UTILIZATION OF FUNCTIONALIZED BIOCHAR DERIVED FROM WATER HYACINTH *EICHHORNIA CRASSIPES* AS GREEN NANO-FERTILIZERS

BY

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CERTIFICATION

This is to certify that this work "Utilization of Functionalized Biochar Derived from Water Hyacinth *Eichhornia Crassipes* as Green Nano-Fertilizers" was carried out by Irewale, Adewale Tolulope (ACE-FUELS/22/PhD/14000128) in partial fulfilment for the award of the degree of Ph.D. in Nanotechnology from Africa Center of Excellence in Future Energies and Electrochemical Systems (ACE-FUELS), The Postgraduate School, Federal University of Technology, Owerri.

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DEDICATION

This work is dedicated to my Lord and Saviour, Jesus Christ, who saved me from my sins.

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ABSTRACT

This study investigated the production, characterization, and application of nano-biofertilizer synthesized from biochar (BC) derived from the aerial tissues of *Eichhornia crassipes* (water hyacinth), an invasive aquatic plant known for rapid growth and nutrient uptake that has adversely affected the aquatic ecosystem. BC was produced from its leaves (D1) and stems (D2) via pyrolysis at 600°C with residence times of 15, 30, 45, and 60 minutes, yielding 31% and 34% respectively. Incomplete carbonization was observed at residence time of 15 minutes while optimum pyrolysis occurred at 30 - 60 minutes. SEM and FTIR analyses revealed porous structures with surface functional groups including –COOH, –OH, C=C, and –S=O in but biomasses, although predominantly in D1. The biochar was alkaline (average pH 10.7), exhibited liming capacities of 14.76–28.94% $CaCO_3$ equivalent, zeta potentials of –34 to –38 mV, and particle sizes ranging from 146 to 583 nm. Although, deficient in copper and zinc, high nutrient availability was recorded with nitrogen (34,550 ppm), phosphorus (56 ppm), potassium (609 ppm), and water holding capacities between 1.58 and 2.26 g/g. BET isotherm modeling showed surface areas of 236.44–249.41 m^2/g and pore volumes of 0.061–0.087 cm^3/g, indicating mesoporous to microporous structures suitable for nanonutrient adsorption. Molecular dynamics simulations revealed thermodynamically favorable adsorptions between BC and ZnO and/or CuO nanonutrients, with a higher affinity for CuO. Comparative pilot field trials with nano-biofertilizer (NF) applied at 10 g, 20 g, and 40 g per 10 kg soil and a conventional fertilizer (CF) at 15g per 10kg soil (per manufacturer recommendation) showed that 10 g NF significantly enhanced growth and physiological parameters over other treatments. CF application resulted in higher plant mortality, indicating possible phytotoxicity of CF under the pilot field conditions. Comparative data obtained for plant height, leaf nitrogen content, chlorophyll concentration, number and surface area of leaves support the potential of biochar-based nanofertilizers as a low-dose, sustainable alternative to chemical fertilizers. Further studies are recommended to validate the predictions from molecular dynamics simulations, as well as to assess the environmental safety and scalability of the nanofertilizer in larger field trials.

Keywords: water hyacinth, functionalized biochar, optimized pyrolysis, nano-nutrient adsorption, molecular dynamics (MD) simulations, nano-biofertilizers, carbon sequestration, smart agriculture

CHAPTER ONE

INTRODUCTION

1.1 Background information

We are now in the era of internet of everything (IoE) and smart everything: smartphones, smart TV, smartwatches, smart eyeglasses, smart speakers, smart cameras, smart refrigerators, smart glucose meters, smart cities and so forth (Kasprzyk-Hordern et al., 2022; Langley et al., 2021; Silva, Khan, & Han, 2018). All these devices are designed to save energy and cost and be customizable to meet the owners' specific needs. Generally, 21st century products are engineered with sustainable development goals in mind by considering their impact on the three P's: the planet, the people, and then of course, the profit to be made. Zeroing in from this perspective, biochar has been seen as a biogenic material and gamechanger of some sort which can be helpful in solving some ecological, environmental and even economic problems confronting humans. For example, biochar can reduce global greenhouse gas emissions by at least 6%, and national emissions by over 10% in 28 countries, making it a viable option for carbon dioxide removal and climate change mitigation efforts (Lefebvre et al., 2023). Also, the European Horizon Program recently inaugurated a multi-million Euro project for circular carbon with customized modular pyrolytic equipment installed in over 130 cruise ships to recover carbon from plastics and other wastes generated on the ships with a target carbon emission reduction of about 80% (Vow ASA, 2022).

Historically, biochar's origins trace back to the ancient Amazon Basin where indigenous people created "Terra Preta" or "black earth" soils by adding charred biomass to the soil, figure 1.1 (Glaser, Haumaier, Guggenberger, & Zech, 2001; McMichael et al., 2014). This soil was exceptionally fertile and have remained so for centuries. The concept of biochar was rediscovered in the late 20th century

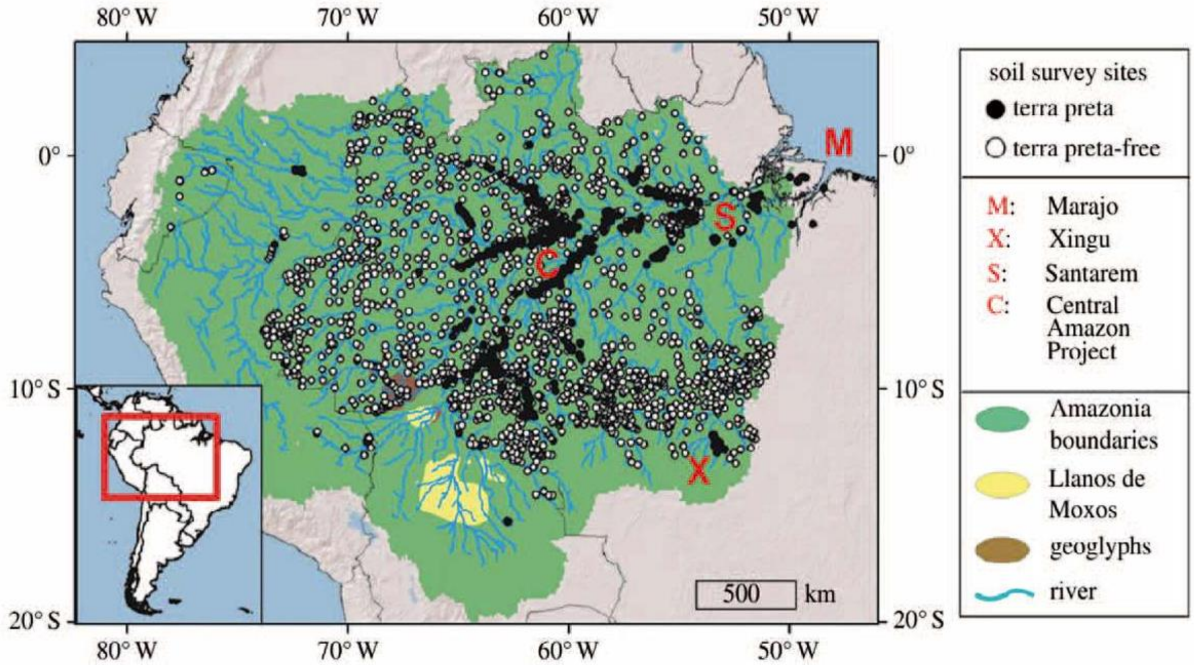

Figure 1.1: Conceptualized Terra Preta within Amazonia basin. (McMichael et al., 2014)

by scientists studying the fertility of Terra Preta soils and this led to renewed interest in this utility product. Similar practices have been found in other parts of the world, such as Japan and West Africa, where charcoal and organic matter were added to soils to enhance fertility.

According to the International Biochar Initiative (IBI), biochar is a fine-grained product of carbonization, characterized by a high content of organic carbon and low susceptibility to degradation, which is obtained through the pyrolysis of biomass and biodegradable waste (Saletnik et al., 2019). Biochar's versatility stems from the cosmopolitan nature of its precursor material including sugar cane bagasse (Iwuozor et al., 2022; Nie et al., 2018; Rahman, Jahiruddin, Kader, Islam, & Solaiman, 2022), bamboo (Alfei & Pandoli, 2024; Chaturvedi et al., 2023), pinewood (Vijayaraghavan & Balasubramanian, 2021), wood (Junhua Jiang et al., 2013; Veksha, McLaughlin, Layzell, & Hill, 2014), sludge (Chen et al., 2015; Huang, Jiang, Huang, & Yu, 2018), animal waste (Côrtes et al., 2019), crop straw (Jun Jiang, Yuan, Xu, & Bish, 2015; Khan et al., 2021), rice husk (Bushra & Remya, 2024; Ma et al., 2022; Nagaraju et al., 2023). The final biochar product's physicochemical properties are therefore dependent on the feedstock as well as the pyrolytic conditions. In general, the key properties include a porous structure, high surface area, high carbon content and chemical stability, which make it beneficial for widescale applications. Researchers have therefore explored various applications of biochar in areas such as agriculture, energy generation, environmental remediation, and climate change mitigation. Some of these include wastewater treatment (Huang, Zimmerman, Chen, & Gao, 2020; Mahmoud & Kathi, 2022; Mohan, Charles, & Todd, 2022), soil remediation (Binda et al., 2022; Bui et al., 2024), supercapacitor electrodes (Chaijak & Michu, 2022; Chu et al., 2022; Junhua Jiang et al., 2013), soil amendments (Khan et al., 2021; Salama, Abdel-Aziz, El-Naggar, Shaaban, & Abdel-Wahed, 2021), and carbon sequestration (Gupta et al., 2020; Rahman et al., 2022; Wijitkosum & Jiwnok, 2019a). Commercial inorganic fertilizers, which is employed to boost agricultural food production, pose significant ecological sustainability and economic problems. This is due to the low nutrient use efficiency (NUE) which makes its application environmentally harmful and economically ineffective. For example, an average NPK fertilizer supplied 20-30% of its nutrient to the plant with the rest leached or washed away in runoff water to aquatic environments (Dimkpa, Fugice, Singh, & Lewis, 2020; Mahanta, Dambale, & Rajkhowa, 2019; Rawal, Pande, Shrestha, & Vista, 2022). The cost associated with such resource wastage is enormous. For example, according to Fertilizer Technical Working Group (Africa Fertilizer, 2024), fertilizer usage in Nigeria has been increasing steadily from 2011 and as of 2023, it stands at 1.64million metric tonnes (Figure 1.2). If 70-80% of this figure is lost due to low nutrient use efficiency, at the current rate of N55,000 per 50kg of fertilizer, it amounts to a whooping loss of 1.8 trillion naira annually (Dimkpa, et al., 2020). This is apart from the colossal environmental and ecological damage such nutrient loss will also cause.

According to Chandini et al. (2019), using fertilizers to improve production is a common factor in soil and water pollution, reduced input efficiency, development of highly resistant weeds, micronutrient deficiency, soil degradation, and raised toxicity in soil. These ecological and sustainability concerns have raised uncertainties about the continued effectiveness of conventional fertilizers, thereby encouraging the exploration of innovative, eco-friendly, nanotechnology-inspired fertilizer alternatives.

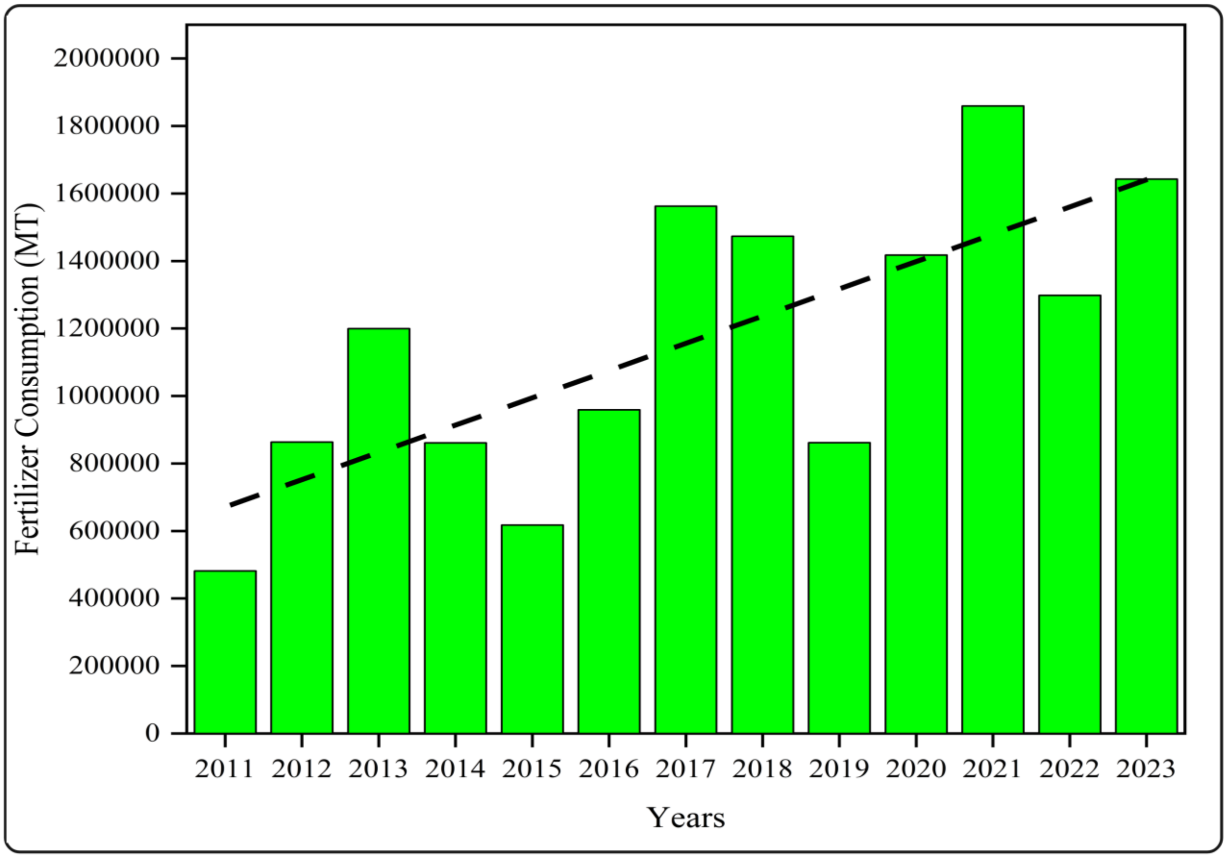

Figure 1.2: Nigeria annual fertilizer consumption from 2011 to 2023. (Data from Africa Fertilizer, 2024)

Water and soil pollution is a significant concern, and environmental activists have called for re-evaluation of agricultural activities to develop more sustainable, smart and innovative farming methods (Garg, Rumjit, & Roy, 2023; Nongbet et al., 2022; Raimondi, Maucieri, Toffanin, Renella, & Borin, 2021). Therefore, precision and smart farming has been proposed to enhance economical, sustainable, and eco-friendly agricultural practices (Ingle, Ingle, Philippini, & da Silva, 2023; Shaikh, Meroliya, Dagade-Gadale, & Waghmode, 2021; Tyagi, Arya, Ramniwas, & Tyagi, 2023; Zhang et al., 2021). These practices involve replacing commercial chemical fertilizers with biomass-based fertilizer. However, partially or totally replacing chemical fertilizers with biomass-derived soil amendments as a source of raw material is an essential alternative for environmental conservation and to promote nutrient recycling (Sayara, Basheer-Salimia, Hawamde, & Sánchez, 2020). Plant biomass upcycling offers several advantages in greener energy production and sustainable agriculture. From reducing environmental pollution, to increasing soil microbial activity, to recycling carbon thereby reducing GHG emission, this innovative technology has the potential for a circular economy and creating sustainable agricultural practices. Nanomaterials have the potential to greatly enhance the quality and nutrient composition of organic biomass (Sonu et al., 2023). This, in turn, allows for the conversion of biomass into nanobiochar and nanofertilizers that are highly efficient.

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic plant known for its rapid growth and abundant biomass. However, its uncontrolled proliferation in infested water bodies has posed significant ecological and economic challenges. In 2021, Lagos State government declared war on water hyacinth due to its negative impact on inland waterways navigation (Vanguard Newspaper, 2021). This research aims to harness the potential of water hyacinth biomass as a sustainable feedstock to produce functionalized nano-biochar fertilizers, offering various benefits including water hyacinth biomass upcycling, improved nutrient delivery to crops and aquatic ecosystem remediation. Together, this work created output values in the three dimensions of environmental, economic, and social benefits.

1.2 Statement of the problem

The rising global human population represents an increasingly significant threat to food security, a key metric for sustainable development in the current century. The United Nations estimates that the human population will approach 9.6 billion by 2050, which signifies mounting pressure on existing resources, including food availability (Antul Kumar, Choudhary, Kaur, Mehta, & Husen, 2021). Driven by limited resources, climate change, and unsustainable economic activities, the threat of food shortage, particularly dire for less economically developed countries such as those in Africa, calls for the design and deployment of innovative approaches to enhance agricultural practices. Against this background, global agriculture continues to struggle with crop yield stagnation, multi-nutrient deficiencies, declining water sources, and inadequate labor (Godfray et al., 2010; Raliya, Saharan, Dimkpa, & Biswas, 2018). Conventionally, efforts to increase agricultural output have relied on chemical fertilizers to deliver needed nutrients to crops, typically applying them through surface broadcasting, subsurface deposition, or dissolution in irrigation water. The reliance on chemical fertilizers has occasioned a sharp rise in global demand, with up to 85% of the mined phosphorus being utilized in food production (Raliya et al., 2018).

The problem statement is therefore, in the face of a global food security crisis exacerbated by unsustainable agricultural practices, particularly the adverse effects of chemical fertilizer application on the environment, can the development and application of a functionalized nano-enabled biochar-based fertilizer derived from water hyacinth offer a viable and sustainable alternative for enhancing agricultural productivity while mitigating environmental degradation and pollution?

Additionally, how can the environmental, social, and ecological challenges arising from the aggressive invasion of aquatic systems by water hyacinth be effectively addressed?

These research problems set the stage for investigating potential solutions and strategies to mitigate the negative impacts of water hyacinth invasion on aquatic ecosystems and the associated environmental and social concerns. The main research questions were:

1. Does water hyacinth biochar contain sufficient nutrients to serve as a feedstock for nano-enabled biofertilizers?
2. How do pyrolytic conditions influence the biochar's physicochemical properties and nutrient availability?
3. Which biochar properties are ideal for nano-biofertilizer formulation?
4. Can water hyacinth biochar adsorb and release nanonutrients effectively to fortify plants?
5. How does experimental biochar-nanonutrient adsorption data compare with computational molecular models?
6. How do nanonutrient concentration, pH, resident time and co-ions affect the biochar's adsorption capabilities?
7. How will the nano-enabled biofertilizer perform compared to conventional fertilizers?

1.3 Objectives of the study

The main objective of this study was to utilize functionalized biochar from water hyacinth (*E. crassipes*) as green nano-fertilizers.

Specific objectives of the study were to:

1. Investigate the effect of furnace residence times (15-60min) on biochar yield at 600°C.
2. Characterize biochar products as per elemental composition, total nitrogen content, functional groups, liming potential, nutrient release rate, carbon storage capacity, hydrodynamic size, zeta

potential, porosity, BET surface area and pore volume, and water holding capacity following the International Biochar Initiative (IBI) protocol.

3. Investigate biochar-nanonutrients adsorption characteristics under varying pH, nanonutrient concentration, contact time and single/co-ion presence.
4. Conduct molecular dynamics and adsorption simulations of biochar interactions with nanonutrients using BIOVIA Material Studio Suite
5. Use nanonutrients to formulate nano-biofertilizer based on biochar nutrient characteristics
6. Carry out pilot field trials to compare formulated nano-biofertilizer with conventional fertilizers.

1.4 Justification of study

The utilization of functionalized biochar from water hyacinth as green nano-fertilizers presents a multifaceted solution addressing environmental, agricultural, economic, and technological challenges. This innovative approach not only manages invasive species but also promotes sustainable agricultural practices, aligning with global sustainability goals. The following areas further breakdown specific areas of justifications for this research.

Technological innovation through advancement in fertilizer technology: The development of green nano-fertilizers from biochar represents an innovative step in agricultural technology. It aligns with the global trend towards precision agriculture, aiming for higher efficiency and sustainability (Anderson, Britt, & Dimkpa, 2023; Mikula et al., 2020; Shaikh et al., 2021; Xia et al., 2022). This study promotes scientific research and innovation in the field of green chemistry and environmental engineering, contributing to academic knowledge and practical solutions for sustainable agriculture

(Izydorczyk et al., 2020; Kopittke, Lombi, Wang, Schjoerring, & Husted, 2019; Patel & Panwar, 2023).

Controlling water hyacinth proliferation: *Eichhornia crassipes* is one of the world's most invasive aquatic plants. It grows rapidly and covers water bodies, disrupting ecosystems, hindering water transport, and promoting mosquito breeding, which poses public health risks (Karouach et al., 2022).

Mitigation of water pollution: The plant's high biomass can be utilized to prevent it from clogging waterways and disturbing aquatic life. Converting water hyacinth into biochar helps manage this waste efficiently (Lubembe et al., 2023; Vanguard Newspaper, 2021).

Nano-fertilizers advantage in sustainable and precision agriculture: Traditional fertilizers can lead to nutrient runoff, causing eutrophication of water bodies, however, nano-fertilizers, due to their small size, offer a controlled release of nutrients, enhancing nutrient uptake by plants and reducing environmental contamination (Kabiri et al., 2017; Khan et al., 2021; Tarafder et al., 2020).

Resource efficiency: Biochar produced from water hyacinth provides a sustainable source of nano-fertilizers. This not only recycles waste but also offers a renewable and environmentally friendly alternative to conventional chemical fertilizers.

Soil amendment properties: Biochar improves soil structure, increases water retention, and promotes microbial activity. Functionalized biochar can enhance these properties, leading to better soil health and increased agricultural productivity (Abd El-Azeim, Salah, & Hammam, 2021; Singh Yadav et al., 2023).

Carbon sequestration: Using biochar as a soil amendment helps sequester carbon, thus contributing to climate change mitigation by capturing atmospheric CO_2 (Gupta et al., 2020; Lefebvre et al., 2023;

Spokas et al., 2012; Tan, 2019). Biochar-based nanoformulation provides a low-cost raw material for fertilizer production, reducing dependency on synthetic fertilizers and lowering agricultural input costs while also helping as a direct atmospheric carbon sequestration material (Preethi et al., 2024; Yaashikaa, Kumar, Varjani, & Saravanan, 2020).

Addressing global food security: By improving soil fertility and crop yields sustainably, biochar-based nano-fertilizers can contribute to food security, especially in low-income regions like Nigeria where soil degradation and nutrient depletion is a major challenge (Godfray et al., 2010; Olaleye & Edje, 2020).

Climate change mitigation: The use of biochar for carbon sequestration and reduced reliance on chemical fertilizers (which have high carbon footprints) contributes to global efforts to combat climate change (Gadzhiev, Vagapova, & Yaumieva, 2023; Nagaraju et al., 2023; R. Xu, Qafoku, Van Ranst, Li, & Jiang, 2016).

1.5 Scope of study

Despite a gamut of research on biochar and the use of the invasive species – water hyacinth (*E. crassipes*) as a possible feedstock, few advances have been made to date in nano-fortification of water hyacinth-derived biochar for plant nourishment. Furthermore, molecular modelling application in the design and study of biochar-nanonutrient adsorption properties as a basis for nanofertilizer formulation has not been adequately elucidated. Therefore, this research work attempts to bridge this research gap by evaluating the potential of water hyacinth-derived biochar for nanonutrient fortification and its broader implications for smart and sustainable agriculture with its attendant economic and ecological impacts.

Therefore in this study, we explored the innovative utilization of water hyacinth (*E. crassipes*), a notoriously invasive aquatic plant, by converting it into functionalized biochar for use as green nano-fertilizers. The process begins with the collection and preparation of water hyacinth biomass, which is then subjected to optimized pyrolytic process to produce functional biochar at 600°C. The research includes a detailed characterization of the functionalized biochar to determine its physical, chemical, and structural properties, ensuring it meets the requirements for agricultural application. Computational molecular dynamics simulation was also employed to understand the atomistic properties that can influence the biochar-nanonutrient adsorption interaction, which is vital in crafting efficient and effective nano-enabled biofertilizers.

Laboratory testing was conducted to evaluate the adsorption properties of the biochar for subsequent formulation. The efficacy of the biochar-based nano-fertilizer in improving soil health and plant growth compared with that of traditional fertilizers was also assessed via a pilot field trial.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual framework

2.1.1 Biochar and nanobiochar

Biochar is a carbon-rich material derived from the pyrolysis of crop residues, wood, or other agricultural wastes. It has gained increasing attention for its potential applications in diverse fields, particularly agriculture, environmental remediation, and carbon management (Bhandari et al., 2023; Singh Yadav et al., 2023). An ancient material dating back to civilizations like the Amazonians exemplified by the creation of the historic *terra preta* anthropogenic dark soils that retained their fertility for centuries, biochar bears unique physicochemical properties central to its versatility and utility in various applications (Shafiq, Anwar, Firdaus-e-Bareen, Zhang, & Ashraf, 2023). Chiefly, biochar has a remarkable surface area to volume ratio, being highly porous and providing an ideal habitat for microbes (Yi et al., 2020). Importantly, its porous microstructure fosters nutrient retention and improves the material's soil water-holding capacity, which is useful in enhancing plant growth and resilience (Bruckman & Pumpanen, 2019; Singh Yadav et al., 2023; P. Zhao et al., 2019). Moreover, biochar's carbon-rich composition not only stores carbon for an extended period but also mitigates greenhouse gas emissions by preventing the rapid decomposition of organic matter in the soil (Ramadan, Asran-Amal, & Abd-Elsalam, 2020).

General properties and physicochemical characteristics of biochar depend on its precursor material, the pyrolytic process conditions, and the type of reactor. The pyrolytic process usually occurs in the absence or near-absence of oxygen and typically at temperatures ranging from 300 to 700°C (Ahmed, 2023). The specific conditions and methods used can significantly affect the properties and effectiveness of the biochar produced for example, slow pyrolysis, flash pyrolysis, carbonization, and gasification (Alfei & Pandoli, 2024). The latter requiring temperatures range between 1000^{0}C to 1600^{0}C. Some of the drawbacks of bulk biochar production include the energy cost for the thermochemical conversion, the high variability in properties due to differences in feedstocks and pyrolytic conditions, possible emissions of pollutants during the production process and so forth. Researchers, however, are seeking various ways to mitigate these disadvantages including conversion to nanobiochar to enhance its value (Ramanayaka, et al., 2020).

The innovation from bulk or conventional biochar to nanobiochar, which entails the preparation of biochar with structural size in the nanoscale (1-100 nanometer), has provided a means of extending its beneficial physicochemical and morphological characteristics by modulating the production methods (Balmuk, Videgain, Manyà, Duman, & Yanik, 2023; Shafiq et al., 2023). Nanobiochar development involves the integration of nanomaterials into the biochar matrix, which allows purpose-driven modifications and incorporation of specific nanoparticles for tailored applications in different industries (Rajput et al., 2022). By incorporating nanoparticles such as graphene or metal oxides, the material gains enhanced reactivity, increased adsorption capacity, and improved electrical conductivity, compared to bulk biochar (Xiang et al., 2020). From an agriculture perspective, nanobiochar production represents an exciting opportunity for engineering purpose-specific, tunable, and environment-friendly solutions. Biochar, which is a carbon-rich material derived from the pyrolysis of crop residues, wood, or other agricultural wastes, has gained increasing attention for its potential applications in diverse fields, particularly agriculture, environmental remediation, and carbon management (Bhandari et al., 2023; Singh Yadav et al., 2023). An ancient material dating back to civilizations like the Amazonians exemplified by the creation of the historic *terra preta* anthropogenic dark soils that retained their fertility for centuries, biochar bears unique physicochemical properties central to its versatility and utility in various applications (Shafiq et al., 2023). Chiefly, biochar has a remarkable surface area to volume ratio, being highly porous and providing an ideal habitat for microbes (Yi et al., 2020). Importantly, its porous microstructure fosters nutrient retention and improves the material's soil water-holding capacity, which is useful in enhancing plant growth and resilience (Bruckman & Pumpanen, 2019; Singh Yadav et al., 2023; Zhao et al., 2019). Moreover, biochar's carbon-rich composition not only stores carbon for an extended period but also mitigates greenhouse gas emissions by preventing the rapid decomposition of organic matter in the soil (Ramadan et al., 2020).

General properties and physicochemical characteristics of biochar depend on its precursor material, the pyrolytic process conditions, and the type of reactor. The pyrolytic process usually occurs in the absence or near-absence of oxygen and typically at temperatures ranging from 300 to 700°C (Ahmed, 2023). The specific conditions and methods used can significantly affect the properties and effectiveness of the biochar produced for example, slow pyrolysis, flash pyrolysis, carbonization, and gasification (Alfei & Pandoli, 2024). The latter requiring temperatures range between 1000^0C to 1600^0C. Some of the drawbacks of bulk biochar production include the energy cost for the thermochemical conversion, the high variability in properties due to differences in feedstocks and pyrolytic conditions, possible emissions of pollutants during the production process and so forth. Researchers, however, are seeking various ways to mitigate these disadvantages including conversion to nanobiochar to enhance its value.

The innovation from bulk or conventional biochar to nanobiochar, which entails the preparation of biochar with structural size in the nanoscale (1-100 nanometer), has provided a means of extending its beneficial physicochemical and morphological characteristics by modulating the production methods (Balmuk et al., 2023; Shafiq et al., 2023). Nanobiochar development can involves the integration of nanomaterials into the biochar matrix, which allows purpose-driven modifications and incorporation of specific nanoparticles for tailored applications in different industries (Rajput et al., 2022). By incorporating nanoparticles such as graphene or metal oxides, the material gains enhanced reactivity, increased adsorption capacity, and improved electrical conductivity, compared to bulk biochar (Xiang et al., 2020). From an agriculture perspective, nanobiochar production represents an exciting opportunity for engineering purpose-specific, tunable, and environment-friendly solutions to longstanding crop productivity and environmental challenges.

Nanobiochar has found several applications capitalizing on the unique combination of its carbon-rich structure and the additional functionalities conferred by the incorporation of nanoparticles. For instance, an attempt was made to find an innovative use for water hyacinth as a green precursor for carbon composite adsorbents and supercapacitor electrodes (Saning et al., 2019). Also, in another study, Mahmoud et al. (2022) investigated the effect of biochar solely or combined with nanosilica and nanopotassium on the agro-physiological and biochemical constituents of potato. The results showed that the biochar with the nano-scale elements significantly improved plants agronomic variables. Generally, the properties of nano-enhanced biochar that confer soil improvement include its high surface area, adsorption, and nanoscale-derived reactivity. These properties also make nanobiochar well-suited for other applications including soil amendments, stabilization of soil organic matter, improved soil fertility, reduced nitrous oxide emissions, carbon sequestration, removal of heavy metals, organic pollutants, and other contaminants from wastewater and drinking water sources (Liu et al., 2024; Mahmoud & Kathi, 2022; Makavana et al., 2021; Muoghalu et al., 2023; Nagaraju et al., 2023; Rawat, Kumar, & Singh, 2020; Sonowal, Koch, Sarma, Prasad, & Prasad, 2023; Yaashikaa et al., 2020; Zuhara, Mackey, Al-Ansari, & McKay, 2022). Recently, nanobiochar has been studied as an emerging ecological remediation tool utilized in waste management processes to adsorb odors, toxic gases, heavy metals, and other pollutants in landfills, water bodies, and wastewater treatment facilities (Elbasiouny et al., 2022; Patel & Panwar, 2023; Xia et al., 2022). In addition, nanobiochar has been used for the remediation of polluted soil profiles. In this role, its ability to adsorb contaminants, such as pesticides, hydrocarbons, heavy metals, and PFAS (per- and polyfluoroalkyl substances), is critical for the restoration of soil quality and productivity (Bui et al., 2024; Yi et al., 2020). In addition, studies have shown that nanobiochar improves soil fertility and plant growth by enhancing nutrient and water retention and promoting crop nutrient uptake (Rajput et al., 2022). Furthermore, the presence of nanoparticles imparts specific beneficial characteristics, including improvements in nutrient release or disease resistance. Like bulk biochar, nanobiochar, is also used for carbon sequestration, which involves burying nanobiochar in soil to reduce the release of atmospheric carbon dioxide and the associated greenhouse effect (Wijitkosum & Jiwnok, 2019a). Altogether, the controlled addition of nanomaterials has extended the utility of biochar in a wide range of applications. (Bayda, Adeel, Tuccinardi, Cordani, & Rizzolio, 2020; Mahmoud et al., 2022; Wijitkosum & Jiwnok, 2019b). Table 2.1 shows a summary of biochar production processes while Figure 2.1 shows the benefits of biochar.

2.1.2 Nanofertilizers

The use of nano-based polymers for fertilizer development represents an innovative and sustainable approach to increasing crop performance and fertilizer use efficiency. Use of various plant-based feedstocks such as wheat straw (Khan et al., 2021), rice husk (Bushra & Remya, 2024; Nagaraju et al., 2023), bagasse (Huang, Chen, Hu, Cheng, & Zhu, 2022; Iwuozor et al., 2022), bamboo (Alfei & Pandoli, 2024; Chaturvedi et al., 2023), pinewood (Vijayaraghavan & Balasubramanian, 2021), corn stalk (Amalina et al., 2022), and saw dust (Jedynak & Charmas, 2024) for production of nanofertilizers have been extensively demonstrated.

Table 2.1: A summary of thermochemical processes for biochar production

Thermochemical Process	Temperature Range (^0C)	Yield (%)	Residence Time	Heating Rate
Slow pyrolysis	100 – 1000	15 – 40	Minutes to hours	Slow (<10 ^0C $mins^{-1}$)
Fast pyrolysis	300 – 1000	10 – 25	<2s	Very fast (~ 1000 ^0C s^{-1})
Torrefaction	200 – 300	61 – 77	Minutes to hours	Slow (<10 ^0C $mins^{-1}$)
Gasification	700 – 1500	~ 10	Seconds to minutes	Moderate – very fast
Hydrothermal carbonization	175 – 300	30 - 72	30mins to 16hrs	Slow
Microwave pyrolysis	550 – 700	34	5 – 20mins	Slow
Flash carbonization	300 - 600	37 - 50	~ 30mins	Slow

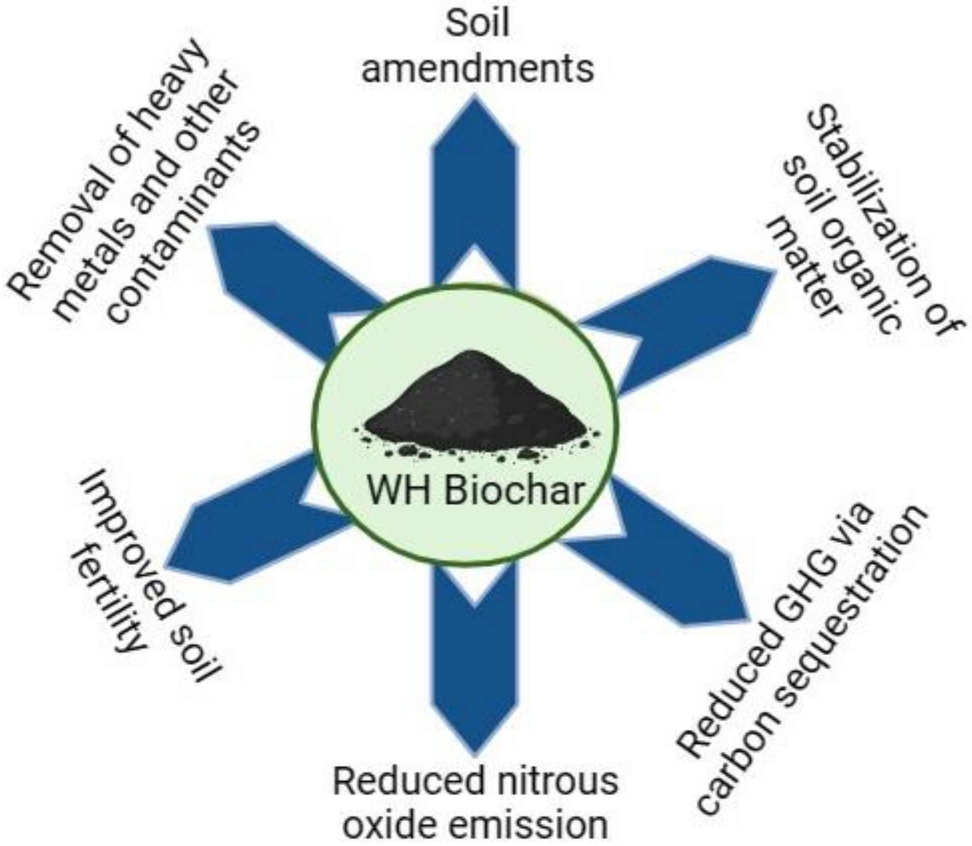

Figure 2.1: Environmental and ecological benefits of biochar

Asides allowing for a high surface area-to-volume ratio, nanotechnology-based fertilizer development also offers the benefits of controlled nutrient release and lower environmental impact, in comparison to conventional fertilizers. According to Rop, Karuku, Mbui, Michira and Njomo (2018), water hyacinth has gained significant attention as an emerging bio-based nanopolymer for controlled-release fertilizer development, where in their work incorporation of water-soluble phosphate fertilizer into a water hyacinth acrylamide polymer increased the nutrient use efficiency and reduced leaching and phosphate toxicity in the local soil (Rop et al., 2018). In another study, Kalaivani and Ravi (2023) synthesized and characterized nanocomposites of ZnO nanopolymers from a water hyacinth extract. The authors found that the nanofertilizer significantly improved root development, enhanced photosynthetic rate, and improved nitrogen use efficiency. Taken together, the adsorption and desorption of nutrients in the plant biomasses by taking advantage of charged functional groups on the biomass surface are major features of the mechanism involved (Izydorczyk et al., 2020; Michalak, Witek-Krowiak, Chojnacka, & Bhatnagar, 2015; Mikula et al., 2020). In this process, nutrient release could be significantly controlled, compared to regular fertilizers of equivalent chemistry. Thus, another potential area of application of water hyacinth as a nanobiofertilizer could be based on the biosorption of nanoscale nutrients such as N (as ammonium), nanohydroxyapatite (P), K, Ca, Mg, S, Zn, Fe, and other nutrients by a slurry of fine powder of the plant biomass derived from milling or grinding. In this regard the nanoscale dimensions can be claimed from either the added nano nutrients or from nano-biomaterials originated from the grinding process. In fact, this strategy can be incorporated into the nanobiochar one described above, where the biochar from water hyacinth can be used for the biosorption process. While these studies highlight the potential utility of water hyacinth as a scaffolding or adsorbent (nano) polymer in nanoscale fertilizers, the concept is still nascent and has received relatively little attention in terms of research and development at scale. Figure 2.2 shows a proposed mechanism of water hyacinth biochar-based nanofertilizer in plant nutrient fortification.

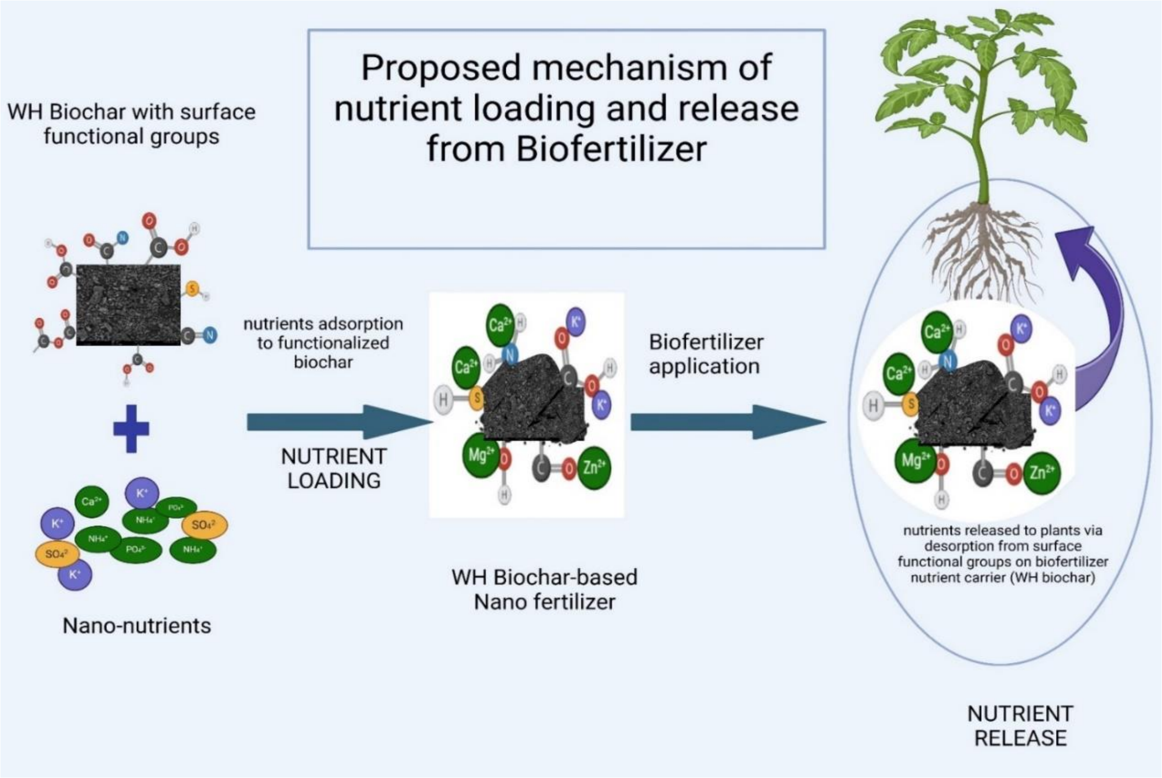

Figure 2.2: Possible mechanism of nutrient loading and release from nano-enabled biofertilizer.

2.1.3 Water Hyacinth (*Eichhornia crassipes*)

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic weed commonly found in water bodies in different parts of the world and reputed for its rapid growth and clogging of waterways (Harun, Pushiri, Amirul-Aiman, & Zulkeflee, 2021). Motivated by the invasive nature of the plant and its detrimental environmental and economic implications, research on water hyacinth has become expansive, covering the entire gamut from the weed's origin, and spread from its native Amazon to its impact on water quality and potential useful applications (Harun et al., 2021). Belonging to the family *Pontoderiaceae*, water hyacinth is a free-floating aquatic plant that thrives in inland freshwater collections, particularly lakes, rivers, and ponds (Ajithram, Winowlin Jappes, Chithra, & Daphne, 2023; Harun et al., 2021). It features broad leaves and purple spiky flowers. With a buoyancy mediated petiolar air sacs, the plant has a variable height that sometimes approaches one meter (Ajithram et al., 2023). Its reproductive cycle comprises both asexual vegetative and sexual, making the plant remarkably invasive, a characteristic that is also assured by its genetic uniformity (Zhang, Zhang, & Barrett, 2010). The plant was introduced from the Amazon as ornamental plants and gifts in the early 20th century, eventually spreading to over 50 countries on five continents (Ajithram et al., 2023; Mujingni, 2012). As shown in Figure 2.2, many countries around the world are affected by widespread water hyacinth infestations. Although, it should be noted that the distribution of water hyacinth may change over time due to efforts to manage and control its spread, as well as changes in environmental conditions.

Specific to Africa, Mujingni (2012) noted that the continent has borne a particularly high burden, following the weed's initial introduction in Zimbabwe in 1937, after which it heavily colonized key water bodies in Mozambique, Ethiopia, Rwanda, Kenya, Tanzania, and Nigeria (Lubembe et al., 2023). In inland water bodies, nutrient-rich agricultural runoff and industrial waste products stimulate the avid growth of water hyacinth, associated with dense overcoats on the water surface (Plate 2.1). The persistence of water hyacinth in tropical waters is enhanced by conducive climatic conditions and hydrodynamic factors that sustain its spread (Djihouessi, Olokotum, Chabi, Mouftaou, & Aina, 2023). Today, water hyacinth is best known for its aggressive invasiveness, water quality degradation, and economic cost, making it a significant environmental and socioeconomic concern in many fishing communities, as well as in communities reliant on water as the principal mode of transportation and recreation.

Several studies have demonstrated the environmental effects of water hyacinth invasion (Bicudo et al., 2007; Harun et al., 2021; Kateregga & Sterner, 2009; Mironga, Mathooko, & Onywere, 2011; Mujingni, 2012; Nega, Ramayya, Afessa, & Manenti, 2022; Villamagna & Murphy, 2010). Patel (2012) noted that the high fecundity exhibited by water hyacinth enables it to outcompete most native species for nutrients, resulting in diminished biodiversity in affected water bodies. In addition to driving biodiversity loss, water hyacinth disrupts local food chains and suppresses the growth of phytoplankton and other resident microbes. Studies have also demonstrated measurable degradation of water quality in areas with dense water hyacinth cover, mostly in the form of sediment and microparticle dissemination and oxygen depletion, which can, in turn, result in asphyxiation of marine flora and fauna (Basaula, Sharma, Belant, & Sapkota, 2021; Basaula, Sharma, Paudel, Kunwar, & Sapkota, 2023; Maulidyna et al., 2021). Unlike submerged vegetation in water bodies, water hyacinth does not release oxygen into the surrounding water, driving its concentration lower and reducing the survival of other plants. In studying the effects of the weed on the water quality and phytoplankton health in Kenya’s lake Naivasha, Mironga et al. (2011) found an inverse relationship between the dissolved oxygen and phytoplankton chlorophyl concentration and water hyacinth cover. Furthermore, Villamagna and Murphy (2010) pointed out that the surface coverage by water hyacinth is associated with a higher rate of evapotranspiration, compared to that recorded in open waters. Furthermore, the dense mat of water hyacinth that forms on rivers can impede navigation and irrigation, as well as increase the risk of flooding of adjacent areas (Sharma, Aggarwal, Saini, & Yadav, 2016).

As outlined earlier, the negative impacts of water hyacinth proliferation include reduction of biodiversity, impeding of water transportation, aquatic recreation and fishing, destruction of bridges and other marine infrastructures, and clogging of power generation dams and irrigation water systems, some of these are summarized in Table 2.2. Water hyacinth proliferation also affects the social well-being of the communities that live around water bodies. These problems have been widely documented in various regions of the globe. For instance, in India, several lakes have been invaded, including among others, the Katraj, Pichola, and Ulsooru lakes in Pune, Udaipur and Bangalore, respectively (Gopal, 2018). In South America, after chemical treatments, the control programs have been contending with re-infestation in the Guadalupe Dam in Mexico. Furthermore, major infestations of water hyacinth invaded Lake Victoria in East Africa, causing negative impacts on fishing communities. Such a bad reputation led the plant to be bestowed with several names in various parts of the world and as indicated in Figure 2.3, for example, the fishing communities in Southwestern Nigeria call it "water terror" (Bolorunduro, 2023).

(a) (b)

Plate 2.1: Early-stage water hyacinth invasion of a Lake in Xochimilco, Mexico (a) and an inland waterway in Lagos, Nigeria (b).

Table 2.2: Summary of modes of water hyacinth menace

Negative effects of water hyacinth	References
Loss of biodiversity: water hyacinth outcompetes native species for nutrients, thereby causing loss of biodiversity	(Bicudo et al., 2007; Harun et al., 2021)
Disruption of local food chain: water hyacinth causes disruption of local food chain via suppression of phytoplankton and other microbes	(S. Patel, 2012)
Degradation of water quality: water hyacinth causes degradation of water quality through deposition of sediments and microparticles in the water.	(Kateregga & Sterner, 2009; Mironga et al., 2011; Mujingni, 2012).
Reduction in dissolved oxygen: In areas of dense coverage, water hyacinth causes reduction in dissolved oxygen by limiting photosynthetic activities within the aquatic ecosystem	(Basaula et al., 2021, 2023).
Impeding of navigational routes and irrigation systems: water hyacinth negatively impacts the use of water for navigation and irrigation purposes	(Nega et al., 2022; Villamagna & Murphy, 2010).
Clogging of dams: hydroelectric power generation systems clogging through WH biomass	(Sharma et al., 2016; Sharma, Sharma, Sharma, Sharma, & Singh, 2020).
Increased risk of flooding in adjacent areas: By impeding free-flow of water and creating excessive sediments, water hyacinth can increase the risks of flooding in adjacent areas.	(Dersseh, Melesse, Tilahun, Abate, & Dagnew, 2019).

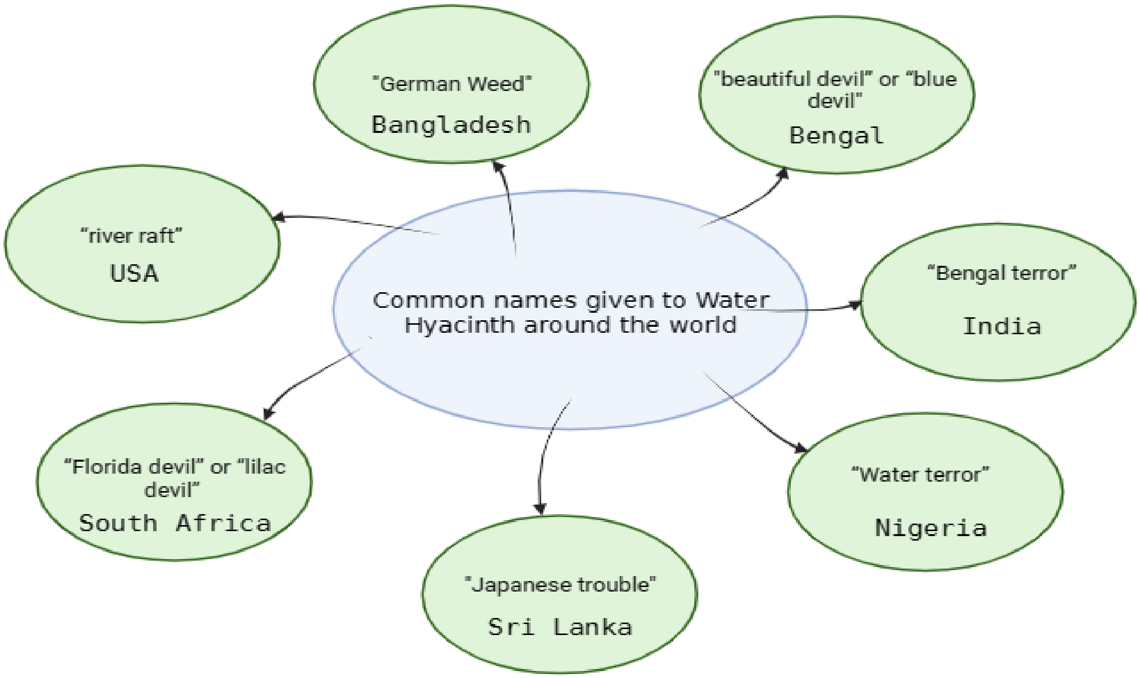

Figure 2.3: Common names for water hyacinth around the world

2.2 Theoretical framework

2.2.1 Biochar production processes

Environmental concerns continue to drive efforts to develop sustainable processes for the development of nanostructured materials, with approaches focusing on low energy consumption, less use of toxic solvents, and utilization of bio-based materials. Bulk biochar, which can be subsequently converted to nano-sized biochar or nano-enabled biochar (commonly called nanobiochar), can be prepared using such feedstocks as animal wastes (Côrtes et al., 2019), rice husk (Bushra & Remya, 2024; Nagaraju et al., 2023), sugar cane bagasse (Nie et al., 2018), wood residues (Junhua Jiang et al., 2013), and sewage sludge (Anupama & Khare, 2021). Conventionally, carbonaceous nanoparticles have been produced using such processes as slow or fast pyrolysis, gasification, laser ablation, arc discharge, or chemical precipitation (Abbas et al., 2018; Ahmed et al., 2023; Rajput et al., 2022; Shafiq et al., 2023). However, the need to limit the material's ecological footprint demands methods that utilize less energy and more readily available precursors. Naghdi et al. (2017) describe the production and characterization of nanobiochar using ball milling, a low-cost top-down method that involves the application of mechanical forces for particle size reduction to nanopowders of different sizes. Table 2.3 shows a summary of various biochar feedstocks, methods of preparation and key applications.

The process utilizes small, spherical grinding media such as steel or ceramic balls that are agitated within a milling chamber containing the feedstock. The repeated impact and shear forces generated by the moving balls effectively break down the biochar into smaller particles, including nanoscale particles, when the milling process is appropriately controlled (Amusat, Kebede, Dube, & Nindi, 2021; Shan et al., 2016). Ball milling has drawn significant scholarly attention as a low-cost approach to producing nanoporous carbon, with the particle size being regulated by varying the

Table 2.3: Biochar feedstock materials, percentage yield, pyrolytic conditions and applications

Feedstock	Biochar yield (wt%)	Pyrolytic conditions	Biochar application	Reference
Animal waste (Bovine bone)	63%	650^0C – 1000^0C 2 – 4hrs resident time	Adsorbent for textile dye contaminated wastewater	(Côrtes et al., 2019)
Animal waste (Fish scale)	43%	650^0C – 1000^0C 2 – 4hrs resident time	Adsorbent for textile dye contaminated wastewater	(Côrtes et al., 2019)
Rice husk	40-45%	500^0C	Agricultural (nutrient release) and environmental (pollutant adsorption) applications for biochar	(Bushra & Remya, 2024; Nagaraju et al., 2023)
sugar cane bagasse	23% and 58%	450^0C for 4hrs and 600^0C	Application in soil enhancement, carbon sequestration and plants (pak choi, maize and groundnut) growth	(Nie et al., 2018; Rahman et al., 2022)
wood residues	21% - 27%	200^0C for 1hr followed by 750^0C for 1hr	Use as supercapacitor electrodes and Cu2+ and Zn2+ adsorbents	(Junhua Jiang et al., 2013; S. Jiang et al., 2016)
sewage sludge	N/A	400^0C - 800^0C	Application as catalyst for pollutant degradation	(Junhua Jiang et al., 2013)

speed of the rotating steel and the milling time. However, Ramanayaka et al. (2020) noted that ball milling renders non-uniform sized nanoparticles, which tend to collide and aggregate, resulting in unintended large particle sizes (Ramanayaka, Tsang, Hou, Ok, & Vithanage, 2020; Ramanayaka, Vithanage, et al., 2020). To overcome these shortfalls, the authors suggest double-disc milling as a more refined method for fabricating nanobiochar with evenly sized particles, higher quantity, and more granular procedural control. A strategic approach to the sustainable synthesis of biochar should entail the selection of non-competing, non-food, and locally sourced feedstock materials. For instance, post-harvest agricultural residues, forestry wastes, grocery wastes, weed species, or invasive plant species such as water hyacinth should be intentionally used as feedstock, reducing the pressure on natural ecosystems, and minimizing transportation-related emissions (Akter et al., 2023). Overall, several sustainable strategies have been developed for green nanobiochar fabrication.

However, regardless of the raw material source, nanobiochar has been prepared using a variety of techniques, ranging from ball-milling to microwave pyrolysis. In addition to direct synthesis of nanobiochar from various raw materials, nanosized biochar molecules are readily formed during preparation of bulk biochar, although its yield is limited to less than 2% (Liu et al., 2018). The common nanobiochar fabrication techniques can be classified into two: top-down and bottom up (Ramanayaka, Vithanage, et al., 2020). In general, the top-down approach involves breaking down bulk feedstocks into smaller structures, and comprises of fabrication techniques such as cutting, grinding, and centrifugation. This approach has been popularized by its relative ease and less energy consumption and cost. In contrast, the bottom-up approach entails up-constructing materials from precursors to the nanoscale, including wet chemistry methods such as precipitation, ionic gelation, and sol-gel methods, among several others. However, the most common and widely studied nanobiochar production approach is ball-milling, a top-down method involving grinding of the feedstock material between stainless steel balls of varying shape and speed. According to Amusat et al. (2021), ball milling is effective as it is capable of nanobiochar fabrication while preserving the material’s crystal structure. Moreover, ball milling is eco-friendly and low-cost. Other top-down techniques for nanobiochar fabrication include disc-milling, chemical treatment, and in-situ pyrolysis. The physicochemical properties of nanobiochar can be varied by changing the feedstock, milling time, and pyrolysis temperature. Zhang et al. (2015) prepared water hyacinth-derived nanobiochar through in-situ pyrolysis at different temperatures between 250ºC - 550ºC, yielding the highest adsorption capacity at 450ºC pyrolytic temperature. Other studies have incorporated a pre-treatment step, such as carbonization or pre-heating, for optimization of feedstock prior to ball-milling, improving particle size and reducing the chance for re-aggregation (Amalina et al., 2022; Junhua Jiang et al., 2013; Liu et al., 2020; Ma et al., 2022; Tian et al., 2022). Sonification is an alternative, high-energy nanobiochar production technique that utilizes ultrasonic radiation for bulk biochar disintegration, which is then suspended in an alkaline solvent (sodium hexametaphosphate) and separated through sieving and centrifugation. Altogether, multiple methods of nanobiochar fabrication have been developed and are applicable to the synthesis of the material from water hyacinth feedstock (Abd El-Azeim et al., 2021; Zhang et al., 2015).

2.2.2 Properties and mechanisms of nano-fertilizers and nano-products

The design of green nano-formulations contributes to their effectiveness. At the nanoscale level, their quantum effects influence the electronic, optical, and magnetic properties, such as tunable fluorescence and superior brightness (Gupta et al., 2024). Nanocomposites exhibit superior mechanical properties such as increased tensile strength, stiffness, and impact resistance. They possess excellent barrier attributes against gases, moisture, and UV radiation, which safeguards farm produce from degradation, spoilage, or environmental damage. The effectiveness of these nanotechnologies arises from their design, which addresses specific agricultural needs and challenges.

Other green nano-innovations have similar sustainable designs. Firstly, nanofertilizers often contain nanoelements with high surface area-to-volume ratios, which increases their solubility in water and soil, compared to bulk materials. They improve nutrient delivery through controlled nutrient release. Nanofertilizers also stimulate microbial activity and reduce environmental impact by minimizing nutrient runoff and greenhouse gas emissions (Dimkpa, Haynes, & White, 2024; Gupta et al., 2024; Shiying et al., 2024). Nanopesticides employ nanomaterials to improve pest control while reducing environmental harm caused by conventional scale pesticides. They increase surface area for better adhesion to pests, utilize nanocarriers for controlled release, target specific pests, and have synergistic effects (Khan & Rathore, 2023). Nanosensors offer high sensitivity and selectivity, miniaturization for on-site monitoring, multiplexed detection capabilities, real-time tracking, and remote sensing. Their durability ensures reliable, continuous monitoring in harsh conditions, aiding precision agriculture and resource optimization for improved productivity (Figure 2.4).

2.2.3 Application of nanotechnology in agriculture

Nanotechnology-based innovative farming solutions include all strategies that encourage higher yields through automated processes that ease cultivation. Ayaz, Ammad-Uddin, Sharif, Mansour, and Aggoune (2019) noted that such growth is evident in using the Internet of Things (IoT) to monitor water, nutrients, disease, soil, machinery, and the environment (Ayaz et al., 2019). The researchers also reported the application of nanotechnology in irrigation, pesticide usage, produce packaging and storage, and crop sensors. A practical example of utilizing this

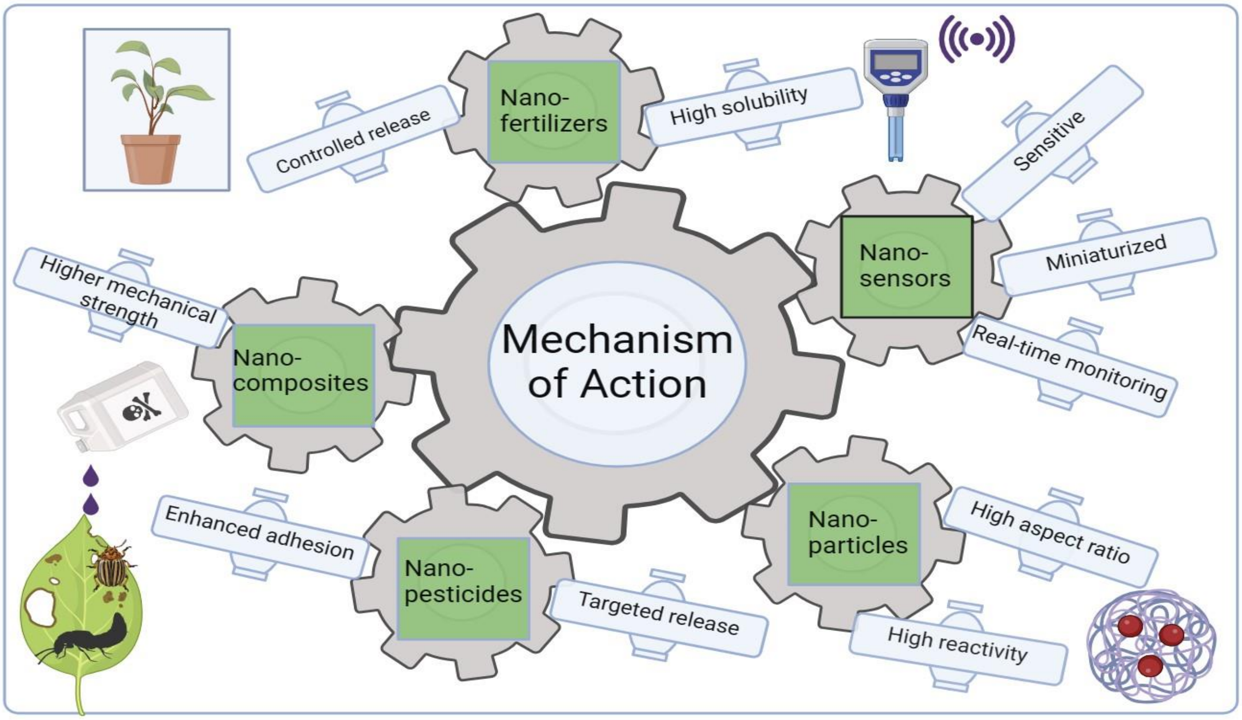

Figure 2.4: Mechanisms of action of nano-enabled products which confer on them unique properties

agricultural advancement is focusing on soil fertility management. Traditional farming involves applying fertilizers by spraying and spreading them evenly on the farm. However, this mechanism causes losses, leading to environmental degradation, herbicide resistance, and poor health for farmers and consumers. A smart and green solution relies on nanofertilizers that directly or indirectly supply nutrients to crops via encapsulation on nanoporous surfaces, polymer coating, or nano-emulsifying deliveries (Pramanik et al., 2020). Soil management improves water retention, benefiting crops by enhancing their resilience and reducing operational costs for farmers over expenses such as irrigation and machinery. A cultivator contemplating watering their plants will not depend on drip bottles or sprinklers. IoT will allow them to utilize the crop water stress index (CWSI) calculated by the air temperature and plant canopy to determine efficient water usage on their farm (Ayaz et al., 2019). Another nanotechnological approach to adopt in agriculture is precision farming, where software such as GIS provides real-time data on plants and their health to guide the decision-making process (summary in Table 2.4). This solution complements genetic engineering, pivotal in breeding healthier crops resistant to diseases due to their improved defense mechanism (Pramanik et al., 2020). After harvest, nanotechnology is still beneficial in packaging the products in airtight containers containing silicate nanoparticles and nanosensors to identify contamination quickly. Refraining from accepting these smart solutions is detrimental to any farmer who seeks to thrive in the modern agricultural industry.

Table 2.4**:** Summary of benefits of nanotechnology applications over conventional practices

Nano-innovation	Method of application or types	Benefits over conventional practices	References
Nanoparticles	Spraying (fertigation)	Energy-saving, reduced waste generation, precise targeting, improved resource use	Khan and Rathore (2023)
Nanocomposites	Material design	Lighter materials, enhanced durability, reduced waste, improved resource utilization	Chakraborty, Mukhopadhyay, Paul, Sarkar and Mukhopadhyay, (2023); Gupta et al. (2024)
Nanofertilizers	Nutrient delivery	Controlled release, reduced energy consumption, minimized environmental impact	Chakraborty et al. (2023)
Nanopesticides	Pest management	Targeted delivery, reduced off-target effects, lower application rates	Preethi et al. (2024)
Nanosensors	Plant, livestock, and soil monitoring	High sensitivity, real-time monitoring, precise adjustments	Tyagi et al. (2023)

2.3 Characterization and molecular modeling of biochar-nanonutrients interactions

2.3.1 Molecular modeling application to nanofertilizer

Researchers and material scientists have applied various atomistic insights and information derivable from the molecular simulation studies to their advantage in fabrication of new products (Mousavi et al., 2023). This has created an opportunity to leverage the computerized modelling software packages (e.g. the BIOVIA Material Studio) to predict, study, analyze and optimize structures at the molecular scales even before they are synthesized. There are different modules which can be applied depending on the type of material being modeled as well as the processes to be investigated with various pre-determined ambient conditions. A quick search of the publications on PubMed database hosted by the US National Center for Biotechnology Information shows the computational molecular dynamics has gained popularity among researchers and material scientists since the turn of the century (Figures 2.5 and 2.6). However, this database search using the root word, "molecular dynamics" with other modifiers, showed it is yet to be fully explored in biochar-nanonutrient interactions at the atomistic levels. This is necessary for effective understanding and analysis of nutrient adsorption and desorption, which can serve as a veritable tool for cost-effective and time-efficient nanofertilizer formulation, as currently being applied in several other fields including molecular biology, drug discovery and designs (Hollingsworth & Dror, 2018). Therefore, this was incorporated into this research work.

2.3.2 Geometry optimization

Geometry optimization in computational chemistry serves as a pivotal step in refining molecular structures to minimize their total energy, ensuring that simulated configurations accurately reflect

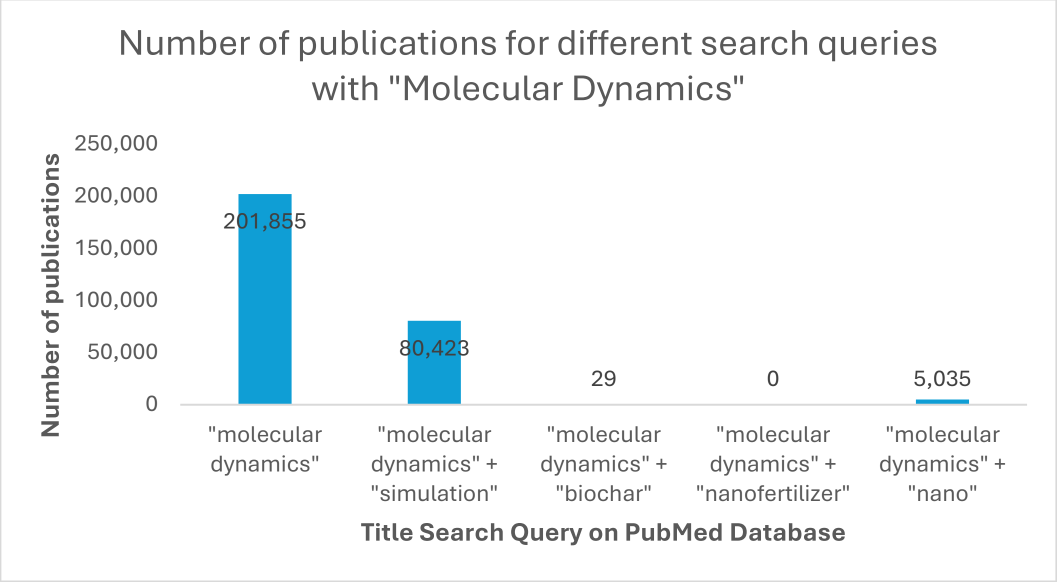

Figure 2.5: Number of publications for different search queries with "Molecular Dynamics" as the root word. (Data generated from PubMed database in May 2024)

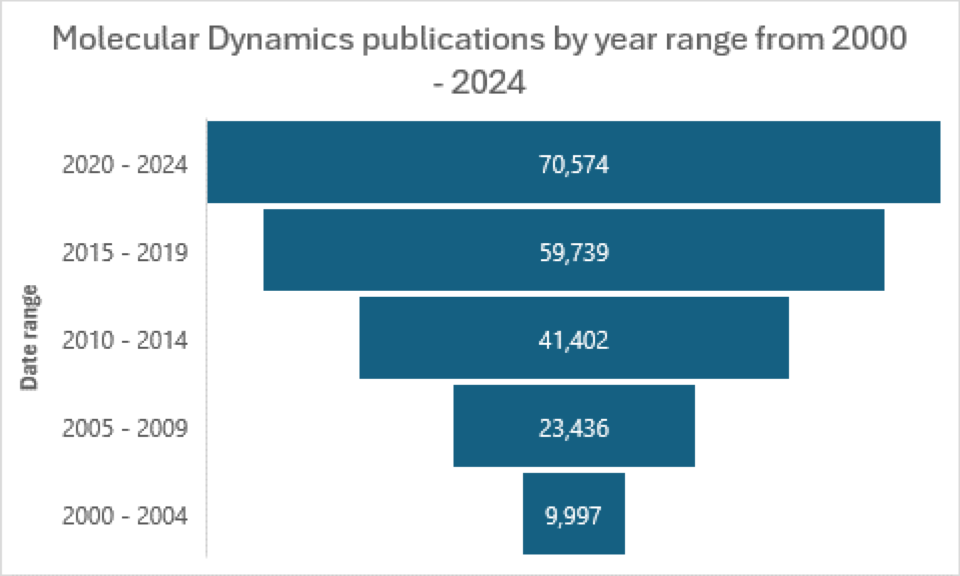

Figure 2.6: Number of publications on Molecular Dynamics by year range showing progressive increase from 2000 – 2024. (Data generated from PubMed database in May 2024)

experimental conditions. According to Bálint and Jäntschi (2021), in the context of molecular orbital calculations, a basis set, comprised of atomic-like functions, forms the foundation for constructing molecular orbitals through linear combinations of atomic orbitals (LCAO). Choices for basis sets, such as Slater-type orbitals (STOs) or Gaussian-type orbitals (GTOs), offer varying levels of accuracy and computational efficiency (Bálint & Jäntschi, 2021). Specifically, STOs offer precise descriptions of atomic shapes, while GTOs are computationally advantageous. In the molecular modeling realm, stationary states, also known as energy eigenstates or molecular orbitals, play a pivotal role, with their accurate depiction being paramount for reliable computational predictions (Sharma, Kumar, & Chandra, 2019b). In molecular dynamics simulations, the time-independent Schrödinger equation serves as a guiding principle for determining the wave function and its dynamic evolution over time (Scherbela, Reisenhofer, Gerard, Marquetand, & Grohs, 2022). While the Schrödinger equation provides an analytical framework for predicting molecular behavior, its exact solutions for molecular systems are generally elusive and require approximation methods (Bálint & Jäntschi, 2021; Chang & Li, 2023). In the case of quantum chemistry calculations, the time-independent Schrödinger equation is often reformulated as an eigenvalue problem, where approximation techniques, such as ab initio or semi-empirical methods, are employed to yield practical solutions (Sharma, Kumar, & Chandra, 2019a). Within this framework, the expansion of unknown one-electron functions, such as molecular orbitals, into a set of known basis functions forms the basis set, allowing for the approximation of molecular wave functions (Bálint & Jäntschi, 2021). Notably, in methods like Hartree–Fock (HF) and Kohn–Sham density functional theory (DFT), coefficients within the basis set are optimized to minimize total energy. The resulting iterative solutions provide self-consistent field (SCF) solutions, which are vital for geometry optimization (Bálint & Jäntschi, 2021; Jäntschi, 2019). By refining molecular structures iteratively, chemists can achieve stable configurations that accurately represent the energetic minima of the system, facilitating the study of biochar-nanonutrient interactions and the design of efficient biofertilizer formulations.

Nanotechnology has revolutionized agriculture through various innovations including nanofertilizers, nanopesticides, nanosensors and so forth (Garg et al., 2023; Hofmann et al., 2020; Neme, Nafady, Uddin, & Tola, 2021; Saritha, Anju, & Kumar, 2022; Singh, Sangwan, Sharma, Devi, & Moond, 2021; Vaidya et al., 2024). Nanofertilizers particularly, with their engineered nutrient delivery systems, offer promising solutions to the challenges of modern agriculture. However, optimizing the efficacy of these nanofertilizers requires understanding their sorption dynamics at the molecular level (Bálint & Jäntschi, 2021; Gautam, 2021; Hu & Wei, 2023; Minervino & Belfield, 2024; Yaphary et al., 2023). BC's intrinsic factors - such as surface chemistry, pore size and volume, shape, and net charge – and extrinsic factors like soil pH, organic matter content, and mineral composition, and water content influence its sorption behavior towards nanoparticles significantly (Dimkpa, 2018). Therefore, understanding these interactions is critical for formulating efficient nanofertilizers that optimize nutrient delivery and promote sustainable agriculture while minimizing environmental impacts.

At the molecular level, interactions between nanofertilizer components and soil constituents govern sorption processes (Bhardwaj et al., 2022; Hu & Wei, 2023; Kabiri et al., 2017; Khan et al., 2021; Tarafder et al., 2020; Yaphary et al., 2023). Therefore, the need for a molecular-level understanding of sorption dynamics in biochar-based nanofertilizer formulation is paramount to leverage the full potential of nanotechnology in agricultural nano-formulations. By integrating interdisciplinary research with advanced analytical tools, we can optimize nanofertilizer designs to address global food challenges while safeguarding environmental health.

In contemporary agricultural practice, reliance on efficient fertilizers has grown significantly. However, Yadav, Yadav and Abd-Elsalam (2023) noted that excessive chemical fertilizer usage, especially nitrogen, contributes to nutrient depletion and environmental degradation. Additionally, studies confirm that overuse of commercial inorganic fertilizers can lead to the loss of between 50% and 70% of potassium in the soil even before the farm is utilized (Dimkpa, Andrews, et al., 2020; Yadav et al., 2023). In contrast, nanofertilizers offer an environmentally friendly alternative by leveraging a different mechanism of action. These engineered biofertilizers containing nanonutrients adsorbed to the surface of a matrix material, can ensure the required amount is cumulatively and progressively released to the plants. This approach leads not only to significant cost savings but also more efficient, environmentally friendly, and economically sustainable farming practices (Dimkpa & Bindraban, 2018; Raliya et al., 2018).

Material scientists have effectively utilized atomistic insights from molecular dynamics (MD) simulations in product developments (Mousavi et al., 2023) using modelling software packages like BIOVIA Material Studio. These tools enable prediction, analysis, and optimization of structures at the molecular scale prior to synthesis. Various modules can be applied based on the material type being investigated under different preset ambient conditions. However, despite their potential, MD simulations have been overlooked in studies involving nano-enabled biochar-based fertilizers. This oversight highlights a significant gap in research that this study aims to address by exploring how intrinsic properties of biochar influence its adsorption interactions with nanonutrients at a molecular level using conceptualized biochar structures within Material Studio Suite software.

2.4.1 Experimental techniques for studying biochar-nanonutrient interactions

The physical properties of the biochar are significantly affected by the chemical properties of the biomass used as a substrate in the overall process. Chemical attributes, such as the cellulose and lignin content that underlie the structural elements and morphology of the biomass, dictate such processes as thermal decomposition (Sun, Hu, Mu, Wang, & Yu, 2022). The thermal stability of the biochar can be associated with the chemical composition of the feedstock, with hemicellulose having the largest impact (Chen et al., 2022; Ma et al., 2019). In some instances, the sorption characteristics of the biochar are increased through amalgamation with nanoparticles. Nanomaterials, such as manganese oxide, have proven efficient in modulating its absorption capacity given the large surface area and polycrystalline structure. (Dhuldhaj, Singh, & Singh, 2022) noted that the conjugation of biochar with MnOx can improve the biochar affinity and adsorption for different materials. Thermogravimetric analysis (TGA) alongside differential scanning calorimetry (DSC) is effective in evaluating the thermal stability and desorption patterns of biochar-nanonutrient conjugates (Li & Chen, 2018). TGA is appropriate when the goal is to assess the thermal degradation properties and stability of biofertilizers, given that it measures the progressive weight loss based on the temperature. Comparatively, DSC offers insight into the phase transition and the thermal activities that are linked to the respective nanomaterials (Li & Chen, 2018; Nyakuma, Syieluing, & Olagoke, 2021).

As stated, the physicochemical aspects of the precursor biomass determine the chemical and structural configuration of the biochar, and subsequently, the ensuing nanoformulations. Therefore, understanding the surface morphology and the structural elements of the material can provide important insights into critical attributes, such as thermal stability. The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can be used in this regard to visualize the respective variables, providing insights into the micro- and nano -scale structures, respectively

(Singh, Amonette, Camps-Arbestain, & Kookana, 2024). SEM characterization is important as it highlights the surface topography, pore structure, and particle size distribution of the biochar (Bushra & Remya, 2024). Information on these attributes is crucial, given that they directly define the adsorption and removal kinetics of the nanonutrients after biochar impregnation. Mercury intrusion porosimetry can be used as a complementary experimental approach as it is effective in determining the specific surface area and pore size distribution in biochar (Zhao, Yang, & Cai, 2021). This technique works by measuring the rate and level of mercury intrusion into the pores of the material, thereby highlighting the volume metrics as well as the number of pores distributed on the given surface. Brunauer–Emmett–Teller (BET) analysis can be utilized in such cases to determine the specific surface area, thereby quantifying the adsorption and desorption isotherms that are necessary for calculating the area (Irewale, Elemike, Shaik, Dimkpa, & Oguzie, 2025; Khalili, Ramjerdi, Boostani, & Ghaderi, 2024).

2.4.2 Characterization of biochar and nanonutrient materials

Characterization of biochar and nanobiochar (NB) requires a series of steps that are not only procedural but also determine the analysis phase. Biochar and the nanomaterial must be prepared to ensure that the respective entity is effectively structured, chemically, and elementally composed to optimize adsorption-desorption rates (Jedynak & Charmas, 2024). Preparation of nanomaterials is crucial because the process may be reiterative, and lack of appropriate samples may lead to negative outcomes. Zhou, Zhang, Zhou and Wang (2019) conducted a study where they used *E. crassipes* stem biochar (ECSBC) to illustrate the optimal process for preparing this biomass. ECSBC was optimized using the response surface methodology (RSM), which is efficient in determining the appropriate interactions among critical processes, including the heating time, the heating temperature, and the heating rate (Zhou et al., 2019). This study aimed to assess the removal of

Cd^{2+} and the conclusion was that under optimal conditions, the biochar depicted maximum removal rates and high adsorption rates meaning that RSM was effective in preparing the condition essential for ECSBC.

There are multiple ways of characterizing biochar and NB. Nanobiochar preparation, as outlined by Song, Chen, Zhao, Qiu and Cao (2019), entails grinding biochar in a mortar and pestle, followed by suspension in distilled water, sonication, and settling overnight to allow particles smaller than 1 micron to precipitate. After centrifugation and drying, the nanobiochar layer is collected and characterized. The yield of both biochar and nanobiochar is determined based on mass ratios before and after pyrolysis. Subsequently, pH and electrical conductivity (EC) measurements are conducted using a 1:10 (w/v) sample-to-water ratio, while Fourier-transform infrared (FTIR) spectroscopy is employed to obtain spectra in the 4000–550 cm^{-1} range (Song et al., 2019). X-ray powder diffraction (XRD) analysis, utilizing monochromatic X-ray radiation, provides insights into crystal structures (Bhandari et al., 2023). XRD is employed to investigate the crystallographic structure and phase composition of nanonutrient materials. XRD patterns reveal characteristic diffraction peaks corresponding to crystalline phases, allowing for the identification of crystal structures and phase transformations during biochar-nanonutrient interactions (Minervino & Belfield, 2024). Finally, the surface morphology of biochar and nanobiochar can be examined using SEM to delineate macro-pore shape. Each characterization technique offers unique perspectives on the structural, chemical, and morphological attributes of biochar and NB, collectively contributing to a comprehensive understanding of their properties and interactions in biofertilizer formulations

2.5 Impacts of nanofertilizers versus conventional fertilizers

2.5.1 Nanofertilizers' performance by soil types

The performance of nanofertilizers is a subject of paramount importance in modern agriculture, with potential implications for crop yield, soil health, and environmental sustainability. The wide variety of soil types used in agricultural settings significantly impacts nanofertilizer efficiency. In many African contexts, the usefulness of traditional chemical fertilizers has been limited by issues such as a lack of soil diagnosis, poor soil fertility, and non-compliance with prescribed fertilizer recommendations (types and dose) (Kihara, Bolo, Kinyua, Rurinda, & Piikki, 2020). It is, therefore, essential to understand how various soil properties can potentially affect the effectiveness of nanofertilizers to optimize their utilization and fully exploit their capabilities (Dimkpa et al., 2023). Soil, a multilayered composition of minerals, organic matter, and microorganisms, displays substantial variation in its physical and chemical characteristics. Several variables may influence the effectiveness of nanoparticles when applied in soil, with soil organic matter composition, texture, ionic strength, presence and form of anions, pH, and soil biology (such as microbial activities) being a few of them (Dimkpa, 2018; Larue et al., 2018). One essential factor is the soil's cation exchange capacity (CEC), which regulates nutrient retention and availability. Undoutedly, the manner in which nanofertilizers interact with the soil matrix and release nutrients to plants is greatly affected by the CEC values of the soil. Similarly, soil pH is critical to the interactions between nanofertilizers and soil. As discussed by Dimkpa (2018), it can influence the aggregation, solubility, and chemical transformation of nanoparticles. The stability of nanofertilizers would be affected differently by acidic versus alkaline soils, which are both common in certain agroecological zones in Africa. Thus, tailoring nanofertilizer formulations to appropriate pH ranges becomes critical to ensure stability and performance across varied soil types in the continent.

Generally, the agronomic effectiveness or the fate of nanofertilizers in the soil depends on the mechanisms of interactions between both the nanofertilizer and the soil. Such mechanisms of action are influenced by the soil properties or soil types, on one hand, and the intrinsic properties of the nanofertilizer, on the other hand (Dimkpa, 2018). For instance, in acidic soil, bionanomaterials may be prone to higher dissolution than in alkaline soil, this partly because in the latter, dissolution is inhibited due to the propensity for particle aggregation. The ionic strength of soil will also affect the particle behavior by influencing aggregation. Hence, soil characteristics such as its chemical properties (e.g. cation exchange capacity, pH, organic matter), biological properties (e.g. microbial activities, plant root exudates, microbiome) and physical properties (e.g. texture, porosity, aggregate stability) can modulate the performance of nanofertilizers (Dimkpa, 2018; Larue et al., 2018) Together with the soil properties, nanoformulation properties play a significant role on its bioactivity and bioavailability mechanisms. These properties include nanonutrient adsorption/desorption properties, solubility, pore sizes, concentration, and nanocomposition (Kihara et al., 2020; Larue et al., 2018). Therefore, by modulating the mechanisms of action, desired outcomes and improved effectiveness of bio-nanofertilizers can be achieved. For example: slow or controlled release of the nutrients, which is achieved by functionalizing the nanoparticles surfaces; developing enhanced efficiency fertilizers (EEFs) which are achieved by balancing their composition; modified pores or composite to promote regulated nutrient release; the tuning of the nanofertilizers to respond to different soil chemistries such as acidity and alkalinity; and designing of the fertilizer to specific crop needs among others (Dimkpa et al., 2023; Larue et al., 2018; Raliya et al., 2018).

2.5.2 Environmental sustainability and benefits of nanofertilizers

While their application in agriculture is relatively novel, nanomaterials have been extensively utilized in such areas as cosmetics, electronics, optics, and sensor technology (Raliya et al., 2013).

In agriculture, nanotechnology promises beneficial applications, including developing effective fertilizers, pesticides, herbicides, and bioconjugated amendments (Giri et al., 2023). The use of nanomaterials in fertilizers leverages such properties as controlled-release dynamics, high sorption capacity, improved bioavailability, large interfacial area, and targeted delivery to boost crop yield with only a minimum of the detrimental environmental effects associated with conventional fertilizers (Basavegowda & Baek, 2021). Many studies have empirically demonstrated the synthesis and utilization of nanofertilizers, with associated benefits including improved crop quality, higher yield, stress tolerance, and robust growth (Dimkpa, Fugice, et al., 2020; Nongbet et al., 2022; Zahra, Habib, Hyun, & Shahzad, 2022). Various nanomaterials have been synthesized for crop production, including metals, metalloids, anions, carbon, and biogenic nanoparticles. Similarly, nanofertilizers are fabricated in varying formulations, ranging from nanoscale macro- and micro-nutrients to nanobiofertilizers and nano-enabled fertilizers (Bairwa, Kumar, Devra, & Abd-Elsalam, 2023). Depending on the formulation and specific nutrient included in nanofertilizers, their application in crop production has been linked to significant beneficial outcomes.

2.5.3 Environmental benefits

Despite delivering the same nutrient elements as conventional fertilizers, nanofertilizers are less injurious to the local soil and water ecosystems. This improved environmental impact arises from the materials' higher nutrient-use efficiency, lower residual accumulation, controlled nutrient delivery, and minimal release of toxic components (Al-Mamun et al., 2021; Khan, Mobin, Abbas, AlMutairi, & Siddiqui, 2017). The low nutrient use efficiency accounts for a major drawback of conventional fertilizers, with N, P, and K fertilizers having estimated maximum nutrient usage efficiencies of 35%, 20%, and 40%, respectively (Al-Juthery, Lahmod, & Al-Taee, 2021). In contrast, the nutrient use efficiencies of macronutrient nanofertilizers have been shown to approach

50% to 70%, representing a significant improvement in delivery (Al-Juthery et al., 2021; Shalaby et al., 2022). Abdel-Hakim et al. (2023) demonstrated that combining conventional fertilizers with nanofertilizers reduced the traditional fertilizer needed for optimum yield in lettuce plants by 50% to 75%. In another study comparing the rate of nutrient delivery between conventional and nano-based fertilizers and pesticides, Chhipa (2017) found a more efficient release of N, P, K, Zn, and Cu from nanofertilizers, compared to conventional fertilizers. Moreover, nanoformulations demonstrated superior capacity for targeted mineral delivery, resulting in smaller amounts of the fertilizers being used and better overall efficiency. Similarly, pesticide nanoformulations showed higher efficiency in broad-spectrum pest protection, primarily as a direct result of a higher compound availability (Abdel-Hakim et al., 2023). The higher nutrient-use efficiency associated with nanofertilizers has been attributed to their higher surface area available for metabolic processes, tunable solubility, and easier uptake into the root and shoot transport systems (Mahanta et al., 2019; D. S. Meena, Gautam, Prakash Patidar, & Meena, 2017). Collectively, these properties increase the proportion of nutrients delivered to the plant, lowering the nanofertilizer requirements, and reducing the environmental impact in comparison to traditional fertilizers.

A notable environmental benefit of nanocomposite fertilizers is the capacity to improve soil quality by amending nutrient cycling and supporting local microbial populations. The loss of soil biodiversity, high acidity, and suboptimal root zone temperature arising from repeated cultivation comprises important mechanisms of fertility loss (Rajput et al., 2021). Besides the use of cover crops and prolonged fallow periods, counteracting these forms of soil quality deterioration is a challenging task. However, the unique surface characteristics that nanomaterials offer have been shown to improve the biological composition and physicochemical features of soil (Rajput et al., 2023). Studies examining the metabolic reactions involving locally applied nanomaterials on plant roots have reported dynamic root exudation of nanoparticles, improvement in soil pH in acidic locales, and regeneration of beneficial soil microbiomes (Bray, Kao-Kniffin, Frey, Fahey, & Wickings, 2019; Oloumi, Soltaninejad, & Baghizadeh, 2015; Zahra et al., 2015). In addition, nanomaterials have been shown to enhance rhizosphere phytostimulation through promotion of phytohormone biosynthesis (Oloumi et al., 2015; Zahra et al., 2017). By influencing the soil pH and microbiome, nanomaterials can improve physicochemical and biological profiles, thereby serving as an effective soil quality restoration strategy. The slow and controlled release of nutrients from nano-dimensional adsorbents confers considerable environmental benefits to nanofertilizers over conventional chemical fertilizers. Several mechanisms have been designed, characterized, and experimentally assessed for controlled and targeted delivery of nutrients in nanofertilizers, including three-dimensional polymeric scaffolds, nanoparticle coating, and green synthesis (Giroto, Fidélis, & Ribeiro, 2015; Madzokere, Murombo, & Chiririwa, 2021; Rop et al., 2018). For instance, Tarafder et al. (2020) synthesized urea-modified hydroxyapatite scaffolds to which copper, iron, and zinc nanoparticles were loaded and their uptake rate was monitored using a model of *Abelmoschus esculentus*. Compared to the conventional fertilizer, the nano-based formulation achieved a sustained, slow release of the nutrients with an application rate 100 times less than that of conventional fertilizer (Tarafder et al., 2020). Polymeric encapsulation of nutrient nanoparticles is one of the commonest techniques used to achieve controlled release (Beig et al., 2022; Guo, White, Wang, & Xing, 2018). By isolating the reactive core from the environmental conditions, the system shields it from rapid degradation and allows for targeted delivery of nutrients. In addition, the polymers used for nanoparticle coating contribute to the slow nutrient delivery through self-degradation (Choudhary et al., 2019). This technique has been applied in the development of targeted-release urea, zinc, and other nanoparticles in fertilizers (Antul Kumar et al., 2021; Zeng, Nyapete, Benziger, Jelliss, &

Buckner, 2019). Besides minimizing nutrient wastage, the slow release prevents the accumulation of leached elements and carriers in the soil, which is a primary environmental concern with conventional fertilizers. Since leached minerals in traditional fertilizers increase soil acidity and are washed into water bodies, the lower rate of chemical accumulation at the application point reduces the buildup of fertilizer components in the environment. Moreover, the capacity for slow degradation lowers the amount of fertilizer required for optimal crop production, further limiting the negative environmental impacts associated with heavy chemical use. Ultimately, the use of nanofertilizers confers significant environmental benefits arising from the lower fertilizer requirements and reduced leaching of materials into the surrounding soil.

The incorporation of naturally occurring biogenic materials in nanofertilizer synthesis represents another dimension of their environmental benefits. Multiple biomaterials have been utilized as polymeric vehicles for nutrients, including chitosan, cellulose, plant extracts, zeolites, and fungi (Angelo, França, & Faez, 2021; Ram, Vivek, & Kumar, 2014). Chitosan is among the most well-established biogenic materials applied for this purpose, with studies reporting the synthesis and field application of copper, NPK, potassium, zinc, and other nanomaterials using chitosan biopolymers. Chitosan is a biogenic polymer derived from chitin, a seafood biowaste (Angelo et al., 2021). Its possession of functional groups, including amine ($–NH_2$) and hydroxyl ($–OH$), lends useful biochemical characteristics that make it useful for several nanomaterial applications, including delivery of specific nutrients as nanofertilizers (Prajapati et al., 2022). Chitosan nanomaterials have been used to achieve efficient slow-release of urea, phosphorus, zinc, and copper, demonstrating the potential to serve as next-generation high-precision biogenic nanofertilizers (Dimkpa et al., 2023; Kumaraswamy et al., 2021). Due to its natural origin, biocompatibility, and biodegradability, the use of chitosan nanopolymers represents a notable step away from the environmental toxicity of chemical fertilizers (Chouhan & Mandal, 2021). A similar trend in the development of nanofertilizers entails the use of plant extracts in the synthesis of metal oxide nanoparticles for crop production improvement. Several studies have demonstrated the synthesis, characterization, and experimentation of such nanomaterials as titanium oxide, zinc oxide, and copper oxide from different plant extracts. Plant-based silver, zinc oxide, and copper oxide nanoparticles have been shown to enhance chlorophyll content, fatty acid composition, flavonoid concentration, disease tolerance, and shoot growth in maize, soybean, sunflower, and other plant species (Batool et al., 2021; Del Buono, Di Michele, Costantino, Trevisan, & Lucini, 2021; Karmous et al., 2023; Salih et al., 2021; Velázquez-Gamboa et al., 2021). In addition to serving as micronutrients in novel nanofertilizers, the nanomaterials developed in this way provide elemental co-factors for plant enzymes in key biochemical pathways occurring in target plants (Karimi, Behbahani, Dini, & Razmjou, 2018). Overall, the biosynthesis of nanomaterials from plant extracts confers biocompatibility and environmental nontoxicity to the resultant nanofertilizers, further lowering the materials' detrimental environmental footprint.

2.5.4 Improved growth and crop yield

Nanofertilizers have been found to induce both rapid plant growth and higher yield across a variety of crops. The combination of higher nutrient-use efficiency, controlled release, greater bioavailability, and effective uptake of nanomaterials used for soil fertility result in rapid biomass gain and productivity of multiple crop varieties. Some studies have compared nanofertilizers with conventional fertilizers, demonstrating superior performance in terms of plant growth rate, biomass, and yield (Drostkar, Talebi, & Kanouni, 2016; Nongbet et al., 2022). In a field study using wheat, millet, mustard, and sesame, Kumar et al. (2022) found that the application of zinc and nitrogen nanofertilizers resulted in higher yields across all tested plant varieties, compared to the use of conventional fertilizers. For instance, as summarized in Table 2.5, in nanofertilizer-treated sesame, yields were 24.24% higher than those in plants treated with conventional fertilizers (Kumar et al., 2022). In another study, foliar-sprayed nanofertilizers induced a 37.7% increase in yield in pearl millet (Tarafder et al., 2020). Similar findings have been reported following the application of zinc oxide, silver, urea, iron, and copper nanoparticle fertilizers in a variety of crops, including maize, cotton, and wheat (Afshar, Rahimihaghighi, & Shirazi, 2014; N. Kumar, Samota, Venkatesh, & Tripathi, 2023; Mahil & Kumar, 2019). In addition to increasing yield, nanofertilizers have been shown to enhance the concentration of crude protein, phosphorus content, and flavonoid in various fruits (Kumar et al., 2023). As a result, the application of nanofertilizers is associated with higher-quality produce, including higher nutritional content and micronutrient value of fruits grown with nanocomposite fertilizers (Davarpanah, Tehranifar, Davarynejad, Abadía, & Khorasani, 2016; El-Ramady et al., 2020). Nanofertilization has also been reported to drive rapid plant growth and maturation based on biomass accumulation and shoot length. Overall, nanofertilizers have the potential to sustainably improve the growth rate and productivity of several crop varieties, as well as serve as a means of fruit fortification for quality improvement. Table 2.5 shows a summary of key findings from some nanofertilizer studies.

2.6 Nanotechnology and agriculture in Africa

2.6.1 Current perspectives on agriculture and nanotechnology

Despite agriculture remaining the mainstay of the economies of most African economies, its development is characteristically lagging those of the more developed countries. A combination of climate change, erratic rains, deteriorating soil quality, and suboptimal farming practices have birthed repetitive cycles of low crop production that poses significant threat to the continent’s food security (Dimkpa et al., 2023; Liverpool-Tasie, 2017). A substantial proportion of African farmers

Table 2.5 Effects of nanofertilization (NF) vs chemical fertilization (CF) for selected crops

Growth Factors	Crop	Specific outcomes
Plant Height	Wheat	NF produced not significantly taller plants than CF
	Sesame	NF produced not significantly taller plants than CF
	Pearl Millet	NF produced significantly taller plants than CF
	Mustard	NF produced significantly taller plants than CF
Growth and Development (e.g. number of tillers or spikelet or ear head or seeds, per plant)	Wheat	NF showed more tillers and spikelets than CF
	Sesame	NF produced more capsules per plant than CF
	Pearl Millet	NF produced more ear heads per plant than CF
	Mustard	NF produced more siliquae per plant than CF
Grain Yield	Wheat	NF produced significantly higher yield than CF
	Sesame	NF produced significantly higher yield than CF
	Pearl Millet	NF produced significantly higher yield than CF
	Mustard	NF produced significantly higher yield than CF

Adapted from Kumar et al. (2022).

practice smallholder subsistence crop production, which is a small-scale practice with little capacity to exploit the economies of scale necessary for profitable crop farming. In much of sub-Saharan Africa, rapid degradation has produced highly acidic soils with low concentrations of phosphorus, nitrogen, and zinc. Consequently, farmers have long relied on chemical fertilizers and composts for soil fertility improvement (Bekunda, Sanginga, & Woomer, 2010; Binswanger-Mkhize & Savastano, 2017; Dimkpa et al., 2023). However, sustained rise in prices and resource limitations have translated to insufficient utilization of chemical fertilizers in crop production, further worsening crop yields in the continent. As Barrett and Bevis (2015) observed, the interplay between low soil quality and low household capital creates a self-reinforcing cycle that continuously lowers crop yields among small-scale farmers. The impact of this cycle of soil infertility and high costs of fertilizers is apparent in many sub-Saharan African countries, where capital restrictions result in inefficient farming practices with low fertilizer utilization (Burke, Jayne, & Snapp, 2022; C. Dimkpa et al., 2023; Hammond et al., 2023; Liverpool-Tasie, 2017; Matsumoto & Yamano, 2011; Sheahan, Black, & Jayne, 2013). This situation has only worsened following the recent war outbreak in Ukraine, which has driven inflation upwards and disrupted global fertilizer and agricultural implements logistics. Coupled with longstanding market imperfections and logistical challenges, the high fertilizer prices have increased concerns over the profitability of the products in commercial farming (Liverpool-Tasie, Omonona, Sanou, & Ogunleye, 2017; Liverpool-Tasie, 2017). Traditionally, African governments have attempted to address the problem of high fertilizer prices through national subsidies and strategic partnerships designed to make the product more affordable for resource-constrained small holder farmers (Boulanger, Dudu, Ferrari, Mainar-Causapé, & Ramos, 2022; Mason & Jayne, 2013; Ricker-Gilbert, Jayne, & Chirwa, 2011; Takeshima & Liverpool-Tasie, 2015). However, nanofertilizer as described in Table 2.6 offers several benefits.

Table 2.6 Key Findings from bio-nanofertilizer studies

Crop Type	Bio-Nanofertilizer Formulations	Key Outcomes	Citation
Chickpea	Fe, Zn, NPK Nano-fertilizers	Improved seed yield and morphological traits.	Drostkar et al. (2016)
Cereal, Oilseed Crops	Nitrogen and Zinc Nanofertilizer with Organic Farming Practices	Positive effects on crop growth.	Kumar et al. (2022)
Agricultural Crops	Nanofertilizers	Review of foliar application in agricultural crops.	Mahil and Kumar (2019)
Wheat	Nano Zinc Oxide and Zinc Oxide	Comparison of effects on wheat.	Afshar et al. (2014)
Pomegranate	Zinc and Boron Nano-fertilizers.	Improved fruit yield and quality.	Davarpanah et al. (2016)
Lettuce	Curtobacterium herbarium strain CAH5	Aluminum bioaccumulation and growth enhancement.	Silambarasan, Logeswari, Valentine and Cornejo (2019)
Citrus	-	Citrus Changing in diverse environments.	Vincent, Morillon, Arbona, and Gómez-Cadenas (2020)
Rice	Nanomaterial Effects on Rice Responses	Benefits of nanotechnology for agricultural applications.	Wang et al. (2021)
Arabidopsis thaliana	Differentially charged Nanoplastics	Accumulation in Arabidopsis thaliana.	Sun et al. (2020)
Hordeum vulgare L.	Nano-Fe2O3	Restoration of plant growth in contaminated soils.	Rodríguez-Seijo et al. (2022)

While such subsidy programs have been somewhat successful in encouraging chemical fertilizer use to increase crop yields in the continent, they have failed to achieve globally competitive fertilizer use and crop productivity. Along with the increasing threat of climate change and progressive soil quality degradation, this gap represents a notable potential for future deployment of nanofertilizers on the African continent.

2.6.2 Current challenges and opportunities

Despite the potential benefits offered by the application of nanofertilizers to address soil quality and crop yield issues in Africa, the technology's realization remains subject to several challenges (Dimkpa et al., 2023). The relatively lagging nanotechnology landscape on the continent comprises a major barrier to its development and eventual deployment in agriculture. While significant progress has been made in promoting the development of the technology in a few countries, most of the continent remains in an early stage characterized by remote interest and theoretical research on the innovation (Lateef, Azeez, Suaibu, & Adigun, 2021). Importantly, the lack of proper regulatory framework and insufficient investment of capital and human resources in nanotechnology will likely limit its development in many African countries. The strain occasioned by fertilizer subsidies and other economic policies aimed at effecting agricultural reforms is likely to contribute to the limited investments in nanotechnology research and development (Irewale et al., 2024). As a cutting-edge innovation, nanotechnology requires significant investments in equipment, human resource, and infrastructure, the absence of which presents an important obstacle to its adoption in Africa. However, many African countries have introduced relevant nanotechnology curricula in educational institutions, a trend that will avail the necessary expertise for development of the innovation (Chugh, Siddique, & Solaiman, 2021; He, Deng, & Hwang, 2019). Finally, the adoption of nanotechnology in Africa and elsewhere is subject to concerns over its potential environmental and human health and safety risks. Several studies have been dedicated to examining the toxicity of nanomaterials in the environment and their potential effects on human health (Bhardwaj et al., 2022; Fu, Xia, Hwang, Ray, & Yu, 2014; Verma et al., 2022). To conclusively determine the balance between beneficial application of nanomaterials and undesirable implications, further research, economic viability analysis, and firm regulatory frameworks are necessary.

The apparent challenges notwithstanding, Africa is faced with significant opportunities for practical application of nanotechnology in agriculture and other fields. Along with the research and regulatory frameworks being established, the continent's vast natural resources place it in a unique position for local synthesis and utilization of a wide range of nanomaterials, especially the mineral and plant-based variants. Africa owns close to 30% of global mineral reserves, with minerals accounting for an average of 70% of total African exports and about 28% of gross domestic product (African Development Bank Annual Meeting, 2023). In addition, most natural reserves of such minerals as phosphorus, copper, zinc, chromium, platinum, cobalt, and diamonds are found in the continent, presenting a vast supply of some of the minerals commonly used in nanoparticle synthesis. Besides its natural resources, Africa has large amounts of arable land and a prominent human resource base. These natural and human resources constitute a solid foundation for the potential establishment of a robust nanotechnology industry. While many African countries' mining industries are largely export-oriented, increasing costs and logistical challenges are driving efforts toward local processing and utilization of natural resources. For instance, Zimbabwe, which holds the world's second-largest chromium deposits, banned the exportation of chrome concentrates and raw ore in 2021 (Mavhunga, 2023). This development mirrored the country's efforts to improve its ferrochrome industry by processing mined resources locally. Similar efforts have been witnessed in the Democratic Republic of Congo (DRC), which is the source of two-thirds of global cobalt supplies. In 2021, the country introduced a ban on the exportation of copper and cobalt concentrates, seeking to increase earnings from the minerals' utilization in the electric vehicle and smartphone markets (Mavhunga, 2023). Coupled with the emerging regulatory and research framework for nanotechnology in such countries as South Africa, these policies present significant opportunities for local synthesis, characterization, and practical application of nanotechnology in multiple fields, including agriculture. Therefore, through enabling policies and capital investments, African countries face considerable opportunities for exploiting their natural resources to develop local nanotechnology solutions to longstanding agricultural challenges.

2.6.3 Compatibility of nanofertilizers with ecological zones in africa

African countries have under-utilized their abundant arable resources due to lack of well-educated and competent human capital within the agricultural and agro-allied sectors. This is also partly due to the lack of availability of fertilizer plants on the African continent making those countries dependent on expensive imported fertilizers (Bekunda et al., 2010; Liverpool-Tasie, 2017; Matsumoto & Yamano, 2011). For example, there are only four ammonia plants in Africa, therefore, the importation, local transportation, and storage costs drive the price of fertilizers up for many African farmers. Thus, the overall agricultural production landscape of Africa fits into the concept of nanotechnology, that can assure improved agricultural outputs with minimal resources utilization, albeit, with negligible ecological and environmental footprints (Ram et al., 2014; United Nations. Economic Commission for Africa, 2020). Although the introduction of nanofertilizers holds promise for transforming agricultural practices in many ecological zones across Africa, the challenge is the critical need to create nano-biofertilizers tailored to the many agroecological regions and soil types found in this vast continent (Gotosa, Kodzwa, Nyamangara, & Gwenzi, 2019). Clearly, establishing the compatibility of such new fertilizers with Africa's different agroecological zones is vital for their effective adoption and sustainable impact.

Africa incorporates many agroecological zones, each characterized by unique climatic conditions, soil types, and crop preferences. Since the nutritional requirements of crops vary, it is crucial to tailor the formulation to the crops grown in each area (Tandzi & Mutengwa, 2020). The ecological variety from the Sahel's dry landscapes to the lush tropical forests calls for a detailed understanding of how nanofertilizers will interact with the unique features of each zone. One crucial factor in assessing compatibility is the climate of a particular ecological zone. As with conventional fertilizers, climatic changes may have a varied effect on nanofertilizers. For example, the solubility and efficacy of nanofertilizers are impacted by the availability of water, which becomes a crucial element in dry environments (Gehlot et al., 2024). Formulations that enhance nutrient release with the water dynamics of these zones are vital for improving crop yields.

Crop variety across ecological zones necessitates flexible nanofertilizer compositions. Arid-adapted crops may have different nutritional needs than their humid-adapted counterparts. It is possible to improve the efficacy and economic feasibility of nanofertilizers by tailoring their compositions to the nutrient demands of crops common in each ecological zone. Additionally, considering the economic and social conditions that exist in various agroecological regions is crucial. Smallholder farmers are more likely to utilize and benefit from nanofertilizers if they are designed to work with local agricultural methods and resources (Verma et al., 2022). Thus, a comprehensive strategy is required to ensure compatibility between nanofertilizers and Africa's many agroecological zones. Analysis of these various agroecological zones in Africa is, however, outside the scope of the current studies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Description of sampling area

Water hyacinth (*Eichhornia crassipes)* used in this research was harvested from Ekpan river, located in the Uvwie Area of Effurun in Delta State, Nigeria (latitude 5^0 33’ 18” and longitude 5^0 44’ 42”). It is a significant waterway that flows through a region known for its urban life activities. Effurun, a prominent town near Warri, is part of the Warri metropolitan area, which is one of the key economic hubs in the Niger Delta due to its oil and gas industries. Ekpan river serves both as a natural resource and as an essential part of the local ecosystem, supporting various forms of wildlife and providing water for domestic and industrial uses. The location where water hyacinth samples were collected is shown in Figure 3.1 and appendix II.

3.1.2 Field materials

1. The location map of the study area and its environs which aided accessibility within and around Effurun Area in Warri.
2. A cutlass for the purpose of harvesting water hyacinth.
3. Set of polythene bags for keeping and safe transportation of the harvested samples.
4. Field notebook and other writing materials for the purpose of writing down anything observed in the field.
5. Global Positioning System (GPS) for geo-referencing points of sampling within the study area.
6. A Camera for photographic and videography data capturing.

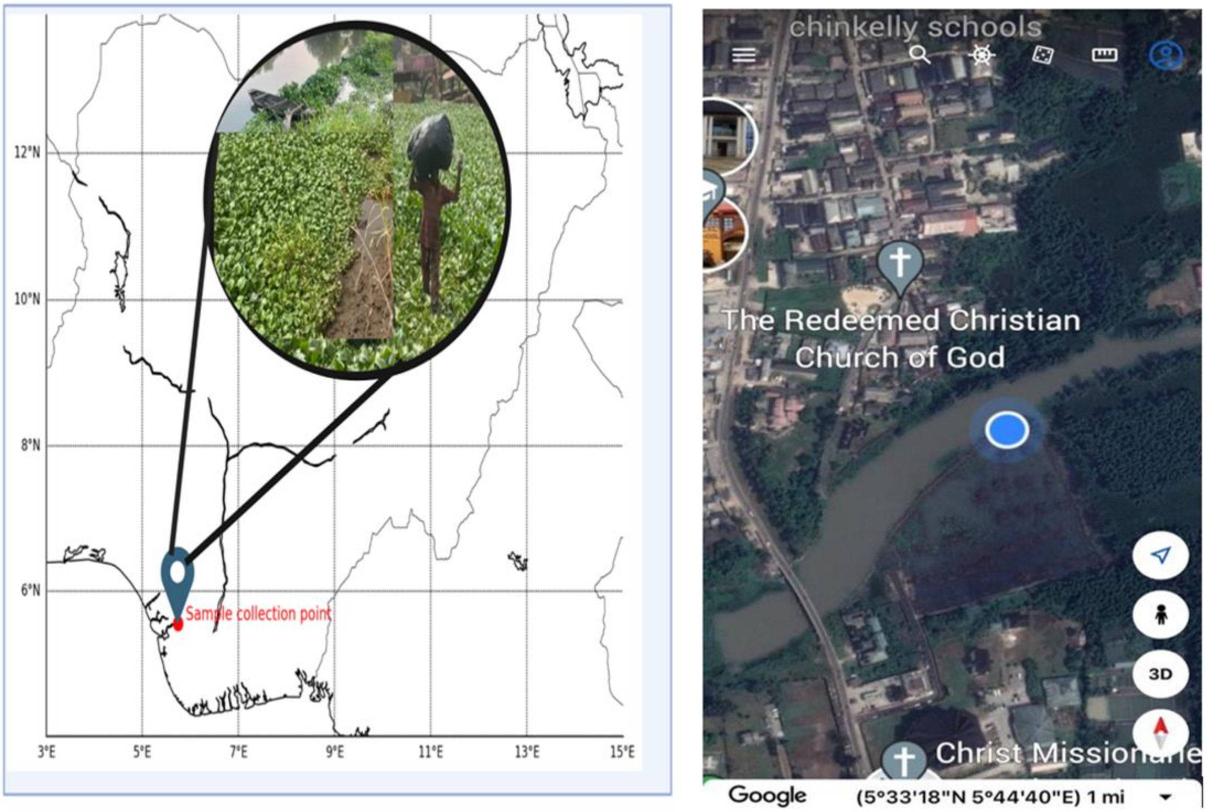

Figure 3.1: Map showing sample location in Delta State with aerial picture from google map.

3.1.3 ZnO and CuO nanoparticles

Commercial zinc oxide (ZnO) nanopowder (99.95% purity, ~18 nm, Stock# US3599) and copper oxide (CuO) nanopowder (99.95% purity, 25–55 nm, Stock# US3063) were purchased from US Research Nanomaterials, Inc. (Houston, TX, USA). The nanoparticles were used as received, without any further modification or purification, for the formulation of the nanofertilizer.

3.1.4 Laboratory equipment

The following major laboratory equipment and resources were used in the study;

1. Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy (SEM-EDX), Hitachi SU3800/SU3900 (Hitachi High-Tech Corporation, Tokyo, Japan)
2. Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) iCap Pro, Qtergra Version 2.14.5122 (Themo Fisher Scientific, MA, USA).
3. Accumet pH meter Accumet Research AR60 (Fisher Scientific, MA, USA).
4. Dynamic Light Scattering (DLS) Malverin Zeta Sizer Ultra (Malvern Panalytical, Malvern, Worcestershire, United Kingdom)
5. High vacuum physisorption analyser Autosorb iQ (Anton Paar) (QuantachromeInstruments, Anton Paar, Graz, Austria)
6. Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR), Bruker Invenio-S (Bruker Corporation, Billerica, Massachusetts, USA
7. Accumet Meter HQ4100 Series multiprobe meter (Hach, Loveland, Colorado, USA).
8. Vecstar muffle furnace model ECF2 (Vecstar Limited, Chesterfield, United Kingdom)
9. LECO FP828 Nitrogen Determinator (LECO Corporation, Michigan, USA)
10. Water Bath with shaker
11. Centrifuge

3.1.5 Data analysis

i. Microsoft Office tools

ii. SPSS software

iii. Origin software

iv. Material Studio 2020 Version

3.2 Methods

3.2.1 Research design

The overall research design workflow is shown in figure 3.2 whereas figure 3.3 shows snapshot of the processes involved in the experimental set-up.

3.2.2 Fieldwork - sample preparation and biochar production

3.2.2.1 Sample preparation

Samples were harvested from aerial tissues of Water hyacinth (WH) from Ekpan River at Uvwie Area of Effurun in Delta State, Nigeria. (Coordinates: latitude = 50 33’ 18” longitude = 50 44’ 42”). They were packaged in polythene bags and transported to Chemistry Department at Federal University of Petroleum Resources (FUPRE), Effurun in Delta State where samples were cleaned and separated into leaf biomass and stem biomass. Air-drying was carried out at ambient atmospheric conditions: temperature 30°C, humidity 71%, and atmospheric pressure of 29.84 inHg until samples were dried to a consistent weight.

3.2.2.2 Biochar production

Dry WH leaf biomass (D1) and dry stem biomass (D2) were pyrolyzed at 600^0C based on the method of Bottezini, Dick, Wisniewski, Knicker and Carregosa (2021) in oxygen deficient atmosphere for resident times of 15mins, 30mins, 45mins and 60mins at Chemistry Department of Federal University of Petroleum Resources Effurun (FUPRE), Nigeria using a Vecstar muffle furnace model ECF2 manufactured by Vecstar Limited, Chesterfield UK. Furnace temperature was set at 600^0C, increasing at 15^0C per minute until the set temperature is attained and maintained for the pyrolysis resident time duration for each treatment. Pyrolyzed biochar samples were cooled to room temperatures in airtight chambers to prevent oxidation. Biochar samples were thereafter milled and passed through a scientific sieve with a 75µm mesh pore to obtain consistent particle size range.

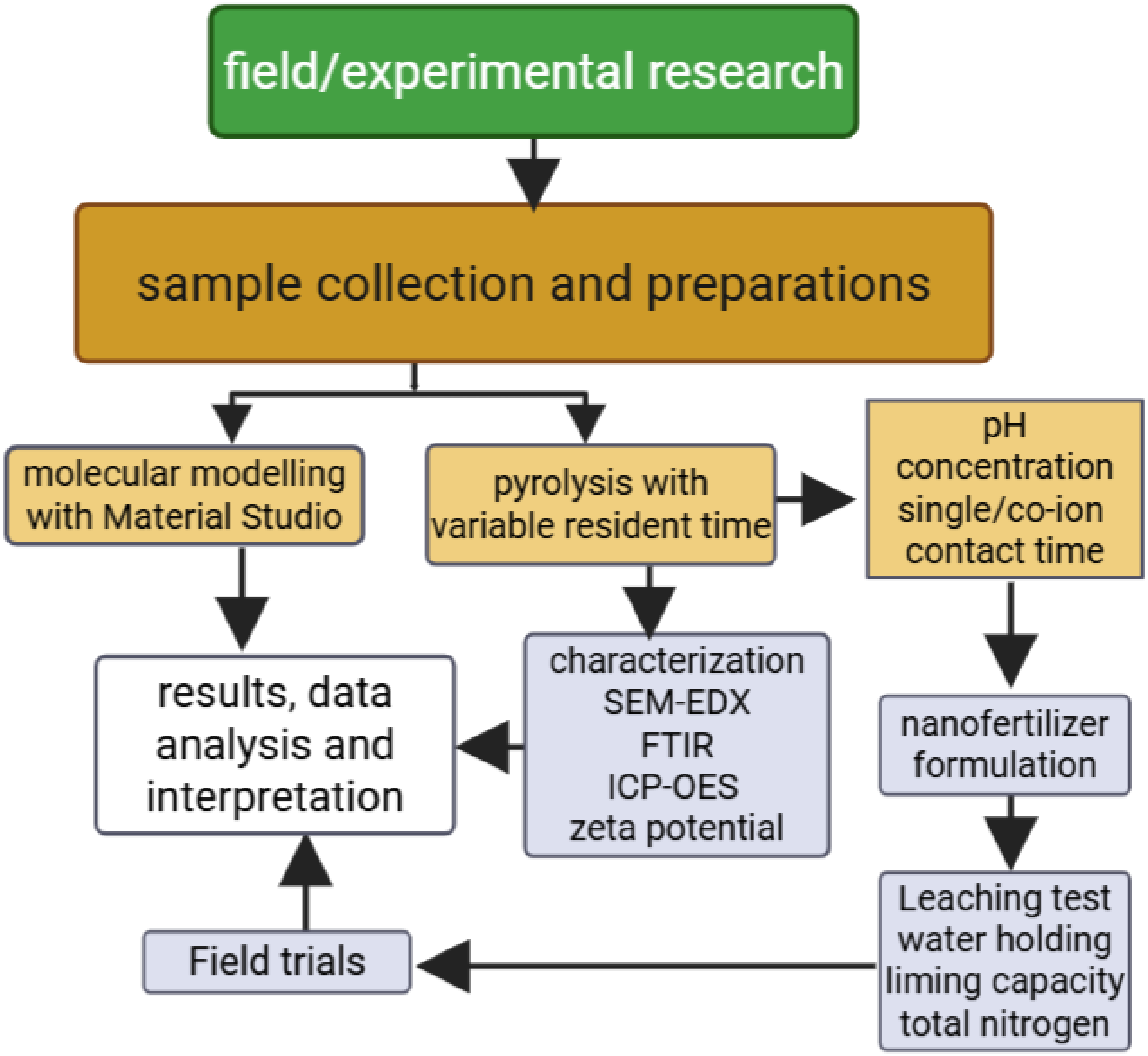

Figure 3.2 Outline for the field and experimental work

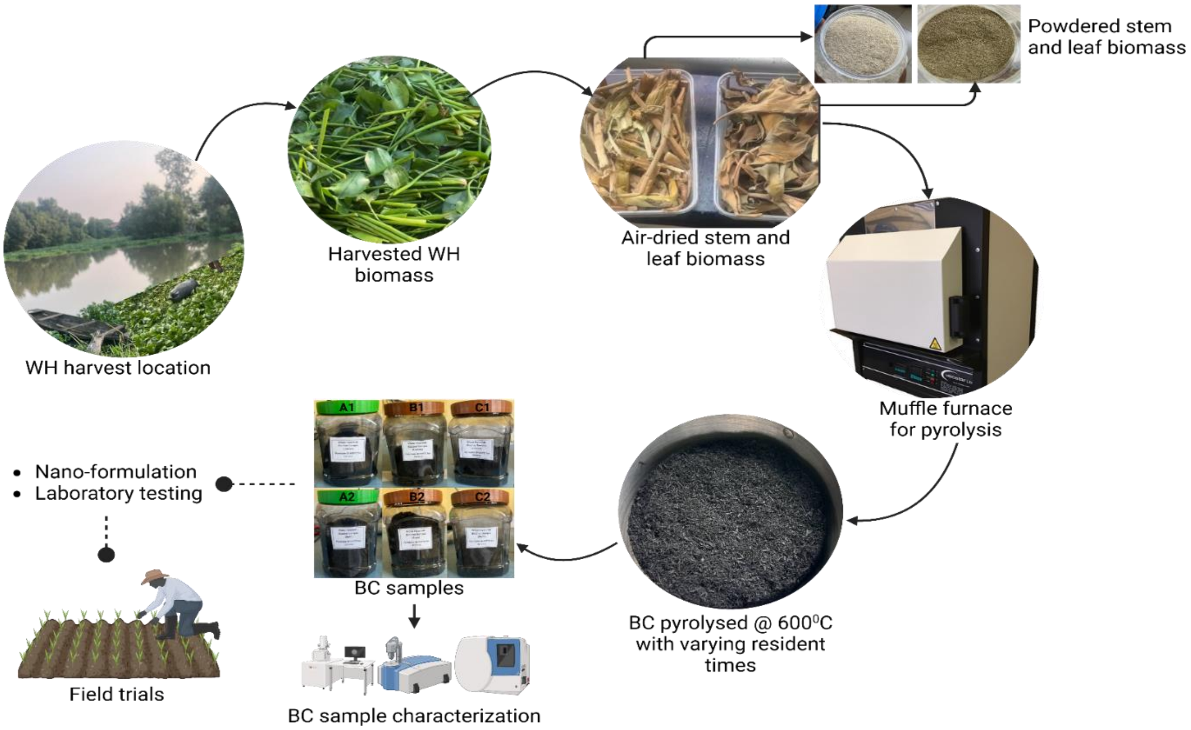

Figure 3.3: Process summary for WH upcycling to produce BC for nano-enabled biofertilizer formulation

Samples were labelled accordingly based on pyrolytic treatments as shown in table 3.1. Biochar samples were kept in sealed labelled plastic containers for further analysis. Biochar yield was calculated for D1 and D2 respectively.

Pyrolysis data were analyzed to determine biochar yields for D1 and D2 according to the equation 3.1 below:

$$Y_b = \frac{M_{bc}}{M_{bm}} \times 100\% \qquad 3.1$$

Y_b = biochar yield in percentage

M_{bc} = mass of biochar in grams

M_{bm} = mass of dry biomass in grams

3.2.3 Characterization of biochar: FTIR, ICP-OES, SEM

Biochar samples were characterized for physicochemical properties and determination of elemental constituents (Chia et al., 2012; Onorevoli et al., 2018). Fourier Transform Infra-Red spectroscopy (FTIR) was carried out with Agilent Cary 630 manufactured by Agilent Technologies, USA. Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) was carried out using Agilent 5800 VDV ICP-OES[Agilent Technologies USA]. Both analyses were carried out at the Connecticut Agricultural Experiment Station, New Haven, and West Campus Laboratory of Yale University, New Haven, USA. Sample morphology and relative elemental concentration were analyzed with Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy (SEM-EDS) using Phenom ProX, manufactured by Phenom World Eindhoven, Netherlands.

Table 3.1 Samples and their name tags

Sample description	Tag
Leaf biochar pyrolyzed at 600^0C for 30mins	A1
Leaf biochar pyrolyzed at 600^0C for 45mins	B1
Leaf biochar pyrolyzed at 600^0C for 60mins	C1
Stem biochar pyrolyzed at 600^0C for 30mins	A2
Stem biochar pyrolyzed at 600^0C for 45mins	B2
Stem biochar pyrolyzed at 600^0C for 60mins	C2
Powdered dry leaf biomass	D1
Powdered dry stem biomass	D2

i. Inductively Coupled Plasma-Optical Emmision Spectroscopy (ICP-OES)

The Agilent 5800 VDV Inductively Coupled Plasma-Optical Emmision Spectroscopy (ICP-OES) manufactured by Agilent Technologies USA was fitted with a SeaSpray nebulizer, double-pass glass cyclonic spray chamber, and semi-demountable dual view (DV) 1.8 mm i.d injector torch. The Agilent SPS 4 autosampler was used for the fast and automated delivery of the samples to the ICP-OES. Instrument operating parameters are shown in Table 3.2. Samples were dried and ashed in a muffle furnace and the residue was dissolved in 5% nitric acid, filtered and analyzed.

ii. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to analyze samples' morphology and microstructure. Before each test, a small layer of platinum was sputter coated onto the samples (Sputter Model: Quorum Q150R S) to counteract the charging effect, shield the samples from harm from the electron beam, and enhance picture quality during SEM (Nyakuma et al., 2021). The samples were then taken out and put in the SEM's sample chamber (Model JEOLJSM IT 300 LV, Germany) for examination. The SEM was operated at 20 kV and 5 mm (working distance). This approach was used to acquire the photographs at a magnification of up to 13,000x which was then used for the SEM examination. By analyzing the surface of the samples with the EDX detector connected to the SEM microscope, the surface morphology and elemental content of the samples was studied. The point ID analysis capability of the AZTEC EDX programme was used to identify and analyse each sample's elemental composition based on the SEM micrographs (Oxford Instruments, UK). As a result, each identified element's magnitude was estimated and expressed in weight percent (wt%).

iii. Fourier Transform Infrared (FTIR) spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was used to analyse functional groups in the biochar sample chemistry (Agilent Technologies, FT-IR Spectrophotometer carry 630, USA). The

Table 3.2 ICP-OES parameters

Parameter	**Axial**
Read time (s)	10
Replicates	3
Sample uptake delay (s)	16
Stabilization time (s)	15
Rinse time (s)	30
Pump speed (rpm)	12
Fast pump during uptake and rinse	Enabled
RF power (kW)	1.2
Auxilliary flow (L/min)	1.00
Plasma Flow (L/min)	12.0
Nebulizer flow (L/min)	0.7
Viewing Height (mm)	16
Sample pump tubing	Nominal size
Internal standard tubing	Nominal
Internal standard	Nominal
Waste pump tubing	Nominal
Background correction	Nominal

samples of powdered biochar were sieved via an analytical laboratory sieve (RetschTM, Germany) to achieve particles smaller than 250 microns. Next, a ZnSe prism plate containing precisely 5 mg of the chosen sample was used to scan for spectra from 4000 to 600 cm^{-1} based on Happ-Genzel apodization. Each sample was imaged 64 times for a total of 5s at a frequency of 16 cm^{-1}. The FTIR-ATR spectra will then be obtained by plotting the raw data.

iv. Zeta potential and hydrodynamic particle sizes

Zeta sizer was used to determine surface charges and particle size ranges of the biochar samples (Song et al., 2019; Suliman et al., 2016). Aqueous suspensions of the samples were prepared with deionized water, and the samples were placed in the Zetasizer for charge determination and hyrodynamic sizes. The data obtained were ploted on a chart.

v. Total biomass and biochar nitrogen determination

Total percentage nitrogen content for the WH dry biomass (D1 and D2) and biochar samples (A1, B1, C1, A2, B2, and C2) was analyzed with LECO FP828 Nitrogen Determinator (Morais et al., 2024). It measures nitrogen content in a sample through combustion of the sample, capturing gaseous nitrogen that is delivered to a detector to ultimately generate percent nitrogen in the sample.

vi. Determination of ammonium- and nitrate-nitrogen in biochar leachates

Determination of the amount of nitrogen in the biochar samples quantitatively and qualitatively were done to aid the necessary nano-fortification necessary for biofertilizer preparation (Cahill et al., 2024; Morais et al., 2024). Tests were carried out for nutrient leaching in the soil amended with the biochar samples . 20g of soil samples obtained from CAES greenhouse was amended with 2g of biochar samples at ratio 10:1 (wt/wt). The soil was thoroughly mixed with the biochar and the mixture placed in a tubular plastic vial with a hole at the base. Cotton wool was used as filter for the leachate and to prevent soil-biochar mixture from falling out (schematics shown in Figure 3.4). 20g of soil without biochar was used as a control. 25ml of deionized water was gently poured on the soil-biochar mixture and the control to obtained total leachate after 24hrs. The experiment was set up in triplicates and leachate samples were collected daily for 5days. The leachates were then analyzed for elemental composition with HQ4100 series accumet nitrogen specific multiprobe meter.

vii. Biochar aqueous nutrient release experiment

Aqueous biochar nutrient release/availability was tested by weighing 1g of biochar sample into a plastic tube. 50ml of deionized water was added and the mixture mechanically mixed. The mixture was left standing for 24hrs according to Singh et al. (2024). It was then placed in a centrifuge at 3,000rpm for 20mins to allow sedimentation of the biochar particles. 10ml of the supernatant solution was removed with a pipette and then the mixture made up to 50ml mark with deionized water. This was repeated daily for 5days and the aqueous extracts kept for elemental analysis.

viii. Nitrate-nitrogen determination in leachate and aqueous release samples

100mg/L, 10mg/L and 1mg/L standards of nitrate solution was prepared using NH_4NO_3 salt (Appendix I). The ammonium nitrate (analytical grade ≥98%) was bought from Sigma-Aldrich, USA. The standards were used in calibrating HQ4100 series accumet meter which was used to determine the nitrate-nitrogen concentration in the samples.

ix. Ammonium-nitrogen determination in leachate and aqueous release samples

100mg/L and 20mg/L standards of ammonium solution was prepared using NH_4NO_3 salt. The ammonium nitrate (analytical grade ≥98%) was bought from Sigma-Aldrich, USA. The standards were used in calibrating HQ4100 series accumet meter which was used to determine the ammonium-nitrogen concentration.

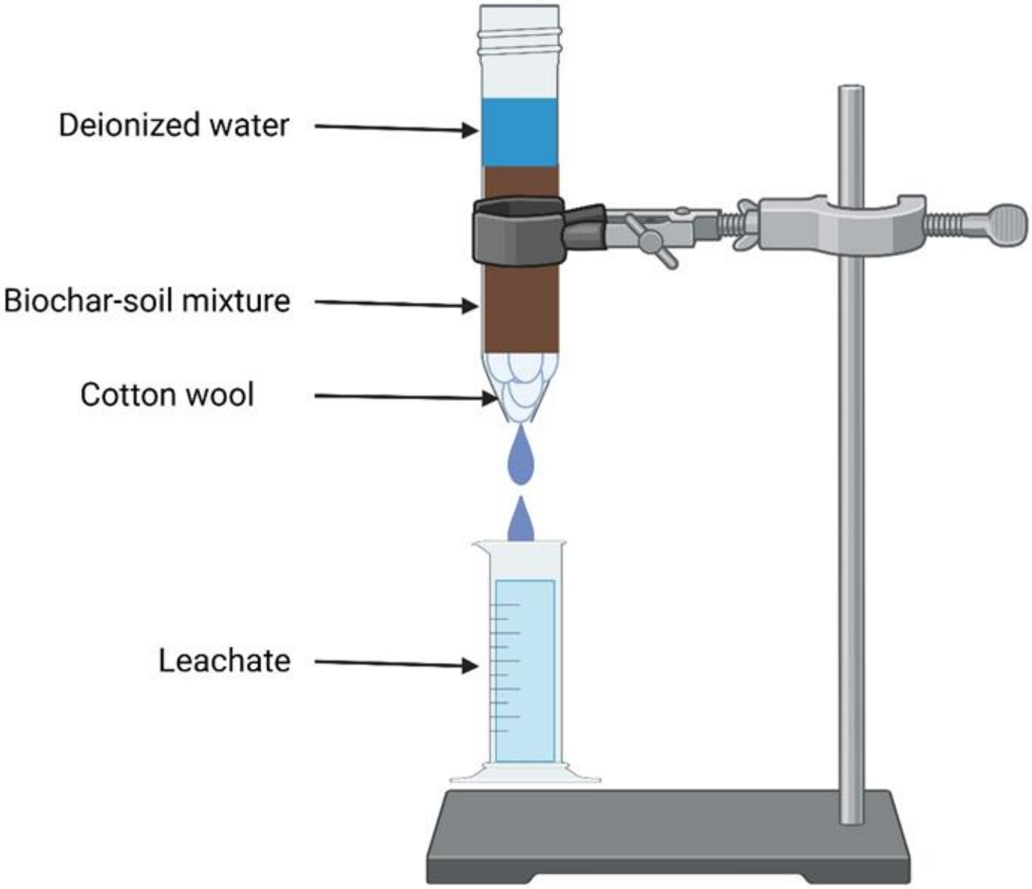

Figure 3.4: Schematic experimental set-up for biochar nutrient soil leaching test

x. ICP-OES analysis of leachate and aqueous release samples

5ml portions from the soil leachates and aqueous nutrient release samples were put into the ICP-OES sample tubes. These were digested with 500µl of 70% nitric acid and then samples were thoroughly mixed and loaded into the ICP-OES for analysis.

xi. Biochar pH determination and liming potential

A 1:20 (w/v) sample/deionized water mixture was made by adding 0.25g of samples (biochar: A1, B1, C1, A2, B2, C2 and biomass: D1 and D2) in test tubes and made up to 5ml with deionized water. The mixtures were thoroughly mixed using a mechanical shaker for 15minutes and allowed to equilibrate for 2hrs (Rajkovich et al., 2012). Then pH determination was done using pH meter Accumet Research (AR60) manufactured by Fisher Scientific, USA.

Liming potential of biochars was determined following the method described by Singh et al (Singh et al., 2024). Briefly, 10ml of a standard 1 mol/L HCl was added to 0.5g of BC. The mixture was mechanically mixed for 30mins. This was allowed to stand for 24hrs. The resulting mixture was then backtitrated against 0.5mol/L NaOH solution ensuring continuous mixing until pH 7 is reached. Blank titration was carried out with 10ml HCl without biochar against the NaOH solution. The volumes of NaOH used were noted and the procedure repeated in triplicates. The liming potential is then expressed as percentage $CaCO_3$-equivalence ($\%cc_{eq}$) according to equation 3.2 (Saritha et al., 2022) and the classification used according to International Biochar Initiative tool (IBI, 2024):

$$\%cc_{eq} = \frac{M\times(b-a)\times10^{-3}\times100.09\times100}{2\times W} \qquad 3.2$$

M = molarity of NaOH (mol/L)

b = volume of NaOH used in blank titration

a = volume of NaOH used in biochar sample titration

10-3 = for conversion of volume from ml to liters

100.09 = molar mass of $CaCO_3$

100 = multiplier for obtaining % $CaCO_3$ equivalence

W = mass of biochar (g)

2 = mole ratio number (1 mole of $CaCO_3$ consumes 2 moles of H^+)

xii. Biochar water holding capacity (WHC)

Biochar Water Holding Capacity (WHC) was conducted based on method described by Singh et al (2024). This was done by weighing 1g of biochar samples into a 14ml plastic tube. 10g of deionized water was gently added to the biochar and allowed to soak completely. Water was allowed to drain out through a filter overnight and the recovered water was weighed and used to calculate the adsorbed water by the BC samples.

xiii. IBI classification of biochar samples

To determine the value (or "grade") of the BC samples based on the International Biochar Initiative (IBI) classification system, the following parameters were inputted into the IBI classification calculator: estimated H/C ratio, organic carbon concentration, total and plant available (aqueous) levels of N, P, K, S, Mg and Ca; liming capacity and average zeta hydrodynamic particle size distribution. The tool automatically computes the classification of the BC samples in terms of fertilizer value, liming capacity, carbon storage potential and particle size range.

3.2.4 Biochar-nanonutrient adsorption experiments

i. Concentration effect on BC-nanonutrient adsorption

To test the effect of nanonutrient concentration on biochar adsorption, 2 grams of biochar samples were mixed with 1000ppm, 800ppm, 600ppm, 400ppm and 200ppm each of CuO and ZnO nanoparticles (procured from US Nanomaterials Research, Inc.) in a 50ml centrifuge tube and made up to 50ml with deionized water to form an aqueous suspension which was thoroughly mixed.

Control experiments were set up without biochar to account for adsorption to the walls of the tube and other losses and biochar without nanonutrients. All experiments were done in triplicates (Jiang et al., 2016; Xu, Cao, Li, Howard, & Yu, 2019; Zhang, Cheng, & Ji, 2022). The samples were kept in a water bath at ambient temperatures (21°C) with the reciprocating shaker set at 90rpm for 24hrs. Samples were then centrifuged at 3,000rpm for 30mins and the supernatant taken to determine the residual nanonutrient ions in solution after the adsorption. The data was used to determine the adsorption characteristics of the biochar.

ii. Effect of pH on BC-nanonutrient adsorption

To test the effect of pH on biochar adsorption, 2 grams of biochar samples were mixed with a fixed concentration of ZnO and CuO nanonutrients (5mg in 50ml each) in a 50ml centrifuge tube and made up to 50ml with deionized water to form an aqueous suspension. The suspensions were mixed and adjusted to pH values 5, 6, 7, 8, 9 using 0.1molar NaOH and HCl solutions. Control experiments were set up without pH adjustment. All experiments and controls were done in triplicates (Jiang et al., 2016; Xu et al., 2019; Zhang et al., 2022). The samples were kept in a water bath at ambient temperatures (21°C) with the reciprocating shaker set at 90rpm for 24hrs. Samples were then centrifuged at 3,000rpm for 30mins and the supernatant taken to determine the residual nanonutrient concentrations in solution after the adsorption. The data was used to determine the adsorption characteristics of the biochar.

iii. Single/Co-ion effect on BC-nanonutrient adsorption

To test the effect of single/co-ions on biochar adsorption, 2 grams of biochar samples were mixed with varying concentrations of CuO and ZnO nanonutrients singly (10mg, 25mg and 40mg) and combined (Cu/Zn: 10/40. 25/25 and 40/10) in a 50ml centrifuge tube and made up to 50ml with deionized water to form an aqueous suspension which was thoroughly mixed. All experiments were done in triplicates (Jiang et al., 2016; Xu et al., 2019; Zhang et al., 2022). The samples were kept in a water bath at ambient temperatures (21°C) with the reciprocating shaker set at 90rpm for 24hrs. Samples were then centrifuged at 3,000rpm for 30mins and the supernatant taken to determine the residual nanonutrient ions in solution after the adsorption. The data was used to determine the adsorption characteristics of the biochar.

iv. Effect of contact time on BC-nanonutrient adsorption

2g of BC samples were added to a 50ml tube containing 50mg of CuO and 50mg of ZnO nanoparticles in aqueous suspension. Deionized water was added to make up the mixture to the 50ml mark. Samples were placed in a Thermo-Scientific water bath set at 90rpm and 21°C. Samples were removed for analysis at contact time = 0, 60, 120, 180, 240, 300, 360 and 1440hrs respectively. All tests were performed in triplicates.

3.2.5 BET analytical procedure

Brunauer-Emmett-Teller (BET) analysis was used to determine the specific surface area and pore volume of the samples. To establish BC's pore size distribution, gas porosimetry was carried out using CO_2 at 273 K on an Autosorb iQ (Anton Paar). Prior to the analysis, the samples were degassed at 200°C for 8 hours under vacuum. The pore size distribution was simulated using Non-local Density Functional Theory (NLDFT) based on the CO_2 sorption isotherms suitable for analyzing micropores. Nominal amounts of samples with a range of 100-150 mg were taken for analysis individually in each cell.

3.2.6 Molecular modelling computational methods

i. Conceptualized biochar structure

The process of BC production can generate a crude mixture of various BC species, depending on the feedstock, pyrolytic conditions and pre- or post-treatments (Bottezini et al., 2021; Chen, Zhou, & Zhu, 2008; Melo et al., 2024; Xu et al., 2021). In this work, molecular dynamics computational modeling was carried out using a typical conceptualized BC (CBC) molecule based on physicochemical data from our ongoing research and the models proposed by other researchers (Dong et al., 2024; Zhao, Lv, & Yang, 2017). This enabled us to explore the CBC's intrinsic molecular and atomistic properties that can influence BC-nanonutrient sorption phenomena, a factor critical in designing a nanonutrient-fortified biochar-based fertilizer. The conceptualized BC optimized structure has a molecular mass of 981.97g/mol (Figure 3.5).

Modelling in this work focused on using the conceptual typical biochar structure (Figure 3.5) to predict its nanonutrient adsorption characteristics necessary for efficient nanofertilizer formulation (Zhao et al., 2017).

Using the Material Studio application, the following parameters were elucidated to understand the atomistic properties of the biochar structure:

a) Geometry optimization using VAMP
b) Electrostatic potential map
c) Electron charge density map
d) Total charge density isosurface
e) LUMO and HOMO
f) Coulson and Mulliken atomic charges assignment
g) Bond order
h) Vis-UV spectra of the molecule
i) ZnO/CuO adsorption characteristics towards the biochar structure.

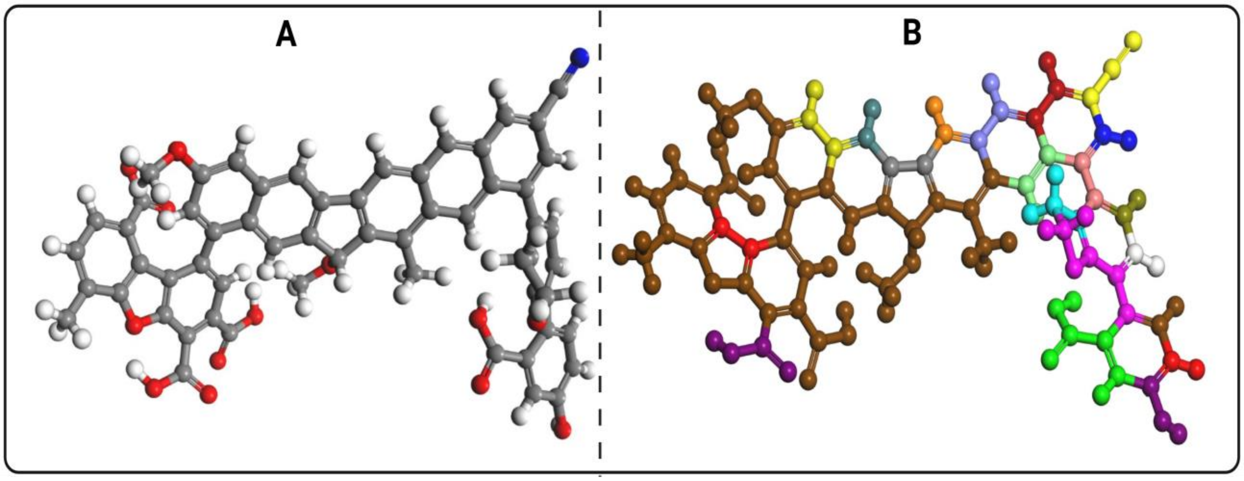

Figure 3.5: Optimized geometry for the conceptualized BC structure **A**=optimized geometry with atom-based coloration **B**=optimized geometry with charged group-based coloration.

ii. Molecular dynamics (MD) simulation with material studio

Among others, the VAMP module was selected on the Material Studio Software (BIOVIA Material Science Studio Academic Research Suite- product no. 5CB-LUR, 2022) because it is capable of rapidly predicting several physical and chemical properties for molecular organic and inorganic systems with molecular orbital program designed using semiempirical methods and optimized for high numerical stability and speed, allowing for interactive computations (Sharma et al., 2019a). Compared to other geometry optimization techniques, VAMP includes several enhancements that enable it to successfully optimize even challenging molecular systems, while offering features such as transition state optimization, solvent model simulations, and the ability to calculate a wide range of electronic properties (S. Sharma et al., 2019a). The module is an advanced semi-empirical quantum mechanics tool designed to model molecular reactions and properties in both gas and aqueous environments. It has been fine-tuned for exceptional numerical stability and speed, allowing for efficient calculations even with large molecular systems. Additionally, the VAMP module includes various improvements for optimizing geometry, transition states, and electrostatic interactions (Pourhakkak et al., 2024; Uwakwe, Okafor, Obike, & Ikeuba, 2017). Geometry optimization was completed with energy minimization using Hamilton set at NDDO (Neglect of Diatomic Differential Overlap) and AM1 (Austin Model 1) with auto multiplicity and RHF (Restricted Hatree-Fock) spin. DMol3 module was also used for molecular parameters of the CBC structure to shed further lights on its intrinsic properties, including highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) and the associated energies E_{HOMO} and E_{LUMO}, respectively, UV-Visible spectrum, bond order and atomic charge assignments, which would help to qualitatively assess the likely adsorption sites on the CBC.

iii. Molecular simulation of CuO and ZnO nanonutrient adsorption on CBC

Adsorption Locator, Forcite, DMol3, and VAMP modules were used to simulate the adsorbent (CBC) and adsorbate (CuO and ZnO) interaction with at least 10 different thermodynamically feasible configurations of the NN-CBC complex. The Adsorption Locator module is designed for investigating individual systems, allowing the determination of the low energy states or adsorption sites on periodic and non-periodic sorbent structures (Sharma et al., 2019b). The Adsorption Locator identifies thermodynamically favourable adsorption configurations by carrying out Monte Carlo searches for the sorbent-sorbate system with decreasing temperatures. This module was specifically used for this task as it can predict with a degree of precision for adsorbates that are either gases or liquid or porous crystals or amorphous structures like silica gel or activated carbon. The parameters used for the adsorption simulations in the material studio is summarized in Table 3.3. The sorbent molecule (CBC) and the sorbates (ZnO and CuO) were optimized with VAMP module before the adsorption simulation. Ten different energetically stable configurational structures or models of the NN-CBC complex were computationally explored for each of ZnO and CuO nanonutrient adsorption process. Hence, parameters such as total energy, adsorption energy, rigid adsorption energy, deformation energy, and change in adsorption energy with increasing number of nanonutrients, representing how changing concentration of adsorbate may influence the stability of the sorbent-sorbate complex were computationally calculated by the Material Studio Suite software modules (Biovia, 2022) to explain the thermodynamic stability of the sorbate-sorbent complex.

Table 3.3: Adsorption simulations parameters for computational studies using the Adsorption Locator module

Simulated annealing calculation parameters	
Loading steps	100000
Heating cycles	5
Steps per cycle	50000
Optimize geometry	Yes
Energy parameters	
Forcefield	COMPASS (Version 2.8)
Charges	Forcefield assigned
Electrostatic terms	
Summation method	Group based
Cutoff distance	15.5 A
Buffer width	0.5 A
Van der Waals terms	
Summation method	Atom based
Truncation method	Cubic spline
Cutoff distance	15.5 A
Spline width	1 A
Buffer width	0.5 A
Simulated annealing	
Automatic	Yes
Adjust Monte Carlo step sizes	Yes
Simulated annealing calculation	
Framework charge	0.000 e
Maximum adsorption distance	5.000 A

Mathematically, these parameters can be defined as (Abeng, Thakur, Anadebe, & Ebenso, 2025; Lan et al., 2023):

$$E_{a\,ds} = E_{total} - E_{CBC} - E_{NN} \qquad 3.3$$

Where:

$E_{a\,ds}$ represents the adsorption energy (in eV) which is the energy change associated with the adsorption of a sorbate onto the sorbent surface.

E_{total} represents the total energy (in eV) of the sorbate-sorbent system, which includes contributions from all components involved in the adsorption process.

E_{CBC} represents energy of the sorbent (CBC) in isolation

E_{NN} represents energy of the sorbate nanonutrient (NN) in isolation

The deformation energy (E_{deform}) can be expressed as (Nugraha et al., 2017; Yan et al., 2025):

$$E_{deform} = E_{total} - E_{rigid} \qquad 3.4$$

Where:

E_{rigid} represents the energy associated with the adsorption when both sorbent and sorbate are treated as rigid bodies where no structural changes occur during adsorption.

E_{deform} represents a measure of the energy change resulting from structural adjustments of the sorbent or sorbate upon adsorption.

The change in adsorption energy with increasing nanonutrients concentration provides insights into system stability and interaction strength, this is defined mathematically as:

$$\frac{\Delta E_{ads}}{\Delta N_i} = f(N_i) \tag{3.5}$$

Where $f(N_i)$ represents a function that captures how adsorption energy E_{ads} varies as nanonutrients number (or concentration) N_i changed in the system.

In the co-presence of both nanonutrients, the overall total adsorption energy, total deformation energy and normalized adsorption energy per site can be expressed as shown in equations 3.6, 3.7, and 3.8 (Uwakwe et al., 2017):

$$E_{ads(combined)} = E_{ads(ZnO)} + E_{ads(CuO)} \tag{3.6}$$

$$E_{deform(combined)} = E_{deform(ZnO)} + E_{deform(CuO)} \tag{3.7}$$

$$\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{combined} = \left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{ZnO} + \left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{CuO} \tag{3.8}$$

3.2.7 Nanofertilizer formulation

In this study, CuO and ZnO nanopowders were used to fortify the biochar into a nano-enabled fertilizer (Appendix IV and V). Based on preliminary studies, WH biochar is rich in other macronutrients but deficient significantly in these important micronutrients. The nanonutrients were supplied by CAES and used in the fertilizer formulation experiments. A 5% wt/wt aqueous salt solution of the desired nano-nutrient was prepared by weighing 5g of each salt and dissolving in 100ml of deionized water (Khan et al., 2021). The biochar was impregnated with 10ml each of the aqueous suspensions stirred together in a conical flask with magnetic stirrer for 3-4hrs to produce the nanobiochar composite slurry. The nanobiochar was then filtered off and dried in an oven at 105°C for 30mins and stored in plastic sample bags for characterization and field trials.

3.2.8 Characterization of nanofertilizer

The characterization assessed the physico-chemical properties of the nanocomposite fertilizer which in turn determines potential to perform the expected activities. The characterization was generally based on the recommendations by Amalina et al. (2022). Colour, surface area, pore volume and size, density and pH of the nanocomposite was determined by suitable physical techniques. The nanocomposite fertilizer was analysed for the elemental composition analysis for carbon, hydrogen, nitrogen and sulphur.

This study utilized a Scanning Transmission Electron Microscopy (S/TEM) to reveal the pore sizes and general structural morphology of the nanocomposite. Fourier Transform Infrared Spectroscopy (FTIR) was used for assessing the organic functional groups including: -COOH, -OH, -NH2, -CN, or -C=O. Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) was used to characterize the synthesized nanobiochar for heavy metals such as chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), and manganese (Mn).

3.2.9 Field trials

The study applied pilot trials to determine the effectiveness of nanoparticle impregnated biochar (nanofertilizer) on crop growth using maize plant. Pilot field trials was conducted using pot experiment to compare nanofertilizer with a conventional commercial fertilizer following the procedure used by Elsabagh, Elkhatib, and Rashad (2024).

The following steps were used as a guide here:

Experimental Design

A randomized complete block design (RCBD) was employed for the pot experiments to reduce variability among treatments.

Number of Treatments Groups:

Nanofertilizer in three doses (40g, 20g and 10g per 10kg of soil respectively)

Conventional inorganic fertilizer (As recommended by manufacturer: 15g per 10kg of soil)

Control (no fertilizer applied)

Group A: 40g of NF per 10kg of soil

Group B: 20g of NF per 10kg of soil

Group C: 10g of NF per 10kg of soil

Group D: 15g of NPK+S per 10kg of soil

Group E: Control group without any fertilizer

Number of Replicates: 15 plants per treatment group to ensure statistical validity.

Test Plant: Zea mays (maize)

Duration: Total duration of field trial was 7weeks.

Experimental Variables

Independent Variables:

Type of fertilizer [nanofertilizer (NF) and conventional fertilizer (CF)]

Nanofertilizer application rates – 10g, 20g and 40g of NF per 10kg of soil in each pot.

Maximum target nanonutrient in soil: NanoCu = 2ppm and NanoZn = 3ppm.

Conventional fertilizer application rates: As recommended by the manufacturer (15g of CF applied per 10kg soil in each pot)

Dependent Variables:

Growth Parameters: Plant height, leaf area, and number of leaves per plant

Physiological Parameters: leaf chlorophyll content and leaf nitrogen content

Experimental Procedure

Soil Preparation: Sandy-loamy soil was used in this experiment. Soil was dried and filtered to consistent sizes with a 2mm sized pore mesh. Equal amounts of soil (10kg each) were measured into each pot. Analysis of the initial soil nutrient content was done to establish a baseline.

Pilot experiment setup: Following Elsabagh et al (2024), the pilot field trial was set up using pots of the same sizes (diameter 23cm and height 23cm) with drainage holes at the bottom (Elsabagh et al., 2024). Pots were filled with the 10kg of soil to a height of 18cm from the base of the pots.

Fertilizers (NF and CF) were applied to the respective treatments and control group was set up without fertilizers.

Fertilizer and nanofertilizer application:

Maize seedlings were grown for 3weeks with baseline parameters obtained. Thereafter, the recommended dosage of CF (NPK+S = 15:15:15+10.2) was applied based on manufacturer's guidelines as stated earlier. Varying dosage of NF was applied in 3 graduating rates as stated earlier.

3.2.10 Data collection/analysis

The collection of field data was conducted using field observations and measurements of plant growth parameters commencing 7days after fertilizer treatments and continued for 20 days thereafter. The determination of nutrient release kinetics was conducted by employing a methodology involving regular sampling and analysis of both soil and plant samples. At 4 days intervals, starting from day 7, post-fertilizer treatment, collection of data on plant growth metrics (height, number of leaves per plant, leaf surface area, leaf chlorophyll and nitrogen content) were carried out.

Assessment of the effects of nano-enhanced fertilizers on plant development and crop productivity was based on the approach of Wang, Yu, and Zhang (2022). Data analysis in this study encompassed the utilization of statistical techniques, specifically analysis of variance (ANOVA) and regression analysis using SPSS software, to assess and compare the efficacy of various treatments in terms of their performance. The analysis focused on examining the patterns of nutrient release and the corresponding responses in plant growth, with the aim of identifying any notable distinctions among the various treatments

3.2.11 Research activity/output indicators

Table 3.4 shows the activities and their outputs in terms of indicators for progress of the research accordingly.

3.2.12 Time Frame

The project was carried out for twenty (20) months as shown in Figure 3.6

Table 3.4 The research activity/output indicators

S/N	ACTIVITY	INDICATORS
1	Field work	Field work shall include initial collection of water hyacinth and the field trials of the nanofertilizer to acquire key primary data needed for the study.
2	Laboratory work	Characterization of the biomass and biochar for preliminary studies to aid the nanoformulation qualitatively and quantitatively. Adsorption experimental study was also used to predict the adsorption characteristics of the biochar. The nanofertilizer was then characterized and tested in the field against a conventional fertilizer. Data from the field trials were also analyzed through laboratory examinations.
3	Molecular Modeling	Determination of adsorption characteristics of the conceptualized biochar structure(s) and its implication on biochar-nanonutrient interactions for efficient biofertilizer formulation.
4	Data Analysis and Discussions	Interpretation and analysis of all data generated throught the studies to draw proper conclusions and engage policy makers for possible upscaling of the process for user acceptance and commercialization.
5	Reports	Complete PhD thesis and a minimum of two review articles (2) and two original research papers (2)

S/N	ACTIVITY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	**Literature reviews**	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
2	**Field/Sample collection**			🟦	🟦																
3	**Laboratory work (Physicochemical characterizations and experimentations)**					🟦	🟦	🟦	🟦												
4	**Biochar-nanonutrient Adsorption Modelling**									🟥	🟥	🟥	🟥	🟥	🟥						
5	**Nanoformulation and Biofertilizer Characterization**													🟩	🟩						
6	**Field Trials and Data Collection**															🟪	🟪	🟪			
7	**Data Interpretation and Analysis**																		🟧	🟧	🟧

Figure 3.6: Gantt chart showing project time frame.

3.2.13 Financial aspects of the project

Table 3.5: Full proposal budget summary

DESCRIPTION OF ITEMS	COST
Data Collection and Analysis	
Field Equipment	N 500, 000.00
Laboratory & IT Equipment	N 2,500, 000.00
Software Acquisition	N 50, 000.00
Sample collection and Field Studies	N 750, 000.00
Travels and Miscellaneous	N 1,500,000.00
Research Printing	N 200,000.00

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Biochar preparation and yield

Data for BC preparation and yield is shown in Table 4.1.

4.1.2 FTIR functional group characterization of WH biomass and biochar

FTIR data (Figure 4.1) indicate that the functional groups present on the WH biomass and BC products varied based on the pyrolytic conditions and precursor biomass.

4.1.3 SEM-EDX characterization of WH Biochar

SEM images of the BC are shown in Figure 4.2 (top 2 rows) indicating variations in surface morphology and porosity with changing pyrolysis resident times for both D1 and D2.

4.1.4 Hydrodynamic particle sizes and zeta potentials of BC

The data for hydrodynamic particle sizes and zeta potential measurements of biochar samples derived from WH biomass are shown in Figure 4.3.

4.1.5 BC pH, liming potential, carbon storage, particle size range and fertilizer value based on IBI classification

The BC pH, liming potential, carbon storage, particle size range and IBI classification is shown in table 4.1

Effect of pyrolytic Treatments on Biochar Nutrient Concentration

The data showing the effect of pyrolytic treatments on biochar nanonutrient concentration is shown in figure 4.4 and table 4.2.

Table 4.1: Pyrolysis yield, pH, liming capacity, carbon storage, particle size range and fertilizer value of BC from WH

Water hyacinth biomass (Sample label)	pyrolysis duration @ 600°C (Sample label)	Biochar yield (%)	pH values	Liming capacity ($\%cc_{eq}$)		Fertilizer Class	Carbon storage capacity (g/kg)		Particle average size (nm)	
				Value	Class		Value	Class	Value	Class
Dry leaf samples (D1)	30mins (A1)	31.49±1.04^{a}	10.77	28.94±0.88^{a}	3	1	553	4	583	Fine powder
	45mins (B1)	31.48±0.83^{a}	10.77	25.39±0.57^{b}	3	1	469	3	354	Fine powder
	60mins (C1)	30.76±0.65^{a}	10.61	23.85±1.10^{b}	3	1	519	4	270	Fine powder
Dry stem samples (D2)	30mins (A2)	34.183±1.28^{b}	10.71	22.60±1.58^{b}	3	1	512	4	304	Fine powder
	45mins (B2)	34.066±1.03^{b}	10.56	16.85±0.96^{c}	2	1	474	3	146	Fine powder
	60mins (C2)	34.360±0.98^{b}	10.72	14.76±0.14^{c}	2	1	517	4	314	Fine powder

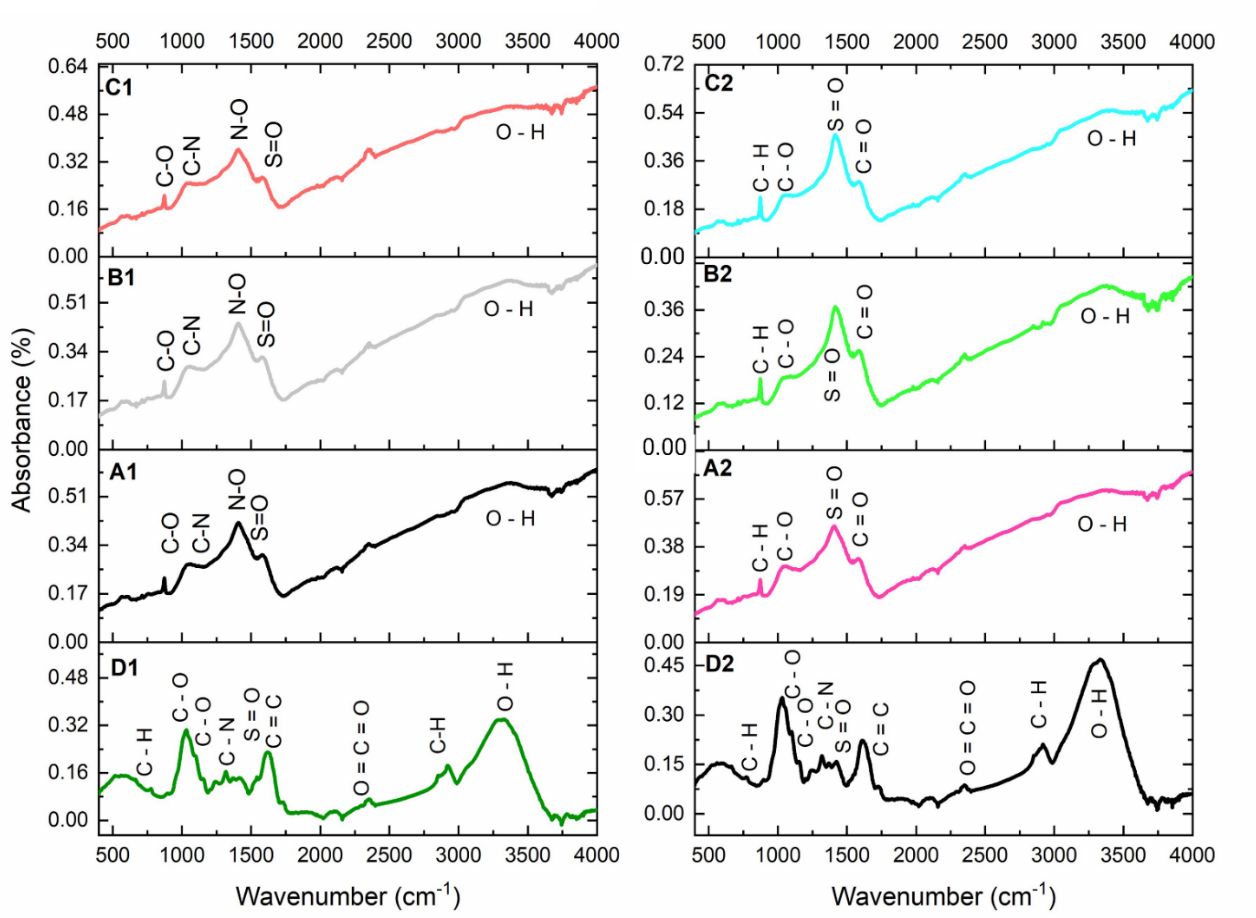

Figure 4.1: FTIR spectra image qualified with absorbance (%) and wavenumber (cm^{-1}) for leaf biomass (D1) and biochar samples (A1, B1 and C1); stem biomass (D2) and biochar samples (A2, B2 and C2).

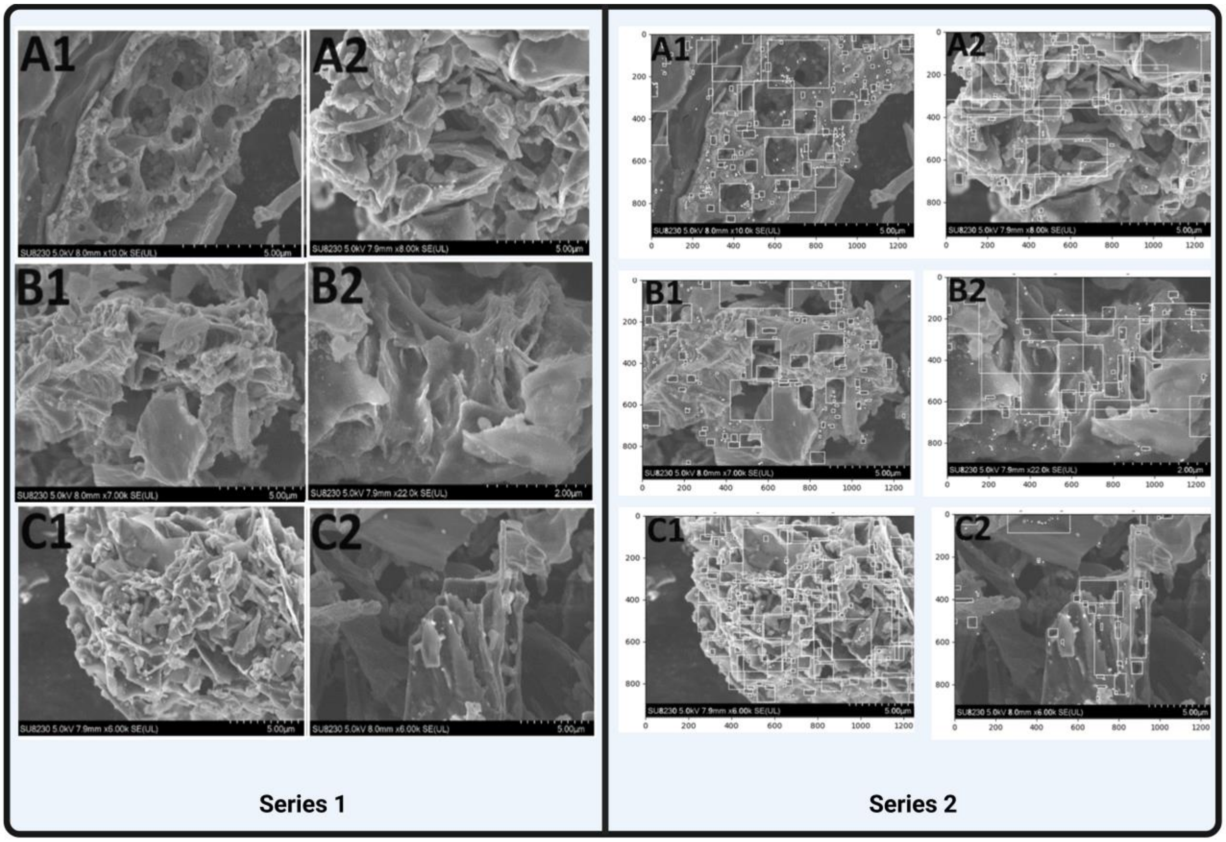

Figure 4.2: Original SEM images taken with magnifications at ≥ 6000x (Series 1) and SEM images with bounding boxes using python codes for pore analysis (Series 2).

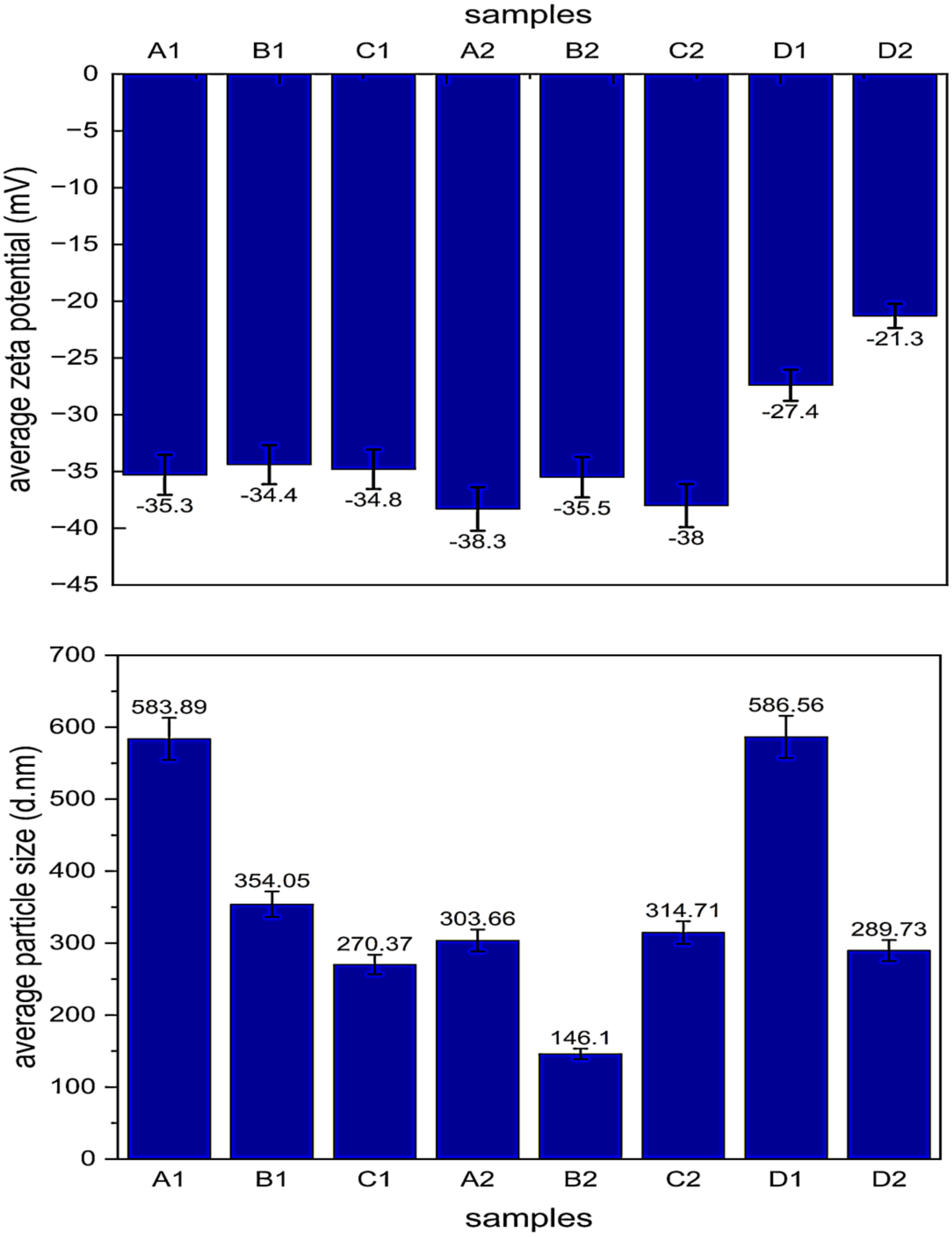

Figure 4.3: Zeta sizes and zeta potentials of leaf and stem WH biochar samples

Figure 4.4: Graph showing N, P, K, S, Cu, Zn, Mg, Fe, B and Ca in different biochar samples

Table 4.2: N, P, K, S, Cu, Zn, Mg, Fe, B and Ca in different biochar samples (ppm): means ± std error *(α =0.05)*

Samples	**N**	**P**	**K**	**S**	**Cu**	**Zn**	**Mg**	**Fe**	**B**	**Ca**
A1	35300.0±57.74^{g}	58.48±0.45^{e}	655.33±4.85^{c}	11.5±0.07^{f}	0.01 ±0.0^{a}	0.37 ±0.0^{e}	63.78 ± 0.5^{e}	4.55±0.03^{g}	0.19 ±0.0^{e}	255.01 ±1.94^{d}
B1	33800.0±23.09^{f}	56.17±0.43^{d}	649.32±5.05^{c}	10.5±0.07^{d}	0.01 ±0.0^{b}	0.36 ±0.0^{e}	56.9 ± 0.12^{d}	3.47±0.02^{e}	0.18 ±0.0^{d}	258.81 ±1.67^{e}
C1	32700.0±57.74^{e}	62.43±0.1^{f}	582.88±2.81^{b}	10.41±0.01^{d}	0.02 ±0.0^{c}	0.46 ±0.0^{f}	62.06 ± 0.4^{b}	2.41±0.01^{c}	0.18 ±0.0^{d}	254.75 ±1.05^{d}
A2	33800.0±57.74^{f}	64.22±0.17^{g}	584.53±1.42^{b}	10.87±0.03^{e}	0.02 ±0.0^{c}	0.46 ±0.0^{f}	59.79 ± 0.36^{b}	2.13±0.01^{b}	0.17 ±0.0^{c}	254.45 ±0.85^{d}
B2	16900.0±57.74^{c}	38.65±0.27^{c}	904.33±4.68^{d}	6.1±0.03^{b}	0.03 ±0.0^{d}	0.29 ±0.0^{d}	56.97 ± 0.17^{c}	1.98±0.01^{a}	0.19 ±0.0^{e}	221.9 ± 1.18^{c}
C2	15700.0±57.74^{b}	38.97±0.17^{c}	950.49±2.3^{e}	6.31±0.03^{c}	0.03 ±0.0^{d}	0.28 ±0.0^{c}	63.89 ± 0.15^{e}	2.94±0.02^{d}	0.2±0.0^{f}	213.18 ±0.56^{b}
D1	30700.0±57.74^{d}	19.6±0.13^{b}	201.22±0.98^{a}	13.95±0.09^{g}	0.04 ±0.0^{e}	0.16 ±0.0^{a}	18.41 ± 0.11^{a}	2.41±0.03^{c}	0.06 ±0.0^{b}	215.31 ±1.01^{b}
D2	11600.0±57.74^{a}	15.22±0.13^{a}	922.28±15.57^{d}	5.77±0.03^{a}	0.06 ±0.0^{f}	0.18 ±0.0^{b}	18.52 ± 0.02^{a}	4.1±0.12^{f}	0.05 ±0.0^{a}	108.82 ±0.24^{a}

4.1.6 Soil leachate and aqueous elemental availability

The results of soil leaching and aqueous elemental availability of BC in the laboratory experiements are presented in Table 4.3 and Figure 4.5.

4.1.7 Water holding capacity (WHC)

Water holding capacity (WHC) data is shown in the Figure 4.6 and the water holding capacity (WHC) was evaluated based on the method earlier described (Singh et al., 2024) according to the equation:

$$WHC = \frac{(w_w - w_f)}{w_b} \times \frac{100}{1}\% \qquad 4.1$$

Where

w_w = weight of water added in g

w_f = weight of drained water after 24hrs in g

w_b = weight of dry biochar sample in g

4.1.8 Adsorption isotherm data and calculation

The adsorption calculations can assume multiple approaches depending on the underlying variables. BET isotherm is commonly used for cases involving monolayer adsorption onto porous surfaces. In this way, BET provides essential information on the material's surface area and the monolayer capacity of the BC. Comparatively, the Langmuir isotherm can also be used (Li et al., 2020). The latter is applicable in cases where a monolayer is assumed to be adsorbed onto a homogenous surface, contrary to the BET isotherm. The Langmuir isotherm assumes that the homogenous surface has finite adsorption sites and adsorption at one site does not interfere with any other site. The Langmuir isotherm model can be expressed by the following equation (Jiang et al., 2016):

$$q_e = \frac{Q_{max} \times bC_e}{1 + bC_e} \qquad 4.2$$

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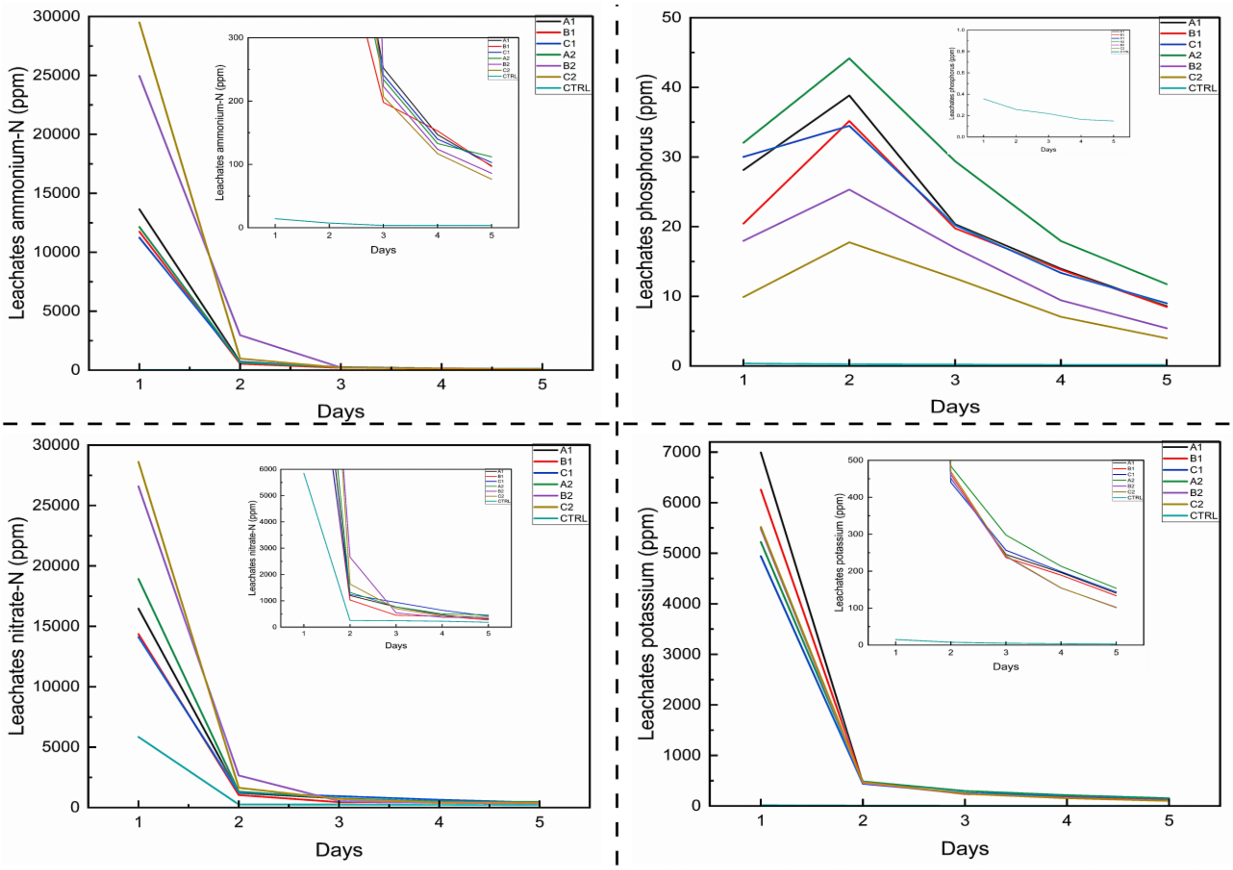

Figure 4.5: Leachate concentrations showing controlled release of NH_4/NO_3-N, P, and K over the period of experiment after Day 1.

Table 4.3: Statistical analysis of nutrient concentrations (ppm) in leachate samples ($\alpha = 0.05$)

Sample	Day 1	Day 2	Day 3	Day 4	Day 5	5-day Total
ammonium-nitrogen concentrations						
A1	14227±213^{a,b,A}	298±27^{a,B}	153±10^{a,B}	134±5^{a,B}	82±2^{a,B}	14,894
B1	14937 ±620^{b,A}	257±63^{b,B}	153±12^{a,B}	133±5^{a,B}	78±2^{a,b,B}	15,558
C1	11483±242^{a,c,A}	269±4^{b,B}	147±13^{a,B}	114±12^{a,b,B}	69±0^{b,B}	12,082
A2	10250±404^{c,A}	316±11^{a,B}	191±9^{b,B}	121±5^{a,B}	90±8^{a,B}	10,968
B2	23110±471^{d,A}	292±69^{b,B}	161±16^{b,c,B}	94±3^{b,c,B}	53±4^{c,B}	23,710
C2	25073±2712^{d,A}	351±69^{a,B}	159±10^{b,c,B}	92±9^{c,B}	51±2^{c,B}	25,726
Ctrl	15±2^{e,A}	10±1^{c,B}	6±1^{d,C}	5±0^{d,C}	4±1^{d,C}	40
nitrate-nitrogen concentrations						
A1	7347±163^{a,A}	322±24^{a,B}	274±2^{a,B}	207±2^{a,B}	161±6^{a,B}	8,311
B1	8503±185^{b,A}	238±9^{b,B}	243±18^{a,b,B}	208±22^{a,B}	171±11^{a,B}	9,363
C1	7173±41^{a,A}	257±17^{b,B}	259±13^{a,B}	199±21^{a,B}	187±18^{a,B}	8,075
A2	4685±101^{c,A}	330±3^{a,B}	314±6^{c,B}	265±7^{b,B}	223±7^{b,B}	5,817
B2	11355± 292^{d,A}	305±16^{a,B}	215±20^{b,d,B}	189±20^{a,B}	164±12^{a,B}	12,228
C2	11143±72^{d,A}	408±11^{c,B}	197±5^{d,C}	174±9^{a,C}	159±4^{a,C}	12,081
Ctrl	701±20^{e,A}	30±4^{d,B}	36±5^{e,B}	52±7$^{c,B.C}$	80±4^{c,C}	899
phosphorus concentrations						
A1	28.15±4.94^{d,e,C}	38.83±0.4^{e,D}	20.36±0.51^{d,B}	13.97±0.36^{d,A,B}	8.61±0.22^{d,A}	109.92
B1	20.45±2.49^{c,d,C}	35.17±0.46^{d,D}	19.76±0.51^{d,C}	13.82±0.32^{d,B}	8.47±0.24^{d,A}	97.67
C1	30.03±0.95^{d,e,D}	34.47±0.47^{d,E}	20.18±1.12^{d,C}	13.37±0.03^{d,B}	9.00±0.47^{d,A}	107.05
A2	32.06±0.24^{e,D}	44.15±0.55^{f,E}	29.40±0.36^{e,C}	17.94±0.17^{e,B}	11.75±0.16^{e,A}	135.3
B2	17.97±4.95^{b,c,B}	25.33±0.12^{c,C}	16.93±0.86^{c,B}	9.44±0.66^{c,A}	5.42±0.47^{c,A}	75.09
C2	9.90±3.20^{b,B,C}	17.75±0.26^{b,D}	12.6±0.68^{b,C}	7.07±0.46^{b,A,B}	3.98±0.25^{b,A}	51.3
Ctrl	0.36±0.03^{a,B}	0.26±0.12^{a,A,B}	0.22±0.01^{a,A,B}	0.16±0.01^{a,A}	0.15±0.0^{a,A}	1.15
potassium concentrations						
A1	6994±200^{e,C}	452±10^{b,B}	245±3^{b,A,B}	196±2^{c,A,B}	141±1^{d,A}	8028
B1	6258±246^{d,B}	462±8^{b,A}	237±3^{b,A}	189±2^{c,A}	133±1^{c,A}	7279
C1	4941±180^{b,C}	441±5^{b,B}	257±12^{b,A,B}	199±1^{c,A,B}	144±1^{d,A}	5982
A2	5222±97^{b,c,D}	484±3^{b,C}	298±1^{c,B}	213±2^{d,A,B}	154±1^{e,A}	6371
B2	5484±62^{c,D}	453±47^{b,C}	240±10^{b,B}	154±6^{b,A,B}	102±5^{b,A}	6433
C2	5519±231^{c,C}	470±61^{b,B}	242±9^{b,A,B}	155±5^{b,A,B}	101±3^{b,A}	6487
Ctrl	15±2^{a,C}	8±0^{a,B}	5±0^{a,A,B}	4±0^{a,A}	3±0^{a,A}	35

Where:

q_e is the amount of absorbate per unit mass of adsorbent at equilibrium (mg/g),

C_e is the equilibrium concentration of solution (mg/L)

Q_{max} is the maximum adsorption capacity of the adsorbent (mg/g)

b is the constant for the adsorption affinity or intensity

The Freundlich isotherm model can be used to predict the characteristics when the underlying process relies on the adsorption of a multilayer on a heterogeneous surface (Chaubey et al., 2024). Thus, Freundlich isotherm model, which is derived by integrating the Langmuir equation over the continuum, captures the varying concentration alongside the correlated adsorption capacity. This model can be expressed by the following equation (Jiang et al., 2016):

$$q_e = K_f C_e^{1/n} \tag{4.3}$$

Where:

q_e is the amount of adsorbate per unit mass of adsorbent (mg/g),

C_e is the equilibrium concentration of solute bulk solution (mg/L)

K_f is an indicator for adsorption capacity

n is an indicator for the adsorption intensity

In essence, the different calculations can be substituted to the diverse isotherm models, including the BET, Langmuir isotherm, Freundlich isotherm, Sips (Langmuir-Freundlich), and Toth isotherm (Chaubey et al., 2024). The Langmuir adsorption isotherm has been touted as providing comprehensive data when dealing with low surface ranges, while the Freundlich, Sips, and Toth amongst other frameworks have proven efficient when working with larger concentration spectrums (Mohan et al., 2022). In summary, the Langmuir isotherm assumes monolayer adsorption on a homogeneous surface, while the Freundlich isotherm describes

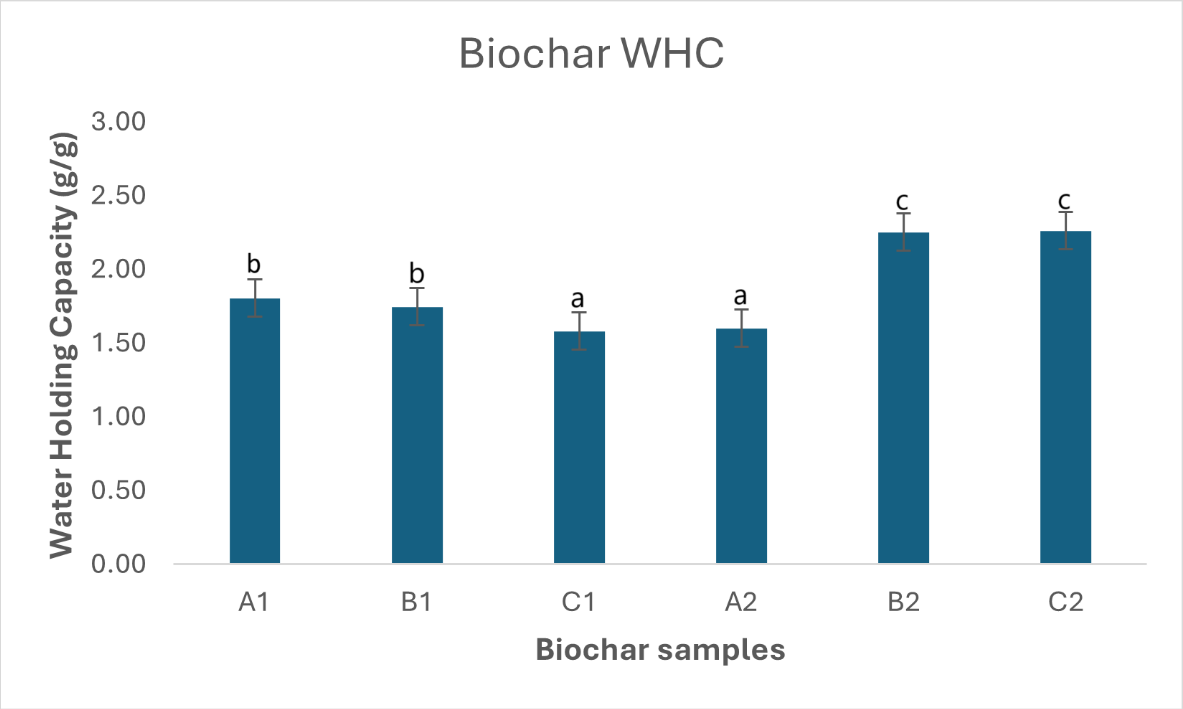

Figure 4.6: water holding capacity (WHC) of BC samples. Lower case letters denote significant differences (α =0.05).

multilayer adsorption on a heterogeneous surface. Additionally, the Freundlich equation exhibits an exponential relationship, while the Langmuir equation follows a hyperbolic curve. By fitting experimental data to these isotherm models, researchers can quantitatively assess the adsorption behavior, elucidate the surface properties, and optimize the design of biofertilizer formulations for enhanced nutrient retention and release kinetics. Isotherm plots are shown in Figure 4.7 whereas plotting a graph of $(P \,/\, P_0) \,/\, V(1 - P \,/\, P_0)$ versus $P \,/\, P_0$, the data was fitted to determine the type of adsorption characteristics of the samples as shown in Figure 4.8 and Table 4.4 shows the specific surface areas and pore volumes of the water hyacinth samples.

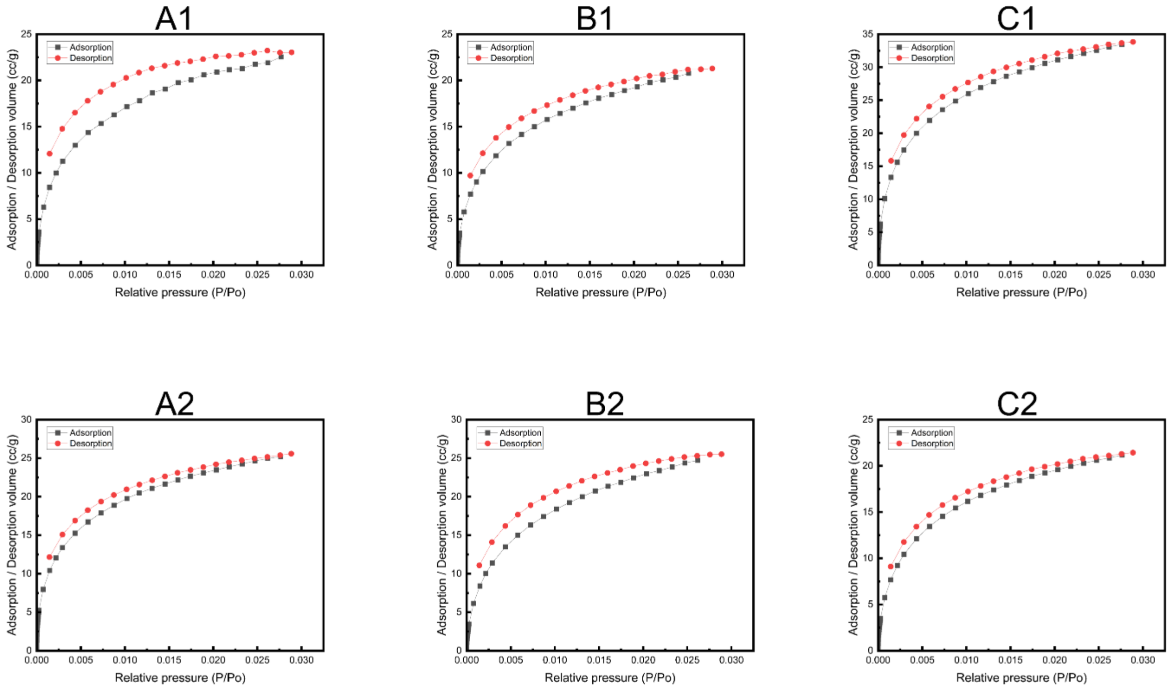

Figure 4.7: Isotherms of adsorption and desorption of CO_2 at STP for water hyacinth BC samples pyrolyzed at 600°C for various residence times.

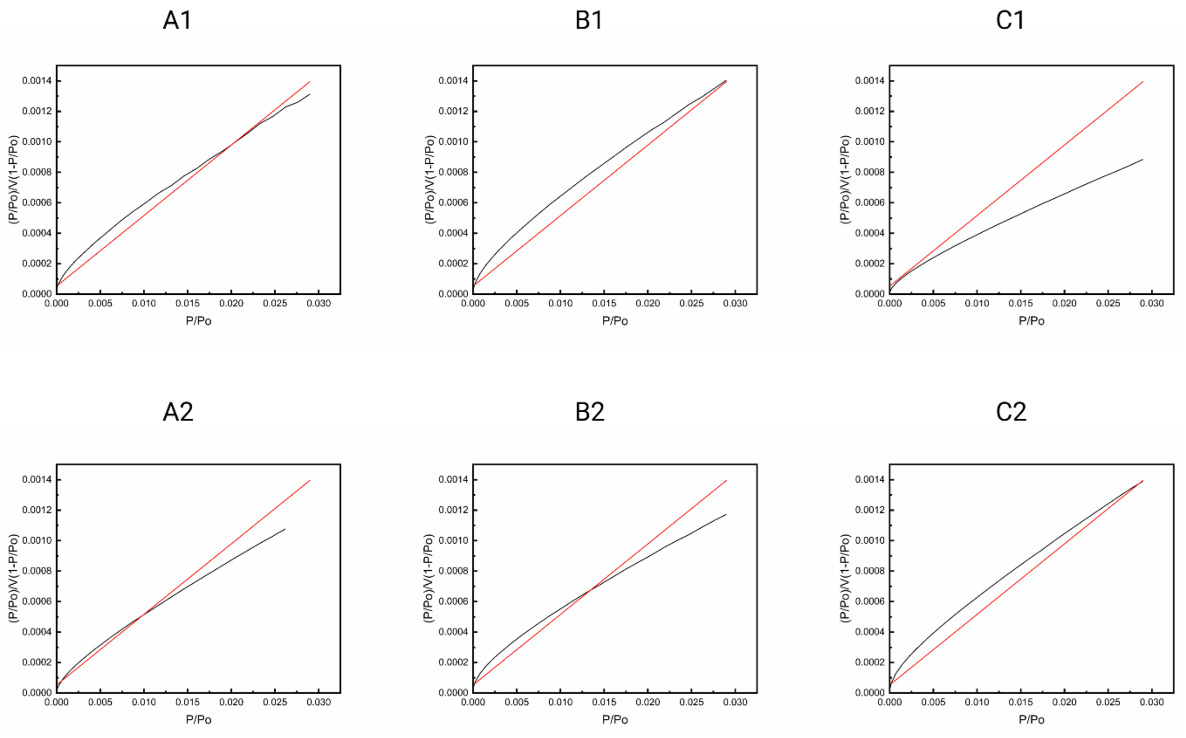

Figure 4.8: Adsorption data fitted with positive intercepts and slopes.

Table 4.4. Specific surface area and pore volumes of WH BC samples (sorbents).

Sample name	CO_2-Specific Surface Area (m^2/g)	CO_2-Pore Volume (cm^3/g)
A1	236.44	0.061
A2	256.71	0.062
B1	250.022	0.061
B2	264.17	0.072
C1	347.264	0.087
C2	249.406	0.067

4.1.9 Conceptualized biochar (CBC) structure: molecular properties

This study aimed to elucidate the sorption interactions between nanonutrients and conceptualized biochar (CBC) through molecular dynamics (MD) simulations. Despite their potential, MD simulations have been underutilized in nanofertilizer formulation research, which is crucial for designing efficient nano-enabled biochar-based fertilizers. Zinc oxide (ZnO) and copper oxide (CuO) were selected as model nanonutrients due to their essential roles as micronutrients for plant health and their typical scarcity in biochar. To address this knowledge gap, we employed MD simulations to investigate the adsorption behavior of ZnO and CuO on CBC. The molecular properties of the optimized CBC structure used in this study are presented in Table 4.5.

4.1.10 Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbitals (LUMO)

According to Koopman’s theory of frontier molecular orbitals (Laplaza, Cárdenas, Chaquin, Contreras-García, & Ayers, 2021), the energies of the highest occupied molecular orbital (E_{HOMO}) and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) can represent the ionization energy (IE) and electron affinity (EA) (Uwakwe et al., 2017) as stated in the equations:

$$\mathrm{IE} = - E_{HOMO} \qquad 4.4$$

$$\mathrm{EA} = - E_{LUMO} \qquad 4.5$$

In this case, ionization energy (IE) represents the energy needed to remove an electron from the HOMO site (Figure 4.9), EA represents the energy needed to add an electron to a LUMO site. Typically, the lower these values, the higher the reactivity of the molecule

Table 4.5: Computational data obtained using BIOVIA material studio software for the optimized CBC using the VAMP (Valence Angle Molecular Orbital Package) module

Parameter	**Values**
Heat of formation	-226.45 kcal/mol
Electronic energy	-149966.37 eV
Core-core repulsion	137380.22 eV
Total energy	-12586.15 eV
Gradient norm	0.38 kcal/mol/Angstrom
Root mean square (RMS) force	0.02
Ionization potential	8.37 eV
filled levels	181
Molecular weight	981.97 g/mol
SCF calculations	823

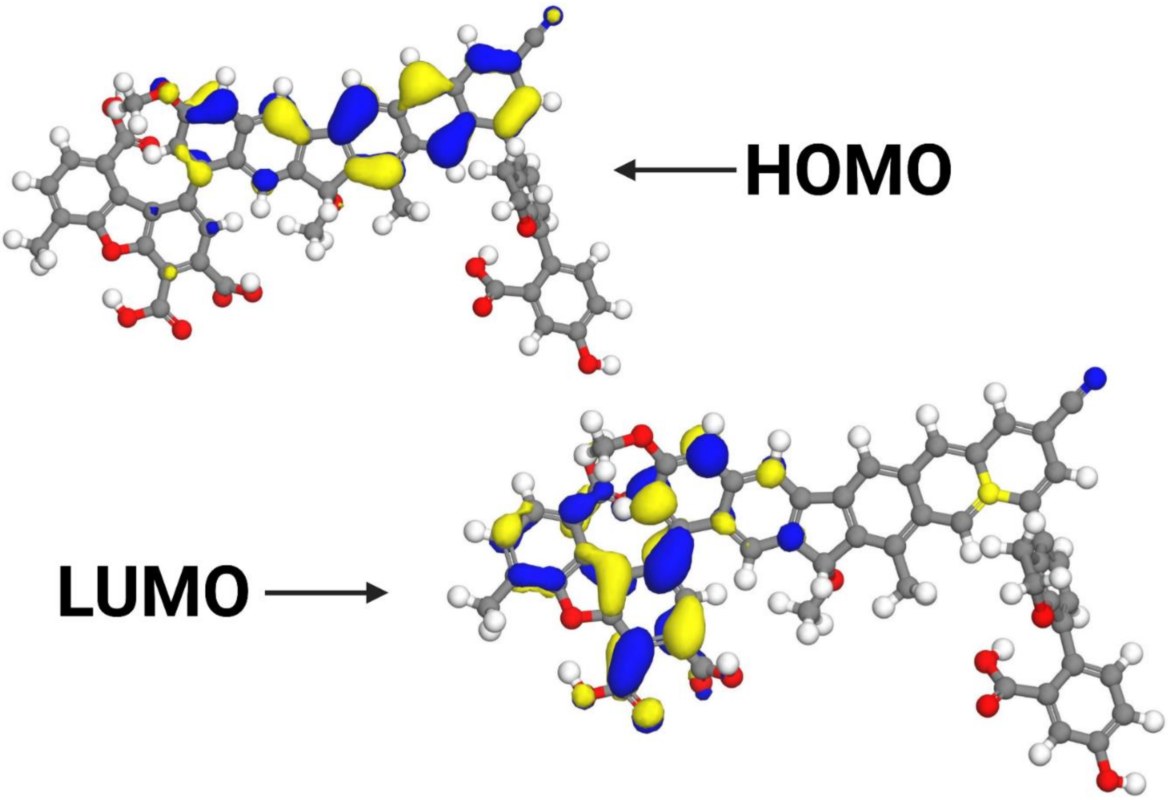

Figure 4.9: CBC structure showing HOMO and LUMO sites from DMol3 module (E_{HOMO} = - 0.184985 eV and E_{LUMO} = - 0.126879 eV).

4.1.11 Computational simulation of CuO and ZnO nanonutrient adsorption on the CBC

The addition of adsorption energies (E_{ads}) of ZnO and CuO when adsorbed independently onto the CBC is significantly dominated by the contribution of ZnO, due to its much larger negative deformation energy compared to CuO, as shown below for structure #1:

$E_{ads(ZnO)} + E_{ads(CuO)} = -3072.698 + (-34.792) = -3107.490$ eV

Whereas, for ZnO and CuO co-presence, the simulated ($E_{ads(combined)}$) = − 2042.611 eV. This implied the presence of ZnO and CuO as single- or co-adsorbates do not change the overall exothermic nature of the adsorption. Although adsorption with either alone is energetically favorable with negative values, ZnO-CBC was significantly accompanied by greater structural deformation than the CuO-CBC complex (Table 4.6 and Figure 4.10).

Although, adsorption energies are consistently negative across all 10 configurational structures for nano-ZnO and nano-CuO independent adsorption simulations, notably, for the ZnO/CuO co-adsorbate simulations, there was a striking difference. This is because in the co-present nanonutrient adsorption simulation for structure #1, ZnO nanonutrients co-presence actually stimulated CuO adsorption to an endothermic process with $\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{CuO}$ value of +9.049 eV whereas $\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{ZnO}$ remain highly exothermic at – 2051.660 (Table 4.6).

Additionally, as you move from configurational structure #1 to #10, both total energy and adsorption energy values become less negative, suggesting that later configurations may be less stable or have weaker interactions with the surface of the CBC. Furthermore, the deformation energy values are relatively small, indicating that significant structural changes are not required for adsorption as shown in table 3. This implies that the adsorption, based on this computational study, is more of physisorption at the surface of the CBC (Figure 4.11a, b and c).

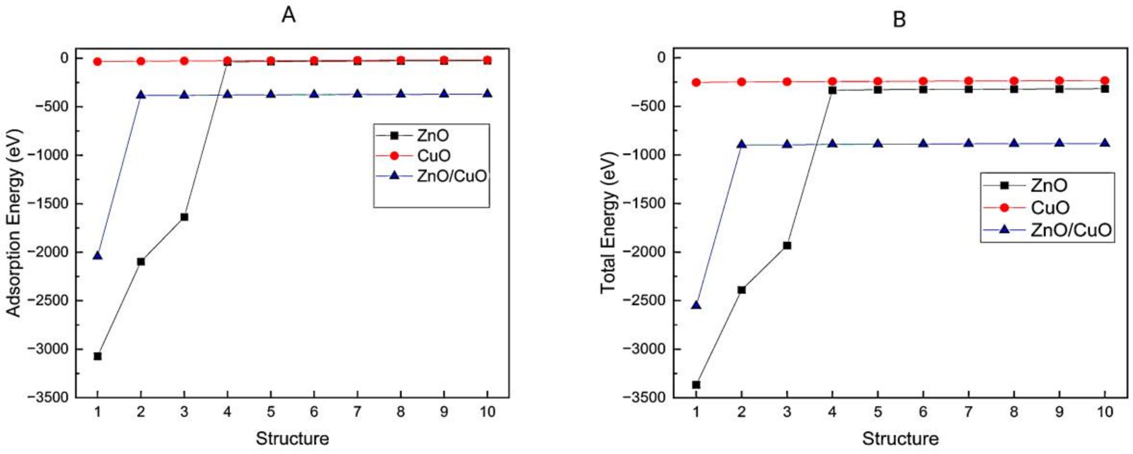

Figure 4.10: Adsorption energy ($E_{a\,ds}$) and total energy (E_{total}) for ZnO-CBC, CuO-CBC and [ZnO+CuO]-CBC complexes for 10 different configurational structures (A = Adsorption energy and B = Total energy)

Table 4.6: Energy thermodynamics of the ZnO-CBC, CuO-CBC and co- adsorption processes.

ZnO-CBC adsorption (independent)

Structures	Total energy E_{total} (eV)	Adsorption energy E_{ads} (eV)	Rigid adsorption energy E_{rigid} (eV)	Deformation energy E_{deform} (eV)	$\frac{\Delta E_{ads}}{\Delta N_i}$
Structure - 1	-3366.827	-3072.698	-1.114	-3071.584	-3072.698
Structure - 2	-2390.131	-2096.002	-0.255	-2095.747	-2096.002
Structure - 3	-1932.231	-1638.103	-2.618	-1635.485	-1638.103
Structure - 4	-332.837	-38.709	-28.355	-10.354	-38.709
Structure - 5	-327.516	-33.388	-22.959	-10.429	-33.388
Structure - 6	-325.888	-31.760	-21.170	-10.590	-31.760
Structure - 7	-323.276	-29.148	-18.478	-10.670	-29.148
Structure - 8	-321.949	-27.820	-17.269	-10.551	-27.820
Structure - 9	-320.743	-26.614	-15.925	-10.690	-26.614
Structure - 10	-318.218	-24.090	-13.385	-10.705	-24.090
Average	**-995.96**	**-701.83**	**-14.15**	**-687.68**	**-701.83**

CuO-CBC adsorption (independent)

Structures	Total energy E_{total} (eV)	Adsorption energy E_{ads} (eV)	Rigid adsorption energy E_{rigid} (eV)	Deformation energy E_{deform} (eV)	$\frac{\Delta E_{ads}}{\Delta N_i}$
Structure - 1	-252.976	-34.792	-28.634	-6.158	-34.792
Structure - 2	-248.153	-29.970	-23.801	-6.169	-29.970
Structure - 3	-245.852	-27.668	-21.387	-6.282	-27.668
Structure - 4	-243.653	-25.469	-19.155	-6.315	-25.469
Structure - 5	-242.572	-24.389	-18.125	-6.264	-24.389
Structure - 6	-240.721	-22.538	-16.185	-6.353	-22.538
Structure - 7	-238.107	-19.924	-13.553	-6.371	-19.924
Structure - 8	-237.877	-19.694	-13.342	-6.352	-19.694
Structure - 9	-236.221	-18.038	-11.668	-6.369	-18.038
Structure - 10	-235.082	-16.899	-10.517	-6.382	-16.899
Average	**-242.12**	**-23.94**	**-17.64**	**-6.3**	**-23.94**

CuO and ZnO adsorption simulation on CBC (co-presence)

Structures	Total energy (eV)	Adsorption energy (eV)	Rigid adsorption energy (eV)	Deformation energy (eV)	$\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{ZnO}$	$\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{CuO}$
Structure - 1	-2554.922	-2042.611	-17.100	-2025.510	-2051.660	9.049
Structure - 2	-896.085	-383.774	-375.973	-7.800	-357.128	-373.991
Structure - 3	-895.823	-383.511	-376.909	-6.602	-376.803	-355.252
Structure - 4	-890.085	-377.773	-370.386	-7.387	-371.155	-354.423
Structure - 5	-889.331	-377.019	-368.627	-8.393	-355.776	-367.992
Structure - 6	-888.417	-376.105	-368.438	-7.667	-370.711	-353.024
Structure - 7	-885.162	-372.850	-364.399	-8.451	-352.472	-367.282
Structure - 8	-884.629	-372.317	-364.724	-7.593	-350.886	-369.252
Structure - 9	-883.495	-371.183	-362.934	-8.249	-356.411	-361.836
Structure - 10	-883.358	-371.046	-363.246	-7.801	-352.168	-366.487
Average	**-1055.1**	**-542.82**	**-333.27**	**-209.55**	**-529.52**	**-326.05**

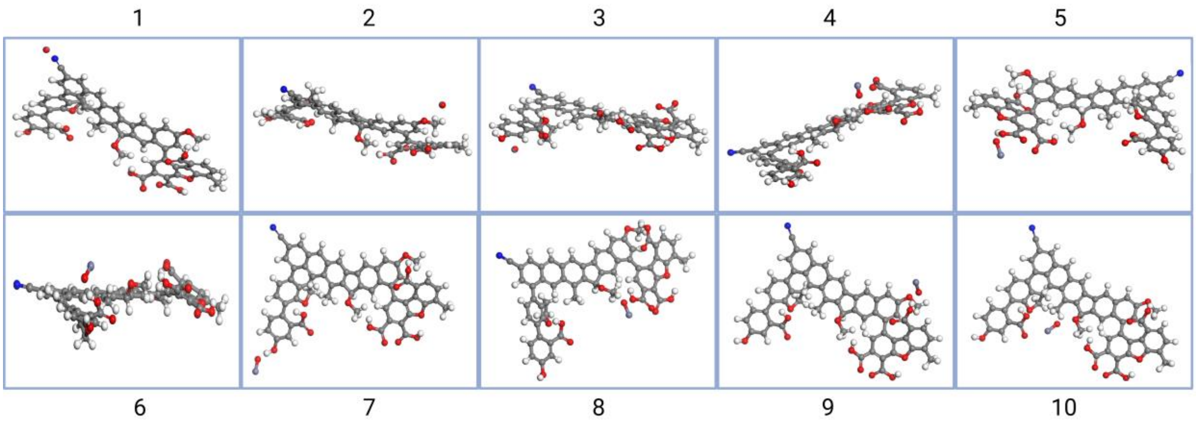

Figure 4.11a: Different configurational structures for ZnO adsorption on CBC

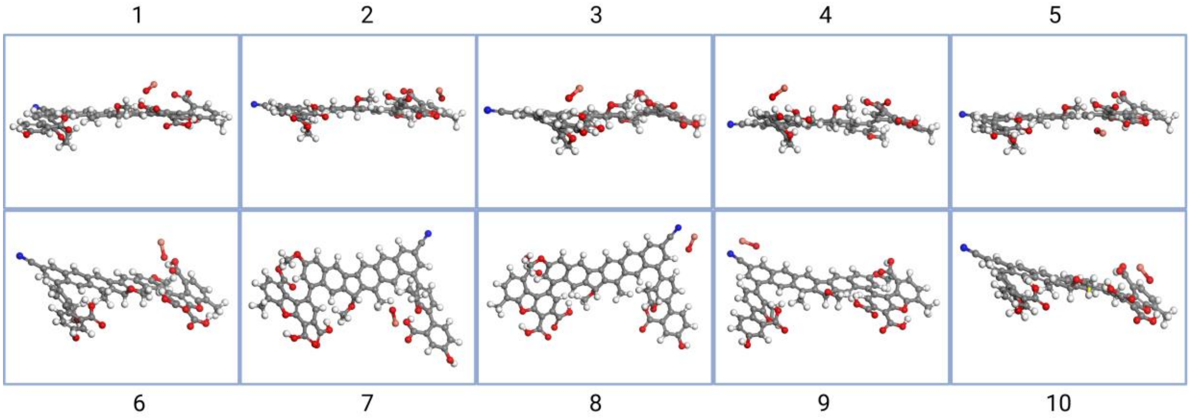

Figure 4.11b: Different configurational structures for CuO adsorption on CBC

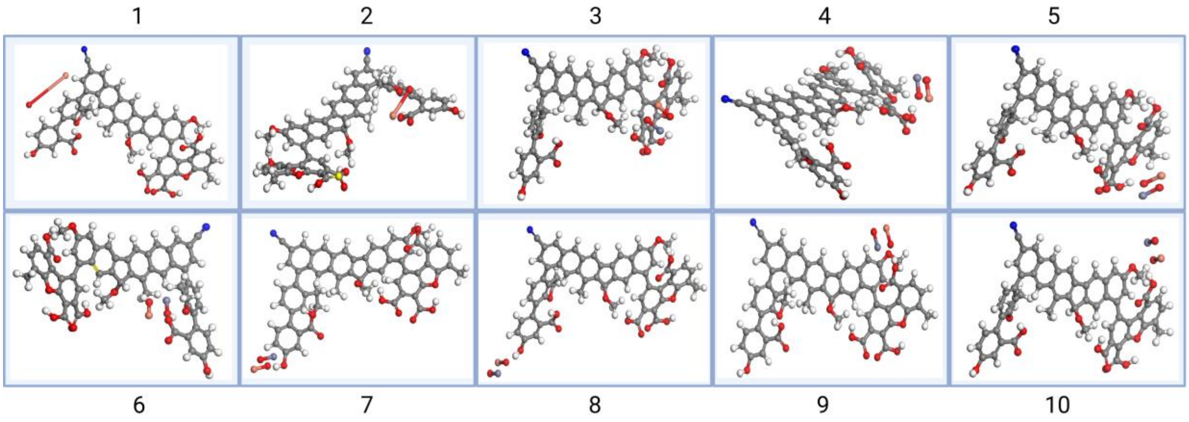

Figure 4.11c: Different configurational structures for [ZnO+CuO] adsorption on CBC (co-presence)

Table 4.7 shows a generalized comparison of the adsorption characteristics of the ZnO and CuO nanonutrients, independently and as co-adsorbates with the CBC. The rigid adsorption energies (E_{rigid}) for ZnO-CBC systems vary between – 1.115 eV to – 28.34 eV across the different configurations whereas CuO-CBC ranged between – 23.80 eV to – 28.63 eV.

4.1.12 Pilot field trials

The pilot study investigated the effect of nanofertilizer applied at three doses (40 g, 20 g, and 10 g per 10 kg of soil), a chemical fertilizer (NPK+S 15:15:15+10.2 at the manufacturer's recommended dose of 15g per 10kg of soil), and a control (no fertilizer) on maize growth over a 20-day period (Figure 4.12 and Appendix III). Five replicates were used for each treatment group, and five growth parameters were measured, namely chlorophyll content, nitrogen concentration, plant height, leaf surface area, and number of leaves per plant. The results revealed clear differences across treatment groups. Chlorophyll content was highest in the 10 g nanofertilizer (Group C) and chemical fertilizer (Group D) treatments, although the latter was accompanied by high plant mortality. Nitrogen concentration was also greatest in Group C, while Group D showed moderate levels but with reduced plant survival. Plant height and leaf surface area were highest in Group C and the control (Group E), whereas Group D again showed poor growth and stunted leaves. The number of leaves was consistently higher in Group C compared to all other groups, indicating balanced vegetative development. Mortality patterns were particularly striking: Group D recorded the highest losses, with multiple plants dying after Day 7, while Group A (40 g nanofertilizer) also showed some withering. In contrast, Groups B (20 g nanofertilizer), C (10 g nanofertilizer), and E (control) exhibited the best survival rates.

Table 4.7: Comparison of ZnO-CBC and CuO-CBC adsorption complexes

Property	**ZnO-CBC**	**CuO-CBC**	**[ZnO+CuO]-CBC**
Average Adsorption Energy	Stronger (−701.83 eV)	Weaker (−23.94 eV)	Stronger than CuO and weaker than ZnO (-542.82 eV)
Variation (Standard Deviation)	High (wide range)	Low (narrow range)	High (wide range)
Deformation Energy	Higher (-687.68 eV)	Minimal (-6.3 eV)	High (-209.55 eV)
Normalized Adsorption Energy	More negative	Less negative	-

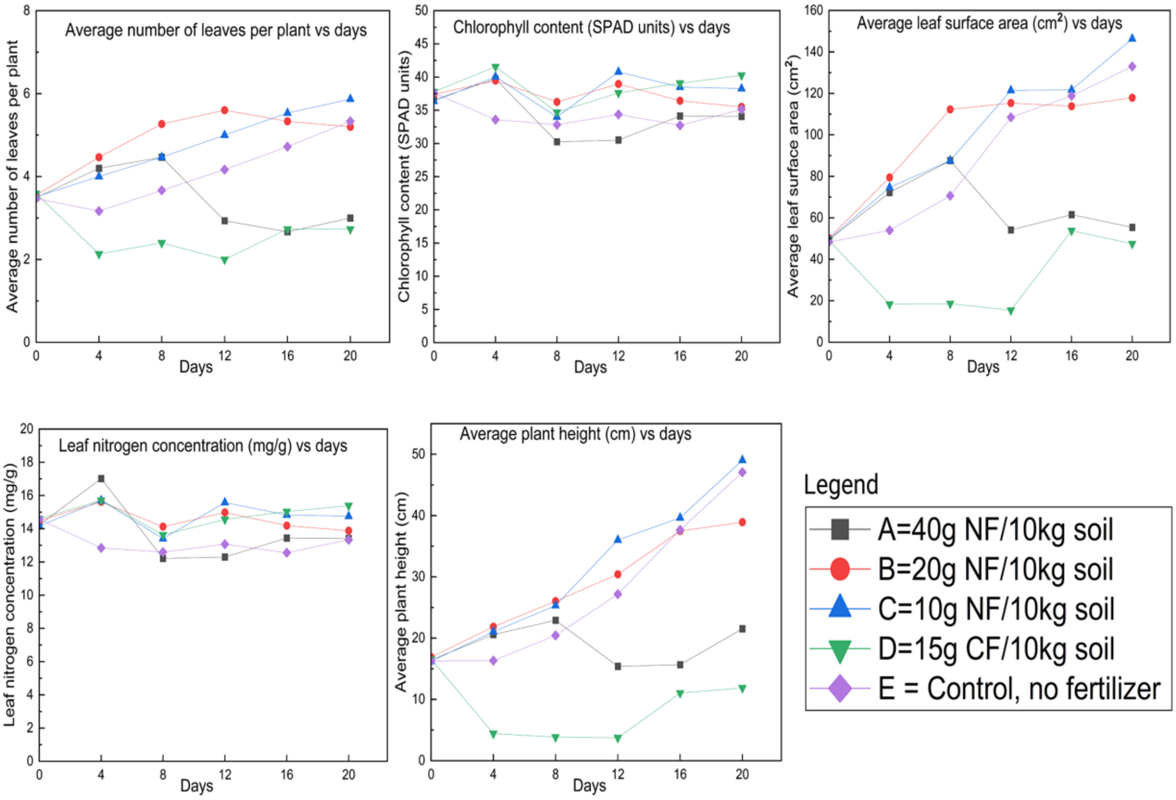

Figure 4.12: Comparative effects of nanofertilizer (10 g, 20 g, and 40 g per 10 kg soil), chemical fertilizer, and control on maize growth parameters (chlorophyll content, nitrogen concentration, plant height, leaf area, and number of leaves) over 20 days. The 10 g nanofertilizer dose consistently supported superior growth and survival, while the chemical fertilizer treatment exhibited poor performance and high mortality.

4.2 Discussion

4.2.1 Biochar preparation and yield

Partial carbonization of the WH biomass was observed for t=15 min, however, total pyrolysis was observed at 30, 45 and 60 min for D1 (leaf) and D2 (stem). Pyrolysis conditions, BC yield, pH and liming capacity data are shown in Table 4.1. BC yields from D1 are significantly lower than D2 ($p<0.05$) for all the pyrolytic treatments indicating different biochemical composition of the WH leaf and stem as indicated by studies by (Lara-Serrano et al., 2016). The higher BC yield observed from D2 can be explained from the presence of more fibrous and woody cellulose in the stem biomass with higher stable carbon content than D1 as previously shown (Lara-Serrano et al., 2016), where WH stem exhibited higher holocellulose, hemicellulose and cellulose than leaf biomass for WH from Yuriria Lake in Mexico. Additionally, both D1 and D2 showed yields consistent with results from Bottezini et al. (2021), Jin et al. (2019), and Narayanan et al. (2021), where yields of between 33.6 – 51.8% were obtained with temperatures between 250°C – 500°C and resident time from 20 to 60 min. However, for D1 and D2, there are no significant differences in yield between the different pyrolytic resident times for each biomass type.

4.2.2 FTIR functional group characterization of WH biomass and biochar

FTIR data in Figure 4.1 and Table 4.8 indicate that the functional groups present on the WH biomass and BC products varied based on the pyrolytic conditions and precursor biomass. For example, D1 spectra showed the presence of only amines, whereas A1, B1 and C1 show weak amine peaks at wavenumber 1036 – 1058 cm^{-1} with nitro- groups being present at 1543 cm^{-1}. This could be due to oxidation of the amines to form nitro-compounds in the resulting BC products. On the contrary, A2, B2 and C2 do not show any peaks in the nitro- or amine-

Table 4.8: Showing FTIR identified functional groups in samples

WAVENUMBER	BOND	CLASS
712	C=C bending	alkene
753	C-H bending	1,2-disubstituted
798	C - O bending	Single bond C-O
872	C-H bending	1,2,4-trisubstituted
1036	C-N stretching	amine
1401	S=O stretching	sulfonyl chloride
1543	N-O stretching	nitro compound
1565	C = O bending	aldehyde
3038	O-H stretching	alcohol
3329	N-H stretching	aliphatic primary amine
3377	O-H stretching	carboxylic acid - OH

region, indicating the difference between the stem and leaf precursors. Alternatively, the amines in D2 could be more heat labile, effectively decomposing during the pyrolytic treatment. A summary of individual spectra is discussed subsequently,

D1: The WH leaf spectra shows a variety of functional groups typical of organic matter, including O-H, C-H, C=O, and C=C stretches, indicating the presence of alcohols, phenols, carboxylic acids, amines, and alkenes.

A1: The BC spectra show similar functional groups but with reduced intensity in the O-H stretch region, indicating some thermal degradation of organic compounds. The presence of sulfonyl and nitro groups suggests thermochemical changes during pyrolysis.

B1: The BC spectra is like A1, but with further reduction in the intensity of O-H stretches, indicating more extensive pyrolysis as resident time increased. The functional groups present indicate the progressive conversion of organic matter into more stable structures.

C1: The BC spectra is like B1 but with further reduced intensity in the O-H region and possibly higher carbonization, as indicated by the C=C stretches.

D2: The WH stem spectra shows a variety of functional groups typical of organic matter, including O-H, C-H, C=O, and C=C stretches, indicating the presence of alcohols, phenols, carboxylic acids, amines, and alkenes.

A2: The BC spectra shows similar functional groups but with reduced intensity in the O-H stretch region, indicating some thermal degradation of organic compounds. The presence of sulfonyl groups suggests chemical changes during pyrolysis.

B2: This spectrum is similar to A2, but with further reduction in the intensity of O-H stretches, indicating more extensive pyrolysis with resident time. The functional groups present indicate the progressive conversion of organic matter into more stable structures.

C2: The BC spectra is similar to B2 but with further reduced intensity in the O-H region and possibly higher carbonization, as indicated by the C=C stretches.

As indicated by Li et al, the FTIR data suggest a rearrangement of molecules from aliphatic CH groups to more condensed, aromatic and thermostable BC products as the pyrolysis resident time increased (Li & Chen, 2018). The pie-bonds in the aromatic species can be desirable for composite fortification especially with electrophilic nanonutrients (Chen et al., 2008). However, adsorption properties of the BC products can diminish as surface functional groups, such as O-H and C-O groups reduce with increase in pyrolysis temperature or resident time (Ahmad et al., 2012), which further underscores the need to select the optimum resident time for effective BC production. In the current study, 30 min treatment could be the most ideal for optimum BC product formation for further nano-fortification.

4.2.3 SEM-EDX characterization of WH Biochar

SEM images of the BC are shown in Figure 4.2 (top 2 rows) indicating variations in surface morphology and porosity with changing pyrolysis resident times for both D1 and D2. Subsequently, SEM images for the BC products at magnifications between 6000x and 22,000x were analyzed using python codes to estimate the pore sizes with bounding boxes (Figure 4.2: bottom 2 rows). Estimated pore area sizes for the samples range from $0.03\mu m^2$ – $0.46\mu m^2$. For a large hypothetical square nanoparticle of 100nm by 100nm size (0.1 μm by 0.1 μm), its area will be $10{,}000nm^2$ ($0.01\mu m^2$). Therefore, as indicated by Chen, Zheng, Xu, Dang and Zhang (2019), and Xing et al. (2023), extensive interfacial interactions both at the BC surface and within the micropores with nanoparticles will be feasible at the pore sizes with increased surface area for physisorption and chemisorption. It has been previously noted that BC structure becomes more ordered with higher porosity and increased surface area as pyrolytic resident time and temperature increases (Claoston, Samsuri, Ahmad Husni, & Mohd Amran, 2014; Sun, He, Pan, & Zhang, 2017; Zhao et al., 2018), this is highly desirable for adsorption of nanonutrients for the purpose of fortifying the BC products for biofertilizer formulation. The micropores in the BC products can also be beneficial as soil amendments for both water holding and aeration which can enhance soil properties for healthy plant growth (Glaser et al., 2024).

4.2.4 Hydrodynamic particle sizes and zeta potentials of BC

The hydrodynamic particle sizes and zeta potential measurements of biochar samples derived from WH biomass are crucial parameters for their potential application in nano biofertilizer formulation (**Figure 4.3**). Although the dimensions of the commercial ZnO (18nm) and CuO (25-55nm) nanoparticles used in the formulation are less than 100nm, the average zeta sizes of the biochar samples indicate significant differences based on the biomass and pyrolytic treatments. D1-derived biochar samples exhibited larger particle sizes, with A1 at 583 nm, B1 at 354 nm, and C1 at 270 nm. Conversely, D2-derived biochar samples showed smaller particle sizes, with A2 at 304 nm, B2 at 146 nm, and C2 at 314 nm. These values are consistent with values obtained by Ha, Nguyen, Wang and Nguyen (2019), when synthesizing NPK nanofertilizers using chitosan as the composite material with a size range between 300 - 700nm. The zeta potential measurements of the biochar samples were consistently negative. Specifically, the recorded values were -35 mV, -34 mV, -35 mV, -38 mV, -36 mV, and -38 mV for A1, B1, C1, A2, B2, and C2 respectively, consistent with the surface functional groups identified by FTIR. Such high negative zeta potential values indicate that the biochar particles possess a surface charge that can contribute to their colloidal stability in aqueous suspensions and subsequent nanoformulations. This stability is crucial for nano biofertilizer, as it will reduce the potential for the aggregation of particles, ensuring a uniform distribution of the biofertilizer when in liquid suspension (Liu et al., 2018; Song et al., 2019).

4.2.5 BC pH, liming potential, carbon storage, particle size range and fertilizer value based on IBI classification

The pH of the biomass was slightly acidic at 6.43 and 6.61 for D1 and D2 respectively whereas all BC samples range between 10.56 – 10.77 (Table 4.1). These data agreed with those obtained by Gezahegn et al. (2024). The alkaline pH can be attributed to the presence of inorganic oxides of K, Ca and Na which are formed with oxygen atoms from the biomolecules during pyrolysis. However, a better characterization is the liming potential of the BC samples, which was expressed as percentage $CaCO_3$ – equivalence $(\%cc_{eq})$. According to Table 4.1, BC products from D1 have the highest liming potential with mean values at 28.94%; 25.39%; 23.85% for A1, B1 and C1 respectively. In contrast, BC samples derived from D2 show significantly lower liming potential with A2 significantly higher than B2 and C2 with values of 22.60%; 16.85% and 14.76% respectively ($p<0.05$). These values are similar to the range of 17.7 to 33.0% reported by Gezahegn et al. (2024). In our study, the 30 min pyrolysis resident time produced the highest liming potential in both D1 and D2 samples ($p<0.05$). The significantly higher values for D1 when compared with D2 can be explained by the presence of alkali metals in the leaves resulting in higher oxides, carbonates, phosphate or silicates formation which confers liming effects on the BC products. Based on the liming potential values, they are classified into class 0 ($\%cc_{eq} < 1\%$), class 1 ($1\% \leq \%cc_{eq} \leq 10\%$), class 2 ($10\% \leq \%cc_{eq} \leq 20\%$) and class 3 ($20\% \leq \%cc_{eq}$) (Singh et al., 2024b) with A1, B1, C1 and A2 in class 3 and B2 and C2 in class 2. This also supports 30 min as the optimum pyrolytic resident time for higher liming capacity, which can be beneficial as amendments for acidic soils (Lewoyehu, Kohira, Fentie, Addisu, & Sato, 2024; Vassilev, Baxter, Andersen, & Vassileva, 2013a, 2013b) found in several agronomic zones around the world especially in parts of Asia, Africa, South America and the United States

(Bekunda et al., 2010; Binswanger-Mkhize & Savastano, 2017; Dimkpa et al., 2023; Dimkpa, 2018). Generally, BC physicochemical properties depend on the pre-, post-, and actual-pyrolytic treatments; and feedstock biomass types (Ippolito et al., 2020; Whalen, Ejack, Gul, & Castro, 2024). These properties modify the overall fertilizer value of the resulting BC, which can also be used to guide the selection of the candidate BC products for further nano-fortification as fertilizers.

In this study, the International Biochar Initiative (IBI) classification system was used (IBI, 2024). This classification uses the percentage concentration of 6 major nutrients (N, P, K, S, Ca, and Mg) in the BC samples as well as the available portions to plants, estimated via total aqueous nutrient release/desorption over 5days. Other factors used in the classification include liming capacity, carbon storage value (based on organic carbon (C) and H/C ratio) and particle size range. Under this IBI system, BC fertilizer values are classified as 4, 3, 2, 1, and 0 with 4 being the best (Table 4.1). Theoretically, fertilizer class higher than 1 is desirable as this implies more nutrients are available to the extent needed by plant, however, field trials was needed to validate this as soil-BC interactions also play a role in qualitative and quantitative nutrient availability (Whalen et al., 2024). However, in terms of liming capacity, D1-derived BC samples and the 30 min treatment for D2 all fall within class 3 and 4. Carbon storage capacity of the BC samples was also highest for A1 samples at 553g/kg of sequestered carbon (Table 4.1).

4.2.6 Effect of pyrolytic Treatments on Biochar Nutrient Concentration

For a BC product to be effective as a nano-biofertilizer composite material, it must retain most of its nutrients for subsequent nano-enabling and eventual nutrient release to nourish plants. To assess the impact of pyrolytic treatments, the percentage nutrient concentration change (N_c) was calculated using biochar yield constant Y_b (0.31 and 0.34 for D1 and D2 biomass respectively) in equation 3, with negative values indicating possible nutrient loss during pyrolytic reactions while equation 4 expresses the comparison of nutrient in biochar directly with biomass for each element. In this instance, for nitrogen concentration for both D1 and D2, the effect of pyrolytic nitrogen loss is in the order 60min > 45min > 30min, indicating that the nitrogen containing biomolecules (such as proteins, amides, pyrimidines, and amines) in the biomass are more labile with increased furnace resident time. This is partially supported by results obtained by Abd El-Azeim et al. (2021) who found that WH pyrolysis at lower temperatures for 30 min is ideal for improvement of soil fertility and organic matter content. Similarly, nitrogen concentration impact for the BC samples is in the reverse order 30mins > 45mins > 60mins. More strikingly, 30 min pyrolytic time produced significantly higher (291% wt/wt) increase in nitrogen content for stem biochar (A2). This indicates that an increase in pyrolysis time at 600°C caused the nitrogen containing species to undergo condensation into heterocyclic nitrogen compounds which are more stable while the heat-labile species are lost via gasification (Gao, Guo, Liang, Liu, & Yang, 2020). Additionally, the increase in the nitrogen concentration can also be due to reactions such as dehydration and decarbonizations (S. Xu et al., 2021). The effect of pyrolysis on selected elements (N, P, K, S, Cu, Zn, Mg, Fe, B and Ca) in the biochar compared to the biomass is represented in Figure 4.4 and Table 4.2.

For the biomasses, D1 has significantly higher concentrations of N, P, S, B and Ca than D2 whereas the reverse is the case with K, Cu, Zn and Fe. There is no significant difference between the concentrations of Mg in both D1 and D2 which agreed with reports from Lara-Serrano et al (Lara-Serrano et al., 2016). Pyrolytic treatments significantly increased P, K, Zn, Fe Mg, B and Ca but significantly decreased S and Cu contents in the BC products for D1. However, for D2, pyrolysis increased concentrations of P, S, Mg, Zn, B, and Ca whereas it reduced concentrations of Fe and Cu. For D2, K concentration was significantly higher in the 60 min pyrolysis, with no significant difference for 45 min and a significant reduction in concentration for the 30 min treatment.

4.2.7 Soil leachate and aqueous elemental availability

Considering that the BC samples were being explored for their suitability for possible nano-augmentation in biofertiliser formulation, it was necessary to assess the availability of nutrients in aqueous and soil media, to ensure their bioavailability. Leaching studies indicated that the BC samples had significantly higher and controlled release of key macronutrients (NPK) from day 2 in the soil medium, over the period of the experiement, compared to soil without BC amendment (Figure 5). As observed by Helal et al. (2023), controlled and sustained release of nutrient from nanofertilizer enhanced plant growth and reduced the consumption of agrochemicals, especially of nitrogen. Results also indicate BC, being alkaline, produced significantly lower NO_3^--N in the leachates extract with higher NH_4^+-N availability, this could be a good application for acidic soils (Zhang et al., 2016). The predominance of NH_4^+-N compared to NO_3^--N in the total amount of N in leachate is a positive point for the BC materials with respect to nitrogen loss from agricultural soils. Several research reports on nitrogen loss from fertilizer application indicated that, nitrogen was mainly leached in the form of NO_{3-}-N rather than NH_4^+-N. Wang and Li (2019) reported the predominance of NO_3-N as the main form of nitrogen loss in soil while Aikpokpodion, Hsiao and Dimkpa (2024) reported 98% of total N loss was in the form of NO_3^--N while NH_4+-N was less than 2% of total N leached. The predominance of NH_4+-N in the leachate infers that, the biochar materials are going to supply nitrogen to the soil in a form that is more retained in soil because of its cationic charge that makes it bind to the negatively charged soil colloids and clay surfaces. On the contrary, NO_3^--N with its anionic charge is highly mobile in soil solution and easily get leached. However, the ultimate fate of the biochar-supplied NH_4^+-N lies on the rate and pathway of nitrification of ammonium to nitrate via ammonium oxidation to nitrite by oxidizing bacteria in soils. Additionally, with a controlled release from the BC, the N-nutrients can be released at rates needed by the plants thereby preventing accumulation and possible losses (Wang et al., 2021).

The trend of phosphorus release from the various BC products show that, except for the control, there was a spike in the release of phosphorus from the various biochar materials during day 2 of leaching exercise before a progressive decline in P release in day 3 to day 5 . Between the first and second day of leaching experiment, sample A1 had a 37.94% increase in P supply from the BC while samples B1, C1, A2, B2 and C2 had 71.98, 14.79, 37.71, 40.97 and 79.29% increase in P supply respectively. The increased release of phosphorus on day 2 of leaching compared to day 1 was an indication of the ability of the micropores within the BC to accumulate nutrient elements which are subsequently released to plants in a controlled manner. Many micropores are present in the BC pore structures resulting in more than 80% of the pore volume (Weber & Quicker, 2018). Phosphorus released on day 1 might have been the portion of P adsorbed on the surface of the biochar particles while the increased P released on day two might have been a combination of desorbed P from the particle surfaces and the portion of P adsorbed within the macro and micropores of the BC. Though the surge in P release from the biochar on day 2 could apparently lead to higher P supply than needed by plant and subsequent fixation of excess P in soil, the alkaline nature of the BC will create an environment unsuitable for P fixation. Phosphorus fixation in soil is mainly due to bonding of P to soil clay and hydr(oxides) of Al, Fe and Mn under acidic soil conditions (Aikpokpodion, 2023). Under acidic soil condition, the hydr(oxides) of Al, Fe and Mn are readily available in soil solution and consequently bind to phosphorus in a chemical reaction which limits the availability of P for plant uptake. With the alkaline condition of the BC, the released P has lesser potential of being fixed and subsequently bioavailable for plant utilization.

For K, release from soil amended with BC follow similar pattern with NO_3^--N and NH_4+-N. Except for the control where the release continue to decrease over the period of the experiment, all BC-amended soil samples had a sharp decrease in K released from day 1 to day 2 of 93.54, 92.62, 91.07, 90.73, 91.74, 91.48% for A1, B1, C1, A2, B2, and C2 respectively as against a decrease of 46.67% by the control. This indicates K is readily available at significantly higher values in amended soils than the control. Doulgeris et al found similarly that increasing proportions of biochar amendment in soils resulted into doubling of available K, suggesting that this is enhance by factors such as cation kinetic and equilibrium exchange (Doulgeris, Kypritidou, Kinigopoulou, & Hatzigiannakis, 2023).

4.2.8 Water Holding Capacity (WHC)

Water holding capacity (WHC) indicate an important property of the biochar for improvement of soil health for improved crop productivity. BC has been demonstrated to help alleviate plant's environmental stressors associated with water household such as drought and salinity (Kammann & Graber, 2024). For the leaf biochar samples, WHC decreased with pyrolysis resident time although A1 and B1 are not significantly different, both were significantly higher than C1. The reverse trend is observed with the stem-derived (D2) biochars where the WHC increased with pyrolytic resident time with A2 significantly lower than both B2 and C2 as shown in Figure 4.6. High WHC is desirable as added benefits for biochar-based formulations and would ensure water and nutrients are available to plants while also enhancing the microbial activities within the soil microbiome as shown by Ghorbani et al who found that biochar amendments enhanced water retention in soils and significantly increased rice yields under evaporation stress (Ghorbani et al., 2023). By reducing the amount of suction force required by plants to extract water from the soil, BC can positively modulate plant's physiological and biochemical process thereby promoting plants' resilience and yields

(Ghorbani et al., 2023; Kammann & Graber, 2024; Mahmoud et al., 2022; Sofy, Heba, Mona, & Abdelghafar, 2021).

4.2.9 BET Surface Area and Pore Analysis of Water Hyacinth Biochar

Table 4.4 shows the surface area and pore volume data for the BC samples. The CO_2-specific surface area and pore volume of BC are heavily influenced by both pyrolysis duration and biomass type (Shen & Wu, 2023; Sun et al., 2017; Zhao et al., 2018). In this study, BCs derived from leaves (A1, B1, C1) and stems (A2, B2, C2) were produced at 600°C with varying pyrolysis times of 30, 45, and 60 minutes respectively, to examine the effect of pyrolysis time on surface area and porosity (Brewer, 2024; Gezahegn et al., 2024).

For the leaf-derived BCs, there is a consistent increase in surface area and pore volume as the pyrolysis time increased (Edeh, Masek, & Fusseis, 2023; Muzyka, Misztal, Hrabak, Banks, & Sajdak, 2023). At 30 minutes (A1), the surface area was 236.44 m^2/g with a pore volume of 0.061 cm^3/g. When the pyrolysis time was extended to 45 minutes (B1), the surface area increased to 250.02 m^2/g, and the pore volume remained at 0.061 cm^3/g, indicating a slight improvement in surface properties. The most significant increase in surface area was observed at 60 minutes (C1), where the surface area reached 347.26 m^2/g and the pore volume increased to 0.087 cm^3/g. This suggests that a longer pyrolysis duration allows for greater volatilization and pore development in leaf BCs, increasing microporosity.

In contrast, the stem-derived BCs displayed a different trend. At 30 minutes (A2), the surface area was 256.71 m^2/g, slightly higher than the leaf BC (A1) at the same time, with a pore volume of 0.062 cm^3/g. After extending the pyrolysis time to 45 minutes (B2), the surface area increased to 264.17 m^2/g, and the pore volume rose to 0.072 cm^3/g, indicating a more pronounced improvement in porosity, compared to the leaf BC over the same duration. However, at 60 minutes (C2), the surface area of stem BC decreased to 249.41 m^2/g, and the pore volume dropped to 0.067 cm^3/g. This decline suggests that longer pyrolysis times can lead to the collapse or densification of pores in stem BC, reducing its overall surface area and porosity.

These results indicate that while leaf BC benefits from extended pyrolysis times (Ji et al., 2024), continuing to develop greater surface area and porosity, stem BC may experience structural degradation and pore collapse after prolonged exposure to high temperatures. Therefore, the optimal pyrolysis time for maximizing surface area and porosity depended on the type of biomass, with leaves benefiting from longer durations, while stems may require shorter pyrolysis times to maintain a high surface area and porosity. This results also align with those obtained by Sigmund et al (Sigmund, Hüffer, Hofmann, & Kah, 2017) indicating that feedstock biomass composition and structure play a key role in carbonization and volatilization of compounds in the biochar product.

4.2.10 Conceptualized biochar (CBC) structure: molecular properties

A negative heat of formation of -226.45 kcal/mol indicates that the CBC molecule is thermodynamically stable compared to its constituent elements in their standard states. This chemically explains why BC is stable under ambient conditions, which is beneficial for applications like long term carbon sequestration and soil amendment (Melo et al., 2024; Ordonez-Loza, Bamdad, Spataro, Papari, & Berruti, 2024). The highly negative electronic energy (-149966.37 eV) typically indicates a stable electronic configuration, suggesting that the molecule has a steady arrangement of electrons. This usually imply less chemical reactivity since chemical reactions generally involve electrons in covalent or electrovalent systems. The ionization potential represents the energy required to remove an electron from the molecule. A higher ionization potential suggests that the molecule is relatively stable and less reactive, which may be advantageous in applications where chemical stability is desired, such as long-term carbon sequestration and soil amendments. Furthermore, the CBC with a total energy of -12586.15 eV, which indicates the total kinetic and potential energies, showed a stable molecular structure since higher negative values indicate higher stability (Ayub et al., 2024). This is further indicated by the core-core repulsion value of 137380.22 eV showing the repulsive interactions between core electrons in the atom. A high core-core repulsion energy indicates that the electronic structure is well-defined, further contributing to overall stability (Abeng et al., 2025).

The gradient norm indicates how close the molecular structure is to an energy minimum during optimization calculations. A lower value (0.38) suggests that the structure is close to a local minimum, indicating stability. Similarly, the root mean square (RMS) force provides insight into the forces acting on atoms in the molecule during optimization. A low RMS (0.02) value indicates that the molecular structure is well-optimized and stable, with minimal forces acting to change its configuration (Tao, Zou, Li, Wang, & Chen, 2024). Generally, these data show that the CBC molecule is thermodynamically stable, well-optimized structurally, and has a relatively high ionization potential, suggesting low reactivity under normal conditions. These theoretical values agree with evidence of biochar's recalcitrant nature as it is not readily mineralized in the soil by either abiotic or biotic processes (Lehmann et al., 2024). These characteristics are favorable for applications in soil amendment and carbon sequestration, where stability and minimal reactivity are desired attributes

4.2.11 HOMO and LUMO

For the CBC, computed values for E_{HOMO} and E_{LUMO} are - 0.184985 and - 0.126879 eV, respectively. Therefore, the HOMO-LUMO energy gap ΔE_{gap}, which also indicates the tendency of the HOMO-LUMO sites to lose or gain electrons in a reaction (Abeng et al., 2025), can be calculated as:

$$\Delta E_{gap} = E_{LUMO} - E_{HOMO} = -0.126879 - (-0.184985) = 0.058106$$

A small HOMO-LUMO gap (e.g., 0.058106 eV in this case) typically suggests high chemical reactivity because electrons require less energy to transition from HOMO to LUMO. This is often associated with materials that are prone to electron transfer, making them reactive. However, biochar is generally known for its stability and low reactivity (Lehmann et al., 2024), as supported by other molecular parameters in Table 4.2, like low RMS force, high heat of formation, and high ionization potential (Loebsack, Yeung, Berruti, & Klinghoffer, 2025; Williamson, Herr, & Mo, 2024). In biochar, the HOMO and LUMO orbitals are usually localized on specific functional groups or unsaturated carbon atoms at edges, which could reduce the energy gap without significantly affecting overall structural stability (Ruiz-Morales, 2002; Su et al., 2024). For example, the HOMO may be localized on electron-donating groups while the LUMO may be localized on electron-deficient regions, such as aromatic rings. Also, oxygen-containing functional groups (e.g., - OH or - COOH) can influence orbital energies, reducing the gap without necessarily increasing overall reactivity (Qureashi et al., 2023; Su et al., 2024). Overall, this localized reactivity may play a role in the adsorption process where high adsorption energies were observed indicating possible chemisorption apart from physisorption through Van der Waals and π-electrons from the aromatic rings in the biochar molecule.

4.2.12 Zno and CuO nanonutrient-biochar adsorption simulations

Table 4.7 shows a broader range for ZnO indicating variability in its interaction with CBC, in contrast CuO exhibited a higher absolute value, suggesting a stronger interaction under conditions requiring less structural distortions. This shows both nanomaterials can effectively interact with the CBC, however the mechanisms and strengths vary based on the structural factors and surface properties. This observation supports findings by Suazo-Hernández et al. (2024) that Zn and Cu availability from soils treated with engineered ZnO and CuO nanoparticles, compared to soils without treatments were 597.7 and 41.8 times respectively.

Availability of nanonutrients in soils is a function of quantity desorbed from the nanoconjugate which also depends on two key factors: the quantity adsorbed and the adsorption energy. However, other extrinsic soil factors like pH, temperature, microbial activities, and cation exchange capacity, could influence the desorption process (Chen et al., 2015). Also, Wei et al. (2019) observed that both nano-ZnO and nano-CuO can be readily adsorbed by activated granular sludge, but CuO-NPs were more effectively adsorbed at 1.31g/g against 0.53g/g for the ZnO-NPs (Wei et al., 2019). The material studio simulated $(\frac{\Delta E_{ads}}{\Delta N_i})$ values show that as more molecules are added, there is a consistent trend toward favorable adsorption, which is crucial for understanding how these materials might behave in practical applications such as biofertilizers or nanonutrient delivery (Bhardwaj et al., 2022). When both ZnO and CuO are adsorbed together, the overall average adsorption energy is – 542.82 eV. However, the individual contributions of each of nano-ZnO and nano-CuO component to the overall adsorption process in the co-presence situation is given by $\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{ZnO}$ and $\left(\frac{\Delta E_{ads}}{\Delta N_i}\right)_{CuO}$ with average values of – 529.52 eV and – 326.05 eV respectively. Therefore, ZnO adsorption energy weakens in the presence of CuO (from – 701.83 eV to – 529.52 eV) whereas CuO adsorption energy strengthens significantly (from -23.94 eV to -326.05 eV). Therefore, although ZnO dominates the adsorption process, its interaction with CBC is reduced in the presence of CuO while the latter benefits from ZnO co-presence by showing a stronger adsorption compared to when independent. The data shows that:

ZnO has the highest adsorption energy, largely due to deformation of CBC when adsorbed alone.

CuO has lower deformation energy but shows overall surface affinity with higher rigid adsorption energy.

In adsorption simulations with ZnO and CuO as co-adsorbates:

ZnO interaction with CBC weakens (less negative adsorption energy).

CuO interaction with CBC significantly strengthens (more negative adsorption energy).

The overall adsorption energy (− 542.82 eV) remains closer to ZnO individual contribution, indicating ZnO still contributes significantly in the adsorption process.

The increase in CuO adsorption energy in the presence of ZnO suggests synergistic effects, possibly due to charge redistribution or structural changes in the CBC substrate that favor CuO adsorption in the presence of ZnO. These computational data support experimental results indicating that adsorbent (e.g activated carbon) behaviour was influenced by the co-presence and ratio of metal oxides especially ZnO and CuO (Balsamo, Cimino, de Falco, Erto, & Lisi, 2016; Yu, Chen, Wang, & Xu, 2021).

Furthermore, observing the structures depicted by the material studio for each of the 10 configurations for the adsorption simulations (independently and co-presently) , it shows that the adsorption of the ZnO and CuO nanomaterials is typically controlled by surface electrostatic and Van der Waals interactions (Chen et al., 2019; Zhao et al., 2017), which indicates a strong physisorption process. Notably, for structures 1 and 2 on the co-present simulation studies, only CuO was adsorbed but for the structures from #3 to 10, both nanoparticles were adsorbed. This could be because ZnO requires high deformation energy when binding with the CBC as against CuO with less deformation energy (Yang, Chen, Gong, & Wang, 2022; Yu et al., 2021).

4.2.13 Pilot field trials

The results of this study demonstrated that biochar-based nanofertilizers, particularly at lower application rates, provide superior agronomic performance compared to conventional chemical fertilizers. The 10 g nanofertilizer treatment (Group C) consistently outperformed all other treatments across multiple physiological and morphological parameters, including chlorophyll synthesis, nitrogen retention, plant height, leaf area, and leaf number. This observation aligns with previous reports that biochar and biochar-based composites improve soil fertility and plant growth by enhancing nutrient retention, increasing water-holding capacity, and stimulating soil–plant interactions (Kumaraswamy et al., 2021; Nagaraju et al., 2023; Raliya et al., 2018; Wang et al., 2022). The balanced performance of Group C suggests that moderate doses of nanofertilizer optimize nutrient availability while avoiding nutrient toxicity, supporting the notion that controlled release of nutrients is a central advantage of nano-enabled fertilizers (Iqbal, Umar, & Mahmooduzzafar, 2019; Sammar Raza et al., 2023; Tariq et al., 2023)

In contrast, the chemical fertilizer group (Group D) exhibited stunted growth and the highest mortality rate (approximately 60%). This indicates a potential phytotoxic effect under the experimental pot conditions, likely due to nutrient overload and salt accumulation in the restricted soil environment (Fageria, Filho, Moreira, & Guimarães, 2009). Excessive use of NPK fertilizers is well-documented to cause osmotic stress, root damage, and nutrient imbalances, particularly in fragile or small-scale systems (Shoukat et al., 2024; Wei et al., 2025). The findings from this study therefore corroborate concerns raised in the literature about the sustainability and environmental risks associated with conventional fertilizers, especially in regions where soils are acidic, low in organic matter, and vulnerable to degradation (Meena et al., 2022; Mishra et al., 2023)

Interestingly, the highest nanofertilizer dose (40 g, Group A) also produced suboptimal outcomes, including withered plants and reduced survival. This may be attributed to the excessive water retention of the biochar matrix, which could have limited root access to oxygen and water, thereby impeding growth. Similar results have been reported in studies where high biochar application rates adversely affected soil aeration and plant performance (Glaser et al., 2001, 2024). This dose-dependent response highlights the critical importance of optimizing nanofertilizer application rates to balance soil moisture dynamics with nutrient delivery.

The consistency of Group C across all measured parameters is particularly significant. Few studies have reported such across-the-board improvements in chlorophyll, nitrogen uptake, and vegetative growth using a single biochar-based formulation. Previous investigations have often emphasized improvements in one or two growth indicators (Dimkpa et al., 2023), but the present findings suggest a broader spectrum of benefits when nanofertilizers are applied at optimal doses. This not only validates the potential of biochar-based nanofertilizers as effective nutrient carriers but also supports their scalability as a sustainable alternative to conventional fertilizers in developing regions.

Overall, these results provide robust evidence that nanofertilizers at moderate concentrations can improve plant growth performance while minimizing the risks of nutrient toxicity and mortality observed with conventional fertilizers. This finding is of particular importance in the Niger Delta region, where soil infertility, environmental pollution, and overdependence on chemical fertilizers threaten agricultural sustainability (Kabiri et al., 2017). By integrating invasive water hyacinth biomass into nanofertilizer production, this research simultaneously addresses ecological management, soil fertility enhancement, and food security, thereby contributing to global calls for sustainable fertilizer innovation (Beig et al., 2022; Channab et al., 2024; Gehlot et al., 2024).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study is novel in highlighting, for the first time, the plant fertilization potential of BC from WH as a function of several inherent characteristics, including liming capacity, nutrient content, and water-holding capacity, among others. The findings show that water hyacinth (WH) can be a useful resource for soil amendment and its value in this regard can be further improved with nano fortification with micronutrients like zinc and copper that are low in the biomass feedstock harvested from Ekpan River in Delta State, Nigeria. These micronutrients are known to have high adsorption ability to plant biomasses. Notably, pyrolytic treatments at 600°C showed 30 min residence time as the optimum for both leaf and stem (D1 and D2) samples, indicating that these biomasses can be pooled during harvest to facilitate operations and subsequent downstream processing. This also is desirable in terms of energy requirements for the thermochemical treatment. With several desirable properties including liming capacity, water holding capacity, high concentration of available N, P, K and highly porous structure of the BC derived from WH, these products show promise for use in amendment in acidic and drought impacted soils. The carbon storage using this invasive plant for nano-biofertilizer formulation may be a way of synchronizing farming operations with natural autotrophic process by using this as a means of mining plant nutrients from water bodies via water hyacinth. This can be a double-edged advantage helping to capture and sequester atmospheric CO_2 through biochar-soil amendments since results in this study showed carbon content in biochar averaged above 70%.

The findings from this research contributed to the development of environmentally friendly and economically viable solutions for sustainable agriculture and ecosystems preservation. Furthermore, WH derived BC can be a more balanced single fertilizer product than most commercial synthetic fertilizers. For example, urea, the most popular N fertilizer contains 46% N; TSP, the most popular phosphorus fertilizer contains 20% P, and MOP, the most popular K fertilizer contains 60% K. Although, effectively, not all these nutrients are available to the plant when applied, however, in contrast, BC products in this study released quantities of these macronutrients in soil medium above the required ppm by plants. Additionally, fortifying the biochar with nanoscale Cu and Zn that are known to be both nutrients and crop protection materials broadened the functionality of the formulated nano-enabled fertilizer.

The findings in the research highlighted the critical influence of pyrolysis duration and biomass on the adsorption properties of water hyacinth-derived BC, as evidenced in specific surface area and pore volume. Leaf-derived BC exhibited a consistent increase in surface area and porosity with longer residence time at 600°C reaching a maximum surface area of 347.26m^2/g and a pore volume of 0.087cm^3/g at 60 minutes. This enhanced microporosity makes leaf BC highly suitable for adsorbing nanonutrients, thereby offering excellent potential for bio-nanofertilizer formulations. Conversely, stem-derived BC displayed an optimal surface area of 264.17m^2/g and a pore volume of 0.072cm^3/g at 45 minutes residence time. Prolonged pyrolysis (60mins) led to structural degradation and pore collapse, reducing adsorption capacity. Collectively, leaf BC is better suited for nanonutrient adsorption due to its ability to modulate porosity over the extended pyrolytic duration, while stem BC requires carefully optimized conditions to retain its structural integrity. However, pooling these two biomasses ensured the resulting biochar combined the essential properties for downstream nanoformulations.

Whereas the computational study aimed to elucidate the molecular foundations of adsorption interactions between biochar and nanonutrients, specifically ZnO and CuO. By analyzing a CBC's properties such as heat of formation, electronic energy, total energy, HOMO-LUMO gap, and bond energy, we established a comprehensive understanding of its structural stability and potential as an adsorbent. The structure was optimized with VAMP module using the Material Studio Suite software.

The adsorption simulations revealed that both ZnO and CuO exhibit energetically favourable interactions with the CBC. Notably, ZnO displayed higher adsorption energy (E_{ads}) compared to CuO due to more negative deformation energy (E_{deform}). This difference suggests that ZnO might exhibit a different mechanism and/or adsorption site on the biochar. Co-adsorption simulations involving both nanonutrients showed significantly more negative average values for total energy (E_{total}) and rigid adsorption energy (E_{rigid}), indicating enhanced stability when both are present. However, ZnO required more energy for the adsorption process due to higher structural deformation energies (E_{deform}). This was reflected in average ($\frac{\Delta E_{ads}}{\Delta N_i}$) values of – 701.83 eV as lone adsorbate and – 529.52 eV with CuO co-presence. Summarily, ZnO exhibits a significantly higher total adsorption energy on CBC compared to CuO, largely due to the high deformation penalties. The adsorption by CuO also improves significantly when ZnO is present, likely due to ZnO-CuO interactions stabilizing the CuO adsorption. Overall adsorption energy under the co-presence scenario is closer to ZnO contribution, which aligns with experimental studies showing ZnO dominates adsorption in a mixed system.

The configurational structures from our simulations suggest strong physisorption as the preferred mechanism over chemisorption for these systems. Physisorption typically involves weaker intermolecular forces such as electrostatic attractions, Van der Waal forces, and hydrogen bonding. Compared to chemisorption, it is usually reversible, and this can be beneficial for applications requiring controlled release or desorption processes.

Taken together, this study contributes valuable insights into how different metal oxides interact at the molecular level with biochar surfaces – a crucial aspect in designing sustainable agricultural practices that integrate nanotechnology with organic amendments in biogenic fertilizers and other applications. Our findings have significant implications for designing efficient nano-biofertilizers and other applications that leverage biochar-nanoparticle interactions. The use of biochar as an adsorbent has implications for nutrient retention in soil by reducing leaching while providing a slow release mechanism beneficial for plant growth. This study demonstrates the potential of biochar-based nanofertilizers derived from water hyacinth biomass as a sustainable alternative to conventional fertilizers for maize cultivation. The findings revealed that lower nanofertilizer doses (10–20 g per 10 kg soil) provided the most favorable outcomes, supporting higher chlorophyll content, nitrogen retention, plant height, leaf area, and leaf number while ensuring high survival rates. In contrast, chemical fertilizer application resulted in stunted growth and significant plant mortality, underscoring the risks of phytotoxicity under small-scale conditions. The results therefore validate the efficiency of nanofertilizers at low concentrations, showing that they can deliver nutrients effectively, minimize toxicity risks, and align with sustainable agricultural practices. These outcomes are particularly relevant to the Niger Delta region, where challenges such as poor soil fertility, environmental pollution, and food insecurity persist. By transforming invasive water hyacinth into an innovative nanofertilizer, this research provides both an environmental remediation strategy and an agronomic solution, contributing to improved food security, reduced reliance on imported chemical fertilizers, and progress towards several Sustainable Development Goals. Future work should focus on scaling up field trials, optimizing dosage under diverse soil conditions, and assessing long-term environmental safety to strengthen the case for adoption and funding support.

This approach aligns with several sustainable development goals, particularly SDG 2 (Zero Hunger) by enhancing food security through improved crop yields and sustainable agricultural practices, and SDG 12 (Responsible Consumption and Production) by promoting environmentally friendly fertilizer systems. Additionally, it supports SDG 13 (Climate Action) through carbon sequestration properties inherent to biochar.

Undoubtedly, foundational knowledge which has been provided in this work, is necessary for optimizing biochar-based, nano-enabled fertilizers with nanonutrients such as ZnO and CuO. Future studies, however, should focus on experimental validation of these computational results alongside investigations into the environmental factors influencing these interactions under real-world conditions.

This research focuses on the valorization of water hyacinth (*E. crassipes*), an invasive aquatic plant that poses significant ecological and socioeconomic challenges globally. By converting this biomass into value-added products, specifically through the production of nanonutrient-fortified biochar, we aim to address multiple sustainability goals aligned with the United Nations Sustainable Development Goals (SDGs).The utilization of water hyacinth not only mitigates the adverse effects of its proliferation—such as reduced biodiversity, impaired water transport, and damage to infrastructure—but also contributes to sustainable, smart and precision agricultural practices. The development of nano-enabled fertilizers from biochar derived from water hyacinth represents a circular economy-approach, transforming an ecological menace into a resource while enhancing soil health and agricultural productivity. This aligns with SDG 2 (Zero Hunger), which aims to end hunger, achieve food security, and promote sustainable agriculture by improving nutrient availability in soils. Our investigation into optimum production temperature and residence time for functional biochar emphasizes environmentally friendly and energy efficient techniques (pyrolysis at 600°C for 15 – 60mins, with 30mins being optimum). These conditions ensure efficient conversion of biomass and enhance nutrient retention and availability in soils, promoting precision agriculture. Theoretically, this research extends to computational modeling using Material Studio Software to unravel the molecular interactions between nanonutrients and biochar, examining their sorption dynamics, a property which is critical for crafting efficient nanobiofertilizer. This work aims to optimize fertilizer formulations for improved plant nourishment, contributing to global food security and ecological balance. Furthermore, this research has the potential to create positive socioeconomic impacts by generating job opportunities in the production and application of these green fertilizers where this invasive plant is present. It aligns with SDG 8 (Decent Work and Economic Growth) by promoting economic development through sustainable agricultural practices and supports SDG 12 (Responsible Consumption and Production) by ensuring that natural resources are used sustainably in agricultural systems.

In conclusion, our work seeks to expand knowledge on the upcycling of non-food crop biomass, particularly using water hyacinth as feedstock. This approach does not only address the ecological impacts of this invasive species but also enhances agricultural sustainability, carbon sequestration with attendant climate bearings, environmental sustainability via reduction in the pollution associated with the use of commercial fertilizers, ultimately further contributing to SDGs #9, 11, 13, 14 & 17. In summary, these conclusions can be itemized as stated below:

1. This study is novel in demonstrating, for the first time, the fertilization potential of water hyacinth (WH)–derived biochar (BC) based on inherent properties such as liming capacity, nutrient content, and water-holding capacity.
2. WH can be valorized as a soil amendment resource, with its nutrient value further enhanced through nano-fortification with zinc and copper—micronutrients deficient in WH biomass but critical for crop nutrition and protection.
3. Pyrolysis at 600 °C for 30 minutes was optimal for both WH leaf and stem biomass, enabling pooled processing that improves energy efficiency and downstream handling.

4. WH-derived BC showed desirable agronomic properties, including liming potential, high water-holding capacity, high available NPK, and porosity, making it suitable for acidic and drought-prone soils.
5. Transforming invasive WH into biochar-based nanofertilizer provides a dual advantage: removing excess biomass from ecosystems and sequestering carbon (>70% C content), aligning with climate-smart agriculture.
6. WH-derived BC provides a more balanced single fertilizer product than conventional fertilizers, releasing plant-available macronutrients (N, P, K) at sufficient levels while reducing leaching losses.
7. Fortification with nanoscale ZnO and CuO enhanced BC's nutrient profile and multifunctionality, broadening its role as both a fertilizer and crop protection material.
8. Pyrolysis duration and biomass type significantly influenced BC adsorption properties. Leaf-derived BC maintained increasing surface area and porosity up to 60 min, while stem-derived BC peaked earlier (45 min) before pore collapse at 60 min.
9. Pooling leaf and stem biomasses combined complementary traits, producing biochar suitable for efficient nanoformulations.
10. Computational modeling confirmed the structural stability of WH biochar and revealed favorable adsorption interactions with ZnO and CuO nanoparticles.
11. ZnO showed higher adsorption energies but incurred higher deformation penalties, while CuO adsorption improved in the co-presence of ZnO, indicating synergistic stabilization.
12. Simulations suggested physisorption as the dominant mechanism, which is advantageous for controlled nutrient release in soil applications.

13. Pilot field trials showed that low doses of nanofertilizer (10–20 g/10 kg soil) outperformed chemical fertilizers, improving maize growth, nutrient retention, and survival rates.
14. Chemical fertilizer treatments caused high plant mortality (~60%), indicating risks of phytotoxicity in small-scale systems, while optimized nanofertilizer doses avoided toxicity.
15. These findings validate WH-based nanofertilizers as effective, safe, and sustainable alternatives to synthetic fertilizers.
16. This research demonstrates that WH valorization into nanofertilizers addresses ecological, agronomic, and socio-economic challenges simultaneously.
17. The approach advances multiple UN Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action), while also supporting goals on decent work, innovation, and sustainable cities.

5.2 Recommendation

Nano-enabled soil amendments represent a paradigm shift in sustainable agriculture, offering solutions to enhance soil health, crop productivity, and climate resilience. However, their scalability and full adoption by stakeholders depend on overcoming infrastructure deficits, cost barriers, and environmental concerns. By fostering multidisciplinary collaboration-spanning policymakers, researchers, and farmers, this technology can bring about equitable food systems and align with global goals such as the United Nations Sustainable Development Goals. Strategic investments, inclusive policies, and rigorous safety protocols will ensure nanotechnology fulfills its transformative potential while safeguarding ecosystems and rural livelihoods.

Further work is therefore, recommended for optimization of pyrolysis conditions, focusing on temperature and biomass residence time, to produce functional biochar with minimal energy consumption and processing time. Additionally, further experimental and computational molecular simulations need to be conducted to study the sorption interactions between biochar and nanonutrients. The nano-enabled biochar-based fertilizers should be tested on various crops under field conditions to evaluate their effectiveness and economic viability. Summarily, the recommendations are:

1. Further optimization of pyrolysis conditions (temperature, residence time) for energy-efficient biochar production
2. Study adsorption interactions between biochar and nanonutrients using experiments and simulations
3. Further studies on dose response of maize to nanofertilizer at < 10g per 10kg soil
4. Field-test nanofertilizer on different crops for growth and yield response
5. Scale up field trials, refine dosage across soil types, and assess long-term environmental impacts to strengthen adoption potential.

5.3 Contributions to knowledge

The key contributions to knowledge among others are:

- The study established for the first time the optimum pyrolytic conditions for the production of functional biochar at 600°C from invasive water hyacinth aerial tissues
- The research is a pioneering work in nanotechnology assisted fertilizer formulation from biochar which was further enhanced through computational molecular modelling.
- The work developed a sustainable biochar-based nanofertilizer for eco-smart and effective crop yield.

- This work has also contributed over 6 high-impact paper publications which has been cited more than 44 times as at the time of this thesis submission.

These contributions are further detailed in the sections below.

5.3.1 Water Hyacinth Biochar (BC) Production

1. Incomplete carbonization occurred at 600°C with 15 min residence time; complete pyrolysis was achieved at 30–60 min.
2. Leaf biomass yielded significantly less BC (31%) than stem biomass (34%) irrespective of the furnace resident time.
3. SEM and FTIR analyses revealed porous structures with surface functional groups including –COOH, –OH, C=C, and –S=O, more prominent in leaf biomass.
4. BC properties (porosity, functional groups, elemental content, liming capacity, nutrient retention/release, water holding capacity) were influenced by both biomass type and pyrolysis duration, with 30 min being optimal.
5. Combining biomass types improved process efficiency and BC balance for nanofertilizer precursor.
6. While deficient in copper and zinc, the BC contained high nutrient availability with nitrogen (34,550 ppm), phosphorus (56 ppm), potassium (609 ppm), hence required CuO and ZnO nanofortification for a balanced biofertilizer.
7. BC showed promising liming potential (15–29% CCeq) and water holding capacity (1.5–2.5 g/g), supporting use in acidic or drought-prone agroecological zones.

5.3.2 Nanofertilizer (NF) formulation, BET Adsorption, and Computational Insights

8. BC impregnation with ZnO (30ppm) and CuO (20ppm) nanoparticles produced nanofertilizers containing 1.51 ppm Zn and 0.50 ppm Cu, enhancing both nutrient value and potential crop protection functionality.

9. Nano-enabled BC released available nitrogen, phosphorus, and potassium in soil at concentrations exceeding plant requirements, offering a more balanced nutrient profile compared to commercial fertilizers like urea (46% N), TSP (20% P), and MOP (60% K)
10. CO_2 adsorption data fit BET models, with monolayer capacity, (21.51–33.37 cc/g) and BET constant, C (254.88–497.53).
11. As shown in SEM images, BET analysis confirmed increases in surface area (up to 347.26 m^2/g) and pore volume (0.087 cm^3/g) with longer pyrolysis.
12. Computational analysis showed stable BC structure (Heat of formation = –226.45 kcal/mol; Total energy = –12,586.15 eV; Ionization potential = 8.37 eV).
13. The rigid adsorption energy (–14.15 eV for ZnO vs. –17.64 eV for CuO) indicates that CuO has a stronger actual binding affinity for the biochar surface which supports literature.
14. Adsorption of ZnO (–529.52 eV) and CuO (–326.05 eV) both strengthened considerably as co-adsorbates.
15. Frontier molecular orbitals, HOMO and LUMO, were spatially distinct with energy gap = 0.058 eV, supporting BC's low reactivity despite a small energy gap.

5.3.3 Pilot Field Trials (NF vs CF)

16. Maize treated with 10–20 g NF/10 kg soil showed superior growth and survival over 40g NF and 15g chemical fertilizer (CF) doses.
17. 60% plant mortality under CF indicated potential phytotoxicity or chemical shock at the recommended dose under small scale systems used in the pilot experiments.
18. 10 g NF/10 kg soil consistently outperformed across most growth metrics, while 40 g NF led to stunted growth and some mortality, highlighting the need for dose optimization.

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APPENDICES

Appendix I: Preparation of standard nitrate (NO_3^-) and ammonium (NH_4^+) solutions from ammonium nitrate (NH_4NO_3) salt.

1 mole of NH_4NO_3 has a molar mass of **80.043g/mol**

Therefore:

80.043g of NH_4NO_3 contains **17.994g** of NH_4^+ and **62.049g** of NO_3^-.

To prepare a standard solution of NO_3^-

62.049g of NO_3^- will be in **80.043g** of NH_4NO_3

1g of NO_3^- will be in 1.29g of NH_4NO_3

0.1g (ie 100mg) of NO_3^- will be in 0.129g of NH_4NO_3

Hence to prepare **100mg/L** of NO_3^- solution, weigh **0.129g** of NH_4NO_3 salt and dissolve in 1 liter (**1,000ml**) de-ionized water.

(**Note:** to prepare 100mg/L in **100ml** of de-ionized water, then weigh **0.0129g** of NH_4NO_3).

Then, from this stock standard NO_3^- solution, **1:10** and **1:100** dilutions can be made to prepare solutions of **10mg/L** and **1mg/L** respectively.

To prepare a standard solution of NH_4^+

17.994g of NH_4^+ will be in **80.043g** of NH_4NO_3

1g of NH_4^+ will be in 4.448g of NH_4NO_3

0.1g (ie 100mg) of NH_4^+ will be in 0.4448g of NH_4NO_3

Hence to prepare **100mg/L** of NH_4^+ solution, weigh **0.4448g** of NH_4NO_3 salt and dissolve in 1 liter (**1,000ml**) de-ionized water.

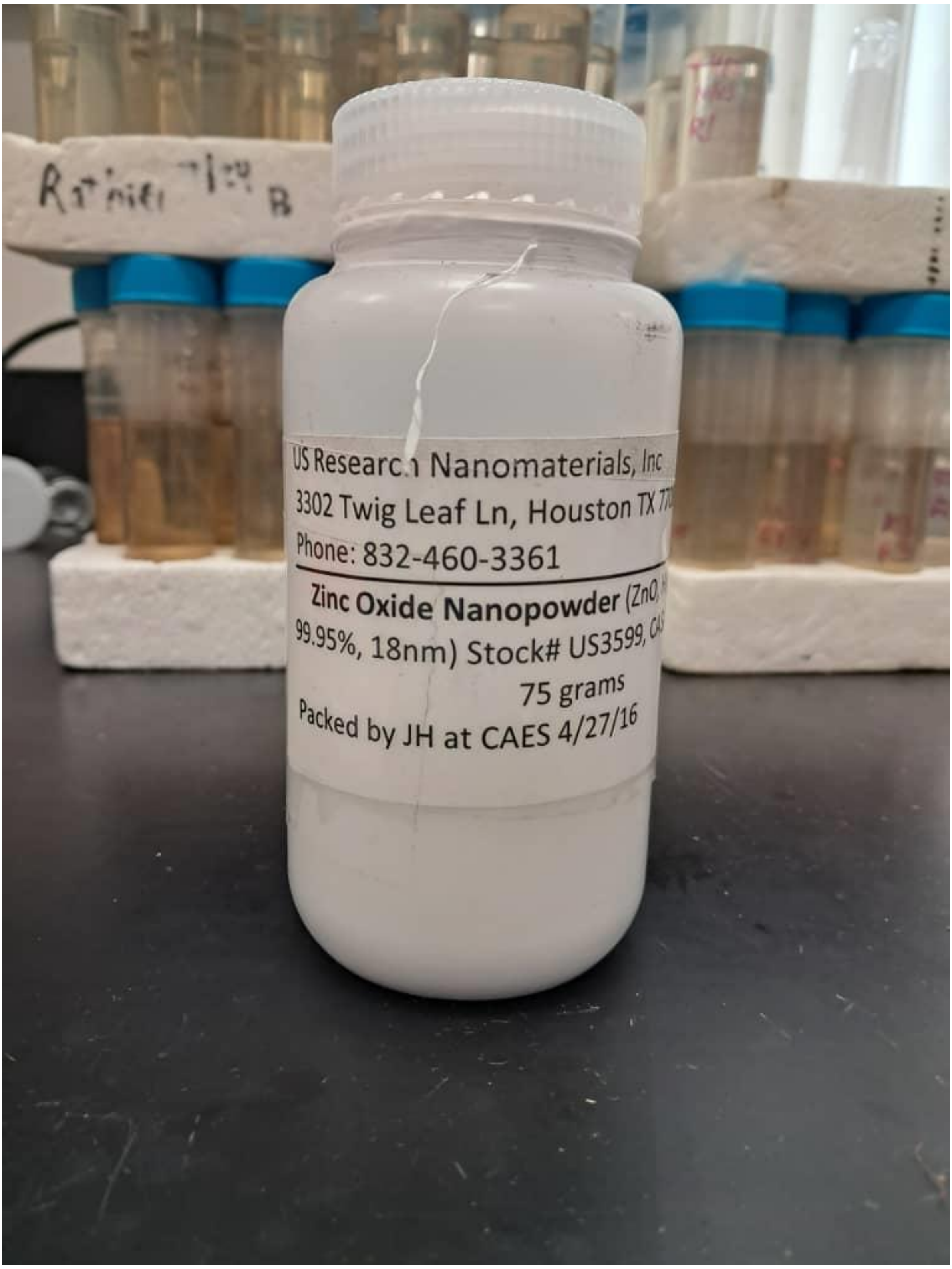
(**Note:** to prepare 100mg/L in **100ml** of de-ionized water, then weigh **0.04448g** of NH_4NO_3).

Then, from this stock standard NH_4^+ solution, **1:5** dilution can be made to prepare a solution of **20mg/L**.

Appendix II: Photographical data showing sample location details

Appendix III: Pyrolyzed water hyacinth biochar samples labelled in plastic containers for further analysis

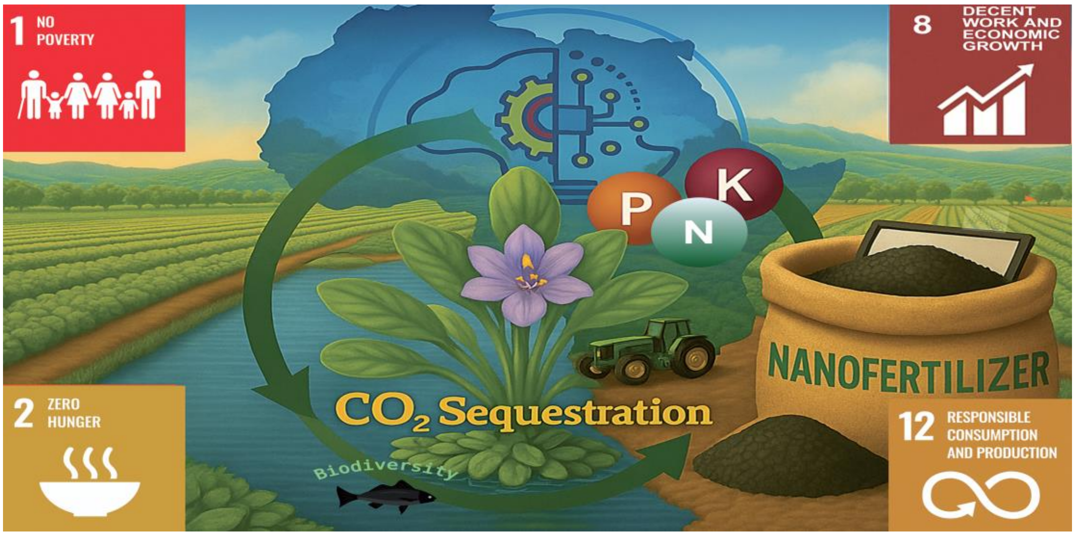
Appendix IV: ZnO nanopowder used in nanofertilizer fabrication



Appendix V: CuO nanopowder used in nanofertilizer fabrication

Appendix VI: Study plants in pilot trials (right to left, groups A – E)

Appendix VII: Accepted artwork showcasing research on the RSC Sustainability journal coverpage



Showcasing work from the African Centre of Excellence in Future Energies and Electrochemical Systems at the Federal University of Technology Owerri, Nigeria. The project is led by Professor Emeka E. Oguzie, with Dr. Elias E. Elemike (Federal University of Petroleum Resources, Nigeria) and Dr. Christian O. Dimkpa (Connecticut Agricultural Experiment Station, USA) as collaborators.

Morphological and chemical profiling of biochar derived from invasive aquatic weed towards bio-nanofertilizer development

This study explores the structural and chemical characteristics of biochar produced from water hyacinth, an invasive aquatic weed. Advanced analyses reveal the biochar's porous architecture, surface properties, and elemental makeup, assessing its viability as a sustainable precursor for bio-nanofertilizers, aligned with the International Biochar Initiative's framework. The findings pave the way for transforming an ecological nuisance into high-value agro-materials, driving eco-smart fertilizer innovation in sustainable agriculture.

Image reproduced by permission of Emeka E. Oguzie from *RSC Sustainability*, 2025, **3**, 3947.

Image created with Biorender.com.

As featured in:

See Christian O. Dimkpa, Emeka Emmanuel Oguzie *et al.*, *RSC Sustainability*, 2025, **3**, 3947.

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Appendix VIII: Certificates awarded

AFRICAN M·R·S

CERTIFICATE OF PARTICIPATION

This Certificate Is Presented To

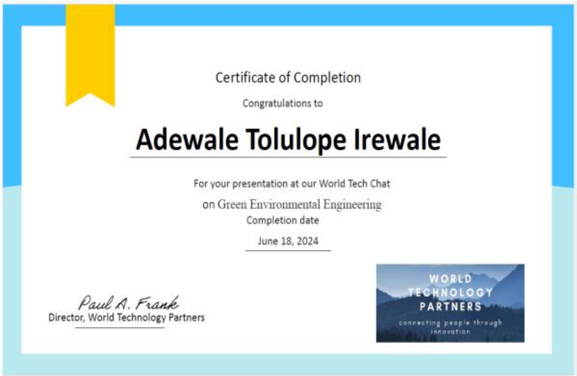
Irewale Adewale

For participating in the 12th International Conference of the African Materials Research Society (AMRS) held in Kigali, Rwanda from 16 to 19 December, 2024

Omololu Akin-Ojo

Dr. Omololu Akin-Ojo
Chair, Conference Organizing Committee
AMRS Vice-President

Prof. Balla Diop Ngom
AMRS President



Certificate of Completion

Congratulations to

Adewale Tolulope Irewale

For your presentation at our World Tech Chat
on Green Environmental Engineering
Completion date
June 18, 2024

Paul A. Frank
Director, World Technology Partners

WORLD TECHNOLOGY PARTNERS
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UNNRG
Nano Research Group

NANO RESEARCH GROUP
University of Nigeria, Nsukka
In collaboration with
OilServ Limited Group of Companies

OILSERV
OILSERV LIMITED
Pipelines and Facilities (EPCIC)

Certificate of Participation
Presented to

Adewale Tolulope Irewale

at
The *6th* Biennial Conference/Workshop on Applications of Nanotechnology to Energy Environment, Agriculture and Health
(Hybrid International Conference 2025)
University of Nigeria, Nsukka
14th – 19th July, 2025

Title(s) of Paper(s) presented:

- Green Nano-Innovations: Current Practices and Tools for Sustainable, Smart, and Precision Agriculture

Prof. Rose U. Osuji
Conference/Workshop Chair

Prof. Fabian I. Ezema
Coordinator, Nano Research Group

UNISA ACE FUELS OILSERV NSF OPTICA

CHISQUARES

Certificate of Attendance

This is to Certify that

Adewale Irewale

Attended the Webinar Titled:

Overview Of How The Chisquares Platform Enhances Research Speed, Quality, And Accuracy

May 7, 2024

Israel Agaku, DMD, MPH, PhD, MBA
Head, Extramural Education

Scan this

Lungile Nkosi, BSc, MPH
Coordinator, Virtual Learning

ACS Student Chapter
JNCASR

CERTIFICATE
of Completion
is presented to

Adewale Tolulope Irewale

for completing a module on
"Understanding Carbon Capture, Utilization and Storage (CCUS)".

Prof. Sebastian C Peter
Jawaharlal Nehru Centre for Advanced Scientific Research,
Convener, National Centre for Carbon Capture and Utilization (NCCCU)

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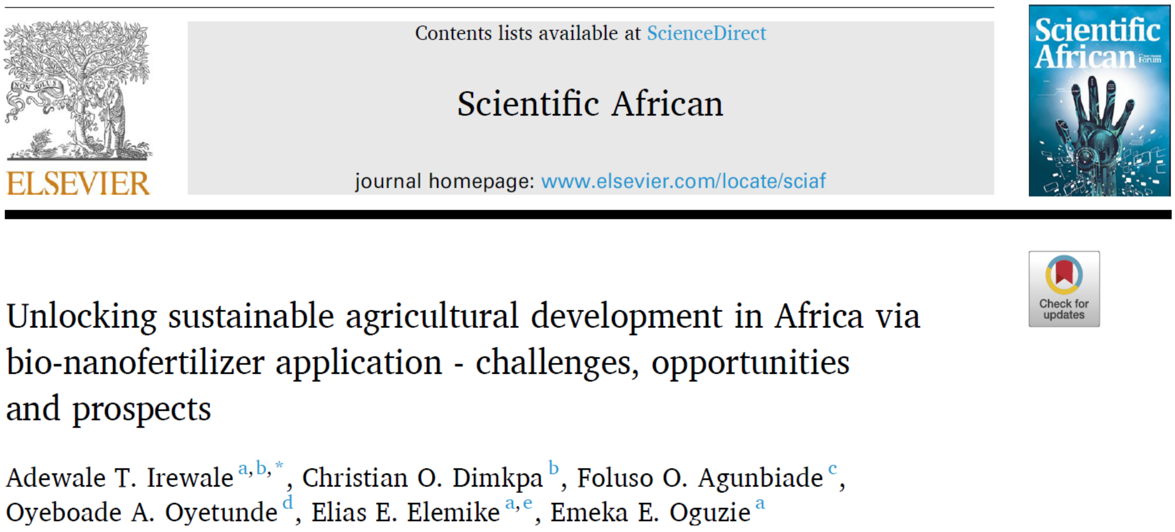
Appendix IX: Some published works from the PhD research and conferences attended

The following peer-reviewed publications were produced from research conducted during this PhD project. Collectively, these articles represent extracted components of the broader research, including experimental investigations, material characterization, and computational modelling of biochar-based nano-enabled fertilizers.

Published journal articles and book chapter

1. DOI: https://doi.org/10.1016/j.sciaf.2024.e02276 **(published June 2024)**



Contents lists available at ScienceDirect

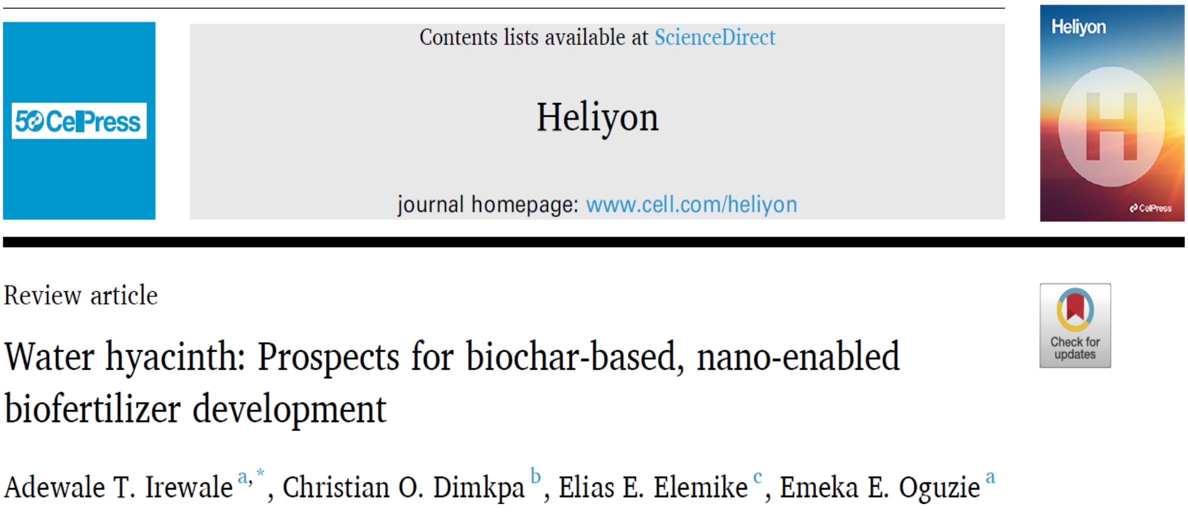
Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Unlocking sustainable agricultural development in Africa via bio-nanofertilizer application - challenges, opportunities and prospects

Adewale T. Irewale[a,b,*], Christian O. Dimkpa[b], Foluso O. Agunbiade[c], Oyeboade A. Oyetunde[d], Elias E. Elemike[a,e], Emeka E. Oguzie[a]

2. DOI: https://doi.org/10.1016/j.heliyon.2024.e36966 **(published August 2024)**



Contents lists available at ScienceDirect

Heliyon

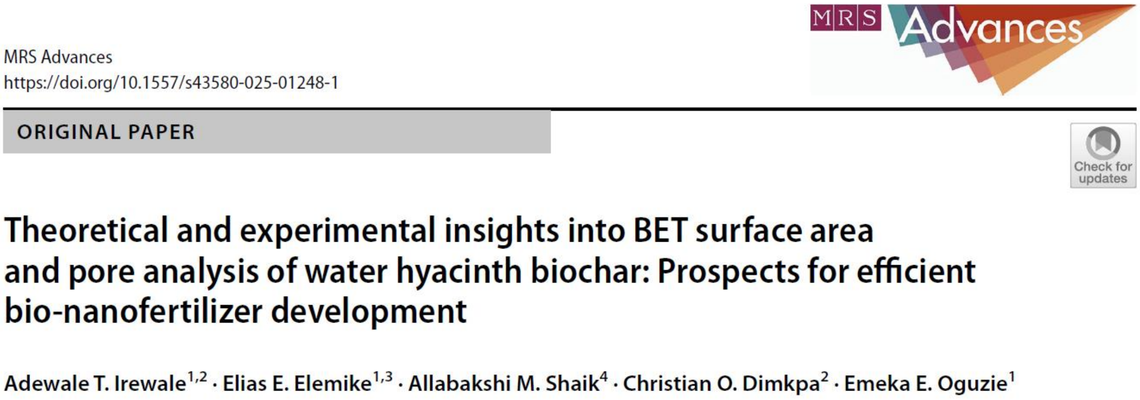
journal homepage: www.cell.com/heliyon

Review article

Water hyacinth: Prospects for biochar-based, nano-enabled biofertilizer development

Adewale T. Irewale[a,*], Christian O. Dimkpa[b], Elias E. Elemike[c], Emeka E. Oguzie[a]

3. DOI: https://doi.org/10.1557/s43580-025-01248-1 **(published March 2025)**


MRS Advances
https://doi.org/10.1557/s43580-025-01248-1

ORIGINAL PAPER

Check for updates

Theoretical and experimental insights into BET surface area and pore analysis of water hyacinth biochar: Prospects for efficient bio-nanofertilizer development

Adewale T. Irewale[1,2] · Elias E. Elemike[1,3] · Allabakshi M. Shaik[4] · Christian O. Dimkpa[2] · Emeka E. Oguzie[1]

4. DOI: https://doi.org/10.1039/D5SU00052A **(published May 2025)**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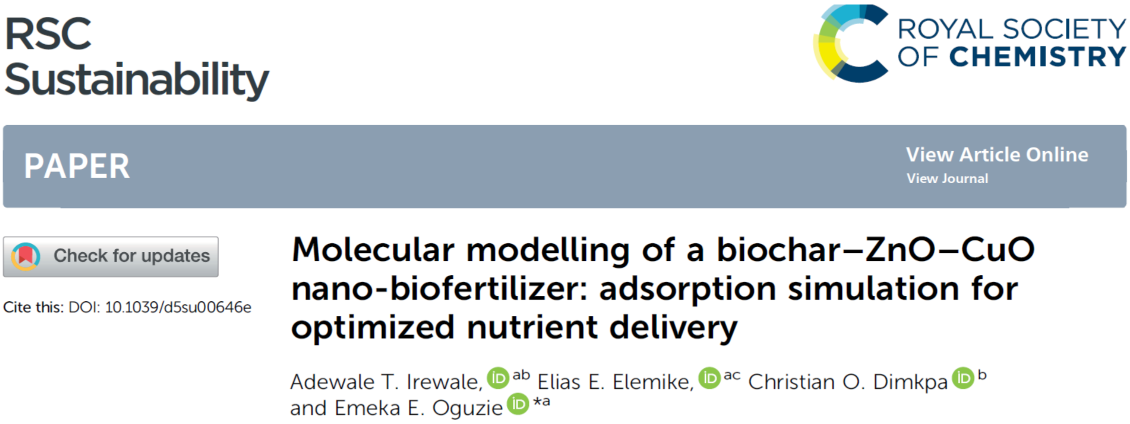
Cite this: DOI: 10.1039/d5su00052a

Morphological and chemical profiling of biochar derived from invasive aquatic weed towards bio-nanofertilizer development†

Adewale Tolulope Irewale, [ab] Elias Emeka Elemike, [ac] Paul Ehimare Aikpokpodion, [b] Raja Muthuramalingam Thangavelu, [b] Christian O. Dimkpa *[b] and Emeka Emmanuel Oguzie *[a]

5. DOI: https://doi.org/10.1039/D5SU00646E **(published September 2025)**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Molecular modelling of a biochar–ZnO–CuO nano-biofertilizer: adsorption simulation for optimized nutrient delivery

Adewale T. Irewale, [ab] Elias E. Elemike, [ac] Christian O. Dimkpa [b] and Emeka E. Oguzie *[a]

6. DOI: 10.1021/bk-2025-1510.ch009 **(Published book chapter October 2025 link)**

Nano-enabled Soil Amendments: Revolutionizing Soil Health and Crop Productivity for Sustainable Agriculture

Adewale T. Irewale*, Elias E. Elemike, Christian O. Dimkpa, and Emeka E. Oguzie

DOI: 10.1021/bk-2025-1510.ch009
Publication Date: October 7, 2025
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Application of Nanotechnology for Sustainable Agriculture and Food Security Volume 2: Innovations in Pre- and Post-Harvest Crop Management
Chapter 9, pp 199-215

ACS Symposium Series, Vol. 1510
eISBN: 9780841295681

Conferences attended and presentations titles

1. 12th Annual International Conference of African Material Research Society, Kigali, Rwanda. Presented paper titled: "Water Hyacinth Biochar as Candidate for Green Nano-innovations: Morphological and Chemical Properties for Bio-Nanofertilizer Development". Dec 2024
2. 6th Biennial Conference/Workshop on Applications of Nanotechnology to Energy Environment, Agriculture and Health held at University of Nigeria, Nsukka. Accepted abstract titled: "Green Nano-Innovations: Current Practices and Tools for Sustainable, Smart, and Precision Agriculture".
3. 19th International Conference on Advanced Materials of the International Union of Materials Research Societies accepted the abstract titled "Molecular Modeling of Biochar–ZnO–CuO Nanobiofertilizer: Adsorption Simulations for Enhanced Nutrient Delivery" for oral presentation, Accra, Ghana, 2025.

Appendix X: Other key milestones in the course of the PhD program

1. PhD research project was awarded 2nd runner-up prize by the World Technology Partners, USA under the Green Environmental Engineering global competition 2024 with a prize honorarium of $125. Other winners are from India, South Africa, Ethiopia, Nigeria. – **3rd June 2024**
2. Showcased PhD research in a presentation at the World Tech Chat Forum 2024 organized by the World Technology Partners, USA on the Theme: Green Environmental Engineering for Sustainability – **Done 18th June, 2024**
3. Received a fully funded grant covering return flights and hotel accommodation for oral presentation of accepted research paper at the Africa Material Research Society (AMRS) 12th International Conference held at Kigali, Rwanda. Title of published abstract: "Water Hyacinth Biochar as Candidate for Green Nano-innovations: Morphological and Chemical Properties for Bio-Nanofertilizer Development" **Dec 13th – 20th 2024.**
4. NRF Concept Notes for possible TETFund grant worth N41million was approved and full proposal submission requested - **31st December 2024.** Concept Notes re-submitted for 2025 grant cycle.
5. Completed and received a certificate for a 6-week Internship Program with the American Chemical Society (ACS) Mentoring Team – **Done Sept 15th – October 30th, 2024.**
6. Convened and organized the Interdisciplinary Network of Researchers in Energy and Sustainable Technologies (INREST) to cater for the needs of students and alumni of ACE-FUELS, FUTO and other scientists in the renewable energy sector. **February 2024**
7. Received a scholarship award from the Management Board of ACE-FUELS to reward my contributions to the establishment of INREST and in recognition of the excellent academic performance and research output. **January 2025.**
8. Completed a 13-module GIFT.ed training program with the theme: "The Global Awareness Programme (GAP): Future-Proofing Nigeria's Next Generation" sponsored by the National Universities Commission and the World Bank – **September 2025.**