan D-550) was used for preparation of biochar. Planetary ball mill (BKBM-V2S) was used for preparation of nano-bonechar, and zeta sizer (ZS-90, Malvern instrument, UK) was used for measuring size of the nano-bonechar (NBC). Analytical weighing balance (Shimadzu ATX 224) was used for weighing of the NBC and soil samples. pH meter (ino-Lab pH-7110) and electrical conductivity (EC) meter (HANNA HI-2300) were used for measuring pH and salt concentration of the soil and NBC, respectively. Fourier transform infrared (FTIR SHIMADZU-8400) was used for identifying presence of functional groups on NBC. Scanning electron microscope (EFI S50 Inspect Netherland) was used to obtain surface morphology whereas, X-ray diffractometer (D/Max-B, Japan)as used for detecting crystalline material in NBC. Energy Dispersive X-Ray (EDX) analysis was used for finding the elemental composition of NBC. Colorimeter (HACH DR-2800) was used for determining fluoride content of soil. The flame photometer (FP910-4, UK) was used to measure sodium (Na), potassium (K) and calcium (Ca) content of the soil and plant. UV spectrophotometer (Thermos Fisher Scientific 51119500) was used to measure bioavailable nitrate-nitrogen and bioavailable phosphorus of the soil, and for measuring the total phosphorus of the plants. A hot plate, hydrometer, plunger, thermometer, water bath and orbital shaker were also used for the conduction of experimental work.

2.3 Feed Stock Collection and Processing

Cow bones were selected for the production of nano-bonechar. Cow bones (~15 kg) were acquired in summer from a slaughterhouse. Cow bones were boiled for 30 min then washed with hot water to remove flesh and fibers. First, bones were sun-dried, and then placed in a drying oven at 110 °C. The whole process of drying was completed in one month. After drying, only metatarsal cow bones were selected due to less moisture content as feedstock. The bones were ground and homogenized before placing in a muffle furnace for biochar production.

2.4 Production of Nano-bonechar

The ground feedstock was pyrolyzed by using a muffle furnace. To ensure a slow pyrolysis process, the heating rate was kept at 5°C min^{-1}. The feedstock (1530 g) was weighed in ceramic crucibles and carbonized at 500 °C for 2 h. All the lids of the crucible were closed for maintaining limited oxygen supply. After weighing, produced bonechar was stored in air tight polyethylene zipper bag for further processing. When bonechar cooled down at room temperature yield was calculated with the following formula:

$$\text{Bonechar yield (\%)} = \text{weight of the bonechar/weight of the feedstock} \times 100$$

After that, bonechar was converted into nano-bonechar with the help planetary ball mill with a capacity of 2 L. The mass of bonechar was allocated to four stainless steel containers of a planetary ball mill according to the ball-to-powered ratio. Three cycles were employed at the mill at a rotating speed of 670 rpm which is the maximum rotating frequency. Each cycle comprises 30 min to prevent the overheating of balls and bonechar (Naghdi *et al*., 2017). The nano-bonechar was stored in polyethylene zipper bag for preventing the clinging effect due to moisture.

2.5 Characterization of Nano-bonechar

The nano-bonechar was characterized for pH, EC, K, Na, organic matter, bioavailable phosphorus and nitrate-nitrogen, proximate and ultimate analyses.

2.5.1 Proximate Analysis

The proximate analysis included the measurement of moisture content, mobile matter, ash content and resident matter. The moisture content of nano-bonechar was determined by keeping the 2 g sample in a ceramic crucible in the oven at 105°C for 24 h without a lid till the achievement of the constant weight. The moisture content of nano-bonechar was calculated by taking the difference in weight loss before and after heating of the samples. Mobile matter which is also known as volatile content that is the non-carbonized nano-bonechar portion was calculated by placing the same crucible with lid in a muffle furnace at 450°C for 30 min. The mobile matter was determined by calculating the instantaneous weight loss difference. The ash content of nano-bonechar was measured by placing the samples in a muffle furnace at 750°C for 30 min (Standard ASTM D2216, ASTM 2010). The non-ash part which is known as resident matter (fixed matter) of nano-bonechar was calculated by the following formula:

$$\text{Resident matter (\%)} = 100 - (\text{mobile matter} + \text{ash} + \text{moisture content})$$

pH and EC of nano-bonechar were measured in 1:20 nano-bonechar and distilled water suspension in replicates. The suspension was shaken at 200 rpm on a shaker for 1 h. The pH and EC were recorded by dipping the electrode of the pH and EC meter in suspension after calibration of the pH meter with pH and EC calibration solutions. Extractable potassium (K), sodium (Na), organic matter, phosphorus (P), and nitrate-nitrogen (NO_3-N) content of nano-bonechar were also measured. Protocols of each parameter are mentioned in Annexure 1.

2.5.2 Ultimate Analysis

The particle size of nano-bonechar was measured by a zeta sizer. For determining the size distribution of NBC, 1 mg of the sample was dispersed in 200 mL of DW along with 1% ethanol and 0.5% Tween 80. After that, the sample was sonicated for 60 min. Ice was provided externally for keeping the sample cool during the process of sonication (Naghdi *et al*., 2017).

A scanning electron microscope (SEM) was used to obtain the surface morphology. The particles of nano-bonechar were visualized on 2500, 10,000 and 30,000 resolutions under vacuum at 20 kV voltage by using SEM. Fourier transform infrared (FTIR) was used for knowing the functional groups present on NBC. X-ray diffraction was used for detecting crystalline material in NBC. Energy Dispersive X-Ray analysis (EDX) was used for finding the elemental composition of NBC. A very thin layer of nano biochar was subjected to TEM analysis for studying the detailed morphology.

2.6. Soil Collection, Study Area and Processing

Naturally fluoride-contaminated soil was collected from the village Kot Maiga, district of Kasur, Pakistan. Geographically, Kasur lies between 31.23157° N and 73.95181° E. Coordinates of each location were recorded at the time of sample collection via GPS. The study area map was then developed using ArcGIS 9.3 as shown in Fig 2.1.

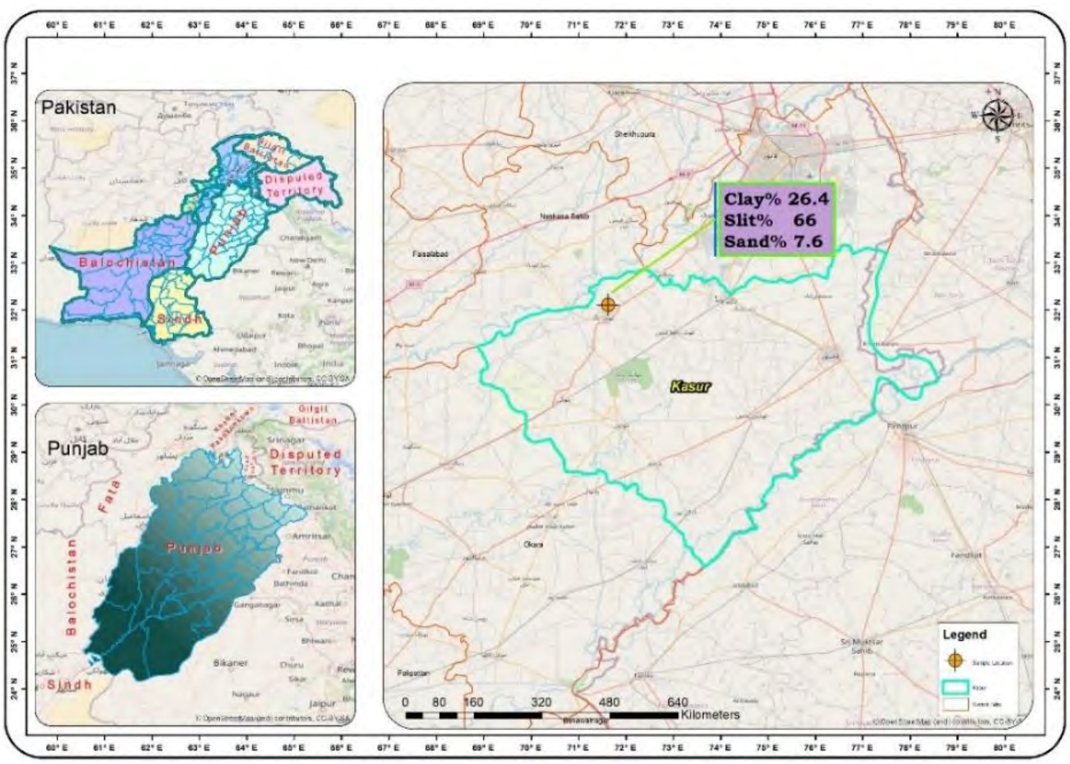

Fig 2.1: Map of fluoride-contaminated natural soil of Kasur.

The soil of Punjab is composed of quaternary alluvial deposits with low organic content (Greenman *et al*., 1967). The aquifer system that lies under Kasur is supported by unconsolidated alluvial deposits that date back to the Quaternary period. The alluvial complex and sand which make up the unconfined aquifer are made up of fine to medium sand, silt, and clay, and they contain varying amounts of apatite ($Ca_5(PO_4)_3F$), fluorite (CaF_2), cryolite (Na_3AlF_6), topaz ($Al_2(SiO_4)F_2$), micas, and clay minerals. These minerals influence the fluoride enrichment in soils and sediments (Younas *et al*., 2019). The aquifer is considered to be homogeneous and has a high transmissivity even though alluvial sediments are present, which gives rise to the aquifer's heterogenic nature (Greenman *et al*., 1967).

After collection, the soil was air dried for one week, and then passed through 2 mm sieve to remove impurities like stone, pebbles, debris and coarse materials to obtain the homogenized soil sample. The processed soil was kept in polyethene bags for further experimentation and analysis.

2.7 Soil Characterization

The soil was characterized for physical i.e. texture, and chemical parameters including pH, EC, K, Na, organic matter, bioavailable phosphorus and nitrate-nitrogen, and water holding capacity. The pH and EC measurements were performed on the aqueous suspension of the soil samples in (1:2). 10 g of each soil sample was taken into the flask, added 20 ml deionized water and shaken for 30 min at 180 rpm (Thomas, 1996). After complete blending, pH and EC were measured on an already calibrated pH and EC meters, respectively. Total organic matter was determined by the method as described by the Walkley-Black (Walkley and black 1934). Soil texture class was determined by the dispersion method (Estefan 2013). Water holding capacity, bioavailable K, Na, PO_4^{-3}, and NO_3-N were also measured (Estefan 2013). A detail of each protocol is given in Annexure 1.

2.8 Soil Incubation Experiment for Fluoride Immobilization

Soil incubation experiment was performed with three distinct rates of nano-bonechar (NBC) (0.5%, 1% and 2% w/w). The experiment was carried out by taking 500 g of soil in a polyethene container and mixed with a particular rate of NBC. In each container, 126 ml distilled water (DW) was added which is 55% according to the water holding capacity of the soil in each container. Each rate was applied in 9 different pots along with three control. After thoroughly mixing the soil, NBC and distilled water, all the containers were weighed and tightly closed with lids before being retained in the dark for incubation. During the incubation period, the moisture content of the soil was maintained (if needed) by adding the DW by comparing the weight of each container with the initial weight. The soil incubation experiment was continued for 80 days. On the 81st day, the soil of each incubated container was air-dried and then analyzed for pH, EC, K, Na, Ca, organic matter, bioavailable phosphorus, nitrate-nitrogen, and different fractions of fluoride.

2.9 Bioavailable and Exchangeable Fluoride Analysis

Incubated and harvested soil samples were analyzed for water-soluble and exchangeable fluoride content. These two forms are potentially bioavailable to soil microbial communities and plants. For assessing water-soluble and exchangeable F^- content, 5 g of incubated soil was mixed with 30 ml DW and 1 M ammonium acetate in falcon tubes, respectively. All the samples were shaken up for 24 h on a shaker at 200 rpm. After that, all the samples were filtered through Whatman filter paper 42 and analyzed on a colorimeter for F^- analysis (Dehbandi *et al*., 2017). For calibration of the colorimeter standards of 20, 40, 60 and 100 ppm were prepared and analyzed on the colorimeter.

2.10 Greenhouse Pot Experiment under Deficit Irrigation System

A pot experiment was conducted for determining the plant production under water stress. The experiment was conducted under 40% 70% and 100% irrigation considering the water-holding capacity of the soil. Approximately, 450 g of air-dried incubated soil was taken in each pot. Seven seeds of *Zea mays* were sowed in three pots at each irrigation rate. When seedlings appear, the thinning of plant was carried out for ensuring the constant seedling in each pot. Each irrigation rate was performed in triplicate along with control (Williams *et al*., 2002). The description of the irrigation rate is given in table 2.1. Variation in the physiological changes of *Zea mays* plants was recorded during 40 days of the experiment.

Table 2.1: Description of irrigation rates for pot experiment.

S. No.	NBC rate	Irrigation Percentage		
		40%	70%	100%
		Irrigation water (mL)		
1	0.5%	25.2	44.1	63
2	1%	25.2	44.1	63
3	2%	25.2	44.1	63

2.11 Carbon Dioxide Efflux Determination

Efflux of carbon dioxide (CO_2) was measured by taking three different rates of NBC i.e. 0.5%, 1% and 2% in triplicates with control (without NBC) and blank (without soil and NBC). Air-dried 250 g soil was taken in a polyethylene container and DI was added according to the water holding capacity of soil. Then, a 10 ml solution of 1 N sodium hydroxide (NaOH) was taken into vials and fixed in each container. After proper labelling of each container, all the containers were kept in the dark. The carbon dioxide release during the incubation period was trapped by the NaOH solution and measured by the gravimetric titration method. The vials having NaOH solution were taken out of each container. Then, 5 ml barium chloride solution plus 2-3 drops of phenolphthalein indicator were added and titrated against 1 M hydrochloric acid (HCl). The first reading was determined after 1 h and then after 1, 2, 3, 5, 7, 15 and 30 days. The efflux of CO_2 was calculated as the rate of CO_2 (mg C g^{-1} soil day^{-1}). After that, cumulative evolved CO_2 was determined in g Kg^{-1} soil. The entire incubation period was consisting of 193 days (Saleem *et al*., 2022).

2.12 Plant Analysis

After harvesting, root and shoot lengths of plants were measured. The plants were then subjected to determine the fresh weight, followed by dry weight, after drying in a hot air oven at 40 °C. After drying, each plant biomass was grounded and the digestion of all plant samples was carried out by the wet-acid digestion method (Glaubig and Poth, 1993). 0.25 g of each plant material and 10 ml mixture of nitric acid and perchloric acid with a 2:1 ratio was added to the digestion flask and placed on a hot plate at 100 °C. Gradually all the plant material dissolved in the acid mixture. The endpoint was determined when dense white cloudy fumes appear in the digestion flask. The flask was then removed from the hot plate, allowed to cool down the sample, and made the volume up to 50 ml. The samples were then filtered by Whatman filter paper 42 and stored in polyethylene storage bottles with proper tagging. Each sample was analyzed for total phosphates, potassium and calcium content (Estefan 2013). Detail of each protocol is mentioned in Annexure 1.

2.13 Total Fluoride Contents of Plants

To assess the total fluoride content in the plant, a 0.5 g shade-dried and grounded plant sample was mixed with 6 ml NaOH solution (670 g pallets of NaOH in 1L) in a ceramic crucible. All the samples were first placed in a drying oven at 150 °C for 1 h. After the solidification of NaOH, all the samples were placed in a muffle furnace at 600ºC for 30 min. After cooling the samples, 10 ml DI water was added for the dissolution of solidified NaOH. Then, 8 ml conc. hydrochloric acid (HCl) was added, and the pH was adjusted to 8-9. Then, samples were transferred to a 100 ml volumetric flask for making volume with DI water, and then filtered all samples through Whatman filter paper 42. All the samples were run on a colorimeter and analyzed for the total fluoride content of the plant (Dehbandi *et al.,* 2017; McQuaker and Gurney, 1977). The uptake of F^- by plants was assessed by calculating the bioconcentration factor (BCF). The equation used for the calculation of BCF is as follows (Yan *et al*., 2022):

$$\text{Bioconcentration factor (BCF)} = (C_{shoot} / C_{soil})$$

2.14 Statistical Analysis

Statistical analysis was performed including one-way ANOVA and two-way ANOVA by using Statistics 8.1 for determining the significance difference in analytical data. Graphs and figures were designed using Origin lab 2019b.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Characteristics of NBC

3.1.1 Proximate and Physiochemical Properties

Physiochemical properties and proximate analysis results of NBC are listed in Table 3.1. The yield of NBC (76±0.25%) was comparatively higher than generally reported biochars because of the hard and inorganic nature of the cow bones from which it was derived (Tomczyk *et al*., 2020). The proximate analysis revealed high ash contents (78.69±1.40%) and relatively low mobile (9.10±0.35%) and resident (9.14±1.72%) matters, which further confirmed the inorganic and stable configuration of the NBC. Patel and his colleagues also detected high ash content in bone char (Patel *et al*., 2015). Moreover, high pyrolysis temperature (500°C) and organic matter combustion residues also contributed to the high ash content in NBC (Tomczyk *et al*., 2020).

Table 3.1: Proximate analysis and physiochemical properties of nano-bonechar (NBC).

Parameters	Results
Yield (%)	76.00±0.25
Moisture (%)	3.07±0.67
Mobile matter (%)	9.10±0.35
Ash content (%)	78.69±1.40
Resident matter (%)	9.14±1.72
pH	8.22±0.02
EC (dS m^{-1})	4.01±0.01
Na^+ (mg kg^{-1})	578±0.01
K^+ (mg kg^{-1})	264±0.05
PO_4^{-3} (mg kg^{-1})	435±0.06
NO_3-N (mg kg^{-1})	3.8±0.01
Organic matter (%)	3.00±0.43

The pH of NBC was 8.22±0.02, which showed the release of inherent alkaline matter during pyrolysis of cow bones. Alkali salts are generally separated from the organic matrix in the feedstocks during pyrolysis, consequently giving biochars an alkaline nature (Rajapaksha *et al*., 2019). The EC of NBC was 4.01±0.01 dS m^{-1}, which indicated the presence of soluble salts in ionic forms. Earlier, the value of EC was also reported in the same range for cow bone char (Amin, 2020). The high EC value was associated with the presence of bioavailable Na^+, K^+, PO_4^{-3}, and NO_3-N, in relatively large amounts in the NBC, i.e. 578±0.01, 264±0.06, 142±0.02, and 3.8±0.01 mg Kg^{-1}, respectively (Table 3.1). The organic matter in NBC was 3.00±0.43%, which could be a good source of organic carbon in the soil.

The physiochemical analysis results revealed that the NBC exhibited mainly a stable inorganic material with large amounts of soluble nutrient ions essential for plant growth.

3.1.2 Particle Size, Crystallinity, and Mineralogy

The average particle size distribution of the NBC was measured by using the zeta sizer. Results are shown in Fig. 3.1a. The highest peak with a relative intensity of 72% showed that particles of 10 nm size were mainly distributed in the NBC. The other particles distributed in the NBC were 306 nm (25%) and 5400 nm (2%). The particle size distribution confirmed the nano-scale size of the NBC. The presence of larger particles might be due to the agglomeration of nano-particles (Ghoshal & Singh, 2022).

XRD was performed to observe the mineralogy and crystalline nature of the NBC. XRD pattern revealed that the crystal structure of the NBC corroborates mainly with the XRD pattern of hydroxyapatite, which is thermodynamically stable and has lower solubility (Fig. 3.1b). The diffraction peaks at 25.8°, 32.1°, 39.95°, 47.15°, 49.4°, and 53.05° were consistent with the crystal structure of hydroxyapatite i.e., (002), (211), (130), (222), (215) and (004) (JCPDS card # 82-1943), respectively (Shahid *et al*., 2020). The peaks observed at 16.9°, 29.25° and 63.8° were related to calcium carbonate (Maeda *et al*., 2019; Shahid *et al*., 2020). Based on the Scherrer equation, the average crystallite size was 10.34 nm for 002, 211, 130, 222, 215, and 004 miller indices (Sahu & Samanta, 2022). The results of XRD are in accordance with the zeta size particle distribution results.

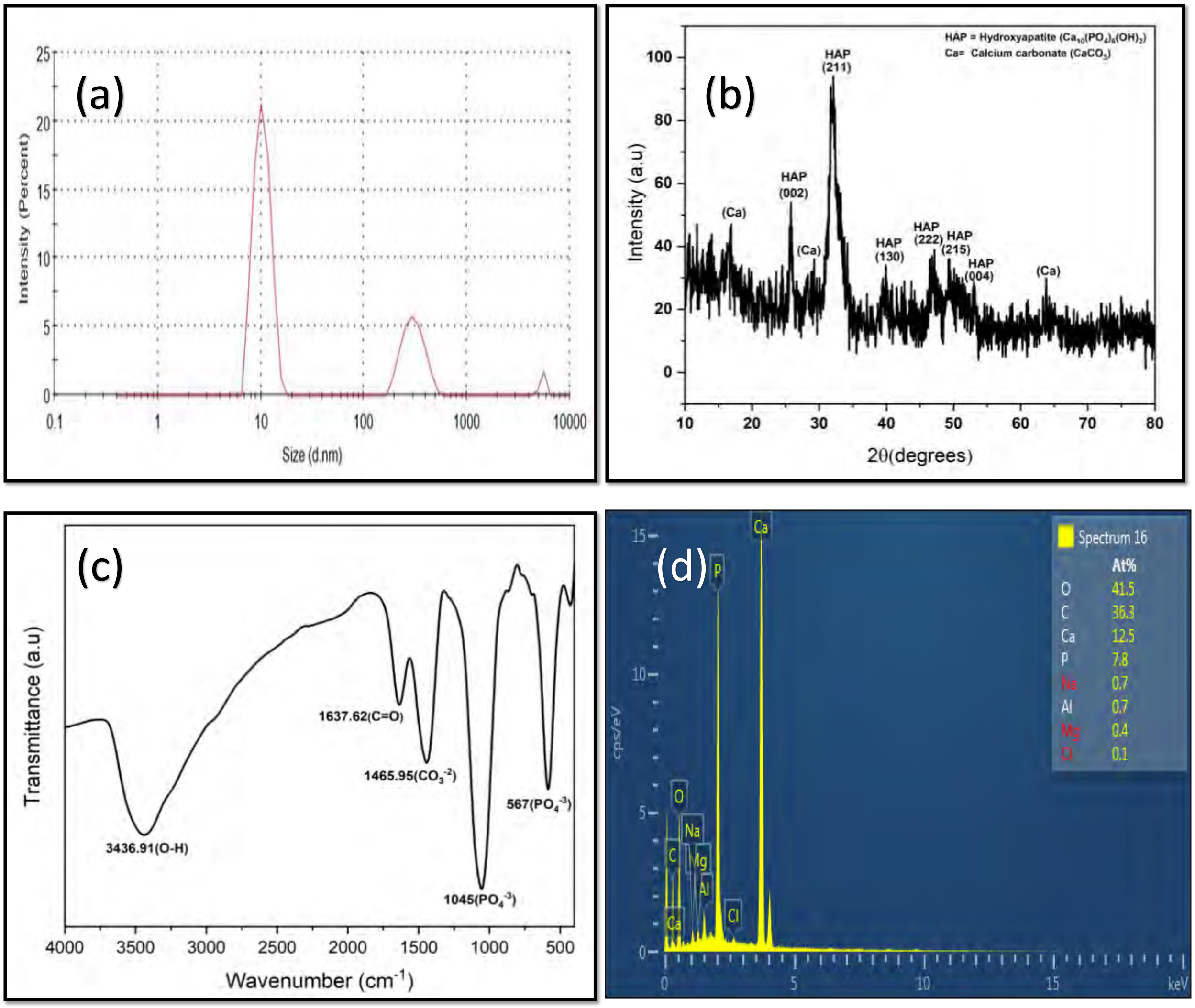

Fig. 3.1: Particle size distribution (a), XRD (b), FTIR (c), and EDX (d) of the nano-bonechar (NBC).

3.1.3 Functional Chemistry and Elemental Composition

The presence of surface functional groups on the NBC was assessed through FTIR spectroscopy and the results are shown in Fig. 3.1c. The bands observed at 3436.91, 1637.62, 1465.95, 1045 cm^{-1}, and 567cm^{-1} reflected the characteristics of hydroxyapatite in the NBC (Shahid *et al*., 2020; Ferreira *et al*., 2022). The first vibration stretch at 3436.91 cm^{-1} revealed the presence of the O-H functional group (Cruz-Briano *et al*., 2021). The band at the second stretch corresponding to the symmetrical mode of the vibration at 1637.62 cm^{-1} (C=O group) shows the presence of organic matter in NBC (Biswas *et al*., 2021; Mei *et al*., 2022). The band at 1465.95 cm^{-1} shows the asymmetric vibration stretch between the carbon and oxygen can be associated with the CO_3^{2-}

group (Shahid *et al*., 2019; Mei *et al*., 2022; Ferreira *et al*., 2022). The sharp bands at 1045 cm^{-1} and 567cm^{-1} showed the presence of symmetric and asymmetric bending modes of vibration stretch between phosphorus and oxygen in (PO_4^{-3}) (Mei *et al*., 2022; Ferreira *et al*., 2022).

EDX spectra were used for determining the elemental composition of the NBC as shown in Fig. 3.1d. The results showed the effective concentrations of O (41.5%), C (36.3%), Ca (12.5%), P (7.8%), Na (0.7%), and Mg (0.4%) in the NBC. These elements could add nutritional value to the NBC for its use as a soil amendment.

From the above results (XRD, FTIR, and EDX), there is sufficient evidence that the chemical makeup of the NBC was similar to that of hydroxyapatite [$Ca_5(PO_4)_3OH$] (Shahid *et al*., 2019).

3.1.4 Surface Morphology

Porous structure and shape of the NBC were investigated by taking SEM and TEM images, respectively. SEM photograph (Fig. 3.2a) revealed the rough texture of the NBC nanoparticles having irregular geometry with random size distribution. This random size distribution is due to agglomeration of nanoparticles, most of them were less than 100 nm in size. It can also be observed that NBC has a nano-granular structure that could provide a larger surface area and favors the complexation reactions of NBC (Shahid *et al*., 2020). TEM photograph showed that the particles of NBC were polycrystalline and irregular polygonal in shape (Fig. 3.2b). The results of the TEM are in accordance with the SEM. Both morphological analyses confirmed the distinctive agglomeration of the NBC, which is consistent with the synthetic crystal structure of hydroxyapatite (Camargo *et al*., 2012; Shahid *et al.,* 2019; Arnich *et al*., 2003).

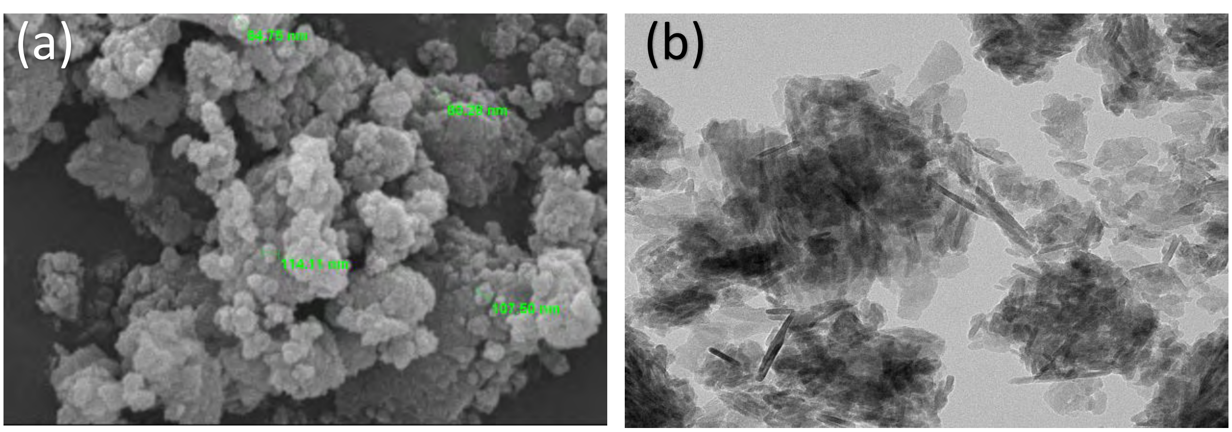

Fig. 3.2: SEM (a) and TEM (b) photographs of nano-bonechar (NBC).

3.2 Soil Properties

The F^--contaminated soil was characterized for basic parameters and results are listed in Table 3.2. The texture of the soil was silt loam with clay, silt, and sand at 26.4, 66.0, and 7.6%, respectively. The soil's water holding capacity was 126 ml per 250 g of soil. The pH of the soil was 7.57±0.03, and EC of the soil was 0.034±0.01 dS m^{-1}, which could be due to the presence of high contents of NaCl (6.86±0.05%) in the soil. Soil organic matter was 1.17±0.02%, and the bioavailable Na^+, K^+, PO_4^{3-}, and NO_3-N were 190±1.43, 438±2.5, 36.78±4.5, and 24.67±2.3 mg kg^{-1}, respectively. The total F^- content was 450 mg kg^{-1} which was higher than for a normal soil (350 mg kg^{-1}) (Hong *et al.*, 2016).

Table 3.2: Physiochemical properties of soil.

Parameters	Results
pH	7.57±0.03
EC (dS m^{-1})	0.034±0.01
NaCl (%)	6.86±0.05
Organic matter (%)	0.47±0.02
PO_4^{3-} (mg kg^{-1})	36.78±4.50
NO_3-N (mg kg^{-1})	24.67±2.30
Na^+ (mg kg^{-1})	190±1.43
K^+ (mg kg^{-1})	438±2.5
Clay (%)	26.4
Slit (%)	66.0
Sand (%)	7.6
Texture	Silt loam
Total fluoride (mg kg^{-1})	450
Water holding capacity (mL 250 g^{-1})	126
Field capacity (mL 250 g^{-1})	63

3.3 Effect of NBC on F^- Bioavailability in Soil

To evaluate the impact of the NBC on the bioavailability of F^-, water-soluble and exchangeable F^- contents were measured in the un-amended and amended soils because both these forms can be taken up by plants. The results are shown in Fig. 3.3. The un-amended soil (control) with 0% application of the NBC had a high concentration of 7.94 and 20.66 mg kg^{-1} of water-soluble and exchangeable F^- contents, respectively. However, the NBC amended soil at 0.5, 1, and 2% application rates showed much lesser contents of water-soluble (2.02±0.22,1.74±0.06 and 0.74± 0.03 mg kg^{-1}, respectively) and exchangeable F^- (8.00±0.19, 4.46±0.15, and 2.22±0.10 mg kg^{-1}, respectively). The 2% application rate was more effective in decreasing the water-soluble and exchangeable F^- contents.

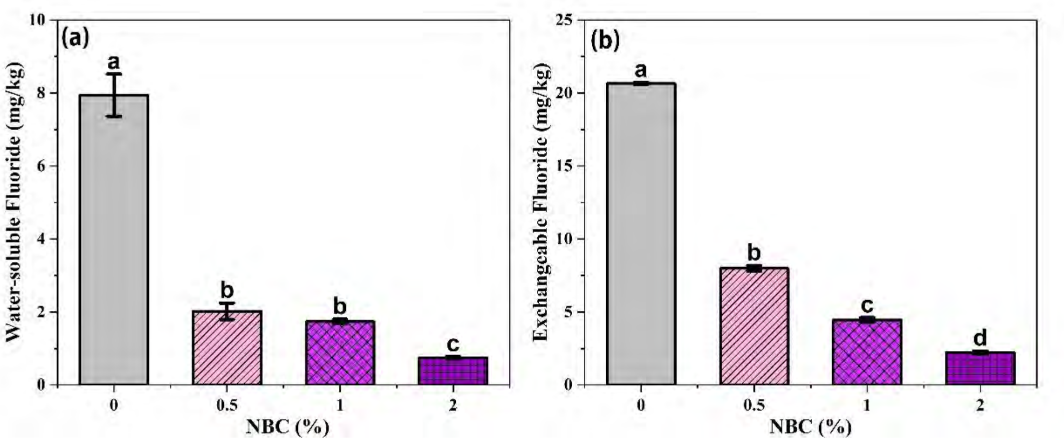

Fig. 3.3: Water-soluble (a) and exchangeable (b) contents of F^- in the incubated soil amended with nano-bonechar (NBC) at different application rates.

3.4 Mechanism of F^- Immobilization in Soil

The decrease in the bioavailability of F^- revealed that the incorporation of NBC induced F^- immobilization in the soil. The hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] mineral present in the NBC may have contributed to the immobilization of F^- (Sawangjang *et al.,* 2021). Additionally, nano-size particles, large surface area, and the presence of nano-hydroxyapatite in the NBC have the potential for binding F^- in the soil (Gan *et al*., 2021). There is sufficient evidence that the O-H functional group of the hydroxyapatite in the NBC could be exchanged with F^- ion in the soil resulting in the formation of fluorapatite [$Ca_{10}(PO_4)_6F_2$] having low solubility in soil solution. This can be explained by the following equation (Sawangjang *et al.,* 2021):

$$Ca_{10}(PO_4)_6(OH)_{2\,(s)} + 2F^-_{(aq)} \rightarrow Ca_{10}(PO_4)_6F_{2\,(s)} + 2OH^-_{(aq)}$$

Gan *et al*. (2021) also reported that the surface O-H of hydroxyapatite being isomorphically substituted for F^- ions in the crystal lattice is likely to be the primary mechanism of F^- immobilization in soil. Another plausible reason of F^- immobilization in soil could be the complexation between F^- and Ca^+ as calcium fluoride (CaF_2), which is insoluble in soil solution. As indicated by the XRD analysis that the NCB contains substantial contents of $CaCO_3$, which upon dissociation in soil-water forms a complex of CaF_2 with F^- ion according to following reactions (Jeong *et al*., 2019):

$$CaCO_{3(s)} + H_2O(aq) \leftrightarrow Ca(OH)_{2\,(aq)} + CO_{2\,(g)}$$

$$Ca(OH)_{2(aq)} + 2F^- \leftrightarrow CaF_{2(s)} + 2OH^-_{(aq)}$$

Overall, enhanced calcium contents in the NBC favor the immobilization of F^- by forming insoluble complexes such as fluorapatite [$Ca_{10}(PO_4)_6F_2$] and fluorite (CaF_2).

3.5 Effect of NBC on Incubated Soil Properties

The quality of NBC amended soil was assessed by analyzing pH, EC, water holding capacity, organic matter, Ca^{2+}, Na^+, K^+, PO_4^{3-}, and NO_3-N. No significant difference was observed in soil pH between un-amended and amended soil at different application rates (Fig. 3.4a).

The NBC at all application rates showed a significant increase in the soil EC as compared to the control (Fig. 3.4b), which could be due to the high EC value (4.01 dS m^{-1}) of the NBC. All the application rates of NBC increased the soil's water holding capacity than that of the control (Fig. 3.4c). An increase in soil's water holding capacity could be attributed to porous structure of the NBC as well as changes in the soil structure (such as particles aggregation) (Rasa *et al*., 2018). Results showed a direct relationship between the application of NBC rates and soil organic matter (Fig. 3.4d). As the rate of NBC increases the organic matter also increases gradually, which is because of the high contents of organic matter (3%) in the NBC.

Effects of the NBC treatment on the availability of cationic (Ca^{2+}, Na^{+}, and K^{+}) and anionic (PO_4^{3-} and NO_3-N) elements are shown in Fig. 3.5 and 3.6, respectively. A significant and gradual increase in the bioavailability of Ca^{2+} and Na^{+} with the increase in application rates of NBC was observed as compared to the control soil (Fig. 3.5a,b). However, the 2% NBC-treated soil showed the highest content of K^{+} in comparison with other treatments (Fig. 3.5c).

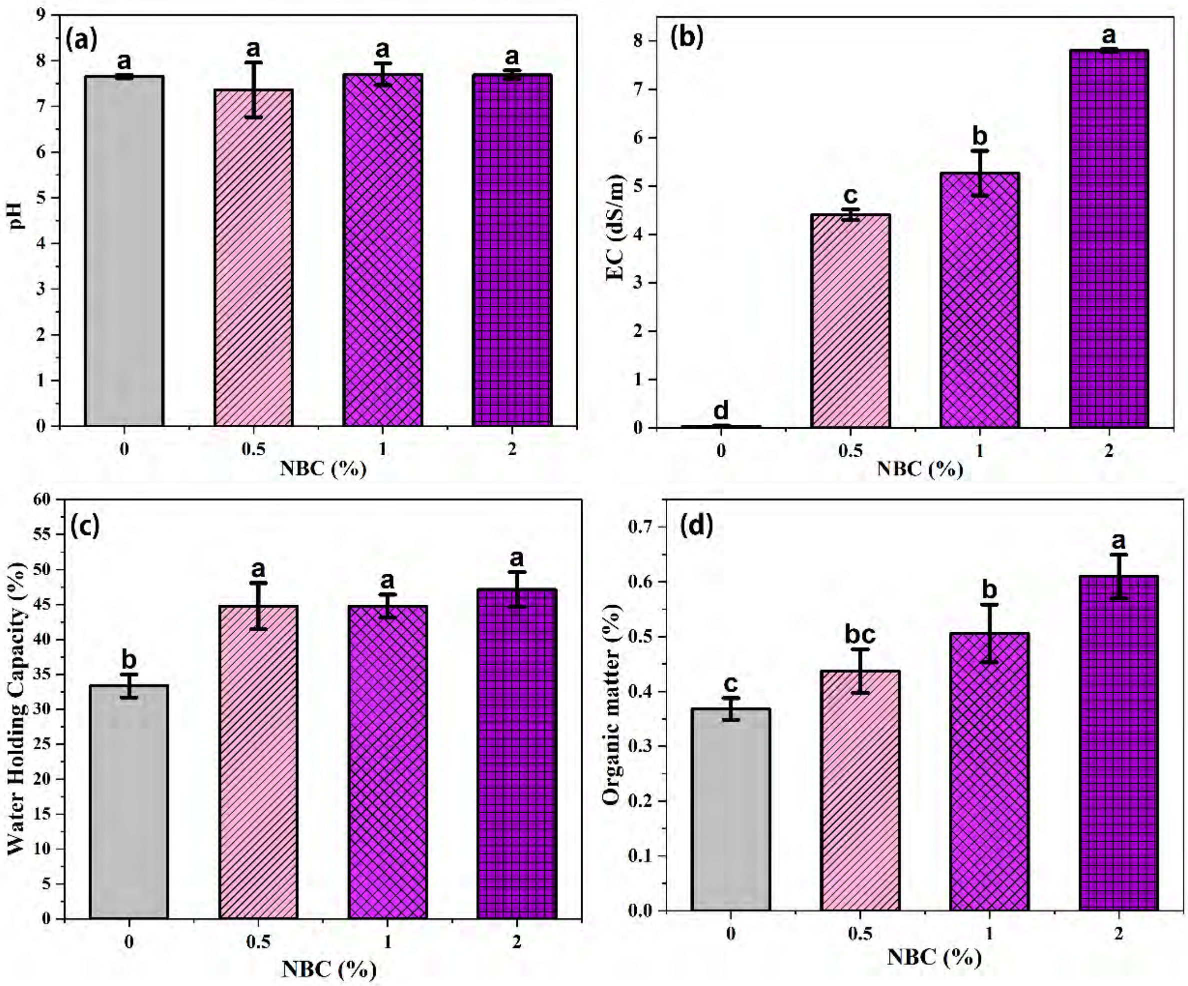

Fig. 3.4: Physiochemical properties: pH (a), EC (b), water holding capacity (c), and organic matter (d), of the incubated soil amended with nano-bonechar (NBC) at different application rates.

Similar results were observed for PO_4^{3-} where maximum contents were observed in 2% treated soil (Fig. 3.6a). Contrarily, the NO_3-N significantly decreased in the NBC-amended soil (at all application rates) as compared to the un-amended soil (Fig. 3.6b), which could be due to the adsorption of nitrates by the NBC (Fatima *et al.*, 2021).

It was clear from the results of amended and un-amended incubated soils that the NBC application improved the soil quality by enhancing the water retention capacity, organic matter, and the availability of essential elements (Ca^{2+}, Na^+, K^+, and PO_4^{3-}). Hence, it can be concluded that the NBC could be used successfully as an effective soil amendment.

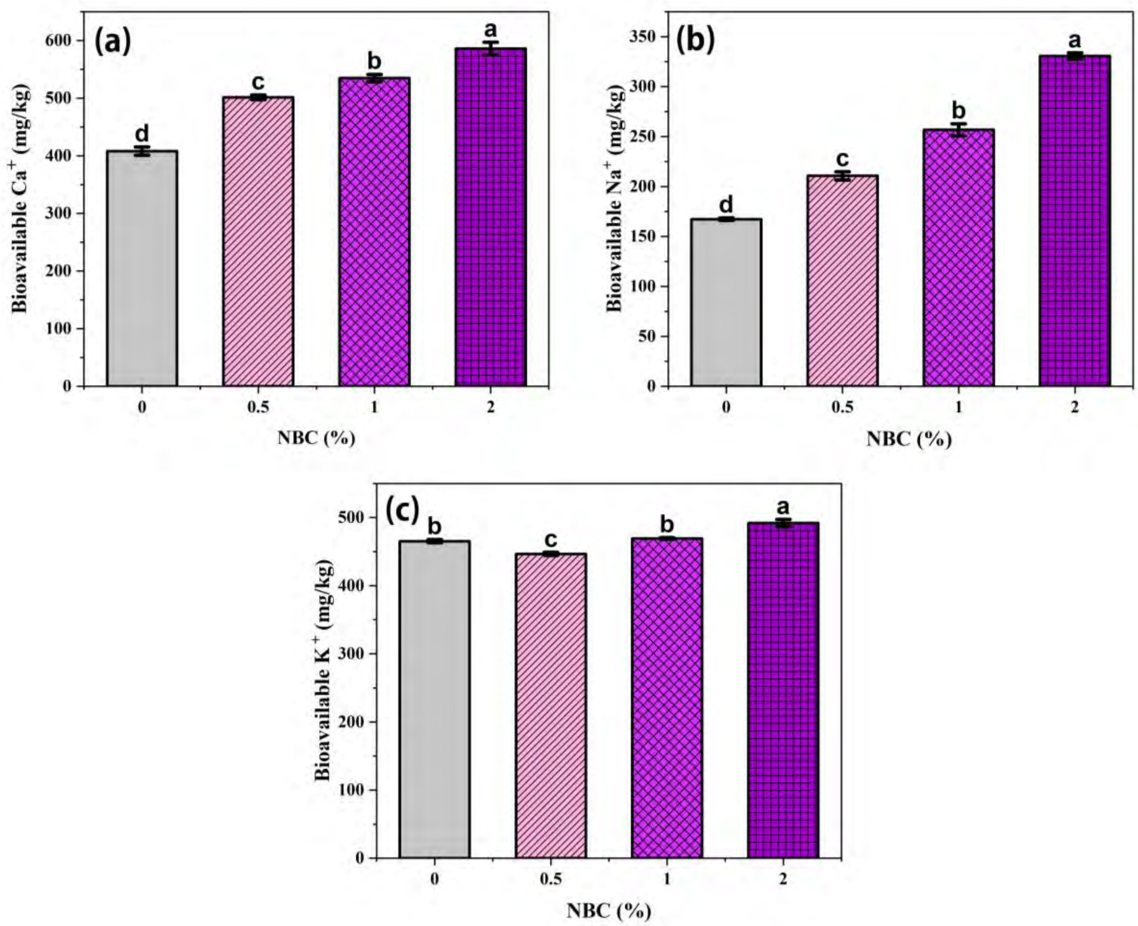

Fig. 3.5: Bioavailable cations: Ca^{2+} (a), Na^+ (b), and K^+ (c), in the incubated soil amended with nano-bonechar (NBC) at different application rates.

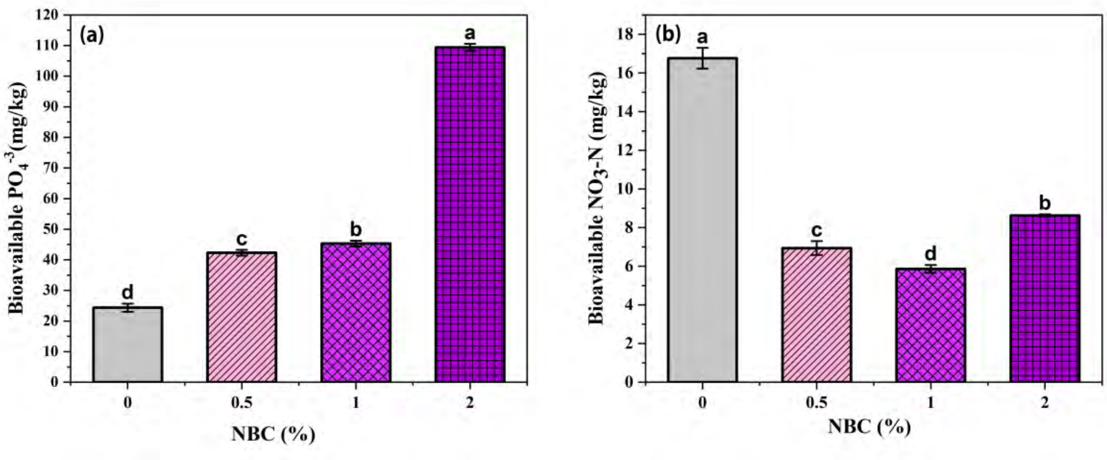

Fig. 3.6: Bioavailable anions: PO_4^{3-} (a) and NO_3-N (b), in the incubated soil amended with nano-bonechar (NBC) at different application rates.

3.6 Role of NBC in Climate Smart Agriculture

3.6.1 Effect of NBC on Plant Productivity under Deficit Irrigation System

One of the goals of achieving a smart agriculture approach is to increase plant yield. The potential of NBC towards goals of climate-smart agriculture (CSA) was assessed by conducting pot experiments under deficit irrigation. *Zea mays* L. was grown under three NBC applications rate including 0% NBC (control), 0.5%, 1%, and 2% respectively. Each rate of the NBC was subjected to 40%, 70%, and 100% irrigation rates. Regarding plant productivity, the results revealed that there was a significant increase in the fresh and dry weights of plants at all three employment rates of the NBC in comparison with the control (Fig. 3.7a,b).

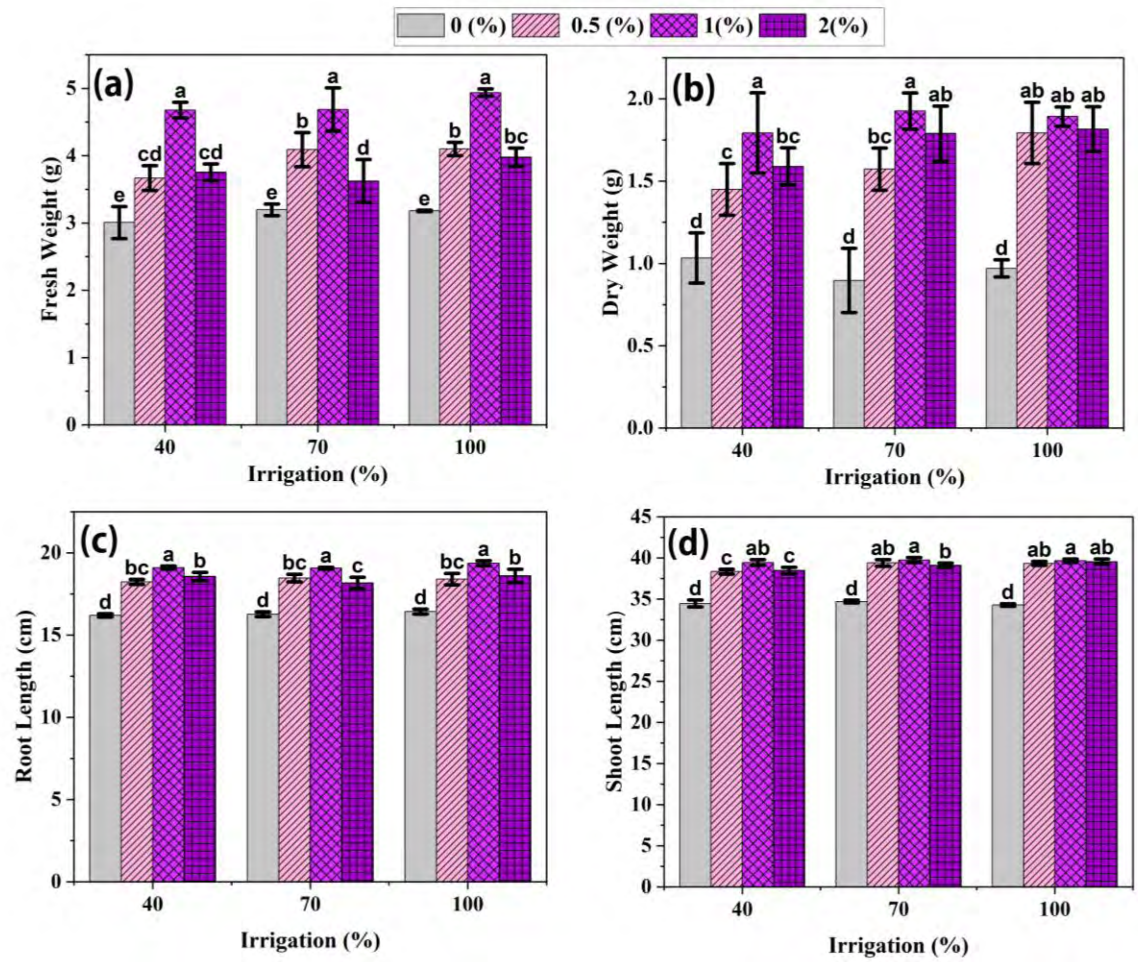

Fig. 3.7: Fresh weight (a), dry weight (b), root length (c), and shoot length (d) of *Zea mays* plants grown in soils amended with nano-bonechar (NBC) at different application rates.

Results also indicated that the NBC aids the increase in root and shoot lengths of maize plants as compared to the control (Fig. 3.7c,d). These productivity results showed that the NBC has a positive effect on improving maize growth. The presence of phosphates in NBC improved the growth by enhancing the bioavailability of phosphorus to plants. The release of phosphorus acted as a natural fertilizer for the maize plant (El Refaey *et al*., 2022). Liu and his colleagues also reported similar results (Liu *et al.,* 2022). Comparing the result of four irrigation rates, the best result was shown by 1% NBC under 70% irrigation in terms of fresh and dry weights, i.e. 4.68±0.32 g and 1.92±0.11 g, respectively. Also, at 100% irrigation, 1% NBC showed a significant increase in fresh weight (4.93±0.05 g) and dry weight (1.89±0.05 g) followed by the 2% NBC (fresh weight 3.97±0.13 g and dry weight 1.81±0.11 g) as compared to control (Fig. 3.7a,b).

NBC aids the increase in root and shoot length as compared to the control (Fig. 3.7c,d). Due to pyrolysis, a high organic matter in NBC is also responsible for magnifying nutrient delivery to plants which ultimately results in the elevation of plant biomass production. The results showed the adaptation of *Zea mays* plants towards a deficit irrigation system in the presence of NBC.

The existence of hydroxyapatite in nano-bonechar, which is a mineral-rich composite, has become one of the reasons for increasing the yield and productivity of *Zea mays* (Raguraj *et al*., 2020). The presence of a huge concentration of minerals like phosphates and calcium carbonates in NBC provides a nutrient-boosted environment for soil and augmented the uptake of nutrients and yield (Glaser *et al*., 2019; Ozyhar *et al* ., 2022; Lee *et al*., 2022). Results showed that NBC has a positive effect on the weight and length of *Zea mays* that enhanced plant yield under deficit irrigation system. This implies that the soil amended with NBC enhanced plant productivity with the conservation of water and adapting to the arid region.

3.6.2 Effect of NBC on Soil CO_2 Efflux Rate

The reduction of greenhouse gas emissions is one of the pillars of smart agriculture because it elevates global temperature leading to global warming. Variations in soil respiration with treatments of NBC throughout 92 days of incubation were traced as CO_2 efflux rate. The incubation experiment was performed with three treatment rates of NBC along with blank in triplicates. After day 1 of incubation, a high CO_2 efflux rate was observed, which might be due to readily available organic matter (Fig. 3.8a). The 0.5% NBC rate showed a slightly high CO_2 efflux rate as compared to control and other treatments. After 92 days of incubation, all the treatment rates of NBC showed a decrease in emissions of CO_2 in comparison to the 1 day but emissions remain slightly low at 0% NBC rate (control) (Fig. 3.8b).

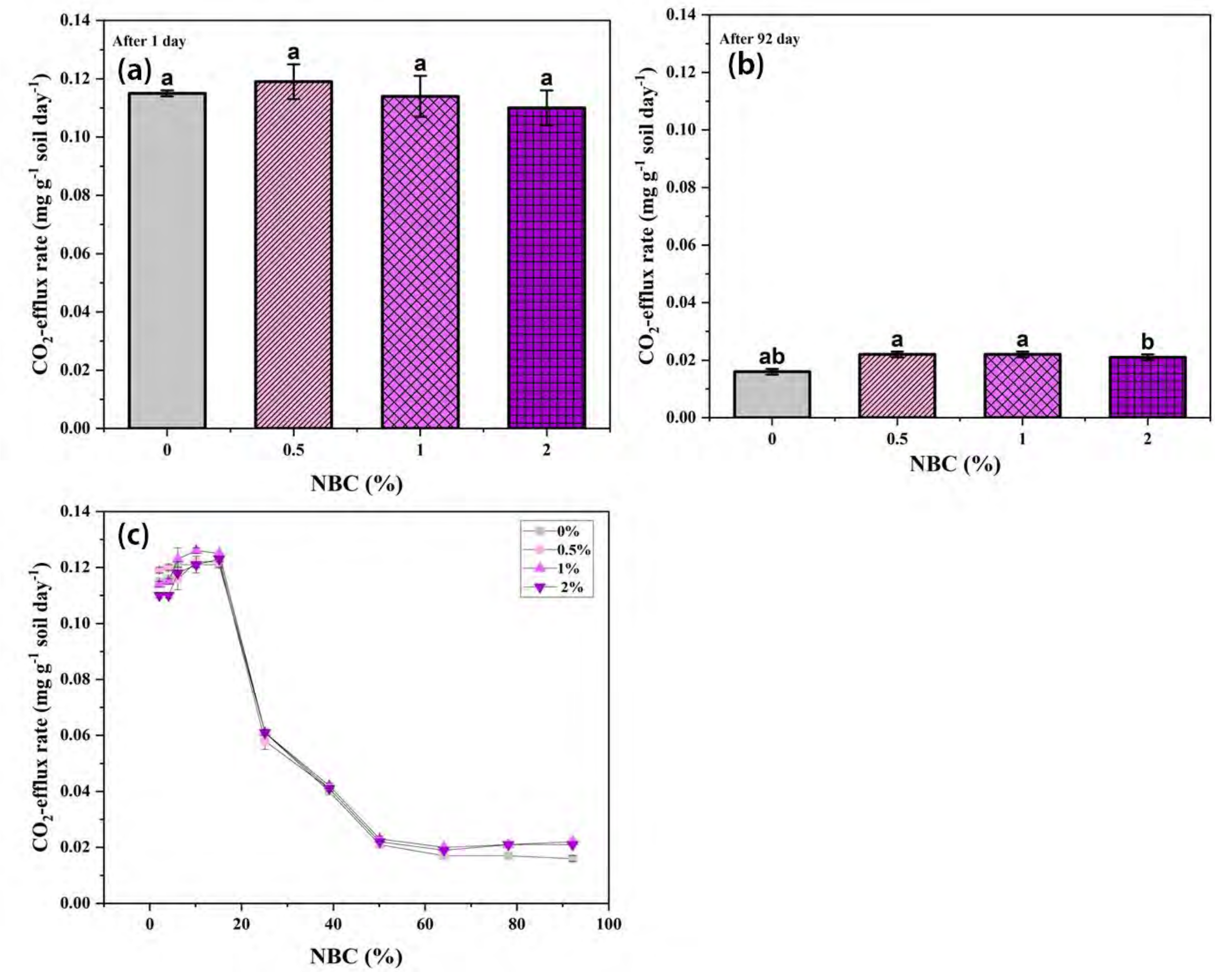

Fig. 3.8: Soil CO_2-efflux rate after 1 day (a), after 92 days (b), and throughout the incubation period (c) with 0%, 0.5%, 1%, and 2% NBC application rates.

The result suggested that at the end of the incubation period, the CO_2 efflux rate declined but slightly high emission was observed in all NBC-amended soil than in un-amended soil. The NBC increased the emission of CO_2 in the atmosphere. It could be due to the addition of organic matter in soil, provided by NBC, to microorganisms, consequently, enhancing the microbial activity due to nutrient availability (Saleem *et al*., 2022). This enhanced microbial activity resulted in high emission of CO_2 in amended soil as compared to unamended soil. It has been stated previously that bonechar with a high employment rate (2.5%) results in high emission of CO_2 efflux (Saleh *et al*., 2020). The results revealed a decline in CO_2 emission on 92nd day than on 1st day, as initial microbial activity was high due to the accessibility of biodegradable organic matter, which declineed steadily over time (Fig. 3.8c). Ardebili and his colleagues also reported that nano-biochars cause a high CO_2 efflux rate (Ardebili *et al.*, 2020).

3.6.3 Effect of NBC on Cumulative Soil CO_2-C

The effect of NBC on the organic C mineralization was recorded as cumulative CO_2-C. Statistical analysis showed that after the first day of incubation, the emissions of cumulative CO_2-C were marginally different in NBC-amended and un-amended soil (Fig. 3.9a).

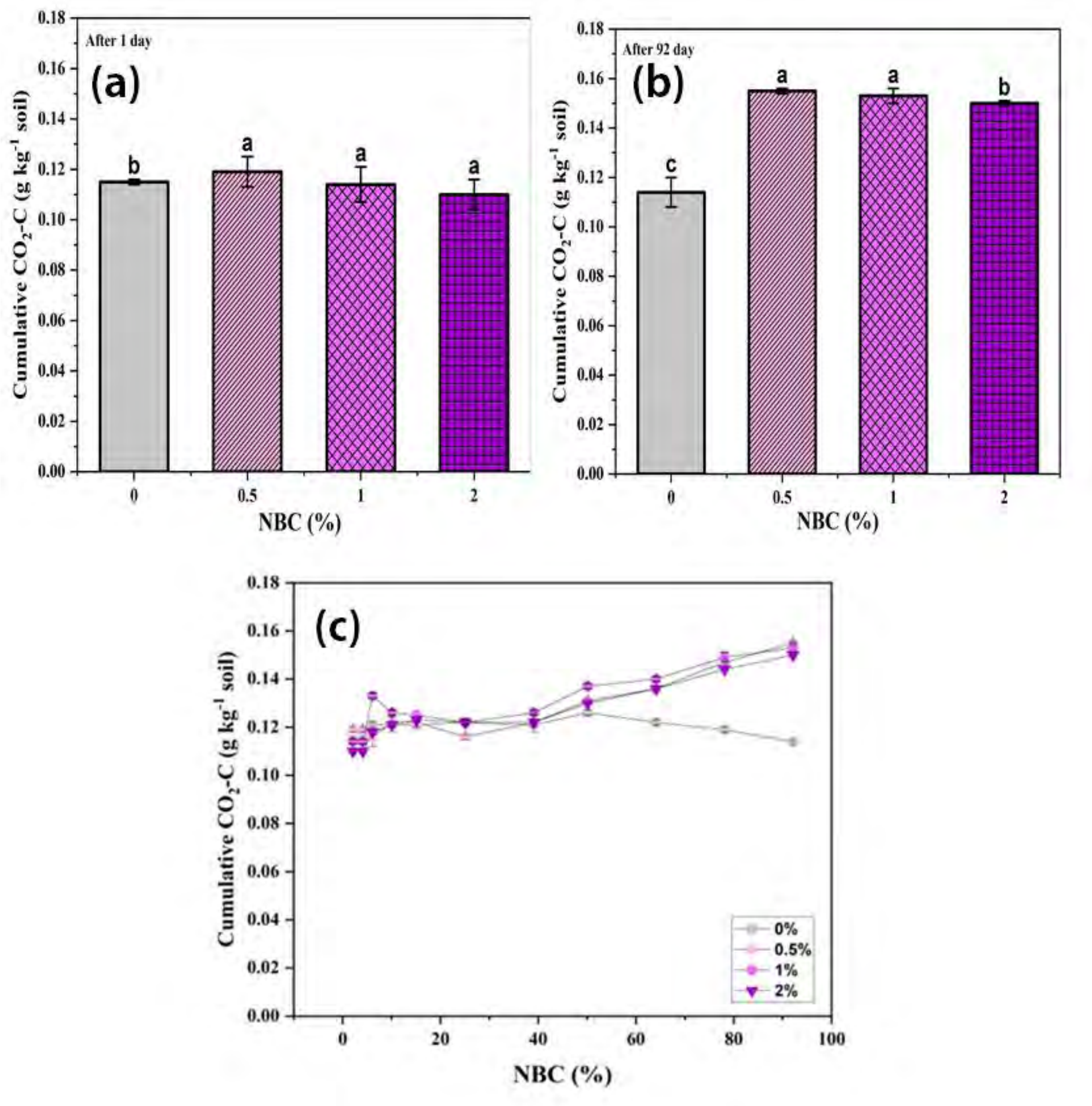

Fig. 3.9: Soil cumulative CO_2-C after 1 day (a), after 92 days (b) and throughout the incubation period (c) with 0%, 0.5%, 1%, and 2% NBC application rates.

This indicates that high pyrolysis temperature (500ºC) of NBC might result in the prevention of cumulative CO_2-C evolution from the soil. It has been reported earlier that biochar prepared at 700ºC prevents the emission of cumulative CO_2-C even after the first day of incubation (Saleem *et al*., 2022). At 92 days, an increase in cumulative CO_2-C was observed in NBC amended soils when compared with the control (Fig. 3.9b,c). This increase might be due to the high surface area of NBC owing to the presence of nano-sized particles, thereby enhancing the microbial activity and ultimately leading to the more emission of cumulative CO_2-C. Furthermore, the organic matter present in NBC is 3±0.43% i.e., two times higher than in the soil (1.17±0.02%). This twice increment of the organic matter in amended soil might be an additional reason for enhancing the emission of cumulative CO_2-C (Mohammed *et al*., 2022). A recent investigation showed that biochar prepared at 500 °C resulted in a high evolution of cumulative CO_2-C as compared to the biochar prepared at lower temperature (Saleem *et al*., 2022).

3.7 Effect of NBC on Total F^-, PO_4^{-3}, Ca^{+2}, and K^+ in Plants

To investigate the effect of NBC application on the total uptake of F^- and other nutrients in plants, a greenhouse pot experiment was conducted by growing maize plants. The results suggest that F^- bioavailability in plants was significantly decreased (Fig. 3.10a). The total F^-content was 322±7.3 mg kg^{-1}in *Zea mays* plants grown in un-amended soil, whereas only 44, 28, and 23 mg kg^{-1} F^- was observed in plants grown in 0.5%, 1%, and 2% NBC-amended soils. Moreover, the values of the bioconcentration factor were 0.06 for 0% (without NBC) and 0.02, 0.01, 0.006 for 0.5%, 1% and 2%, respectively (Fig. 3.11). The BCF values indicated a significant decrease in accumulation and uptake of F^-. Hence, NBC proved successful in providing an uncontaminated soil environment to plants. This showed that the presence of NBC helped in lowering the bioavailability of F^-, as confirmed by its immobilization in the incubated soil.

The effect of NBC on total PO_4^{3-}, Ca^{2+}, and K^+ in maize plants is shown in Fig. 3.10b,c,d. The PO_4^{3-} and Ca^{2+} contents were significantly higher in plants grown in amended soils than those of un-amended soil, and the result confirmed the direct relation with NBC application, as a substantial difference in the total PO_4^{-3} and Ca^{2+} contents can be seen between all three application rates of the NBC. The K^+ contents in maize plants gradually and significantly increased with the application rate of the NBC at 0.5%,whereas K^+ decreased with the increase in NBC application.

According to Graber et al. (2017), high concentration of biochar results in holding cations and binding from the soil solution.

Deficit irrigation system, however, did not show any significant effect of NBC treatment on above mentioned parameters.

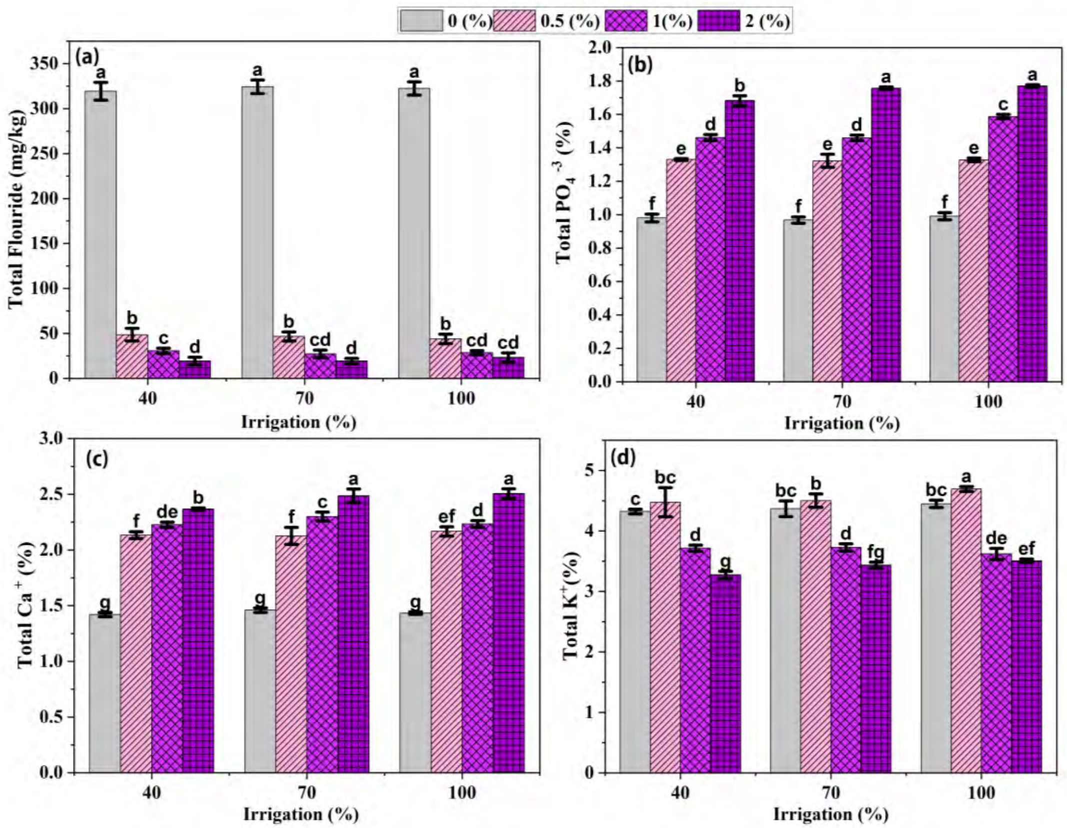

Fig. 3.10: Total F^- (a), PO_4^{3-} (b), Ca^{2+} (c), and K^+ (d) in shoots of *Zea mays* plants grown in soils amended with nano-bonechar (NBC) at different application rates.

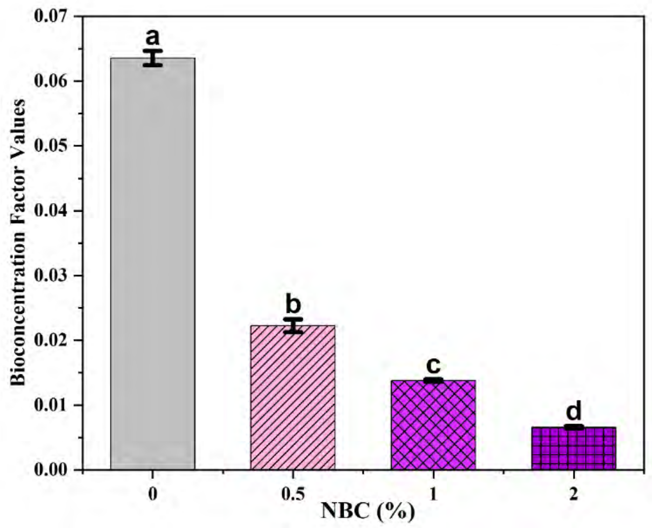

Fig. 3.11: Bioconcentration factor values of *Zea mays* for F^-.

Overall, the results showed decrease in the fluoride uptake and increase in the phosphates and calcium uptake by *Zea mays* plants grown in NBC-amended soil. This proved that the presence of NBC help in plant growth, yield, and productivity. The target of the experiment was to provide a polluted-free and climate-smart environment for plant growth. Results indicated that all application rates of NBC provided promising results.

3. 8 Assessment of Soil Quality

After harvesting *Zea mays* L., the soil was characterized for F^-, physicochemical properties, bioavailable cations, and anions, to assess the soil quality. Nano-bonechar particles contribute toward improving soil fertility and yield of the plant due to their unique physical and surface properties. The fluoride contents including water-soluble and exchangeable remained low in harvested soil, which showed that NBC amendment succeeded in immobilizing F^- (Fig. 3.12).

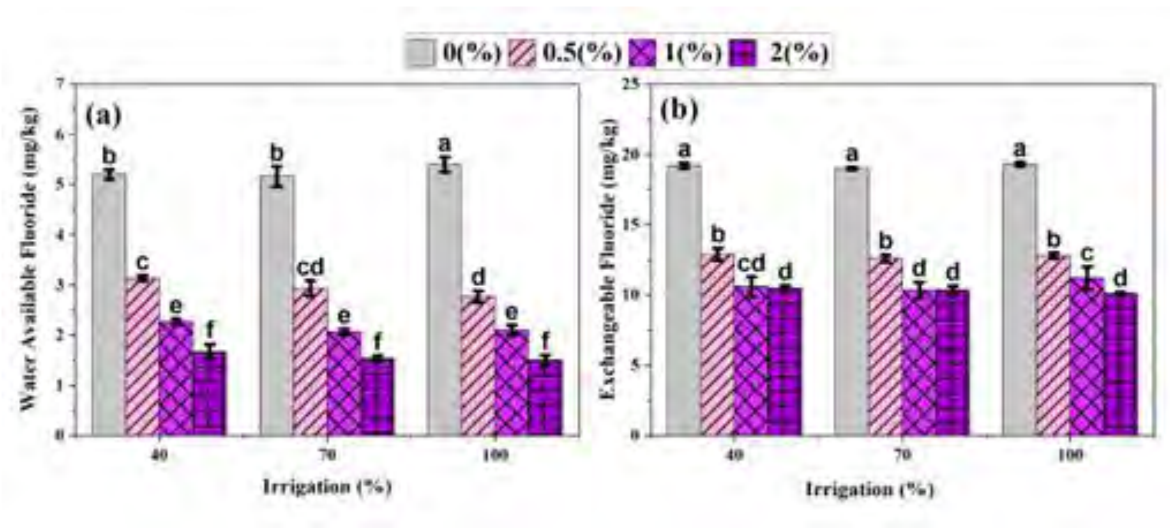

Fig. 3.12: Water soluble (a) and exchangeable (b) fluoride contents in harvested soil amended with nano-bonechar (NBC) at different application rates.

There was no significant difference observed in pH value in amended and un-amended soil (Fig. 3.13a). The EC increased as the rate of the NBC increased (Fig. 3.13b), which might be due to the presence of a huge concentration of minerals in the NBC. A similar trend was found regarding water holding capacity of the (Fig. 3.13c). As organic matter is the source of nutrients for microbes and plants, the results were positive because organic matter increased in all the amended soils. Nevertheless, 2% NBC with 70% irrigation showed the best results regarding organic matter (Fig. 3.13d).

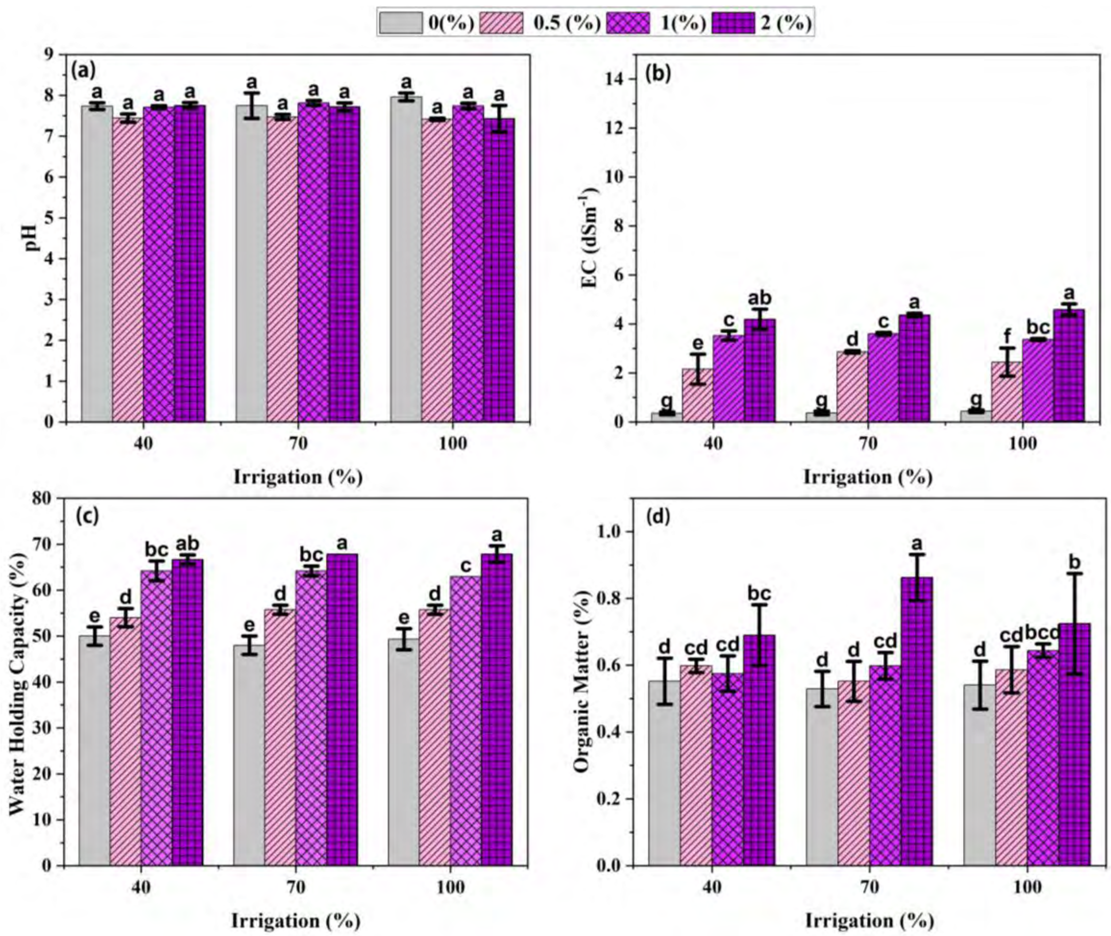

Fig. 3.13: Physiochemical properties of harvested soil: pH (a), EC (b), water holding capacity (c) and organic matter (d), amended with nano-bonechar (NBC) at different application rates.

Bioavailable cations including Ca^{+2}, Na^{+}, and K^{+} increased except by 0.5% and remained slightly below the control regarding K^{+} in all the irrigation rates (Fig. 3.14). Results indicated that there is a direct correlation between the concentration of phosphates and the employment rate of NBC as phosphate increased significantly in all the amended soil as compared to the un-amended soil (Fig. 3.15a). However, nitrate-nitrogen was found to decrease in amended soil as compared to the un-amended soil (Fig. 3.15b). This might be due to the adsorption of nitrate-nitrogen with NBC (Fatima *et al*., 2021).

Overall, results suggested that the concentration of NBC has more impact on physiochemical properties and enhancing nutrients regardless of the irrigation rate. This proves that NBC can provide nutrients under a deficit irrigation system.

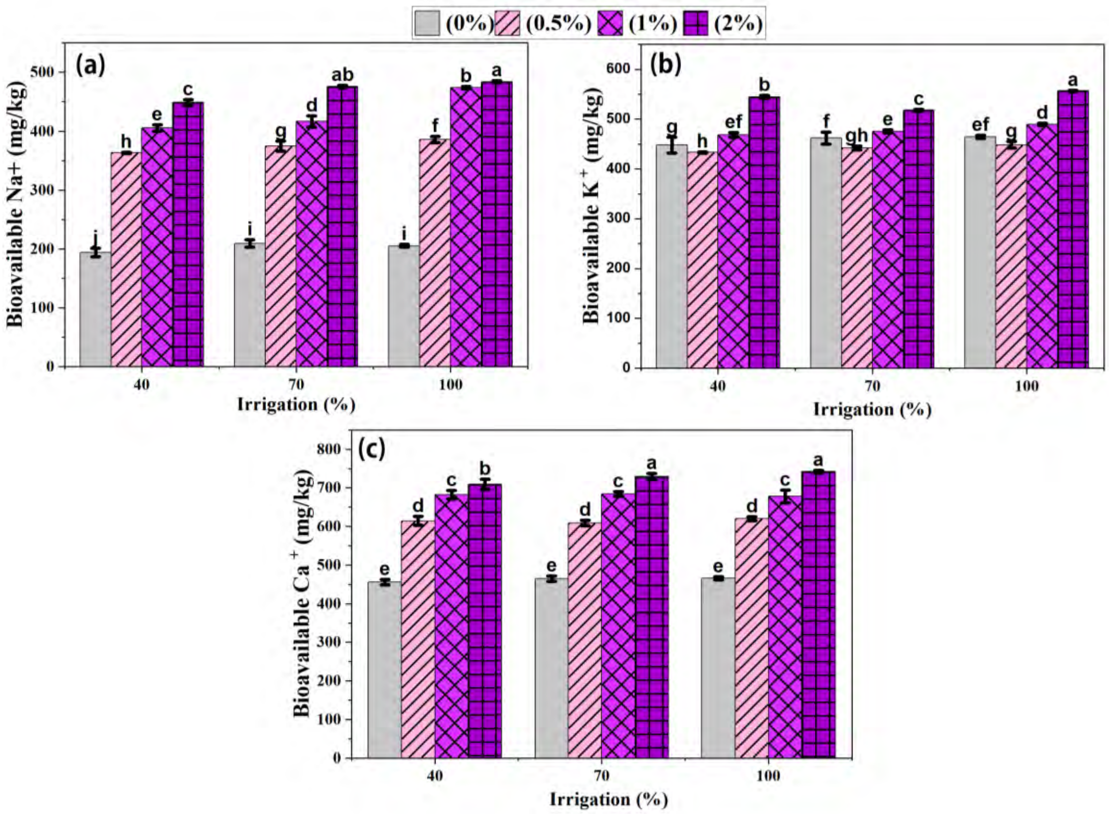

Fig. 3.14: Bioavailable cations: Ca^{2+} (a), Na^{+} (b), and K^{+} (c) in the harvested soil amended with nano-bonechar (NBC) at different application rates.

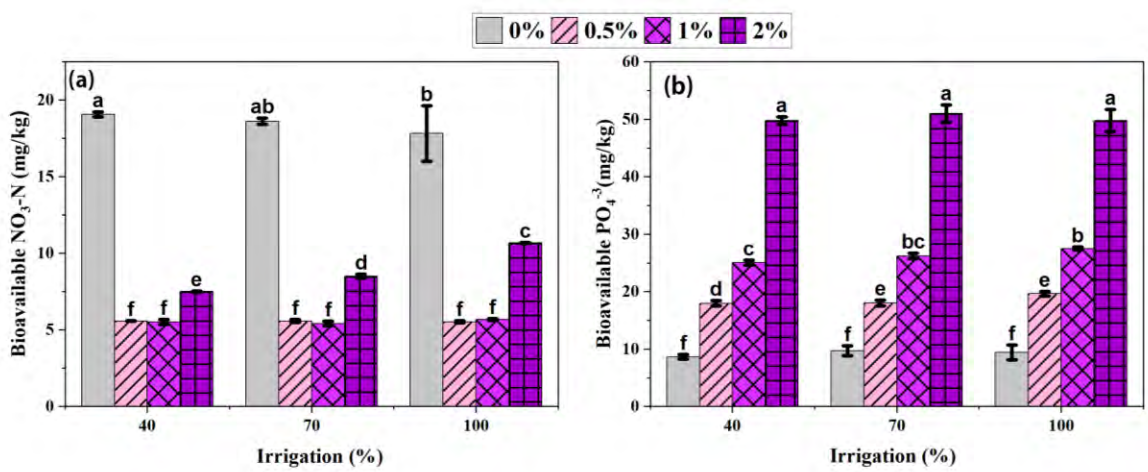

Fig. 3.15: Bioavailable anions: PO_4^{3-} (a) and NO_3-N (b) in the harvested soil amended with nano-bonechar (NBC) at different application rates.

Conclusions

The aim of this study was the production of nano-bonechar (NBC) from cow bones to immobilize fluoride in naturally contaminated soil and assess the potential of NBC to climate-smart agriculture. Results revealed that NBC successfully immobilized water-soluble and exchangeable fluoride content. Soil incubation experiment concluded that NBC effectively lowered the bioavailability of fluoride in soil due to complex formation, ion exchange, and adsorption. Due to threatening climate disasters globally, it is necessary to find out economic ways to cope with the issue of climate change. Therefore, a 92 days incubation test was carried out to determine the trend of CO_2 efflux rate and cumulative CO_2-C emission from the soil in the presence of NBC at three different rates. Due to the presence of high organic matter and microbial activity, the efflux rate of CO_2-C and cumulative CO_2-C emissions was higher in NBC-treated soil than in unmodified soil. A greenhouse pot experiment by growing *Zea mays* L. plants in NBC amended soils showed an increase in overall plant biomass. Results confirmed that NBC was effective in enhancing plant productivity, and water stress adaptation. It is concluded that NBC successfully attenuated F^- and showed positive effects on *Zea mays* L. productivity and nutrient enhancement. Therefore, it is recommended that NBC is an appropriate and economical soil ameliorant for recycling food waste and progressively significant practice for climate-smart agriculture under-limited resources.

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