

# EFFECTS OF LEAD (Pb) POLLUTION ON SOIL FERTILITY CHARACTERISTICS OF INLAND VALLEYS OF ISHIAGU, SOUTHEASTERN NIGERIA

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## ABSTRACT

The research work was conducted at two locations in 2012, 100 meters away from mining pits to determine the effects of lead, slope position and depth on the variation of soil chemical properties. Random sampling method was used to collect soil samples from the study area. Samples were collected from upper-slope, middle-slope and bottom slope positions at depths 0 – 15 cm and 15 – 30 cm. Results indicated that the soils are generally clayey to clay loam and poorly drained in all the depths. The upper slope position in the two locations gave the highest contents of sand, while the highest percent of clay and silt were obtained from the bottom slope position in both Amaeze and Ihietutu locations. Results showed a significant difference among the chemical properties of upper, middle and bottom slope soils. Soil pH of the upper-slope (6.2) was the highest followed by middle (5.9) and bottom slopes (5.8). Soil pH in 15 – 30 cm depth gave the highest significant ( $P = 0.05$ ) value (6.2) than the 0 – 15 cm depth (5.8). Exchangeable calcium and magnesium were highest at bottom slope followed by middle and upper-slopes. The results indicated that soil pH, CEC and available phosphorous increased as lead concentrations increases, total nitrogen decreases with increase in Pb, especially in Amaeze location. The soil in all the locations sampled is marginally fertile, as organic carbon and nitrogen of the fertility parameters are within the low- medium range when compared with the standard values. Exchangeable Mg, Ca and CEC are within medium and high range compared with the standard values.

**Key words:** chemical properties, floodplain, hydromorphic, leaching, Marginal fertility

## INTRODUCTION

Soil degradation is a major threat facing many agricultural soils in West Africa. This is as a result of high annual rainfall, leaching, high soil acidity, deforestation, and poor management culture obtained in the area. The obvious effect of these factors range from landslide, soil fertility depletion, loss of biodiversity and soil erosion putting agricultural ecosystem at a risk [1]. Soil fertility is an important factor which determines the growth and productivity of plants. It is determined by the presence or absence of macro or micronutrients.

Variation of soil properties within a defined climatic region may also result from topographic heterogeneity [2, 3, 4, and 5]. The resultant soil-vegetation and soil-landscape interrelationships therefore should be expected to be more complex than either of the two considered separately.

Nejad and Nejad [6] reported the effect of topography on soil genesis and development of soils and observed that slope gradient and slope length had direct and indirect effect on calcification, mineralization and soil physical and chemical properties.

The metals that are considered as heavy are those with a “density greater than a certain value, usually 5 or 6gcm<sup>3</sup>” [7]. Heavy metals agreeably are one of the major pollutants that are encountered in the soil. Most readily cited examples of these substances as shown by Wild [7], include Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni) and Zinc (Zn). As Aydinalp and Marinova [8] observed, a precise knowledge of heavy metals concentration and the forms in which they are found, their dependence on soil's physico-chemical properties provide a basis for careful soil management, which will limit as far as possible, the negative impact of heavy metals on the ecosystem. Ahmad *et al.* [9] reported that increased heavy metal content negatively affects soil microbial population, which may have direct negative effect on soil fertility.

The major effects of heavy metals pollutants amongst others is their effects on microbial activities. Higher concentration of heavy metal pollutants have been reported to have reduced soil microbial population, hence reduction in organic matter decomposition [10]. Other negative effects of heavy metals, especially as they are being discharged through industrial effluents include negative effects on porosity and water holding capacity, CEC, mineral composition and seed germination as established on an Indian soil contaminated by discharges from fertilizer factory [11].

All heavy metals are toxic at soil concentrations above normal level of 35 mg/kg [12]. The CEC of the soil is a key factor in determining heavy metal concentration and even availability in the soil. As CEC is determined by organic matter content and clay type and quantity, one is invariably saying that organic matter content and clay content affect concentration of heavy metals in the soils. Aydinalp and Marinova [8] explained the influence of these two factors on the concentration of heavy metals in the soil as follows; heavy metals tend to form complexes with organic matter in the soils which are different for each metal. In addition to forming complexes, organic matter also retains them in exchangeable forms. These two properties affect each heavy metal differently.

In general, the higher the CEC of the soil, the higher the ability of the soil to retain heavy metals, and therefore the higher the concentrations of the metals. Soil pH has direct impact on heavy metal concentration, thus, at high soil pH, heavy metals are retained in soils if the buffering capacity is high enough to resist the acidic input solution and at low levels of soil pH, cation exchange capacity becomes the more dominant process in heavy metals retention [13]. Fertilizers contain heavy metals such as lead and arsenic. Pesticides contain lead, arsenic and mercury. Sewage sludge contains cadmium, arsenic and lead [14].

Lead is certainly the most common contaminant of and permanent resident in soils [15]. Organic matter, can bind to heavy metals very effectively; for example, the number one source of lead contamination is lead-based paint, which chipped or scraped off building exteriors over periods of decades or centuries. Plant and soil microorganisms must cope with the resulting elevated levels of heavy metals in the soil. They have evolved complex systems for surviving and coexisting in such environments [13].

This research aimed at investigating the effects selected heavy metal (Pb) on the fertility indices of the soil. The objectives of the research included the determination of the concentrations of the heavy metal (Lead) in the soil, the fertility status of the soil in terms of the amounts of some of the exchangeable basic cations, the (CEC) of the soil, organic carbon, total nitrogen and available phosphorus, and the effects of the metal on the fertility parameters.

## 2. MATERIALS AND METHODS

### 2.1 Location of the Study

The study was conducted at Amony and Ihietutu villages in Ishiagu, Ivo Local Government Area of Ebonyi State, Southeastern Nigeria. Ishiagu is located between latitude 5° 55' N and 6° 00' N and longitudes 7° 30' E and 7° 35' E. The relief of the study area is low-lying and undulating [16].

The geology of the area comprises sequences of sandy shales, with fine grained micaceous sandstones and mudstones that is Albian in age and belongs to the Asu River Group. Generally they are dark coloured shales and mudstones. The dark coloured shales are believed to have formed in stagnant

marine basins and are dark coloured because they contain sulphide minerals and large quantities of organic matter [16].

Soils in the area comprise reddish brown gravelly and pale clayey soils derived from shales and shallow pale brown soils derived from sandy shales. All the soils are residual. The red yellow soils are derived from the red and reddish-yellow earth formed by the weathering and subsequent ferruginisation of underlying sandstone units, the shales and igneous rocks which form the bedrock [16]. The soil classification is Ultisol, which is hydromorphic, of shale parent material with underlying impervious layer at about 40 cm depth. It is characterized by rampant flooding and water logging which is a precipitate of poor drainage resulting from the impervious layer, high soil bulk density and crusting [17]. The flooding is experienced about the peaks of the rainy season (July and September) and covers the basins and floodplains around the middle and lower courses of the river and the streams [18].

## 2.2 Collection of Soil Samples

Random **sampling** method was used to collect soil samples from the study area. Twelve (12) auger samples were collected from each sampling location at 0-15 cm and 15 – 30 cm depths at the upper, middle and lower (bottom) courses of the streams at both east and west sides of banks. This means that two (2) points were sampled from each slope position **with two soil samples from each**. The auger samples were stored in labeled polythene bags. They were dried under shade for three days, crushed, sieved with a 2 mm sieve and taken to the laboratory for the determination of particle size distribution and **some chemical properties**.

## 2.3 Laboratory Methods:

Particle size distribution using hydrometer method according Gee and Bauder [19]. Soil pH was measured in a 1:2.5 soil:**0.4: 0.1** M KCl suspensions (**H<sub>2</sub>O and KCl**) [20]. The soil OC was determined by the Walkley and Black method described by Nelson and Sommers [21]. Total nitrogen was determined by semi-micro kjeldahl digestion method using sulphuric acid and CuSO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub> catalyst mixture Bremner and Mulvaney [22]. Exchangeable bases were determined by the method of Thomas, [23]. CEC was determined by the method described by Rhoades [24]. Available phosphorus was measured by the Bray II method [25]. The double acid digestion technique [26] was used in sample extraction using HCl.HNO<sub>3</sub> to digest the soils for the heavy metal analysis. The lead concentration was determined by using an Instrumentation Laboratory IL251 Atomic Absorption Spectrophotometer equipped with two hollow cathode lamp holders and Rank-Hilger slotted cathode lamps.

## 2.4 Data Analysis:

Data analysis was performed using **GENSTAT 3** 7.2 Edition. Significant treatment means **were** separated and compared using Least Significant Difference (LSD) and all inferences were made at 5% **Level** of probability.

# 3. RESULTS AND DISCUSSION

## 3.1 Variations on the Physical Properties of Studied Soil

Results of physical and chemical properties of the soils samples studied are shown on (Table 1 and 2). The soils are generally clayey to clay loam. Poorly drained in all the depths, this could be as a result of the influence of parent materials, which is clayey in texture. The clay content of the soils were generally medium to high, ranging between 29 and 57% in the **0 – 15 cm and 15 – 30 cm** depths. This could be as a result of geological fertilization of inland valleys (the transportation of clay and other finer materials on the upland soils by serious sheet erosion down the lowland) observed in the study areas. Moreso, the clay percent was greater within 15 – 30 cm than 0 -15 cm soil depth in both locations. This could be as a result of the transportation of clay by leaching observed in the study area. Silt content was also very high in the two soil depths, ranging between 25 and 43%. In few cases the values did not follow a definite trend, showing little fluctuations within depths of **0 – 15 cm and 15 – 30 cm**. However, soil depth of 0 – 15 cm contained higher percent of silt particles.

The sand content of the soils were generally low to medium, ranging between 4 and 44% in the soil depths. This could be attributed to the parent material which are poorly leached followed by the continuous accumulations of clay and silt contents of the soil thereby increasing the aggregate stability of the soil. The value did not follow a defined trend within the depth.

The results also indicated that there were variations on the percent sand, clay and silt soils among the slope positions. It was obtained that upper slope position in the two locations recorded the highest contents of sand, while the highest percent of clay and silt were obtained from the bottom slope position in both Amaeze and Ihietutu locations. The could be attributed to the geological fertilization of inland valleys and transportation of clay from upper slope positions by leaching which allows continual accumulation of clay and silt materials at the bottom slope positions in the study area.

**Table 1: Physical properties of studied soils**

Location	Slope	Depth (cm)	Sand	Silt	Clay	Textural Class
				%		
Amaeze	Upper	1	34	33	33	CL
Amaeze	Upper	2	44	25	31	CL
Amaeze	Middle	1	38	33	29	CL
Amaeze	Middle	2	38	31	31	CL
Amaeze	Bottom	1	24	33	43	C
Amaeze	Bottom	2	20	35	45	C
Ihietutu	Upper	1	4	43	53	SC
Ihietutu	Upper	2	4	41	55	SC
Ihietutu	Middle	1	18	33	49	C
Ihietutu	Middle	2	4	39	57	C
Ihietutu	Bottom	1	18	29	53	C
Ihietutu	Bottom	2	8	35	57	C

### 3.2 Variations in the Chemical Properties of the Studied Soil

The variations in the distribution of the soil chemical properties in the studied locations was shown in Table 2. The results showed that the soil pH measured in water varied significantly ( $P = 0.05$ ) between the two locations. It was recorded that the highest pH mean value (6.2) was obtained from Ihietutu location. The results indicated that the pH decreased with slope position with the highest value obtained from the upper slope of ihietutu location. This result did not conform to the findings from the work of Garcia *et al.* [27] who reported highest  $\text{Na}^+$  concentration at bottom slope position of 30 eroded sites. Hendershot *et al.* [28] also reported slightly higher pH at the down slope positions. The result indicated that soil depth gave significant ( $P = 0.05$ ) variation on the pH with 15 – 30 cm soil depth giving the highest significant value, while the least pH was obtained from the 0 – 15 cm depth. The increase in soil pH down the profile could be attributed to the downward movement of Ca and accumulation therein the 15 – 30 cm depth. Previous researches also reported a sharp increase in soil pH with increasing soil depth [29, 30] due to higher accumulation of  $\text{Ca}^{2+}$  in the sub-surface soil [31]. Hao and Chang [32] reported similar results and revealed that in irrigated soils  $\text{Ca}^{2+}$  decreased in surface soil (0-15 cm) but increased at depths below 30 cm due to the downward movement of lime with percolating water to subsurface soil that cause an increase in soil pH. However, the decrease in the pH as the slope decreases followed the lead concentration in the slope positions. It was recorded that lead concentration decreased as the slope decreases with the highest concentrations on upper slope position of Ihietutu location that also recorded the highest pH and CEC values (Table 3). This is in conformity with the submission that the higher the CEC of the soil, the higher the ability of the soil to retain heavy metals, and therefore the higher the concentrations of the metals. Also, at high soil pH, heavy metals are retained in soils if the buffering capacity is high enough to resist the acidic input solution and at low levels of soil pH, cation exchange capacity becomes the more dominant process in heavy metals retention [13].

The results also indicated that organic carbon (OC) was affected positively ( $P = 0.05$ ) by both location and depth. It was obtained that Ihietutu site with 2.12 % OC was significantly higher than Amaeze site

with mean value of 1.41 %. Results regarding soil organic carbon and total nitrogen, though not significant, revealed an increasing trend from upper to bottom slope position which might be due to their downward movement with runoff water from upper slope and accumulation there at the bottom slope position. Previous researchers [33] argued that the amount of soil organic matter in the semi-arid region is the main factor controlling soil available phosphorous and other soil fertility parameters. Thus decrease in soil organic matter content at upper slope (and vice-versa), with erosion hazards, might have decreased the available P and K in soil at upper slope position [34].

The result showed much significant soil organic carbon pool on soil depth 0 – 15 cm. This could be attributed to high organic matter or finer soil particles that accumulate on the top soil due to litter fall or plant stubbles decomposition.

The results equally indicated that exchangeable sodium, potassium, calcium and CEC only varied significantly ( $P = 0.05$ ) among the locations with the highest exchangeable sodium obtained from Amaeze site. However, the highest mean value (8.90 me/100g) of calcium was obtained from Ihietutu location, as against 4.07 me/100g obtained in Amaeze site. The soil CEC (45.0 me/100g) was significantly higher in Ihietutu location than at Amaeze site (29.9 me/100g). Exchangeable sodium, potassium, calcium and CEC are almost equally distributed across the whole slopes and depths in both locations. The result agrees with Barthold *et al.* [35] whom their result clearly show that topography does not control the spatial variation of exchangeable K and Mg in the tropical forest soil-scape.

It was obtained (Table 2) that exchangeable magnesium concentration varied among the locations and slopes. The downward trend with decreasing significant concentrations of exchangeable Mg agrees with the findings of Tsui *et al.* [36] that the differences in soil properties along the transect decreased from gentle slope to very steep slope, were also attributable to slope processes. The results showed that there were no significant variations on the soil base saturation, exchangeable acidity and available phosphorous among the slopes and soil depths studied in the two locations. It was observed that the soil available phosphorous, though not significant, increased down the slope position in Amaeze location.

The mean values for all the fertility parameters measured are shown in Table 2. It could be said that the soil in all the locations sampled is only marginally fertile, especially as some of the parameters (organic carbon and nitrogen), are only within the low-medium range when compared with the standard values given by Landon [37]. Exchangeable Mg, Ca and CEC are within medium and high range compared with the standard values given by Landon [37]. Marginal fertility is a characteristic of many tropical soils mainly because of the high rate at which organic matter is lost, high rate of leaching, highly weathered mineral and low input agricultural practices. Results shows that in all the samples the total nitrogen values were very low to high ranging between 0.11 – 1.36% and there was a decrease with depth in all the slope with Amaeze middle, recording the highest value of 1.36%.

The values recorded at Ihietutu for most of the parameters may even be regarded as the only values that can be described as reasonably above marginal level. This may probably be due to the fact that higher levels of organic wastes are incorporated into the cultural practices of the areas. This is clear from the difference in the organic matter values of the area compared to the other area. The phosphorus levels in the two areas are drastically lower than even the values suggested by Landon [37] as low.

**Table 2: Mean values of the fertility indices determined for the different locations**

Location	Slope	Depth (cm)	pH	OC (%)	TN (%)	Na (Me/100 g)	K Me/10 0g	Mg Me/10 0g	Ca Me/10 0g	CEC (Me/100 g)	Avail. P (mg/kg)
Amaeze	Upper	1	5.7	1.82	0.29	0.15	0.53	4.2	4.2	30.0	3.73
Amaeze	Upper	2	6.4	1.11	0.27	0.11	0.27	2.8	3.4	23.6	3.73
Amaeze	Middle	1	5.7	1.02	1.36	0.13	0.57	1.8	2.0	26.8	3.73
Amaeze	Middle	2	5.8	0.75	0.24	0.16	0.61	1.4	2.4	17.6	3.73
Amaeze	Bottom	1	5.6	2.04	0.11	0.15	0.55	1.8	6.6	42.0	5.6.0
Amaeze	Bottom	2	5.7	1.73	0.35	0.19	0.73	3.6	5.8	39.2	5.6.0
Ihietutu	Upper	1	6.4	2.75	0.25	0.08	0.44	6.2	10.6	53.6	7.46

Ihietutu	Upper	2	6.8	1.42	0.29	0.13	0.57	5.2	8.6	45.6	6.53
Ihietutu	Middle	1	5.7	3.23	0.46	0.11	0.5	3.8	9.4	52.4	7.46
Ihietutu	Middle	2	6.2	1.51	0.28	0.10	0.42	2.0	8.2	48.8	3.73
Ihietutu	Bottom	1	5.7	2.21	0.38	0.13	0.61	4.6	7.8	26.0	4.66
Ihietutu	Bottom	2	6.3	1.59	0.29	0.08	0.38	4.0	8.8	43.6	4.66
<b>Mean</b>			<b>6.0</b>	<b>1.77</b>	<b>0.38</b>	<b>0.127</b>	<b>0.52</b>	<b>3.45</b>	<b>6.48</b>	<b>37.43</b>	<b>5.05</b>
<b>CV %</b>			<b>3.0</b>	<b>29.8</b>	<b>84.8</b>	<b>21.9</b>	<b>25.9</b>	<b>23.1</b>	<b>22.2</b>	<b>30.6</b>	<b>28.7</b>
<b>LSD<sub>(0.05)</sub> Location</b>			<b>0.243</b>	<b>0.718</b>	<b>NS</b>	<b>0.0379</b>	<b>NS</b>	<b>1.09</b>	<b>1.97</b>	<b>15.65</b>	<b>NS</b>
<b>LSD<sub>(0.05)</sub> Slope</b>			<b>0.298</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>1.33</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>LSD<sub>(0.05)</sub> Depth</b>			<b>0.243</b>	<b>0.718</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>Standard (Landon, 1991)</b>											
	<b>High</b>			<b>3.35</b>	<b>0.30</b>	<b>2.00</b>	<b>15.00</b>	<b>1.20</b>	<b>8.00</b>	<b>30.00</b>	<b>140</b>
	<b>Medium</b>			<b>2.00</b>	<b>0.15</b>	<b>0.70</b>	<b>5.00</b>	<b>0.60</b>	<b>3.00</b>	<b>15.00</b>	<b>60</b>
	<b>Low</b>			<b>0.75</b>	<b>0.05</b>	<b>0.30</b>	<b>2.00</b>	<b>0.20</b>	<b>0.50</b>	<b>6.00</b>	<b>20</b>

Depth 1 = 0 – 15 cm, Depth 2 = 15 – 30 cm, NS = Not significant, OC = organic carbon, TN = total nitrogen, Na = exchangeable sodium, K = exchangeable potassium, Ca = exchangeable calcium, CEC = cation exchange capacity, BS = base saturation, EA = exchangeable acidity, Avail. P = available phosphorous.

Table 3 shows the comparison of the concentration of the metal (Lead) in the sites investigated and the minimum approved values under European regulations and American literature. The result indicates that despite the variability in the metal values between the depths and slopes, and the fact that the two locations are close to mining pits, mean values of the metal investigated for both sites studied were lower than both the Bowen (1979) in Aydinalp and marinova [8] and the EU recommended means. Therefore, lead concentration in the soils of the locations studied has no much significant impact on the fertility decline of the area. Despite the variability, the results are in somewhat not in close agreement with the findings of Anonymous [38] in the soils of the Jakara dam irrigation site in which case the concentration of lead was found to be appreciably high (up to  $27.9\mu\text{g g}^{-1}$ ).

**Table 3: Mean concentrations of lead for the two studied sites**

Sample site	Slope	Depth	Lead concentration (mg/kg)	Soil pH	Soil CEC (me/100)
Amaeze	Upper	1	0.286	5.7	30.0
Amaeze	Upper	2	0.326	6.4	23.6
Amaeze	Middle	1	0.401	5.7	26.8
Amaeze	Middle	2	0.571	5.8	17.6
Amaeze	Bottom	1	0.526	5.6	42.0
Amaeze	Bottom	2	0.553	5.7	39.2
Ihietutu	Upper	1	0.841	6.4	53.6
Ihietutu	Upper	2	0.828	6.8	45.6
Ihietutu	Middle	1	0.705	5.7	52.4
Ihietutu	Middle	2	0.649	6.2	48.8
Ihietutu	Bottom	1	0.839	5.7	26.0
Ihietutu	Bottom	2	0.668	6.3	43.6
<b>LSD<sub>(0.05)</sub> Location</b>			0.1633		
<b>Minimum allowable concentration of lead in soils (mg/kg)</b>					
Bowen (1979)			35		
(Aydinalp and Marinova (2003))					
EU Values (Wild, 1996)					

Sources: Lab. Analytical data and Bowen, (1979) in Aydinalp and Marinova (2003)

### 3.3. Effects of Lead (Pb) Concentration on the Soil Fertility Parameters

The result (Figure 1) showed that the soil pH increases as the concentration of Pb increases in the soil. The result agrees with the findings of Sharma and Agrawal [13], that at high soil pH, heavy metals are retained in soils if the buffering capacity is high enough to resist the acidic input solution and at low levels of soil pH, cation exchange capacity becomes the more dominant process in heavy metals retention. It was also obtained that the highest soil organic carbon pool was recorded in areas with moderated concentrations of Pb (Figure 2).

The result indicated that the soil total nitrogen was negatively affected, as higher concentration of lead in the studied soils reduced the levels of nitrogen availability in the soil (Figure 3). The result in Figure 4 had the same trends as was obtained in the soil pH. The soil CEC increased with increase in the concentrations of Pb in the soil (Figure 4). This is in agreement with the submission that, the higher the CEC of the soil, the higher the ability of the soil to retain heavy metals, and therefore the higher the concentrations of the metals [13].

The result of Figure 5 indicated that the soil available phosphorous increases with increase in lead concentration in Amaeze site.

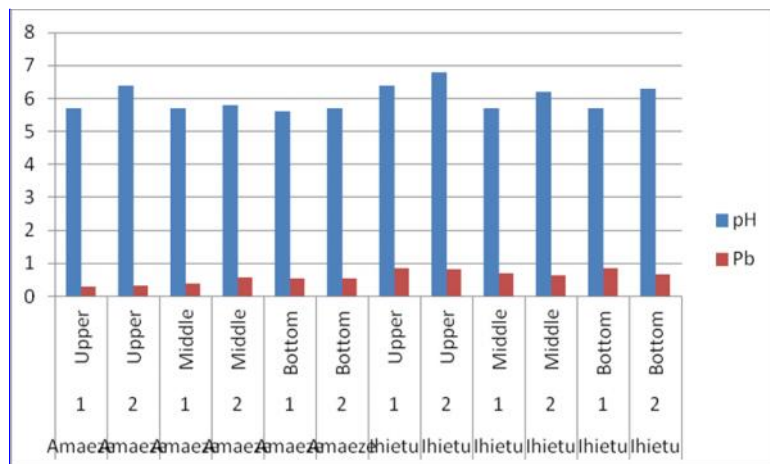


Figure 1: Effect of lead concentration on the soil pH

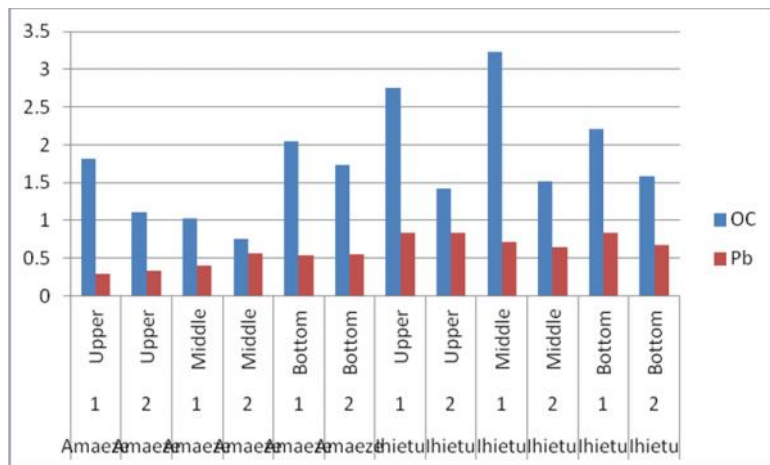


Figure 2: Effect of lead concentration on the soil organic carbon

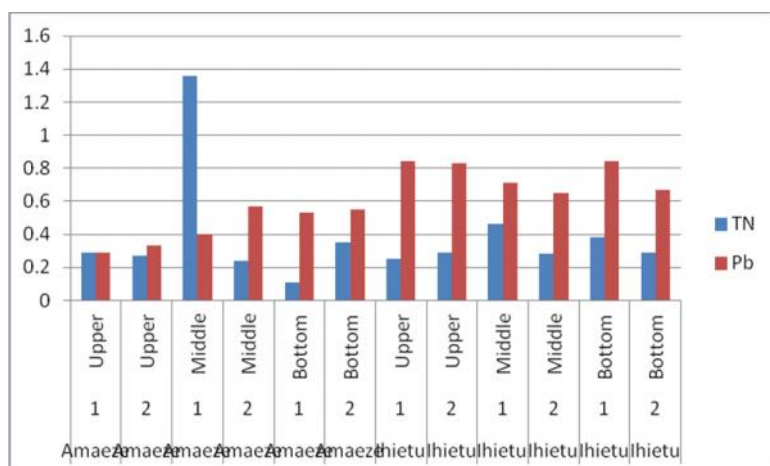


Figure 3: Effect of lead concentration on the soil total nitrogen



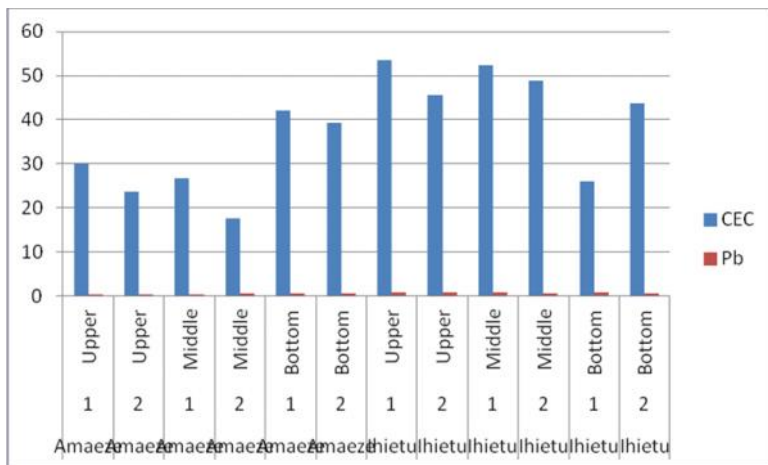


Figure 4: Effect of lead concentration on the soil CEC

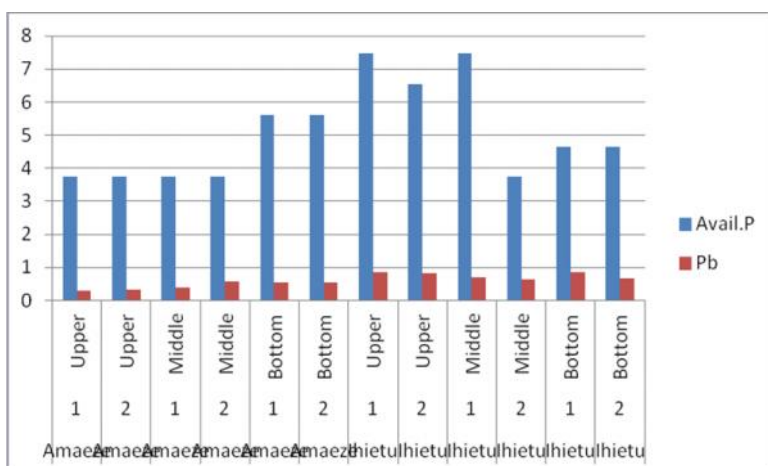


Figure 5: Effect of lead concentration on the soil available phosphorous

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#### 4. CONCLUSION

Soils of the studied areas are generally clayey to clay loam and poorly drained in all the depths. The upper slope position in the two locations gave the highest contents of sand, while the highest percent of clay and silt were obtained from the bottom slope position in both Amaeze and Ihietutu locations. Consequently, a significant difference among the chemical properties of upper, middle and bottom slope soils. The soil pH, CEC and available phosphorous increased as lead concentrations increases, while total nitrogen decreases as the Pb increases. Despite the variability in the metal values between the depths and slopes, and the fact that the two locations are close to mining pits, the mean values of metal investigated for both sites studied were lower than both the Bowen (1979) and the EU recommended means. Therefore, lead concentration in the soils of the studied locations has no much significant impact on the fertility decline of the area. It could safely be concluded that the quality of the soil for production, although not immediately under threat especially with the very low mean values of the pollutant and the lack of significant effects it exert on many of the fertility indices determined. However such safety cannot

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be guaranteed forever. This is because the pollutant **may be** gradually building up, because of its nature of forming complexes and not being easily leached out.

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## AUTHORS' CONTRIBUTIONS

" 'Author 1' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author 2' and 'Author 3' managed the literature searches. 'Author 4 and 5' managed the analyses of the study ... All authors read and approved the final manuscript."

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