

FUNCTIONAL EFFECT OF WOOD ASH ON SOIL QUALITY

Comment [H1]: The MS describing the effect of wood ash application on the soil properties after its application. The MS need through revision to clear all lacunas.

Abstract

The functional effect of wood ash (WA) amended soil was investigated to ascertain the effect on soil quality. Physicochemical parameters, heavy metals, microbiological, and enzyme activity status of the WA amended soil were ascertained using standard methods. Soil samples treated with 5% and 10% WA were set-up against the untreated control for the study. The samples were ~~analysed~~analyzed in two stages, the first was immediately after the treatment, and the second was after mineralization. The results observed revealed a shift in the pH of the treated soil samples against the control before and after mineralization. Electrical Conductivity (EC) ranged from 220.29±0.14 to 240.82±0.14 µs/cm before mineralization and 220.29±0.14 to 240.82±0.14 µs/cm after mineralization. The soil samples treated with 5% and 10% WA had significantly ($p < 0.05$) increased EC when compared to the control both before and after mineralization. The observed increase in EC was concentration dependent on the presence of WA. The temperature ranged from 29.80±1.41 to 30.70±2.83 °C before mineralization and 29.80±1.41 to 30.70±2.82 °C after mineralization. The %Nitrogen reduced significantly ($p < 0.05$) in the treated soil samples when compared to the control both before and after mineralization. Copper ranged from 0.05±0.03 to 0.48±0.01 mg/kg before mineralization and 0.18±0.42 to 0.40±0.03 mg/kg after mineralization. The soil sample treated with 10% WA had significantly ($p < 0.05$) reduced copper before and after mineralization unlike the soil sample treated with 5% WA which had significantly ($p < 0.05$) reduced copper before mineralization and significantly ($p < 0.05$) increased after mineralization. The soil samples treated with WA had insignificantly ($p > 0.05$) reduced nickel after mineralization. The soil samples treated with WA had profound microbial load reduction when compared to the control. Before and after mineralization, soil alkaline phosphatase reduced significantly ($p < 0.05$) in the treated samples against the control while acid phosphatase increased significantly ($p < 0.05$) in the treated soil samples when compared to the control. These observations could be linked to the presence of WA in the treated soil samples before and after mineralization. In conclusion, WA treatment increased electrical conductivity, reduced copper and nitrogen concentrations, reduced microbial load and increased some enzyme activities. .

Comment [H2]: The EC values and temperature before and after mineralization given in the abstract were same; how they are different from each other? Abstract is lengthy and need to reduce; Avoid writing of non-significant results in the abstract and write only significant and important findings.

Keyword: wood ash, physicochemical properties, microbial loads; enzyme activity.

INTRODUCTION

The importance of the soil and its quality to humanity cannot be overstated (Else *et al.*, 2016). The quality of the soil plays important role in numerous practices in civil engineering and agriculture (Lal, 1998; Brady, 1988). Soil quality determines a lot in the construction of civil engineering structures such as bridges, dams, roads, and canals (Lal, 2001). In agriculture, soil is regarded as the outermost part of the earth crust on which plants grow (Shivanna and Nagendrappa, 2014). Its quality is the soul of crop and food production in the area of agricultural practices (Doran and Parkins, 1996). Mbah *et al.* (2018) described soil as a fundamental agricultural resource, which serves as a reservoir of nutrients for plants. The quality of the soil reveals the capability of a soil sample to sustain the production of

Comment [H3]: Write the need of adding amendments in soil for maintaining its fertility condition in 1-2 sentences with 1-2 references. Avoid citing old references until it is necessary and no any substitutes are available. Write the research gap in more elaborative manner.

Formatted: Font: (Default) Times New Roman

plants and animals, support the health of humans, and as well maintain the air and water quality of the soil (Balet *et al.*, 2018). The physical, chemical, and biological properties of the soil are among the measurable parameters that reveal the capacity of the soil to perform a specific function, and can be used to predict the future sustainability of the soil (Doran and Parkins, 1996). According to Doran and Parkins(1996), electrical conductivity (EC), soil nitrate (SN), and soil pH are among the chemical indicators; aggregated stability, water capacity and water holding ability, bulk density, and soil structure, are among the physical indicators; whereas soil micro and macro organisms, organic matter, mineralization of nitrogen, total organic carbon and soil enzymes are among the biological indicators of the soil. Uptake of the soil nutrients by plants as they grow and mature is among the ways through which nutrients of the soil are lost (Oliver and Gregory, 2015). Some of the known soil nutrients share relationships with some of the soil indicators that can be used to evaluate the sustainability of the soil (Else *et al.*, 2016; Jency *et al.*, 2023). Agricultural soil is often boosted with organic and inorganic supplements, or both combined together, to ensure that the required agricultural soil quality is sustained for the growing of crops, food and animal production (Raghavendra *et al.*, 2020). The use of fertilizer constitutes the inorganic source. Different studies have reported the importance of fertilizer in the growing of crops to maximum use (Stewart *et al.*, 2005; Mohamed *et al.*, 2008). Write here with need of adding amendments in soil for maintaining its fertility condition in 1-2 sentences with 1-2 references.

In recent years, there is a renewed interest in using ash to enhance soil quality for agriculture purpose (Jonna, 2017). Jonna(2017) described ash as combustion residue of organic materials, with trace elements from its biomass and inorganic nutrients. Wood ash (WA) is among such ash with inorganic nutrients and trace elements of its biomass. It is a residue of powdery remains from the burning or combustion of wood and possesses the materials used to maintain the soil quality through the replenishment of lost nutrients from the soil (Serafimova *et al.*, 2011; Nweke, 2018). The burning or combustion of wood for ash could be done using power plant of an industry, wood stove, fireplaces or even bonfire (Bang and Andreasen *et al.*, 2021). The WA contains many elements required for forage and crop production, and serves as a soil conditioner or stabilizer (Nweke, 2015). It possesses the ability to bring the coagulation of soil with loose structure (Kanuet *et al.*, 2021). It has been reported that the species of the tree used in the production of WA, the process, and nature of the burning, are the key determinants of the chemistry of the ash produced (An and Park, 2021). Consequently, WA from hardwoods contains higher micronutrients and lower silica than WA from softwoods (Nweke, 2018). Studies on the potential (Bonfim-Silva *et al.*, 2017), and complexity of WA as fertilizer, utilization of WA as manure (Gómez-Rey *et al.*, 2012; Adekayode and Olojugba, 2019), and using WA as guide for agricultural production in home garden (Kelvin *et al.*, 2020) have all been reported. Previous studies by Mbah and Nkpaji (2009), and Nottidge *et al.* (2005) reported that WA produced positive effects to plant growth and crop yield when used for maize production. The WA has been classified as a liming agent and noted that care should be taken when using it for amendment (Nweke, 2018). Füzesi *et al.* (2015) regarded WA as a non-hazardous waste of non-agricultural origin.

There is need to fill up the gap created by the existing research studies on WA. The present study evaluated the effects of WA on the quality of soil with a view to ascertain the status of some physicochemical, microbial, and biological indicators of the soil.

Formatted: Font color: Auto

Formatted: Font color: Auto

MATERIALS AND METHODS

Procurement of WA and soil samples: The WA sample used in the present study was procured from Ogbo-osisi in Owerri, Imo State, Nigeria. The WA was produced by combusting the wood of Velvet Tamarind tree (*Dialium guineense*), known as “Ncheleku”, “Awin” and “Tsamiyarkurm” by the Igbos, Yorubas, and Hausa tribes in Nigeria, in an industrial oven at a very high temperature till a complete combustion was achieved. The soil sample used was procured from Imo State University School Farm.

Soil preparation and treatment with WA: Five kilogram of dried soil sample was collected from Imo State University School Farm, and sieved (6.87 mm hole size). The soil sample was collected at a depth of 0.15 cm from the earth surface within the farm into a plastic container. The soil was properly sieved (6.87 mm hole size) and prepared by removing all unwanted materials. It was then distributed equally into three perforated plastic buckets; two of the plastic buckets were treated with WA at the concentrations of 5% for one bucket of soil and 10% for the other bucket of soil. One of the buckets of soil was given zero treatment and it served as the control. The treated soils in the buckets were analyzed in two stages; the first was immediately after the treatment and the second was two weeks after the treatment which was allowed for mineralization.

Physicochemical parameters determinations: Soil pH was determined using the method of Bates (1954). The temperature of the soil was taken in-situ with the help of mercury in glass thermometer. Electrical conductivity (EC) was potentiometrically estimated. Soil organic matter (OM) content was determined by using the as described by Tyurin method. Cation exchangeable capacity (CEC) was determined using Brown method (Sumner and Miller, 1996). % Base saturation (BS) was estimated as described by Nweke *et al.* (2018). Exchangeable acidity (EA) was determined by the titrimetric method of Mclean (1982). The concentration of Mg, K, Na, and Ca concentrations were evaluated with the Atomic Absorption Spectrophotometer (AAS). The % nitrogen was measured in a gram of the soil with the micro-Kjeldahl method (Bremner, 1996). Phosphorus was quantified with the method as described by Bray and Kurtz (1945). Heavy metals such as copper, iron, zinc, lead, chromium, and nickel were evaluated using AAS.

Microbiological evaluations: The soil bacterial organisms were isolated and grouped using the methods as described by Chessbrough (1984). The methods as described by Agu and Chidozie (2021) were used for isolation of the fungal organisms and grouping.

Estimation of soil enzyme activity: Soil alkaline phosphates (ALP) and acid phosphatase were estimated using the method as described by Tabatabai (1982). Urease was estimated with the method as described by Tabatabai and Bremner (1972). Colorimetric method was used in the determination of soil lipase activity (Margesin *et al.*, 2002). SOD was estimated following the method of Bergmeyer (1984).

Statistical Analysis: Results were presented as mean and standard deviation. Data obtained in this study were subjected to statistical analysis using statistical software version of 7.2. Significant difference was established using least significant difference (LSD) at 5% significant level.

Comment [H4]: Check the depth of soil sample collection:
Is it open field experiment (pot were kept in open environment) OR pots were kept in controlled environment: Mention clearly.
Write about the climatic condition of the location of experimentation.

Comment [H5]: ??

RESULTS AND DISCUSSION

Saniet *et al.* (2012) noted that the physical and chemical properties largely determine the suitability of the soil for its planned use and management requirements to keep it most productive. The soil fertility also determines its possible uses and to some extent its yields (Saniet *et al.*, 2012). Table 1 shows the physicochemical parameters of the treated soil samples. According to Kekaneet *et al.* (2015), soil pH is considered as the most significant parameters of the soil because it affects other soil parameters. The pH of the soil samples treated with WA ranged from 7.16-7.24 before mineralization and 7.16-7.24 after mineralization. Soil samples treated with 5% and 10% WA had pH that reduced against the control before mineralization. The same trend was also observed after mineralization but only the soil sample treated with 5% WA had pH value that reduced against the control. The soil sample treated with 10% WA did not change in pH when compared to the control after mineralization. According to Dandwate (2020), the soil pH range from 6.00-8.5 is regarded as a normal soil. The wood ash tended to reduce the pH of the soil as observed in the present study before and after mineralization. In related study, Ulery *et al.* (1993) noted that calcite, a major component of wood ash maintained moderate alkaline pH in the surface soils that are normally neutral to strong acids. The observation of the study was in line with the work of Füzesi *et al.* (2015) who noted that the application of WA enhanced the pH of the top soil by 0.3-2.4 units. Electrical conductivity (EC) is an important soil constituent, which indicates the total soluble salts constituent of the soil (Dandwate, 2020). The EC of a soil solution is directly related to its ion concentration (Kekaneet *et al.*, 2015). The EC in the present study ranged from 220.29 to 240.82 $\mu\text{s}/\text{cm}$ before mineralization and 220.29 to 240.82 $\mu\text{s}/\text{cm}$ after mineralization. Soil samples treated with 5% WA and 10% WA had reduced EC against the control both before and after mineralization. The WA may have influenced the soluble salts present in the soil samples. The energy absorbed and lost is a factor unto which the soil temperature depends (Kekaneet *et al.*, 2015; Onwuka and Mang, 2018). The soil temperature ranged from 29.80-30.70 °C before mineralization and 29.80 to 30.70 °C after mineralization. The soil samples treated with 5% WA and 10% WA did not change in temperature when compared to the control both before and after mineralization. It could be that WA had no influence on the temperature of the treated soil samples. Organic matter (OM) is a very valuable property of the soil, which adapts the soil to stability to agricultural practices and against erosion. Soil that is poor in OM is susceptible to soil erosion (Ku and Sangita, 2015), and will not be good for agricultural practices while the reverse is the case for soil that is enriched with OM. The OM of the WA amended soil samples in the present study ranged from 1.07 to 1.60 % before mineralization and 1.07 to 1.60 % after mineralization. The soil sample treated with 5% WA had OM that reduced against the control both before and after mineralization. The soil sample treated with 10% WA had no reduction OM before and after mineralization. It could be that WA affected OM of the soil negatively by relatively small quantity. The cation exchange capacity (CEC) of a soil is related to the texture and soil minerals (Onwuka and Mang, 2018). It is among the characteristics of the soil that is determined by the parent materials. The CEC show the ability of a soil sample to hold positively charged cations by electrical attraction (Nweke, 2018). The soil samples amended with WA to the tone of 5% and 10% had reduced levels of CEC against the control before and after mineralization. The soil samples with 10% WA had the highest CEC reduction followed by the soil sample treated with 5% WA. The influence of WA on CEC of the soils samples could be concentration-dependent. Onotima and Okibe (2021) noted that reduced CEC does not favour fertile soil due to its ability in limiting available positively charged nutrients. The percentage of CEC occupied by bases is given as base saturation (BS). The % BS shares a direct relationship with the pH

Comment [H6]: Avoid explaining the non-significant results and discussion for the same parameters: Write only significant findings. Both N and P showed reduced due to 5 5 AND 10 5 WA application; What author want to convey is not clear? Is WA application is good or not. Avoid unnecessary citing of references and discussion should be for the study and not citing the findings of other researchers.

Comment [H7]: This is well known and no need to cite these references; The results and discussion should be start with the findings of study followed by reasoning/ fact/logics followed by references to support your findings.

Comment [H8]: Both figures are same;

Comment [H9]: Both figures are same;

of the soil. The availability of Mg^{2+} , Ca^{2+} , and K^+ increases with increase in % BS (Adam *et al.*, 2016). Soil samples treated with 5% and 10% WA had % BS that increased before and after mineralization. It has been reported that both CEC and % BS can help assess soil fertility. They are particularly important in highlighting differences in the fertility of the soil between soil samples from a source, and lime requirement determinations in the soil. The Total-total Base-base Saturation saturation (TBS) is regarded as a fraction of the negative binding sites occupied by bases. The TBS of the present study showed no difference in TBS of the soil samples treated with WA when compared to the control before and after mineralization. The amount of acid cations such as hydrogen, and aluminum that occupied on the CEC is the exchangeable acidity (EA). The EA of the soil samples treated with 5% had no reduction in EA against the control before and after mineralization while the soil treated with 10 % WA increased when compared to the control before and after mineralization. The ions of Mg, K, Na, and Ca are alkaline which are known to bring about raise in pH value of the soil (Kanu *et al.*, 2021). The samples treated with 5% and 10% WA had Mg, K, and Ca concentrations that reduced when compared to the control, before and after mineralization. The soil sample treated with 5% WA had no change in Na against the control before and after mineralization while the soil sample treated with 10% WA had increased Na when compare to the control. Soil nitrogen and phosphorus are known for the roles they play in enriching the soil as nutrients for plant growth and maturing. The deficiency of nitrogen or phosphorus in the soil always manifests as deficiency diseases in plants. The soil sample treated with 5% WA had unchanged % nitrogen when compared to the control, both before and after mineralization. The soil sample treated with 10% WA had reduced % nitrogen against the control, both before and after mineralization. This observation was in line with the report of Füzesiet *al.* (2015) which said that WA behaves like fertilizers with low nitrogen on its application to the soil. Johan *et al.* (2021); noted that soil phosphorus is a macronutrient required by plants for optimum growth and development. The same authors noted that soil phosphorus is limited in availability due to fixation. It has been reported that in alkaline soils phosphorus readily reacts to form soluble calcium phosphates that are sparing in form (Johan, *et al.*, 2021). Phosphorus in the soil sample treated with 5% WA reduced when compared to the control before and after mineralization but at increased concentration of 10% treatment of WA, there was increased phosphorus against the control both before and after mineralization.

Comment [H10]: This is well established; Why author want to cite its again?

Table 1: Physicochemical parameters of the treated soil samples.

Formatted: Font: (Default) Times New Roman

Physicochemical Parameters	Control (Before)	WA-5% (Before)	WA-10% (Before)	Control (After)	WA-5% (After)	WA-10% (After)
pH	7.24±0.04 ^b	7.16±0.03 ^a	7.23±0.01 ^{ab}	7.24±0.04 ^b	7.16±0.03 ^a	7.23±0.01 ^{ab}
Electrical Conductivity (µs/cm)	220.29±0.14 ^a	228.48±0.14 ^b	240.82±0.14 ^c	220.29±0.14 ^a	228.48±0.14 ^b	240.82±0.14 ^c
Temp. (°C)	29.80±1.41 ^a	30.70±2.83 ^a	30.00±1.41 ^a	29.80±1.41 ^a	30.70±2.82 ^a	30.00±1.41 ^a
OM (%)	1.60±0.14 ^b	1.07±0.03 ^a	1.41±0.03 ^b	1.60±0.14 ^b	1.07±0.03 ^a	1.41±0.03 ^b
CEC (Cmol/kg)	0.17±0.03 ^c	0.11±0.03 ^b	0.01±0.00 ^a	0.17±0.03 ^c	0.11±0.03 ^b	0.01±0.00 ^a
% BS	84.06±0.07 ^a	87.35±1.41 ^b	88.95±0.14 ^b	84.06±0.07 ^a	87.35±1.41 ^b	88.95±0.14 ^b
TBS (Cmol/kg)	0.40±0.14 ^a	0.13±0.14 ^a	0.39±0.14 ^a	0.40±0.14 ^a	0.13±0.14 ^a	0.39±0.14 ^a
EA (Cmol/kg)	0.90±0.21 ^a	0.80±0.14 ^a	4.00±0.28 ^b	0.90±0.21 ^a	0.80±0.14 ^a	4.00±0.28 ^b
Mg (Cmol/kg)	0.08±0.01 ^a	0.06±0.03 ^a	0.07±0.03 ^a	0.08±0.01 ^a	0.06±0.03 ^a	0.07±0.03 ^a
K (Cmol/kg)	0.03±0.01 ^a	0.01±0.00 ^a	0.02±0.01 ^a	0.03±0.01 ^a	0.01±0.00 ^a	0.02±0.01 ^a
Na (Cmol/kg)	0.02±0.01 ^a	0.02±0.01 ^a	0.26±0.04 ^b	0.02±0.01 ^a	0.02±0.01 ^a	0.26±0.01 ^b

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Ca (Cmo/kg)	0.07±0.03 ^a	0.03±0.01 ^a	0.05±0.01 ^a	0.07±0.03 ^a	0.03±0.01 ^a	0.05±0.01 ^a
%Nitrogen	5.30±0.28 ^b	4.90±0.28 ^b	1.50±0.28 ^a	5.30±0.28 ^b	4.90±0.28 ^b	1.50±0.28 ^a
Phosphorus (mg/kg)	11.31±0.28 ^b	9.95±0.04 ^a	12.79±0.28 ^c	11.31±0.28 ^b	9.95±0.04 ^a	12.79±0.28 ^c

Results are mean and standard deviation of triplicate determinations. Values with similar letters of alphabet along the same row are not statistically significant at 5% levels WA= Wood ash; CEC: Cation exchangeable capacity; %BS= Percentage base saturation; TBS=Total base s saturation; EA= Exchangeable acidity.

According to Violante *et al.* (2010), the bioavailability of heavy metal, toxicity, and leaching in the soil are affected by pH and other factors. Füzesi *et al.* (2015) noted that the application WA can bring about rapid changes in the chemical properties of the soil. The heavy metals of the treated soil samples (Table 2) showed the soil sample treated with 5% WA had reduced copper before mineralization but increased after mineralization when compared to the control. The soil sample treated with 10% WA showed reduced copper both before and after mineralization. The soil sample treated with 5% WA showed increase in iron concentration before mineralization and decrease in iron after mineralization when compared with the control but the soil sample treated with 10% WA had increased iron concentration both before and after mineralization. The zinc in soil samples treated with 5% and 10% WA respectively increased against the control before mineralization. However after mineralization, the zinc in the soil sample treated with 5% WA reduced while the one treated with 10% WA increased. The impact of WA on the zinc content in the present study seemed to be concentration-dependent. Rodríguez *et al.* (2019) noted that lead and chromium are among the possible harmful components found within WA as heavy metal (oids) without any known biological role. The relevance of these elements is that they persist within the environment to bring about bioaccumulation as they enter the food chain with detrimental impact on living organisms including humans (Rodríguez *et al.*, 2019). Lead in the soil samples treated with WA increased against the control before mineralization but reduced when compared to the control after mineralization. Chromium in the soil sample treated with 5% WA increased when compared to the control before mineralization but after mineralization, the soil samples treated with WA had reduced chromium against the control. Nickel increased in the soil sample treated with 5% WA but did not change in the soil sample treated with the 10% WA when compared to the control before mineralization. Nickel in the treated samples did not change against the control after mineralization. The WA ultimately showed its effect on the heavy metal contents of the treated soil samples in the present study. Rodríguez *et al.* (2019) reported on effects of WA on nutrients and heavy metal (oid)s mobility in an ultisol stating that all the WA doses that were used in the study made heavy metals such as copper and zinc available but that was not the case with the present study. The nature of the wood used in the preparation of the WA used in the present study may have been the source of the different observation made in the present study.

Table 2: Heavy metals of the treated soil samples.

Heavy metals (mg/kg)	Control (Before)	WA-5% (Before)	WA-10% (Before)	Control (After)	WA-5% (After)	WA-10% (After)
Copper	0.48±0.01 ^c	0.47±0.03 ^{cd}	0.05±0.03 ^a	0.36±0.03 ^c	0.40±0.03 ^{de}	0.18±0.42 ^a
Iron	2.08±0.04 ^a	3.79±0.03 ^c	5.78±0.01 ^e	3.90±0.28 ^c	3.28±0.01 ^b	5.08±0.01 ^d
Zinc	1.05±0.03 ^a	1.28±0.14 ^b	3.96±0.04 ^d	1.28±0.14 ^b	1.08±0.91 ^{ab}	3.08±0.01 ^c
Lead	0.11±0.03 ^a	0.22±0.03 ^{bc}	0.22±0.01 ^{bc}	0.27±0.01 ^c	0.21±0.01 ^b	0.17±0.01 ^b
Chromium	0.01±0.05 ^a	0.03±0.00 ^c	0.01±0.00 ^a	0.02±0.00 ^b	0.01±0.00 ^a	0.01±0.01 ^a

Nickel	0.14±0.01 ^b	0.17±0.01 ^c	0.12±0.01 ^b	0.08±0.01 ^a	0.06±0.00 ^a	0.06±0.00 ^a
--------	------------------------	------------------------	------------------------	------------------------	------------------------	------------------------

Results are mean and standard deviation of triplicate determinations. Values with similar letters of alphabet along the same row are not statistically significant at 5% levels.

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

The microbial activities of the soil ~~is among its~~ bio-indicators of fertility. Table of microbial load of the treated soil samples (Table 3) revealed that the soil sample treated with 5% WA had TVBC that increased against the control before mineralization but decreased when compared to the control after mineralization. The soil sample treated with 10% WA had TVBC that decreased against the control, both before and after mineralization. The TFC of the treated soil samples reduced when compared to the control both before and after mineralization. The reduction of TFC in the present was dose-dependent. This was in agreement with the report of Ewaet *al.* (2023) who stated that the addition of WA in the soil shapes the composition of the soil fungi. Perucciet *al.* (2008) opined that addition of WA to the soil increases the microbial activity and changes the microbial structure but noted that the effects are related to the doses and form of WA applied. The WA in the present study only increased TVBC at 5% WA treatment with the soil before mineralization. However, a change in the microbial structure may have been introduced based on the reduced microbial load observed in the samples; especially after mineralization. The nature of the wood use in the production of the WA of the study could be the reason behind the observed deviation from the observation of Perucciet *al.* (2008). Bang-Amdreasenet *al.* (2017) reported that WA induced pH changes strongly affect soil bacterial numbers and value may have been behind the changes observed in the microbial load.

Table 3: Microbial load of the treated soil samples.

Microbial Load (CFU/ml)	Control (Before)	WA-5% (Before)	WA-10% (Before)	Control (After)	WA-5% (After)	WA-10% (After)
TVBC ×10 ²	2830.00 ±39.60 ^f	1290.00 ±21.21 ^d	855.00 ±16.97 ^{de}	321.00 ±8.49 ^c	164.00 ±7.07 ^b	94.00 ±4.24 ^a
TFC ×10 ²	865.00 ±32.53 ^d	265.00 ±21.21 ^c	165.00 ±1.45 ^c	94.00 ±11.31 ^b	90.00 ±4.24 ^b	23.00 ±4.24 ^a

Results an mean and standard deviation of triplicate determinations. Values with similar letters of alphabet along the same row are not statistically significant at 5% levels. TVBC=Total Viable Bacterial Count; TFC=Total Fungal Count.

The WA has been reported to affect both the chemistry and biology of the soil in complex ways. This has been attributed to its effects on pH and other soil parameters. The enzyme activity of the treated soil samples (Table 4) showed that ALP of the soil samples treated with WA had reduced activity against the control before and after mineralization. However, the soil sample treated with 5% WA had the lowest ALP activity after mineralization unlike the soil sample treated with 10% WA that had the lowest ALP enzyme activity before mineralization. According to Pieroet *al.* (2006), it is well established that phosphatase activity depends on available phosphorus. It could be that mineralization made WA to release its phosphorus to the soil hence the increased activity observed after mineralization with 10% WA amended soil. Acid phosphatase activity of the soil samples treated with WA had increased activity against the control both before and after mineralization. The observed increase in acid phosphatase activity was concentration-dependent. It has been reported that several phosphatases are linked with the hydrolysis of organic phosphorus. The relationship between phosphate activity is usually very complex with available phosphorus but studies have reported an inverse

Formatted: Font color: Auto

relationship between the two (Orczewska *et al.*, 2012). The soil samples treated with WA showed increased acid phosphatase activity. Lipase activity in the treated soil samples did not change when compared to the control before mineralization and after mineralization. Lipases are enzymes associated with the degradation of molecules of lipids in the soil (Ogbolosingha *et al.*, 2015). Judging from the way its activity increased in the treated soil samples after mineralization, WA may have aided the activity of the enzyme in the soil after mineralization. Urease activity in the soil sample treated with WA showed no change in activity against the control both before and after mineralization. It could be that WA had no influence on the urease activity of the soil in this study. The SOD is among the marker indicators for stress (Rose *et al.*, 2021). The SOD is found as metalloenzymes in prokaryotes and some eukaryotic organisms. Some of the organisms use the soil as their natural habitat (Rose *et al.*, 2021). The SOD of the treated soil sample increased against the control before mineralization while the soil sample treated with 5% WA had reduced SOD activity when compared to the control after mineralization. The soil sample treated with 10% WA had SOD that increased against the control after mineralization. It could be that WA at different concentrations had different effects on the soil microbes saddled with the responsibility of producing the enzyme.

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: Font: (Default) Times New Roman

Table 4: Enzyme activity of the treated soil samples.

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

Enzyme(Conc. unit/gram of sample)	Control (Before)	WA-5% (Before)	WA-10% (Before)	Control (After)	WA-5% (After)	WA-10% (After)
ALP	4.72±0.14 ^c	4.25±0.14 ^d	4.16±0.14 ^{ab}	3.88±0.14 ^c	0.50±0.07 ^a	3.31±0.14 ^b
Acid phosphatase	0.95±0.03 ^a	1.21±0.14 ^b	1.17±0.14 ^{ab}	14.04±0.03 ^c	16.82±0.14 ^d	17.32±0.01 ^e
Lipase	0.02±0.01 ^a	0.03±0.01 ^a	0.04±0.00 ^a	3.60±0.14 ^b	4.01±0.04 ^c	6.27±0.14 ^d
Urease	0.12±0.01 ^a	0.11±0.03 ^a	0.15±0.04 ^a	0.24±0.03 ^b	0.27±0.03 ^b	0.25±0.03 ^b
SOD	0.13±0.03 ^a	3.48±0.14 ^b	4.30±0.14 ^c	16.31±0.14 ^c	10.61±0.14 ^d	23.28±0.01 ^f

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, 11 pt

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, 11 pt

Formatted: Font: 11 pt

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, 11 pt

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman, 11 pt

Comment [H11]: Write the conclusion in quantifiable terms such as % increase/decrease in selected parameters due to treatment applied. Avoid writing generalized statement in conclusion. Address the objectives of the study in conclusion.

Values with similar letters of alphabet along the same row are not statistically significant at 5% levels. ALP= Alkaline Phosphatase; SOD=Superoxide Dismutase

CONCLUSION

The soil samples treated with WA changed the in pH value and some other physicochemical parameters, reduced the microbial population before and after mineralization, and increased the activity of most enzymes investigated in the treated samples after mineralization were profoundly affected. These observations could be due to the presence of the WA added the soil samples. \

REFERENCES

Comment [H12]: Strictly follow the reference writing style of the journal for both citing the references in the text and enlisting them in the reference list. References are too many in number, reduce it to the needful (25-30). Avoid citing too old references until is necessary.

Adam, P. ,Gaspar, A. P. and Labosk, C.A.M. (2016): Base saturation: what is it? should i be concerned? does it affect my fertility program? Proc. of the 2016 Wisconsin Crop Management Conference, Vol. 55, pp. 55-62.

Comment [H13]: ??

Adekayode, F. O. and Olojugba, M. O. (2019): The utilization of wood ash as manure to reduce the use of mineral fertilizer for improved performance of maize (*Zea mays* L.) as measured in the chlorophyll content and grain yield. *African Journal of Soil Science* **7**(3): 001-006.

Agu, K.C. and Chidozie, C. P. (2021): An improved slide culture technique for the microscopic identification of fungal species. *International Journal of Trend in Scientific Research and Development* **6**(1):243-254.

Comment [H14]: ??

An, J. Y. and Park, B. B. (2021): Effects of wood ash and N fertilization on soil chemical properties and growth of *Zelkova serrata* across soil types. *Scientific Reports* **11**:14489. <https://doi.org/10.1038/s41598-021-93805-5>

Bai, Z., Casparia, T., Gonzalez, M. R., Batjes, N. H., Mader, P., Bünemann, E. K., de Goede, R., Brussaard, L., Xu, M., Ferreira, C. S. S., Reintam, E., Fan, H., Mihelič, R., Glavan, M., and Tóth Z. (2018): *Effects of agricultural management practices on soil quality: a review of long-term experiments in Europe and China*. *Agriculture Ecosystems and Environment* **265**: 1–7.

Comment [H15]: ??

Bang-Andreasen, T., Nielsen, J. T., Voriskova, J., Heise, J., Rønn, R., Kjoller, R., Hansen, H. C. B. and Jacobsen, C. S. (2017): Wood ash induced pH changes strongly affect soil bacterial numbers and community composition. *Front Microbiol.* **8**: 1400-1423. doi: 10.3389/fmicb.2017.01400

Comment [H16]: ??

Bang-Andreasen, T., Peltre, M., Ellegaard-Jensen, L., Hansen, L. H., Ingerslev, M., Ronn, R., Jacobson, C. S. and Kjoller, R. (2021): Application of wood ash leads to strong vertical gradients in soil pH changing prokaryotic community structure in forest top soil. *Scientific Reports* **11**:742 | <https://doi.org/10.1038/s41598-020-80732-0>

Bergmeyer, H. V. (1984): Methods of enzyme analysis. Weinheim Verlag Chemie. Third edition. p.92.

Bonfim-Silva, E., Pereira, M., Da Silva, T. and Fenner, W. (2017): Potential of wood ash as a fertilizer in BRS Piatã grass cultivation in the Brazilian Cerrado soil. *American Journal of Plant Sciences* **8**: 2333-2344. doi: 10.4236/ajps.2017.810156.

Comment [H17]: ??

Bremner, J. M. (1996): Nitrogen-total. In Methods of Soil Analysis Part 3—Chemical Methods, SSSA Book Ser. 5.3 (eds Sparks, D. L. et al.) 1085–1121 (Soil Science Society of America, American Society of Agronomy, 1996) <https://doi.org/10.2136/sssabookser5.3.c37>.

Bates, R. G. (1954): Electrometric pH determination. John Wiley and Sons Inc, New York.

Brady, N.C.(1988): The nature and properties of soils. Macmillan Publishing Co., Inc.

Bray R.H. and Kurtz L.T. (1945): Determination of total, organic, and available forms of phosphorus in soils. *Soil Science* **59**: 39–45.

Chessbrough, M. (1984): Medical laboratory manual for tropical countries (volume II). Dodington Butter Worth & Co Ltd UK. 58-69.

Dandwate, S. C. (2020): Analysis of soil samples for its physicochemical parameters from Sangamner city. *GSC Biological and Pharmaceutical Sciences***12**(2):123-128.

Doran, J.W. and Parkins, T. B. (1996): Quantitative indicators of soil quality: a minimum data set. In J.W. Doran and A.J. Jones, eds. *Methods for Assessing Soil Quality*. SSSA, Inc., Madison, Wisconsin, USA. <http://soilquality.org/indicators.html> (accessed on the 6/Feb/2024).

Else, K. B., Paul, M., Jens, W., Lijber, B., Giulia, B., Ron, D., Violette, G., Luuk, F., Wijnand, S., Zhanguo, B. and Thomas, C. (2016): Concepts and indicators of soil quality – a review. www.iSQAPER-project.eu (Accessed on the 5th of Feb., 2024).

Ewa, B., Wojciech, P., Piotr, B. and Lasota, J. (2023): Effects of wood ash on the soil properties and fungal community structure in a beech forest in Poland. *Geoderma Regional* 34:1-10.

Füzesi, I., Heil, B. and Kovács, G. (2015): Effects of wood ash on the chemical properties of soil and crop vitality in small plot experiments. *ActaSilv.Lign. Hung.* 11 (1):55–64.

Jency, J., Dhivya, K. and Rajeshkumar, S. (2023): Studies on physico-chemical parameters in different soil samples from Erode District, Tamil Nadu, India. *GSC Biological and Pharmaceutical Sciences* 24(2), 29–39.

Johan, P.D.; Ahmed, O.H.; Omar, L.; Hasbullah, N.A. (2021): Phosphorus transformation in soils following co-application of charcoal and wood ash. *Agronomy* 11(10). <https://doi.org/10.3390/agronomy11102010>

Johansen, J. L., Nielsen, M. L., Vestergård, M., Mortensen, L. H., Cruz-Paredes, C., Rønn, R., Kjøller, R., Hovmand, M., Christensen, S. and Ekelund, F. (2024): The complexity of wood ash fertilization disentangled: Effects on soil pH, nutrient status, plant growth and cadmium accumulation. *Environmental and Experimental Botany***185**: 1044241..

Jonna, W. (2017): Effects of wood ash on soil fertility and plant performance in southwestern Kenya. <https://core.ac.uk/download/pdf/211580566.pdf> (Date accessed 7th Feb, 2024).

Kanu, M. O., Gabriel, W and George, I. (2021): Measurement of physicochemical properties, electrical and thermal conductivity of wood ash for effective soil amendment. *Indonesian Journal of Applied Physics* 11(2): 176-188.

Kakkar, P., Das, B. and Viswanathan, P.N. (1984): A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics***21**:130-132.

Kekane, S. S., Chavan, R. P., Shinde, D. N., Patil, C. L., and Sagar, S. S. (2015): A review on physico-chemical properties of soil. *International Journal of Chemical Studies* **3**(4): 29-32.

Kelvin, S., Extension, B., Sawyer, C. and Washburn, C. (2020): Using Wood Ash in the Home Garden. <https://hort.extension.wisc.edu/articles/using-wood-ash-in-the-home-garden/> (Accessed on 7th Feb., 2024).

Ku, S. T. and Sangita I (2015): A Review on role of physico-chemical properties in soil quality. *ChemSci Rev Lett* **4**(13):57-66.

Lal, R.(1998): Soil quality and agricultural sustainability. Ann Arbor Press, Michigan

Comment [H18]: ??

Lal, R. (2001): Managing world soils for food security and environmental quality. *Advances in Agronomy*, Volume 74.

Margesin, R., Feller, G., Hämmerle, M. Stegner, U. and Schinner, F. (2002): A colorimetric method for the determination of lipase activity in soil. *Biotechnology Letters* **24**:27–33. <https://doi.org/10.1023/A:1013801131553>

Mbah, C. N., Dada, O. A., Okoro, T. N., and Ifejimalu, A. (2018): Use of poultry droppings and Wood ash soil amendment and its effect on soil properties and yield of maize. *International Journal of Advances in Scientific Research and Engineering* **4**(7): 74-78.

Mbah, C.N. and Nkpaji, D. (2009): Response of maize (*Zea mays* L.) to different rates of wood ash application in acid Ultisol in Southeast Nigeria. *Journal of American Science* **5**(7): 53 – 57.

Mclean E. O. (1982) .Soil pH and lime requirements. In Page A. L. (ed) *Methods of soil analysis part 2 chemical and microbial properties Agronomy Series No. 9 ASA, SSSA Madison WI, USA*

Mohamed, S.A., Ewees, S.A., Sawsan, A., Seaf, E.Y. and Dalia, M. S. (2008): Improving maize grain yield and its quality grown on a newly reclaimed sandy soil by applying micronutrients, organic manure and biological inoculation. *Research Journal of Agriculture and Biological Sciences* **4**:537–544.

Nottidge, D. O. (2005): An investigation in the leguminous crop residue and wood ash for improving soil condition and productivity of maize (*Zea Mays* L) in south east Nigeria. Ph.D dissertation Michael Okpara University of Agriculture, Umudike.

Nweke I. A. (2015): Physical and chemical properties of four contrasting soils under different landuse systems. *Adv. Res.* **3**(2): 236- 243.

Nweke, I. A. (2018): Contrasting tillage systems and wood ash effect on soil chemical properties. *British Journal of Environmental Sciences* **7** (1): 8-25.

Ogbolosingha, A.J., Essien, E.B.2 and Ohiri, R.C. (2015): Variation of lipase, catalase and dehydrogenase activities during bioremediation of crude oil polluted soil. *Journal of Environment and Earth Science* **5** (14): 128-141.

Oliver, M.A. and Gregory, P.J. (2015) Soil, food security and human health: a review. *European Journal of Soil Science* **66**: 257-276.

Orczewska, A., Piotrowska, A. and Lemanowicz, J. (2012): Soil acid phosphomonoesterase activity and phosphorus forms in ancient and post-agricultural black alder [*Alnus glutinosa* (L.) Gaertn.] woodlands. *Acta Societatis Botanicorum Poloniae* **81**(2): 81-86.

Onotima, C. C. and Okibe, F. G. (2021): Multivariate analysis of the physico-chemical properties of soils in selected locations of the floodplain of river Kaduna in Niger State, Nigeria. *Global Journal of Pure and Applied Sciences* **27**: 279-288.

Onwuka, B. M. and Mang, B. (2018): Effects of soil temperature on some soil properties and plant growth. *Adv. Plants Agric. Res.* **8**: 34–37.

Perucci, P., Monaci, E., Onofri, A., Vischetti, C. and Casucci, C. (2008): Changes in physico-chemical and biochemical parameters of soil following addition of wood ash: A field experiment. *European Journal of Agronomy* **28**(3): 155-161.

Piero, P., Elga, M., Cristiano, C. and Costantino, V. (2006): Effect of recycling wood ash on microbiological and biochemical properties of soils. *Agronomy for Sustainable Development*, **26** (3): 157-165.

Raghavendra, M., Sharma, M. P., Ramesh, A., Richa, A., Bilore, S. D. and Verman, R.K. (2020): Soil health indicators: methods and applications. In: Rakshit, A., Ghosh, S., Chakaroborty, S., Philip, V., Datta, A (eds). *Soil Analysis: Recent Trend and Applications*. Springer, Singapore. https://doi.org/10.1007/978-981-15-2039-6_13

Rodriguez, Y., Maudier, B., Zagal, E. and Hernández, P. (2019): Effects of wood ash on nutrients and heavy metal(oid)s mobility in an ultisol. *Int J Environ Res Public Health*. **16**(7): 1246. doi: [10.3390/ijerph16071246](https://doi.org/10.3390/ijerph16071246)

Rose, A. C., Corsi, D., Cavi, N., Bruni, N. and Dosio, F. (2021): superoxide dismutase administration: a review of proposed human uses. *Molecule* **26** (7): 1894. doi: [10.3390/molecules26071844](https://doi.org/10.3390/molecules26071844)

Sani, U., Uzairu, A. and Abba, H. (2012): Physico-chemical parameters of soil in some selected dumpsites in Zaria and its environs. *Chemsearch Journal* **3**(1): 1 – 6,

Serafimova, E., Mladenov, M., Mihailova, I. and Pelovski, Y. (2011): Study on the characteristics of waste wood ash. *Journal of the University of Chemical Technology and Metallurgy* **46** (1): 31-34.

Gómez-Rey, M. X., Madeira, M. and Coutinho, J. (2012): Wood ash effects on nutrient dynamics and soil properties under Mediterranean climate. *Annals of Forest Science* **69**: 569–579. <https://doi.org/10.1007/s13595-011-0175-y>

Stewart, W. M., Dibb, D.W., Johnston, A. E. and Smyth, T. J. (2005): The contribution of commercial fertilizer nutrients to food production. *Agronomy Journal* **97**: 1 – 6.

Sumner, M. E. and Miller, W. P. (1996): Cation exchange capacity and exchange coefficients. In *Methods of Soil Analysis Part 3—Chemical Methods, SSSA Book Ser. 5.3* (eds Sparks, D. L. et al.) 1201–1229 (Soil Science Society of America, American Society of Agronomy, 1996).

Tabatabai (1982): Soil enzymes In: *Methods of soil analyses Part 2. Agronomy monograph. Vol. 9* pages. American Society of Agronomy, Madison. Pp. 903-904.

Tabatabai, M. A. and Bremner, J. A. (1969): Use of p-nitrophenylphosphate for assay of soil phosphatase activity. *Soil Biol Biochem.* **1**: 301-307

Tabatabai, M.A. and Bremner, J.M. (1972): Assay of urease activity in soils. *American Journal of Soil Science Society* **41**: 350-352.

Shivanna, A. M. and Nagendrappa. G. (2014): Chemical Analysis of Soil Samples to Evaluate the Soil Fertility Status of Selected Command Areas of Three Tanks in Tiptur Taluk of Karnataka, India *Journal of Applied Chemistry*. Ver. I. 7(11):01-05.

Ulery, A. L., Graham, R.C. and Amrhein, C. (1993): Wood-ash composition and soil pH following intense burning. *Soil Science* 156(5): 358-354.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. & Pigna, M. (2010): Mobility and bioavailability of heavy metals and metalloids in soil environments. *Journal of Soil Science and Plant Nutrition* 10(3):268-292.

Formatted: Font: Not Bold

UNDER PEER REVIEW