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Immobilization of copper, cadmium and lead using NaOH and H_2SO_4 modified biochar and *Pseudomonas aeuroginosa*

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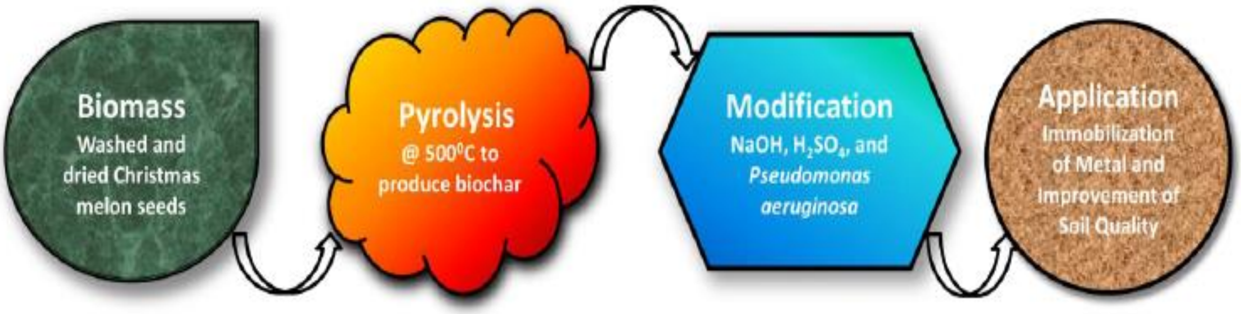
Abstract: Pollutants in soil have become a major threat to man and its environment, leading to damage to human health and an increased death rate. This study was therefore aimed at remediating Copper (Cu), Cadmium (Cd) and Lead (Pb) using Sodium Hydroxide (NaOH) and Tetraoxoulphate (vi) acid (H_2SO_4) modified biochar produced from the seed of Christmas melon (*Adenopusbreviflorus)* fruit, and a microorganism (*Pseudomonas aeruginosa*). Findings revealed that the modified biochars and microorganism affected the distribution of heavy metals in soil. Cd and Cu showed a decrease in concentration from 3.4-51% and 8.6-57%. Meanwhile, the soil O.M, pH, CEC, EC, Nitrogen content and Phosphorus content across all treatments indicated a good immobilization of Cu, Cd and Pb in soil thereby reducing their bioavailability in soil. It is concluded that modified biochar derived from the seed of Christmas melon can reduce the bioavailable heavy metals in soil and improve soil quality.

Keywords: *Biochar; Remediation; Microorganism; Heavy metals; Christmas melon*

Graphical Abstract



1. Introduction

Pollutants constitute an environmental health problem because of their toxic characteristics (Sarkar *et al.*, 2022). They build up in soils and water from both atmospheric and human activities, such as mining and smelting operations, wastewater irrigation, agro-chemical application, and traffic emissions (Joshi *et al.*, 2022). These pollutants have the potential to accumulate and thereby contaminate soil, crops, water and sediments. And through food chain, can be transferred to humans and animals causing deleterious health risks (Zaynab *et al.*, 2022; Karim *et al.*, 2019; Belbachir *et al.*, 2013). Precisely, pollutants in soils are not only harmful to humans but also pose a serious threat to ecosystems and agricultural production (Prithvi *et al.*, 2022).

Studies have focused on different methods of remediating pollutants and reducing their accumulation in soils using different organic and inorganic amendments, some of which include thermal soil remediation, air sparging, encapsulation and bioremediation (Rajkovich *et al.*, 2022, Hussain *et al.*, 2022). However, bioremediation through the adsorption process has gained more popularity due to the use of natural materials, its cost effectiveness and itseco-friendly nature. Bio-remediation through the process of adsorption has been used to remediate pollutants in the environment using microbes, plants, and microbial or plant enzymes to remove environmental pollutants through the use of activated carbon, biochar, etc.

Heavy metals are ubiquitous in the environment and as micronutrients, some are required for metabolic activities in biological systems but when nutritional level is exceeded, they become toxic. Cd and Pb bind with metalloproteins causing malfunctioning of cells and toxicity. Heavy metal toxicity can lower energy levels and damage the functioning of major organs in the body including; the brain, lungs, kidney and liver (Engwa *et al.*, 2019). Long-term exposure can lead to deteriorating physical, muscular, and neurological processes that imitate diseases such as multiple sclerosis, Parkinson's disease and Alzheimer's disease. Repeated long-term exposure to Pb, Cd and their compounds may even cause cancer (Mitra *et al.*, 2022). Several works proposed techniques to eliminate heavy metals using adsorption processes (Jodeh *et al.*, 2015).

Biochar is a porous, carbonaceous substance that is produced from waste biomass through the pyrolysis technique and has been used extensively as an eco-friendly amendment for the treatment of wastewater and soil pollution due to its unique surface properties (porous structure, micropores, etc.), the abundance of microchannels, and stable aromatic chemical structure (such as alkaline pH and rich organic functional groups) (Harvey, 2022).

Biochar has also been added to soil as an amendment to improve soil quality and immobilize soil contaminants, reducing their bioavailability and increasing the capacity of the soil to retain nutrients and water (Saffari *et al.*, 2022; Zheng *et al.*, 2022), and thus increase crop yield (Zheng *et al.*, 2022). The addition of microorganisms into soil can improve soil quality and reduce the availability of toxic metals in soil, this is thereby regarded as bio-indicators of soil health (Antonangelo *et al.*, 2021; Haroun *et al.*, 2024).

Despite the potential benefits of biochar and microbial interventions, there remains a need for further research to optimize their application in pollutant remediation. This study seeks to address this gap by investigating the synergistic effects of biochar derived from *Adenopus breviflorus* seeds modified with NaOH, H_2SO_4, and *Pseudomonas aeruginosa* on soil physicochemical properties and the immobilization of heavy metals such as Cu, Cd, and Pb. By elucidating the mechanisms underlying biochar-microbial interactions, this research aims to develop more effective and sustainable solutions for mitigating soil pollution and safeguarding environmental and human health.

2. Methodology

2.1 Biochar production, modification and characterization

Christmas melon (*Adenopus breviflorus)* fruit was collected from a local farm in Ago-Iwoye town of Ijebu-North Local Government Area of Ogun State, Nigeria. The plant material was washed and opened to collect the seeds. The seeds were then washed with de-ionized water and dried at room temperature. The seed was pyrolyzed in a furnace at 500 ^{0}C at a heating rate of 20 ^{0}C min^{-1} for 5 hours.

The products were ground and sieved to <2 mm after cooling to room temperature. Modification of biochar was carried out in order to enhance its adsorption efficiency. Acidic (2.0 M of H_2SO_4), basic (2.0 M of NaOH) and microbial (*Pseudomonas aeruginosa*) modification of the biochar was carried out. Each was agitated for 5 hours and allowed to settle for 24 hours. Thereafter, the mixture was filtered using a 2mm pore-size filter paper. The biochar residue was dried in oven at 100 ^{0}C for 2 hours. The unmodified and modified biochars were characterized using FTIR spectroscopy in order to identify the functional groups present. The FTIR spectra were captured using a Bruker FTIR spectrometer, taken in transmittance, with 225 scans and a resolution of 4 cm^{-1}. The samples were made into pellets after being processed in KBr (10 wt%) (Yang *et al.*, 2022). The unmodified was labeled BO; the H_2SO_4 modified biochar was labeled BA and the NaOH Modified biochar was labeled BB.

2.2 Source and application of pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium belonging to the genus Pseudomonas, known for its remarkable adaptability and wide-ranging metabolic capabilities. It is considered an opportunistic pathogen, capable of causing severe infections in immunocompromised individuals, particularly those with cystic fibrosis, burn wounds, or undergoing prolonged hospital stays. However, beyond its pathogenic potential, Pseudomonas aeruginosa is also recognized for its significant ecological importance and versatile metabolic activities.

The bacterium was isolated from the Olabisi Onabanjo University College of Agricultural Sciences Tree Crop Nursery Development Project farm in Ago Iwoye, Ogun State, Nigeria. Through phenotypic and molecular analysis conducted in the Microbiology Laboratory of the institution, the bacterium was identified as *Pseudomonas aeruginosa*. Our unpublished data showed that the strain showed a high tolerance to Cadmium, Copper and Lead. The organism after resuscitation was mixed thoroughly in the different treatments with the artificially heavy metal-contaminated soil in designated pots.

2.3 Soil collection and incubation experiment

The surface soil sample (0-20 cm) used for the pot experiment was collected from a farm settlement in Ago-Iwoye, Ogun State. 5.0kg of the air-dried soil was carefully weighed and transferred into eight (8) buckets. The soil was spiked with 100 mL of 30ppm of Copper Sulphate ($CuSO_4$), Lead Nitrate [$PbNO_3)_2$] and Cadmium Nitrate [$CdNO_3)_2$]. After this, the following treatments were added to the spiked soil in the buckets and labeled respectively: H_2SO_4 modified biochar only (BA); NaOH modified biochar only (BB); H_2SO_4 modified biochar and *Pseudomonas aeruginosa* (BAPs); NaOH modified biochar and *Pseudomonas aeruginosa* (BBPs); *Pseudomonas aeruginosa* only (Ps). Each of these treatments was mixed thoroughly with the soil and was left for fourteen (14) days to attain equilibrium. The pot experiment was run in duplicate and was conducted in the Department of Plant Science Screening House of Olabisi Onabanjo University, Ago-Iwoye. The soil moisture was stabilized at 70% of the field water holding capacity (Kang *et al.*, 2022). Each of the experimental buckets was aerated and routine management was carried out for the period of incubation. After 14 days, the soil samples were collected to determine the concentration of Cu, Cd and Pb. Similarly, the soil samples were collected at a depth of 10cm for heavy metal analysis in order to obtain available Cu, Cd, and Pb concentrations present in the soil, after thirty (30) days of incubation.

2.4 Laboratory analysis

Physicochemical properties (pH, total organic carbon, total Nitrogen and Phosphorus, ash and moisture content, pore size) of the soil sample and biochar before and after treatments were determined using the procedures described by (Mujtaba *et al.*, 2021). The concentration of available Cu, Cd and Pb in soil samples was estimated by diethylenetriaminepentaacetic acid (DTPA) solution extraction (Campillo-Cora *et al.*, 2021). 2g of soil sample was weighed into an extraction tube; 20mL of DTPA was added. The mixture was then shaken in an end-over-end at 30rpm for 2hours at ambient 10min temperature. About 12mL of the mixture was decanted into a centrifuge tube and centrifuged for ten minutes (Campillo-Cora *et al.*, 2021), and was analysed by atomic absorption spectrophotometry (model 210A).

3. Results and Discussion

3.1 Physicochemical parameters of soil sample and biochar before incubation

The results of the physicochemical properties of soil and biochar before treatment are presented in **Table 1**. The soil had a pH of 6.6 and the percentage composition of the total organic compound, total nitrogen and percentage composition of phosphorus reached 1.42%, 0.58%, and 0.28% respectively. This indicated that the nutrition present in the soil was deficient. The results further showed that the pH of the soil is slightly acidic while that of the unmodified biochar is basic. Of all the physicochemical properties determined, only the moisture content and pore size of BO are higher than that of the soil. Meanwhile, the large surface area and pore size may favour the adsorption of heavy metal contaminants.

3.2 Characterization of BO, BA and BB

The peaks observed at 1558, 1364, 1110, 700 and 663 cm^{-1} in the BO spectrum as shown in **Figure 1**, could be attributed to the vibration of C=O or C=C, C-OH (phenolic), C-O (carboxylic), C-O (alkyl) and C-C respectively (Mujtaba *et al.*, 2021). In the BA spectrum as shown in **Figure 2**, the peaks observed at 1155, 1435, 1699, and 2911 cm^{-1} could be attributed to C-O, C-N, C=C, and C-H stretching respectively. Similarly, the BB spectrum, observed at the following peaks: 1028, 1371, 1867, and 2914 cm^{-1} are attributed to C-O, C-N, C=C, and C-H stretching respectively in **Figure 1**.

Table 1. The physicochemical properties of soil and biochar

Parameters	Soil	Biochar
pH	6.60±0.15	9.0±0.16
% Ash	2.40±0.05	1.8±0.02
% Moisture	1.20±0.11	5.45±0.05
Pore Size	0.32±0.10	0.54±0.09
% Nitrogen	0.58±0.14	0.42±0.01
% Phosphorous	0.28±0.07	0.18±0.02
% Total Organic Compound	1.42±0.03	1.05±0.01

Furthermore, the peaks observed at 3119 and 3459 cm^{-1} in BB, and 3336, 3399 and 3481 cm^{-1} in BA were a result of the O-H stretching. The sharp peak observed at 1600cm^{-1} in all the adsorbents was due to the ketonic group (C=O) (Khawkomol *et al.*, 2021). Comparing the spectra observed before and after modifications, there were changes observed. In the BA and BB spectrum as shown in **Figure2** and **Figure3**, the broad adsorption at 3481 and 3459 cm^{-1}can be ascribed to the O-H stretching vibrations while the band reduced to 3358 cm^{-1} in the BO spectrum. This indicated that the modified biochars has an increased adsorption capacity than the unmodified biochar, and the tendency to adsorb more pollutants.

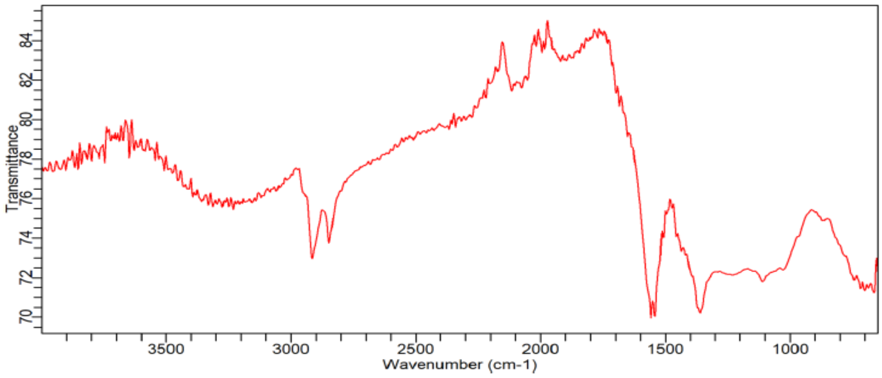


Figure 1. Spectrum of Unmodified Biochar (BO)

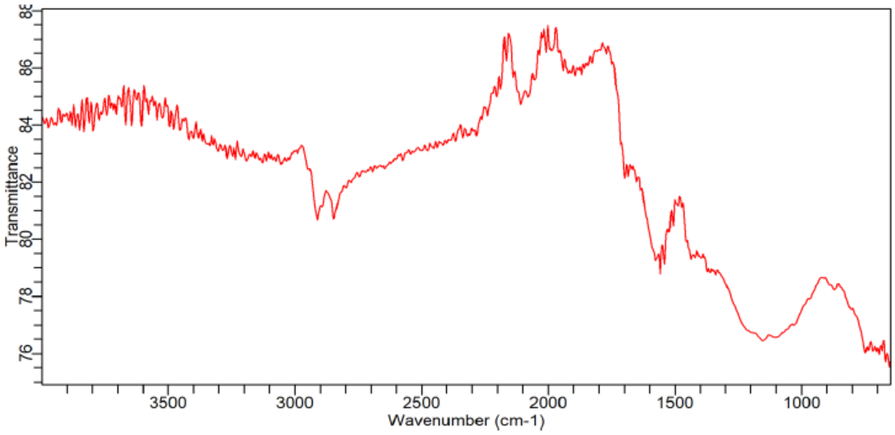


Figure 2. Spectrum of H_2SO_4 Modified Biochar (BA)

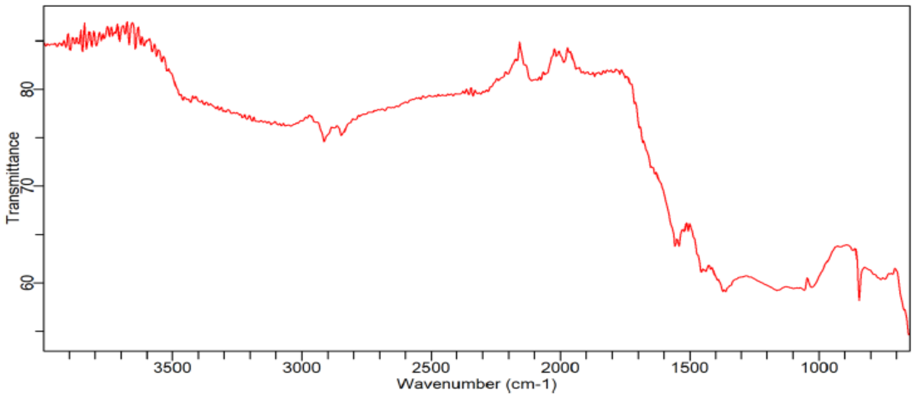


Figure 3. Spectrum of NaOH Modified Biochar

3.3 Effect of modified biochar on bio-available Cu, Cd and Pb in Soil

The effect of modified biochar in immobilizing spiked heavy metals was assessed by determining the concentrations of bioavailable metals after the14th and 30th application days and the results are presented in **Figures 4, 5 and 6**. Their concentrations in bio-available forms were found to decrease in all the treated soils. As observed, the concentration of Cu (0.338ppm) reduced by 31% (0.233ppm); Cd (0.027ppm) by 19% (0.012ppm); and the concentration of Pb (0.326ppm) by 9% (0.298ppm). Also, the application of BAPs showed that the concentrations in bioavailable form, of Cu (0.137ppm) reduced by 11% (0.122ppm); Cd (0.023ppm) by 8.6% (0.021ppm); while that of Pb (0.262ppm) was reduced by 4% (0.252ppm).

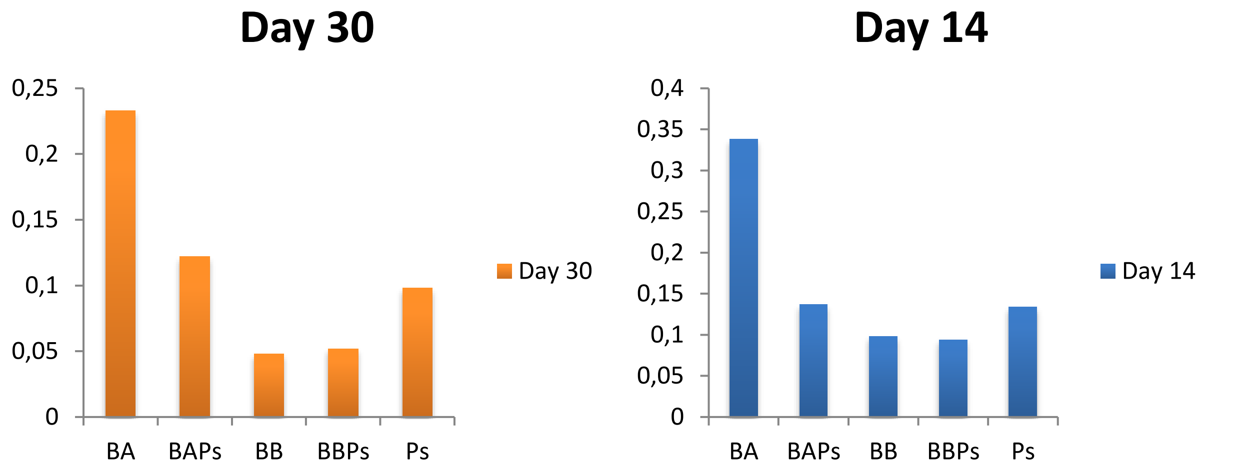


Figure 4. Bioavailable Cu (ppm) after Incubation

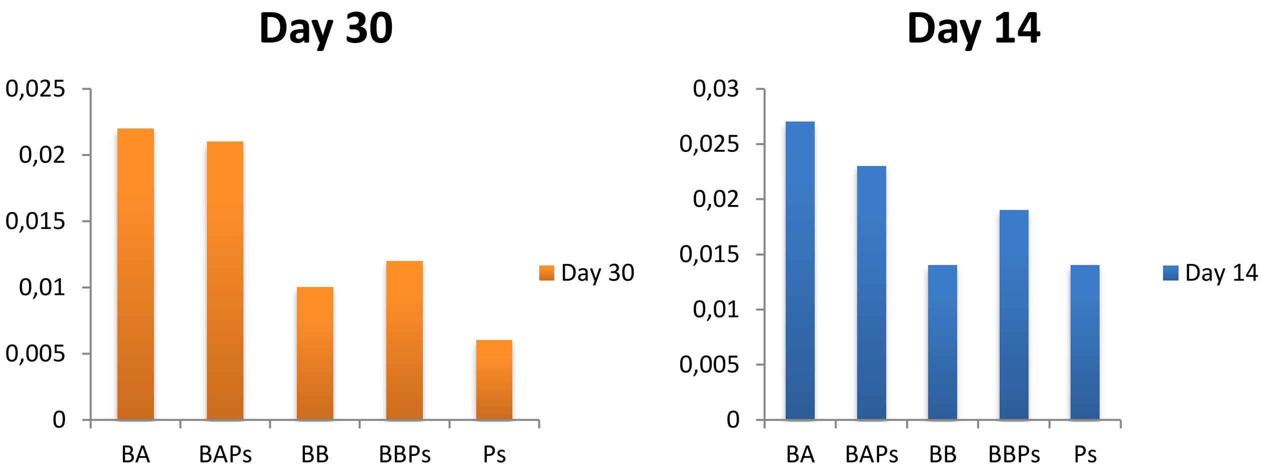


Figure 5. Bioavailable Cd (ppm) after Incubation

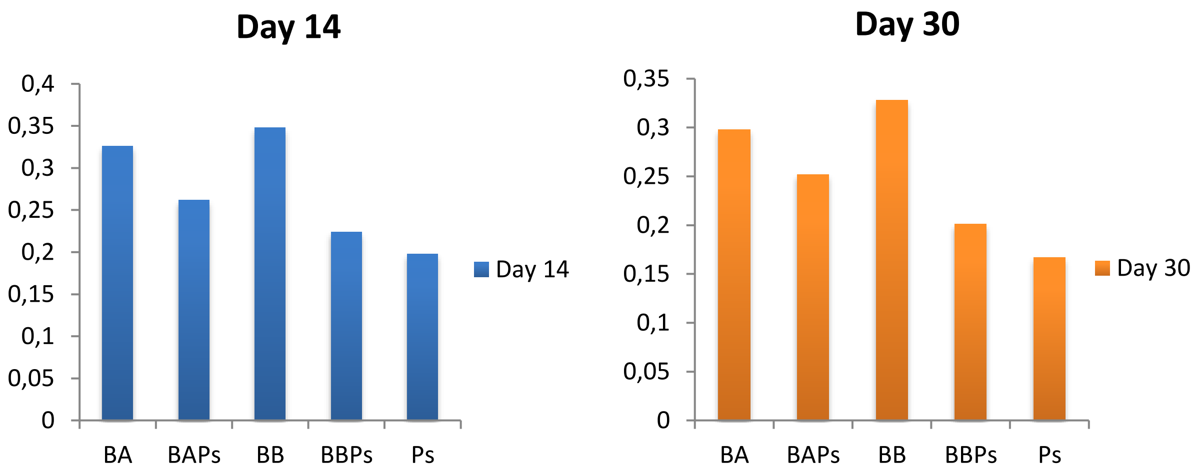


Figure 6. Bioavailable Pb (ppm) after Incubation

Furthermore, the application of BB reduced the concentrations in bioavailable form, of Cu (0.098ppm) by 51% (0.048ppm); that of Cd (0.014ppm) by 29% (0.01ppm); and that of Pb (0.348ppm) by 5.7% (0.328 ppm). Similarly, the application of BBPs reduced the concentrations in bioavailable form, of Cu (0.094 ppm) by 45% (0.052 ppm); of Cd (0.019 ppm) by 37% (0.012 ppm); and that of Pb (0.224 ppm) by 10.3% (0.201ppm). Finally, the application of Ps decreased the concentrations in bioavailable form, of Cu (0.134 ppm) by 27% (0.098 ppm); that of Cd (0.014 ppm) by 57% (0.006 ppm); as well as Pb (0.198 ppm) by 16% (0.167 ppm). Findings confirmed that Cd showed a good percentage decrease in concentration followed by Cu. This is a result of the efficiency of the co-application of biochar to immobilize Cd and Cu cations in polluted soil (Haider *et al.*, 2021). This finding supports that of Mehdizadeh *et al.* (2021) who noted that biochar can reduce the activities of cadmium in soil and as well improve crop yield. Similar to this was the findings of Nobaharan *et al.* (2021) who asserted that the addition of soil increased dry matter yield and Zn and Fe concentrations but significantly decreased the concentrations of Cd and Cu concentrations in plants. The exchangeable Cd fraction was also reduced to a less available Cd in the soil. In addition, findings from Haider *et al.* (2022) showed that the co-application of biochar and microorganisms significantly improved the growth of plants, transpiration rate, water use efficiency, photosynthesis rate and reduced Cd accumulation in plant root, shoot as well as soil.

3.4 Effect of soil treatments on soil physicochemical properties and metal immobilization

The study investigated the impact of soil treatments involving NaOH and H_2SO_4 modified biochar, as well as *Pseudomonas aeruginosa*, on key soil physicochemical properties, including pH, organic matter (O.M.), Cation Exchange Capacity (CEC), and Electrical Conductivity (EC). These parameters play crucial roles in determining the chemical behavior of heavy metals in soil environments. Understanding how heavy metals interact with these soil properties is essential for elucidating the mechanisms by which they are immobilized and rendered less bioavailable.

Soil pH serves as a fundamental indicator of soil acidity or alkalinity, influencing various chemical and biological processes in the soil. Modifications to biochar with NaOH and H_2SO_4 can potentially alter soil pH, thereby affecting the solubility and mobility of heavy metal ions. Organic matter content in soil, often referred to as soil organic matter (O.M.), contributes to soil fertility, structure, and nutrient availability. Changes in O.M. levels can impact heavy metal binding capacity and the formation of metal-organic complexes, influencing their mobility and bioavailability.

Cation exchange capacity (CEC) represents the soil's ability to retain and exchange cations, including heavy metal ions. Modifications to biochar and the introduction of microbial agents such as *Pseudomonas aeruginosa* can influence CEC, and therefore the binding and retention of heavy metals in the soil matrix. Electrical conductivity (EC) reflects soil salinity levels and ion mobility, which can have an impact on the leaching and transport of heavy metals.

3.4.1 Effects of biochar treatment on soil pH

According to Gondal *et al.* (2021) Soil pH has the potential to modify metal solubility and availability by controlling metal dissolution and precipitation. Soil pH can also regulate the ionization of pH-dependent ion-exchange sites on organic matter and metal oxide clay minerals affecting cationic metal availability (Kicinska *et al.*, 2022). Our finding showed that the addition of H_2SO_4 modified biocharto Cu, Cd and Pb contaminated soil resulted in the decreased of soil pH across all acidic biochar treatment (BA=5.8, BAPs=6.2) after 30days incubation as shown in **Table 2**, compared with the untreated soil (pH= 6.6). For the Ps experiment, the soil pH showed a decrease to 6.1. On the other hand, the addition of NaOH-modified biochar to Cu, Cd and Pb-contaminated soil significantly increased the pH of soil across all basic biochar treatments (BB=8.1, BBPs=8.0) after 30days of incubation. The decrease in soil pH across all treatments with H_2SO_4-modified biochar is likely caused by the acidity of H_2SO_4. Thus, indicating the acidity of the soil. On the other hand, the increase in soil pH across all treatments with NaOH is likely caused by the alkalinity of NaOH, indicating the alkalinity of the soil. This finding supports that of (Chen *et al.*2021) who confirmed a significant localized effect on soil pH as a result of the induced biochar application. Meanwhile, the reduction in concentration of extractable Cd and Cu can be attributed to increasing soil pH possibly due to the application of biochar Albert *et al.* (2021), Similarly, our findings agreed with Calcan *et al.* (2022); Lu *et al.*(2022) and Meng *et al.* (2022), who asserted that the increase in soil pH after the application of biochar is attributed to the alkaline nature of the biochar, and tends of decreasing the concentration of heavy metals such as Zn and Pb in soil.

Table 2. Change in the soil pH after biochar treatment

Treatment	14days incubation	30days incubation
Ps	6.0	6.1
BA	5.6	5.8
BAPs	6.2	6.2
BB	8.0	8.1
BBPs	8.1	8.0

3.4.2 Effects of biochar treatment on organic matter (OM)

Organic matter plays a major role in soil biological and chemical processes. It can participate in the primary reactions involving proton reaction and complexation in soil solution which are responsible for trace metal immobilization (Sun *et al.* 2021). The application of H_2SO_4 and NaOH-modified biochars and *Pseudomonas aeruginosa* to Cu, Cd and Pb contaminated soil significantly increased the OM of soil across all treatments between 14-30days incubation as shown in **Table 3**. This indicated good retention and immobilization of heavy metals in soil thereby reducing their bioavailability.

Findings from this study correlate with (Rombola *et al.* 2022) who reported that organic matter in soil can work as a factor which causes heavy metal immobilization and reduces their bioavailability. Similar to this was the report of De la Rosa *et al.* (2018) who asserted that organic matter derived from brown coal was effective in immobilizing cadmium, lead and zinc thereby decreasing the concentration of the metals in the soil as a result of its high carbon content (Shentu *et al.* 2022).

Table 3. Change in soil organic matter (OM) after biochar treatment

Treatment	14days incubation	30days incubation
Ps	2.37	4.44
BA	2.99	3.94
BAPs	5.4	7.08
BB	3.37	3.77
BBPs	4.91	7.58

3.4.3 Effects of biochar treatment on cation exchange capacity

One of the major roles of CEC in soil is that it affects how well the soil can retain important nutrients and acts as a buffer against soil acidity (Wu *et al.* 2021). The addition of H_2SO_4 and NaOH-modified biochars, and *Pseudomonas aeruginosa* to Cu, Cd and Pb contaminated soil significantly increased the CEC of soil across all biochar and microorganism treatments between 14-30days incubation (see **Table 4**) except in the case of BB which showed a decrease in CEC between the incubation periods. The increase in soil CEC is expected due to the considerable increase in soil nutrient content as a result of the presence of N and P in the biochar (Antonangelo, Culmand and Zhang, 2024). Similarly, the immobilization of metal as a result of an increase in soil CEC supports the findings of (Yang *et al.* 2021) who noted that increase in cation exchange capacity corresponds to a larger capacity for metal adsorption. Similarly, findings from Cui *et al.*(2021) indicated that soil cation exchange capacity affected plant growth and the amount of total Cd concentration. On the contrary, the use of thiol-modified biochar could result in a low and no significant difference in soil CEC (Fan *et al.* 2020).

Table 4. Change in soil cation exchange capacity after biochar treatment

Treatment	14days incubation	30days incubation
Ps	12.01	12.36
BA	11.76	12.76
BAPs	14.32	16.62
BB	14.64	14.24
BBPs	12.76	14.86

3.4.4 Effects of biochar treatment on soil Electrical Conductivity

The capacity of soil water to transmit electrical current is measured by the term soil electrical conductivity (EC). Electrical conductivity is an electrolytic process that mostly occurs through pores filled with water. Thus, the addition of biochar to soil has the tendency to increase the salinity of such soil, and inhibit the growth of halophyte plants and the activity of metal accumulation in plants (Zhang *et al.* 2021). In this study, application of H_2SO_4 and NaOH-modified biochars, and *Pseudomonas aeruginosa* to Cu, Cd and Pb contaminated soil showed a decrease in the electrical conductivity of soil across all treatments after 30days of incubation except in *Pseudomonas aeruginosa* treatment which showed a slight increase in EC as shown in **Table 5** (Khadem *et al.* 2021).

Table 5. Change in soil electrical conductivity after biochar treatment

Treatment	14days incubation	30days incubation
Ps	0.25	0.28
BA	0.44	0.34
BAPs	0.32	0.27
BB	0.36	0.26
BBPs	0.38	0.28

This implies that modified biochar contributed to the reduction of the electrical conductivity of heavy metals in the soil. Thus, confirming that the higher the EC value, the higher the concentration of metals in the soil and vice versa (Othaman *et al.* 2020). This finding also corroborates with that of (Tang *et* *al.* (2021) who found that high EC levels increased Cd accumulation and decreased Zn accumulation in the soil and shoots of plants.

3.4.5 Effects of biochar treatment on soil Nitrogen

The presence of nitrogen in soil has the tendency to improve soil quality and possibly reduce the activity of heavy metals (Zhang *et al.* (2021). In this study, there was an increase in nitrogen content of the soil after 30days of incubation, on the application of H_2SO_4 and NaOH-modified biochars, and *Pseudomonas aeruginosa* to the contaminated soil as shown in **Table 6**. This shows the ability of the modified biochars in the improvement of soil quality and immobilization of heavy metals in the contaminated soil. This is because nitrogen is an essential nutrient for plant growth. It assists plants in processing their food and creating chlorophyll which is required by plants to obtain food from sunlight (Salem *et al.* 2023). However, there are possibilities of a negative correlation to be identified between the availability of NPK and bioavailable Cd, Pb and Cr as reported by (Zhang *et al.* (2021). Meanwhile, our findings correlate with Qi *et al.*(2022) who reported that MgO-loaded nitrogen and phosphorus biochar were responsible for the removal of heavy metal ions in soil.

3.4.6 Effects of biochar treatment on soil phosphorus

Phosphorus is said to be an essential component of nucleic acids, phospholipids and energy-rich phosphate compounds (Peiris *et al.*, 2023). It therefore plays an important role in plant growth, and fruit and seed development.

Table 6. Change in soil Nitrogen after biochar treatment

Treatment	14days incubation	30days incubation
Ps	0.32	0.42
BA	0.26	0.38
BAPs	0.29	0.40
BB	0.18	0.25
BBPs	0.32	0.42

Notably, biochar as a source can supply nutrients such as nitrogen (N), phosphorus (P), potassium (K), and other trace elements inherently present in the original feedstock used for biochar production (Ahmed *et al.*, 2021). This makes biochar one of the promising amendments for plant growth and soil remediation. The application of H_2SO_4 and NaOH-modified biochars, and *Pseudomonas aeruginosa* to contaminated soil showed an increase in soil-phosphorus content after 30 days incubation across all biochar treatments as summarized in **Table 7**. This indicated that phosphorus played a role in the immobilization of Cd, Cu and Pb in the contaminated soil (Ghodxzad *et al.*, 2021; Errich *et al.*, 2021). In addition, biochar rich in phosphorus has excellent adsorption capabilities for heavy metals and could be used as soil-remediation agents as noted by (Peiris, 2023; Hayyat et al., 2016).

Table 7. Change in soil phosphorus after biochar treatment

Treatment	14days incubation	30days incubation
Ps	0.26	0.32

BA	0.28	0.36
BAPs	0.23	0.35
BB	0.20	0.26
BBPs	0.32	0.42

Conclusion

This study assessed the effect of modified biochar treatment on soil physicochemical properties and metal immobilization. Modification especially with BB and BBPs was found to increase the soil O.M, CEC, N and P content which have been found to enhance the adsorptive capacity of the soil and improve crop yield. The FTIR spectrum of the NaOH and H_2SO_4-modified biochar showed the presence of C-O, C-N, C=C, O-H, C=C and C-H stretching which aids adsorption. Modified biochars especially NaOH-modified biochar (BB) and NaOH-modified biochar and *Pseudomonas aeruginosa* (BBPs) enhance the immobilization of Cu and Cd by reducing their bioavailability in soil. Therefore, biochar derived from the seed of Christmas melon especially if modified with BB and BBPs can reduce the bioavailable heavy metals in soil, and improve soil quality.

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