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Heavy Metal and Microbial Composition of Soil Around Sawmilling Sites in South East Nigeria

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Abstract: Human activities in urban settings contribute in no small measure to the contamination of soils and thus poses major health concern. The levels of different soil metals associated with sawmill environs including chromium (Cr), Cadmium (Cd), Zinc (Zn), Lead (Pb), Copper (Cu) and Iron (Fe) was evaluated using Atomic Adsorption Spectrophotometer (AAS) following the digestion of the soil samples whilst the microbial composition was determined using standard techniques. The concentrations of the different studied metals in the soils decreased with depth in both sites. More concentration of heavy metals was observed in the 0m soil depth. The studied heavy metals recorded varying concentration in the soil with the highest values of 345.6mg/kg, 25.3mg/kg, 0.75mg/kg, 4.96mg/kg and 4.40mg/kg respectively for Iron, Zinc, Chromium, Lead and copper. The distribution of the studied metals by level of abundance were Fe > Zn > Ni > Cr > Pb. The concentration of copper was found more on the edge (0m) and decreased as the distances studied. Decreased concentrations of the metals at distances far off the sawmilling site (500m) strongly highlights the contribution of sawmilling activity to the accumulation of these heavy metals in such environments. The bacterial counts at both sites show that soil from Okigwe had slightly higher load. It had 5.4×10^7cfu/g, 5.0×10^7cfu/g and 4.4×10^7cfu/g at 0m, 50m and 100m respectively. It also had 4.9×10^7cfu/g, 4.6×10^7cfu/g and 4.4×10^7cfu/ at 0m, 50m and 100m respectively for total fungal load. The samples of soil obtained from 500m distance away from sawmilling operations had the least number of the bacterial counts at both sites with counts 4.4×10^7cfu/g and 4.1×10^7cfu/g for the Okigwe and Ahiaeke sawmills respectively. In this study gene sequencing of the bacterial 16SrRNA was adopted to characterize associated bacterial communities within sawmilling soils. Our results show that the isolates were predominantly Proteobacteria, including *Enterobacter*, *Alcaligenes,* and *Bacillus* species. Gene sequences of bacterial 16S rRNA gene fragments retrieved from bacterial isolates in this study were deposited in the GenBank nucleotide sequence database under accession nos. MK621199, MK621103, MK621201, MK640631, MK640622 MK640642 and MK640638 (www.ncbi.nlm.nih.gov). The concentrations of the metals as observed from this study may not portray significant exposure risks, however there are fears that accumulation of these metals over time might be of concern in future. Hence this study calls for continued assessment and monitoring of activities within sawmills and it's surrounding to protect the environment from imminent harm from excessive accumulation.

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

1. Introduction

Several anthropogenic activities including sawmilling contributes to the contamination of soils with Heavy metals at varying concentrations. Pollution of the soil takes its toll when the concentrations of these metals reaches remarkably high levels than those of the background thus deteriorating the environment [1] Elevated concentrations of these heavy metals impacts the activities of soil microorganisms with substantial effect on crop yield and quality.

Contamination with heavy metal is a disturbing environmental dilemma due to their non-biodegradability [2] and are thus not likely to be rendered them less toxic. The resultant effect is buildup of these metals to harmful threshold levels as they remain in the environment for a long time [3]. These heavy metals also subject to dispersal from soils, especially by rainfall which can carry them over protracted distances hence attaining pollution levels higher than is stipulated by regulatory agencies [4].

On the other hand, a metal polluted soil could present inherent negative effects on the surrounding microbial flora. These metals subsequently are absorbed by plant tissues and animals that feed on such tainted plants can potentially lodge these metals in their tissues or even the milk in the case of lactating animals [5]. Humans may also be at the receiving following the consumption of such plants and animals, which could result to different biochemical dysfunstion. Invariably, all living organisms within a sawmilling ecosystem are affected through food chain [6, 7].

Metals contribute significantly to microbial existence. Many of these metals including calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc, are essential, providing the organisms with micronutrients [8]. However, several other perform no biological role (e.g. silver, aluminium, cadmium, gold, lead and mercury), and are [8] potentially toxic to the microorganisms. Despite the transformatory abilities of microorganisms on metals, significant amounts of these metals possess damaging effects on the soil microbial activity and functioning. Previous studies have earlier described the negative effects of metals on the soil ecosystem. Also, remarkable decrease in microbial biomass [9] have been found in metal contaminated soils compared to uncontaminated soils. Also, many studies have shown that metal contamination causes a shift within the soil microbial community from sensitive to less sensitive microbes [10]. Heavy metals reduce the biomass of microorganisms and lower their soil activity and even if they do not reduce their number, they depress their biodiversity [11, 8].

The measurement of heavy metal accumulation in soil appears to be a useful tool for evaluating the potential heavy metal hazards of the environment [12]. Hence this study evaluated the concentrations of heavy metals and microbial flora in the soils around sawmilling sites.

2. Methods

Digestion of soil: 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).

2.1. 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 in [13]. Dilutions from 10^{-3} and 10^{-4} were inoculated onto fresh Tryptone Soya Agar and Sabouraud Dextrose Agar Plates. The Spread plate method of inoculation was used where 0.1ml of the respective dilutions (10^{-3} and 10^{-4}) were plated on various agar plates and evenly spread over the entire plate using a flame sterilized glass rod. The inoculated plates were incubated at 35°C for 24hrs for bacteria and at room temperature (25+2°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.

2.2. Molecular Characterization

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. 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.

3. Results

The levels of lead observed at the Ahiaeke sawmill during the rainy seasons at distances of 0m - 500m were 4.25mgkg to 1.24mgkg. The values recorded for the soil samples collected at the okigwe sawmill also was in the range of 4.96mgkg to 0.88mgkg. The levels of lead across the different distances and seasons differed significantly at $p<0.05$.

Iron had the highest concentration among all the metals studied Iron concentration for the sawmill soil samples from Ahiaeke were: 244.60mg/kg to 106.60mg/kg. The levels of iron across the different distances had significant differences at $p<0.05$. The concentration range of Iron for the Okigwe sawmill were: 345.60mg/kg to 210.10mg/kg respectively. Also there was a significant variation between distances of 0m (244.60mg/kg) and 100m (116.50mg/kg) at the Ahiaeke site (Table 2). The concentration of Iron was found more on the edge (0m) and decreased as the distances increased. The concentration range of copper in the soil samples from Ahiaeke sawmilling site across the different distances were between: 1.14mg/kg to 0.168mg/kg. Also, concentration range at Okigwe were between 2 4.40mg/kg to 0.39mg/kg (Tables 1 and 2). The high concentrations of copper (4.40mg/kg and 1.14mg/kg for Okigwe and Ahiaeke respectively) as obtained during the rainy season in the surface soil horizons (0m) are mainly of anthropogenic origin. There was a significant variation between distances of 0m (1.14mg/kg) and 50m (0.84mg/kg) at the Ahiaeke site during the rainy season (Table 2). Significant differences ($p<0.05$) was also observed for the samples from Okigwe at 0m (4.40mg/kg) and from that of 500m (0.39mg/kg).

The microorganisms isolated from the study sites as identified through sequencing of the 16SrRNA gene included species of *Enterobacter hormachei*, *Alcaligenes faecalis* and *Proteus* species. The distribution of bacterial and fungal isolates across both sites revealed *Bacillus circulans* as the most predominant bacterial isolate. Other isolates with high level of prevalence included *Alcaligenes faecalis* and *Pseudomonas aeruginosa*.

Table 1. *Metallic Ion Content of Soil Sample of the Various Study Locations during Rainy Season.*

Parameter	Okigwe				
	Om	50m	100m	500m (Control)	LSD
Zinc (mg/kg)	20.30^{a}	15.10^{c}	18.60^{b}	12.20^{d}	1.70
Iron (mg/kg)	345.60^{a}	200.40^{c}	196.70^{d}	210.10^{b}	3.70
Cadmium (mg/kg)	1.025^{a}	0.930^{b}	0.216^{c}	0.064^{d}	0.15
Chromium (mg/kg)	0.750^{a}	0.110^{d}	0.135^{c}	0.145^{b}	0.02
Copper (mg/kg)	4.40^{a}	3.10^{b}	1.86^{c}	0.39^{d}	1.30
Lead (mg/kg)	4.96^{a}	4.00^{b}	3.10^{c}	0.88^{d}	0.90

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 Rainy Season.*

Parameter	Ahiaeke				
	Om	50m	100m	500m (Control)	LSD
Zinc (mg/kg)	16.30^{b}	25.38^{a}	14.25^{c}	10.20^{d}	2.05
Iron (mg/kg)	244.60^{a}	202.80^{b}	116.50^{c}	106.60^{d}	9.90
Cadmium (mg/kg)	0.081^{a}	0.038^{c}	0.036^{c}	0.047^{b}	0.01
Chromium (mg/kg)	0.039^{a}	0.022^{c}	0.029^{b}	0.044^{a}	0.01
Copper (mg/kg)	1.14^{a}	0.84^{b}	0.346^{c}	0.168^{d}	0.18
Lead (mg/kg)	4.25^{a}	2.45^{b}	0.86^{d}	1.24^{c}	0.38

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

Table 3. *Microbial Counts of Sawmill and Control Soil Samples at different distance at Okigwe in cfu/g.*

	0m	50m	100m	500m
THBC	$5.4x10^{7}$	$5.00x10^{7}$	$4.7x10^{7}$	$4.4x10^{7}$
TCC	$2.1x10^{4}$	$1.9x10^{4}$	$1.7x10^{4}$	$1.5x10^{4}$
TPSBC	$2.3x10^{4}$	$2.5x10^{4}$	$1.8x10^{4}$	$2.0x10^{4}$
TNBC	$2.7x10^{6}$	$2.5x10^{6}$	$2.6x10^{6}$	$2.4x10^{6}$
TAC	$2.5x10^{5}$	$2.4x10^{5}$	$2.2x10^{5}$	$2.1x10^{5}$
TFC	$4.9x10^{7}$	$4.6x10^{7}$	$4.4x10^{7}$	$4.7x10^{7}$

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 4. *Microbial Load of Sawmill and Control Soil Samples at different distance at Ahiaeke in cfu/g.*

	0m	50m	100m	500m
THBC	$4.60x10^{7}$	$4.70x10^{7}$	$4.40x10^{7}$	$4.10x10^{7}$
TCC	$1.6x10^{4}$	$1.3x10^{4}$	$1.5x10^{4}$	$1.2x10^{4}$
TPSBC	$2.0x10^{4}$	$1.7x10^{4}$	$2.1x10^{4}$	$1.8x10^{4}$
TNBC	$2.3x10^{6}$	$2.6x10^{6}$	$2.4x10^{6}$	$2.1x10^{6}$
TAC	$2.2x10^{5}$	$2.4x10^{5}$	$2.3x10^{5}$	$2.1x10^{5}$
TFC	$4.5x10^{7}$	$4.3x10^{7}$	$4.6x10^{7}$	$4.4x10^{7}$

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.

4. Discussion

All the studied metals (Cr, Pb, Cd, Cu, Fe and Zn) investigated were found to be present in the soil samples. The elevated concentration level of lead (Pb), at other distances studied relative to the 500m distance as witnessed from both Sawmill sites might be attributed to the deposition from the automobile exhaust fumes used at the sites as an earlier studes has reported that Nigeria gasoline is usually leaded. Similar observation was reported by Ene *et al.* [14] on soil sample in Romania. Furniture makers within these sites most often rely on generators for operation thus increasing the amount of lead released into the surrounding environment. These are important contributory factors for the elevated level of (Pb) recorded in this investigation. The range of values of Pb observed at both sites (4.25-4.96mg/kg) were lower compared to average Pb level of 63.69±27.31mg/kg reported at Osogbo [15], 15.28-76.92µg/g at Kaduna [16] 58.56 – 342.67 µg/g 11.00 – 32.00mg/kg at Owerri [17] in Nigeria.

The consistently high load of iron (244.60-345.6mg/kg) recorded in all the sites is expected because iron is a major constituent of saws used in sawmill operations or wood processing. This finding is in line with what was recorded earlier for iron and lead concentrations in soil samples from sawmill sites in Sapele [18]. The Value of Zn (16.30-20.30mg/kg) obtained is higher than all other metals investigated except Fe, but lower than 25.68± 4.67mg/kg reported for 10m for road side soil in Osogbo [15].

The value of copper obtained in this study from the Okigwe soil sample (4.40mg/kg) during the rainy season is within the range (2.44 and 4.21mg/kg) reported by Niedzwiecki *et al.* [19], in a previous investigation of heavy metal levels in Bauchi, Nigeria. The concentration of this metal might be connected to the deposition of the chemicals used in wood preservation on the receiving soil. The concentration of copper was found more on the edge (0m) and decreased as the distances studied. The findings of this study were also confirmed by the studies of Nabulo et al. [20], with their reported higher values of copper obtained on the edge of the soil. The concentrations of cadmium from soil samples at the Okigwe site were significantly different at $p<0.05$. The values of cadmium as obtained in this study (0.064-1.025mg/kg) could be compared with 0.85mg/kg reported for soil by Fakayode and Olu-Owolabi [15] and 0.35mg/kg reported for roadside topsoil by Reimann *et al.* [21].

The obsersed reuction of the concentrations of the heavy metals at 500m distance away from operational site suggests possible inputs from the sawmill operation to the metal content in the study sites, thereby, confirming sawmill operations as potential source of soil contamination.

The high number of bacterial count as obtained in this study could be attributed to anthropogenic activities by workers at the various sites. The counts as observed could probably be because of runoff water that carried dirt and debris from nearby surroundings to the study sites. The bacterial count of soil has previously been reported to be affected by high concentrations of lead and other heavy metals [22]. The more the lead concentration in a soil samples, the less the number of bacterial count and vice versa. This finding agrees with work of Wyszkowska [23] where it was reported that "In a soil contaminated by higher doses of heavy metals regardless of the soil use, cadmium, copper and lead significantly reduced the count of heterotrophic bacteria".

The bacterial counts at both sites show that soil from okigwe had slightly higher load. It had 5.4 × 10^7cfu/g, 5.0×10^7cfu/g and 4.4×10^7cfu/g at 0m, 50m and 100m respectively. It also had 4.9× 10^7cfu/g, 4.6×10^7cfu/g and 4.4×10^7cfu/ at 0m, 50m and 100m respectively for total fungal load. The soil samples from 500m distance which was used as control had the least number of the bacterial counts at both sites with counts 4.4×10^7cfu/g and 4.1×10^7cfu/g for the Okigwe and Ahiaeke sawmills respectively. Significant reductions in microbial biomass was found in uncontaminated soils (500m) compared to metal contaminated soils (0m). Adaptation could thus be the mechanism behind the responses of microbes to the presence of increased heavy metals concentration at the lower distances studied (100m); as well as in variations of metal bioavailability.

The samples from Ahiaeke and Okigwe sites in the present study harbored coliforms (including *Escherichia coli, Proteus* species and *Enterobacter* species). The coliform load were, however, not significantly different among the samples. The presence of coliforms in the samples could be attributed to exposure to conditions favorable for the introduction and growth of this group of organisms [24].

5. Conclusion

The level of these metals especially Pb and Cd contents in Ahiaeke-Ndume Ibeku and Okigwe Sawmill is a pointer to pollution of these sites with heavy metals. The concentrations of the metals as observed from this study may not portray significant exposure risks, however there are fears that accumulation of these metals over time might be of concern in future. Hence this study calls for continued assessment and monitoring of activities within sawmills and it's surrounding to protect the environment from imminent harm from excessive accumulation.

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