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PHYSICOCHEMICAL, HEAVY METAL AND MICROBIAL COMPOSITION OF SOIL WITHIN SAWMILLING SITES IN DRY SEASON IN SOUTHEAST NIGERIA

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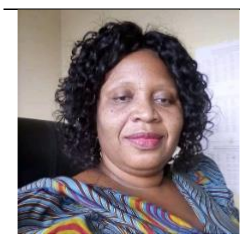

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ABSTRACT

The growing demand for wood and wood products for use in furniture making has necessitated an upsurge in sawmilling activity with its deleterious environmental pollution attributes. This study assessed the effect of heavy metals introduced into the soils through sawmilling activities as well as the impact of sawmilling activities on physiochemical and microbial properties of such soil. Soil samples were collected from selected sawmill sites in two timber markets (Ahiaeke and Okigwe) both in Nigeria at depths 0m, 50m, 100m and 500m. Physicochemical parameters were analyzed in soils using standard methods. The concentration of heavy metals in sawmill soil was determined by Atomic Adsorption Spectrophotometer (AAS) after digestion of the soils. The total sodium in the soil samples during dry season from both sites ranged from 0.252% to 0.174% and 0.24% to 0.19% for Ahiaeke and Okigwe respectively. A significant difference was observed in the amount of potassium content of the soil samples at different distances ($p<0.05$). Accumulation of potassium was highest in the Ahiaeke milling site with an average of 1.189 % a distance of 50m. The samples collected at a distance of 100m showed the least potassium accumulation (0.154%). The total organic carbon (TOC) in the soil samples during the dry season from both sites ranged from 2.27% to 1.19% and 2.73% to 1.15% for Ahiaeke and Okigwe respectively. The electrical conductivity (EC) of the soil samples from the Okigwe site ranged from 0.64cmolkg to 1.68cmolkg with samples from the edge (0m) distance recording the highest value whilst the values were within 0.88cmolkg to 1.36cmolkg for samples from Ahiaeke site. In both sawmill sites, Zn and Fe was quite high in concentration at 0m depth. The average Zn concentration at this depth was 29.60 and 38.50mgkg^{-1} respectively for the Okigwe and Ahiaeke sites. It decreased with depth to reflect concentration of 27.10mgkg^{-1} at 50m depth and 8.30mgkg^{-1} at 100m depth for the Ahiaeke site. Average iron concentration of soil from the Okigwe sawmill site varied between 214.50mgkg^{-1} to 340.80mgkg^{-1} whilst mean Pb concentration varied between 1.88 to 3.51mgkg^{-1}. The concentration range of copper in the soil samples from Ahiaeke sawmilling site and Okigwe sawmilling site across the different distances were between: 0.55mg/kg to 0.38mg/kg and 2.24mg/kg to 0.21mg/kg respectively. This findings indicated that soil qualities varied between slightly contaminated to severely polluted status. This showed that proactive measures must be taken to minimize accumulation of these metals.

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Keywords- Heavy Metals, Wood, Sawmill, Ahiaeke, Pollution

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INTRODUCTION

The demand for wood products globally for industrial and domestic purposes is the premise that has necessitated the establishment of Sawmill industries (Ajibulu *et al*., 2013). However, it is becoming increasingly alarming that sawmilling activities are characterised by series of environmental pollution.
Soils normally contain low background levels of heavy metals. However, in areas where agricultural, industrial or municipal wastes are land-applied as fertilizer, concentrations may be much higher. Heavy metal contamination of soil occurs when heavy metals are introduced to soil through human activities, leading to the gradual deterioration of the ecology and environment (Basumatary *et al*., 2012). Major chemicals used in the wood processing industry are creosote, copper, chromium, arsenic (CCA) and pentachlorophenol (PCP). Soil contamination problem with one or more of these chemicals can often be expected at wood preservation site, particularly at old sites (Adelekan and Abegunde, 2011). Machines and other auxiliary equipment used by sawmill operators are powered by diesel or gasoline generator occasioned by inaccessible power supply. These activities send heavy metals into the air and are subsequently deposited into nearby soils which are absorbed by plants (Ashraf *et al*., 2014).
Heavy metals can have inhibitory effects on the development of bacterial, fungal and actionmycetes. They reduce biomass of microorganisms, reduce their soil activity and even if they do not reduce their number, they depress their biodiversity (Ahemad, 2012; Sobolev and Begonia, 2008). High levels of toxic elements in the soil samples lead to destabilization of ecological balance as they find their way into the food chain thus posing health hazard to public health and the environment at large (Chukwuma *et al*., 2010).
The trends of environmental abuse in most local settings suggest that humans are probably heading towards environmental degradation, hence this study aims at evaluating environmental public health implications of indiscriminate sawmill waste disposal.

METHODS

Physiochemical analysis was carried out as described by UNEP (2002); Udo *et al.*, (2009) and AOAC (2005). The following parameters were determined: pH, Electrical conductivity (EC), Exchangeable cations (K, Na, Ca, and Mg), Total Nitrogen (TN), Available Phosphorus (Av. P), Total Organic Carbon (TOC) Exchangeable Acidity (EA).

DETERMINATION OF HEAVY METALS

Digestion of soil: The air- dried 2mm sieved soil sample (1.0g) was measured into a 100ml of 6M HCL. The mixture was heated to paste on a hot plate. The paste so formed was leached with 10ml of 4M HCL, filtered into 20ml standard flasks and the residues washed with more acid and make up 20ml.

The concentration of heavy metals such chromium (Cr), Cadmium (Cd), Zinc (Zn), Lead (Pb), Copper (Cu) and Iron (Fe) in sawmill soil was determined. This was done by Atomic Adsorption Spectrophotometer (AAS) after digestion. Reagent blank was prepared, the same way as earlier describe, but without sample and the volume made up to 20ml. the extracts and the blank were analyzed for trace elements using Atomic Adsorption Spectrophotometer (UNICAM AA919).

MICROBIOLOGICAL EVALUATION

The serial dilution technique was employed in the inoculation of the soil samples. Each of the samples was diluted in the 10-fold serial dilution technique described by Gurung *et al.* (2009). Dilutions from 10^{-3} and 10^{-4} were inoculated onto freshly Tryptone Soya Agar and Sabouraud Dextrose Agar Plates. The Spread plate method of inoculation as described by Prescott *et al.*, (2007) was used where 0.1ml of the respective dilutions(10^{-3} and 10^{-4}) were plated on

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various agar plates and evenly spread over the entire plate using a flame sterilized glass rod. The inoculated plates were incubated at 35^{0}C for 24hrs for bacteria and at room temperature (25+2^{0}C) for fungi.

Isolated organisms were identified by standard microbiology identification techniques including Grams staining, catalase test, citrate, hydrogen sulphide test, methyl-Red test, voges-proskauer test as well as the urease and indole tests.

The identity of each isolates was further authenticated using standard DNA Sequencing protocols. DNA was extracted from the isolates at molecular biology laboratory of Niger Delta University, Bayelsa state Nigeria and the extracts sequenced for their nucleotide sequences for use in identification.

RESULTS AND DISCUSSION

Table 1: Metallic Ion Content of Soil Sample of the Various Study Locations during Dry Season

Parameter	Okigwe				LSD
	0m	50m	100m	500m (Control)	
Zinc (mg/kg^{-1})	29.60[a]	20.20[c]	24.26[b]	15.40[d]	4.06
Iron (mg/kg^{-1})	340.80[a]	214.50[c]	224.10[b]	196.00[d]	9.60
Cadmium (mg/kg^{-1})	0.090[b]	1.020[a]	0.059[c]	0.027[d]	0.03
Chromium (mg/kg^{-1})	1.150[a]	0.096[c]	0.140[b]	0.059[d]	0.04
Copper (mg/kg^{-1})	2.24[a]	0.59[c]	0.66[b]	0.21[d]	0.07
Lead (mg/kg^{-1})	3.51[a]	3.06[b]	1.88[c]	0.96[d]	0.45

Values with different superscript across a row are significantly different from each other.

Table 2: Metallic Ion Content of Soil Sample of the Various Study Locations during Dry Season

Parameters	Ahiaeke				LSD
	0M	50m	100m	500m (Control)	
Zinc (mg/kg^{-1})	38.50[a]	27.10[b]	8.30[c]	4.50[d]	3.80
Iron (mg/kg^{-1})	516.30[a]	335.80[b]	106.75[d]	115.40[c]	8.60
Cadmium (mg/kg^{-1})	1.035[b]	1.160[a]	0.064[c]	0.033[d]	0.03
Chromium (mg/kg^{-1})	0.060[a]	0.038[c]	0.044[b]	0.035[c]	0.01
Copper (mg/kg^{-1})	0.55[b]	0.86[a]	0.45[c]	0.38[d]	0.07
Lead (mg/kg^{-1})	6.10[a]	4.25[b]	2.10[c]	0.87[d]	1.23

Values with different superscript across a row are significantly different from each other.

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Table 3: Physiochemical Properties of Soil Samples of the Various Locations during Dry Season

Parameters	Okigwe				
	OM	**50M**	**100M**	**500M (Control)**	**LSD**
Temperature	31.0^{b}	32.8^{a}	32.5^{a}	30.0^{c}	1.00
pH	6.64^{a}	6.17^{c}	6.46^{b}	5.15^{d}	0.18
EA ($cmolkg^{-1}$)	1.68^{a}	0.80^{c}	1.12^{b}	0.64^{d}	0.16
ECEC	10.61^{a}	9.69^{b}	7.53^{c}	6.62^{d}	0.91
Total Phosphate ($Mgkg^{-1}$)	29.50^{b}	22.10^{d}	36.70^{a}	25.40^{c}	3.30
Total Nitrate ($Mgkg^{-1}$)	0.266^{a}	0.140^{b}	0.070^{d}	0.113^{c}	0.03
Total organic carbon ($mgkg^{-1}$)	2.73^{a}	1.35^{b}	1.00^{d}	1.15^{c}	0.20
Total Calcium ($Mgkg^{-1}$)	6.40^{a}	5.20^{b}	4.00^{c}	3.60^{d}	0.40
Total Magnesium ($Mgkg^{-1}$)	2.40^{b}	3.20^{a}	2.00^{c}	2.00^{c}	0.40
Total Potassium ($Mgkg^{-1}$)	0.328^{b}	0.266^{c}	2.05^{a}	0.184^{d}	0.06
Total Sodium ($Mgkg^{-1}$)	0.244^{b}	0.278^{a}	0.226^{c}	0.191^{d}	0.05

Table 4: Physiochemical Properties of Soil Samples of the Various Locations during Dry Season

Parameters	Ahiaeke				
	OM	**50M**	**100M**	**500M (Control)**	**LSD**
Temperature	30.7^{a}	30.2^{a}	30^{ab}	28.0^{c}	0.20
pH	6.90^{a}	6.45^{b}	5.60^{c}	4.89^{d}	0.45
EA ($cmolkg^{-1}$)	0.88^{b}	0.72^{c}	0.42^{d}	1.36^{a}	0.16
ECEC	9.2^{a}	7.87^{b}	7.91^{b}	7.30^{c}	0.57
Total Phosphate ($Mgkg^{-1}$)	25.70^{b}	30.70^{a}	19.50^{d}	19.70^{c}	0.20
Total Nitrate ($Mgkg^{-1}$)	0.196^{a}	0.182^{b}	0.140^{d}	0.154^{c}	0.01
Total organic carbon ($mgkg^{-1}$)	2.27^{a}	1.54^{b}	1.39^{c}	1.19^{d}	0.15
Total Calcium ($Mgkg^{-1}$)	5.60^{a}	4.40^{c}	5.20^{b}	4.00^{c}	0.40
Total Magnesium ($Mgkg^{-1}$)	2.00^{b}	2.40^{a}	2.00^{b}	1.60^{c}	0.40
Total Potassium ($Mgkg^{-1}$)	0.292^{b}	1.189^{a}	0.154^{d}	0.164^{c}	0.13
Total Sodium ($Mgkg^{-1}$)	0.252^{a}	0.165^{c}	0.131^{d}	0.174^{b}	0.01

Values with different superscript down a column are significantly di fferent ($P<0.05$) from each other.

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Table 5: **Microbial Counts of Sawmill and Control Soil Samples at different distance at Okigwe in cfu/g**

	0m	50m	100m	500m
THBC	$3.8x10^7$	$3.30x10^7$	$3.6x10^7$	$3.3x10^7$
TCC	$1.3x10^4$	$1.1x10^4$	$1.4x10^4$	$1.3x10^4$
TPSBC	$1.6x10^4$	$1.3x10^4$	$1.2x10^4$	$1.4x10^4$
TNBC	$1.9x10^5$	$1.6x10^5$	$1.4x10^5$	$1.5x10^5$
TAC	$1.5x10^6$	$1.4x10^6$	$1.6x10^6$	$1.3x10^6$
TFC	$3.9x10^7$	$3.3x10^7$	$3.5x10^7$	$3.7x10^7$

Key:
THBC: Total Heterotrophic Bacteria Count
TCC: Total Coliform Count
TFC: Total Fungal Count
TPSBC: Total Phosphate Solubilization Bacteria Count
TNBC: Total Nitrifying Bacteria Count
TAC: Total Actinomycetes Count

Table 6: Microbial Counts of Sawmill and Control Soil Samples at different distance at Ahiaeke in cfu/g

	0m	50m	100m	500m
THBC	$3.5x10^7$	$3.60x10^7$	$3.3x10^7$	$3.1x10^7$
TCC	$1.2x10^4$	$1.2x10^4$	$1.1x10^4$	$1.0x10^4$
TPSBC	$1.4x10^4$	$1.1x10^4$	$1.2x10^4$	$1.3x10^4$
TNBC	$1.7x10^5$	$1.5x10^5$	$1.6x10^5$	$1.3x10^5$
TAC	$1.3x10^5$	$1.3x10^6$	$1.1x10^6$	$1.2x10^6$
TFC	$3.4x10^7$	$3.5x10^7$	$3.1x10^7$	$3.3x10^7$

Key:
THBC: Total Heterotrophic Bacteria Count
TCC: Total Coliform Count
TFC: Total Fungal Count
TPSBC: Total Phosphate Solubilization Bacteria Count
TNBC: Total Nitrifying Bacteria Count
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DISCUSSION

Sawdust is a wood waste containing a very rich carbonaceous component that can be used as soil amendment. However, it is also important to understand the adverse impact that sawdust might have on soil and crops. Phosphate values varied with the highest value recorded for 50m soil sample collected at Ahiaeke during the dry season. The values recorded in this study are higher than those reported by Iwegbue *et al.* (2006), Chaudhare (2013). The level of phosphate recorded in this study could be attributed to some decayed sawmill wastes at the sites. According to Isirimah *et al.* (2003), phosphorus is essential for the seeds and development of fibrous root system in plants. The levels of phosphate were significantly different at $p<0.05$ across the sites, indicating a common source of pollution. The relative level of available P in the soil samples could be linked with the chemical composition of sawmill waste especially when it is burnt.

The highest concentrations of nitrate in sawmill soils were recorded at 0m distance Okigwe site. Many studies conducted on physicochemical properties of waste soils on refuse dumpsites documented Total Nitrate at different levels (Okunola *et al*., 2011; Gasu and Ntemuse, 2011). The ranges in this study were higher than those reported by Awode *et al*. (2008) who reported lower ranges of 0.10 to 2.20%. The levels of Total Nitrate for soil samples from the Okigwe site obtained at 100m distance were significantly lower than those of the 0m. Overall, the concentrations of nitrate were higher in the rainy season than in the dry season.

Variations in magnesium levels were detected in the soil samples from Ahiaeke. Accumulation of magnesium was highest in the Okigwe sawmill site at 0m and 100m distances. The samples collected at a distance of 100m and 500m at the Ahiaeke site in the rainy season showed the least potassium accumulation. A significant difference was observed between the samples collected at a distance of 0m and 500m during the rainy season. Similarly, the observed values during the dry season differed significantly from each other with the values recorded for the 50m soil sample at the Okigwe site differing from that of the 100m sample. Accumulation of potassium was highest during the dry season in the Okigwe sawmill site at a distance of 100m. The samples collected at a distance of 500m showed the least potassium accumulation. There was significant differences ($p<.05$) in the amount of potassium recovered from the various distances. To compare the soil potassium content during rainy season at the various distance from Okigwe site, potassium decreased with increase in the distances.
Heavy metal contaminated soils impose various adverse effects on composition and activity of the soil microbial community (Fagbote and Olanipekun, 2010). Previous studies have demonstrated the adverse effect of heavy metals on soil microbial community structure and activity (Ahemad, 2012; Yao *et al.*, 2003)

Generally, the concentrations of the metals were highest at the edge (0m) soils. This is expected since the edge (0m) of the soil is the point of contact at the sawmill site. The metal levels at both sites were significantly higher than the levels observed in the control sites.
These heavy metals when present in solid wastes have been known to produce major environmental impacts (Suman *et al*., 2011; Ebong *et al*., 2007). In both sawmill sites, Zn and Fe was quite high in concentration at 0m depth. The average Zn concentration at this depth was 29.60 and 38.50mgkg^{-1} respectively for the Okigwe and Ahiaeke sites. It decreased with depth to reflect concentration of 27.10mgkg^{-1} at 50m depth and 8.30mgkg^{-1} at 100m depth for the Ahiaeke site. Average iron concentration of soil from the Okigwe sawmill site varied between 214.50mgkg^{-1} to 340.80mgkg^{-1} whilst mean Pb concentration varied between 1.88 to 3.51mgkg^{-1}. The values obtained for both heavy metals differed remarkably from the control (Table…). Heavy metals studied were found to decrease with depth as the heavy metals were more concentrated in the upper layers (0–50m) of both sites. Among the heavy metals the least accumulated metal in the soils were Chromium and cadmium. The Cu content of the soil sample taken at 0m depth was found to be significantly different ($p>0.05$) relative to the rest of the depths.

The most predominant bacteria among the isolates were *Bacillus* species, *Kiebsiella pneumoniae* and *Enterobacter* sp which all recorded 100% occurrence. This predominance of *Bacillus* species may be attributed to their ability to resist

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harsh environmental conditions and heavy metal contamination in soils. *Bacillus* species are known to produce spores that enable them with stand environmental harshness. This finding agrees with Laugauskas *et al* (2005), who found *Bacillus* species as the most bacteria in the soils contaminated with lead. This finding is also in agreement with Ezejiofor, (2013), in which they reported *Bacillus* species among the organisms that resist lead highest in their work, *Serretia marcescens, Micrococcus sp, Bacillus cirulans* and *Escherichia coli* were found to have moderate distribution among the bacteria isolated with percentages of 92%, 83%, 58% and 50% respectively.

Gene sequences of the various isolates obtained from their 16S rDNA revealed a high sequence similarity to bacteria belonging to the families Enterobacteriaceae, Moraxellaceae, Pseudomonadaceae, Nocardiaceae, and Bacillaceae. The partial sequences of the isolates were compared with closest relatives from GenBank. The sequence analysis of 16S rRNA revealed the isolates to be closely related to family Enterobacteriaceae, Pseudomonadaceae, and Bacillaceae. The DNA sequence and subsequent BLAST analysis indicated a high similarity of the obtained sequence corresponding to the respective isolates. The sequences for the respective isolates were deposited in GenBank database under the accession numbers (MK621103, MK621201, MK640631, MK640622, and MK643270).

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