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# INFLUENCE OF HOSPITAL WASTES ON SOIL ECOSYSTEM AND ITS IMPLICATIONS FOR AGRICULTURAL ACTIVITIES IN NIGERIA

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The recent proliferation of agricultural activities (farming and grazing of animals) within hospital premises is a major concern, leading our team of researchers to conjecture that such an environment may have adverse effects on plants, animals, and human health. This research sought to assess the microbial and heavy metal status of soils under cultivation within hospitals in Calabar, with a view to determining the implications for food and farmer's safety. Four hospitals with open dumps and one with an incinerator, all with ongoing farming activities within one meter from the hospital dump-sites were identified within Calabar metropolis for the study. Crops were cultivated less than 1 m distance around the open dumps and incinerator within the hospital premises. Forty-eight composite top (0-15 cm) and sub (15-30 cm) soil samples were randomly obtained from farms within the five hospitals and a control site within Calabar metropolis and analyzed for microbial properties and heavy metals zinc (Zn), lead (Pb), copper (Cu), chromium (Cr), and iron (Fe) contents. Two edible plants from each sampling site were also collected for heavy metal detection. The results showed that the soils were sandy loam in texture. The pH of soils in the study site ranged from strong to slightly acid in reaction. The results for microbial analysis indicated a proliferation of mostly pathogenic organisms at the hospital sites. The following bacterial isolates were identified from the hospital sites: *Clostridium spp., Bacillus spp., Pseudomonas spp., Micrococcus spp., Corynebacterium spp., vibrio spp, E.coli, Streptococcus spp., Salmonella spp., Staphylococcus spp., klebsiella spp., Nocardia spp., Enterococcus spp.* and *Mycobacterium spp.* Probable fungal isolates from the hospital sites include *Aspergillus spp., Mucor spp., Scopulariopsis spp., Verticillium spp., Paccilomyces spp., Phoma spp., Rhizopus spp., Alternaria spp. Drechslera spp, and Microsphaeropsis spp.* The metal pollution index (MPI) of the hospital sites indicates slightly to moderate pollution with Cu, Pb, Zn and very severe pollution of Cr and Zn at hospital 1. Generally, the hospital soils were excessively polluted with Fe. Based on the metal pollution index (MPI) ranking these levels of heavy metals can exert negative effects on plants, soil ecosystem and the environment in general. This was evident in the accumulation of heavy metals in soil and plant tissue grown at the hospital sites than those from the control site. However, the bio-accumulation of Zn, Cu, and Fe in plants from the hospital sites were below the standards by FAO/WHO. Owing to the preponderance of pathogenic organism observed and the increasing levels of heavy metals in soil and plants recorded in this research, it is recommended that agricultural activities should not be carried out at hospital sites to prevent contamination of pathogenic organisms by farmers and to ensure food safety.
**Keywords**: Medical waste, microbes, heavy metals, food and farmer safety.

## INTRODUCTION

Humans at some point in their life develop different health challenges and as such medicare is vital for human well-being. Hospital is a place with trained staff that provide superior and quality healthcare services to people with health challenges (Fatima *et al.,* 2018). However, as important as health care services are, their delivery process generates a lot of solid and liquid wastes commonly referred to as biochemical or hospital wastes. The various forms of hospital waste as outlined by Parn (2007), Nasima (2000) and Lekwot (2012) include: (1) Biological/pathological wastes. (2) Infectious wastes containing pathogens in sufficient concentration or large quantity that could cause disease e.g. culture and stock of infectious agents from laboratories, surgery waste, originating from infectious patients. (3) Pharmaceutical wastes. (4) Cytotoxic wastes made up of expired or left over cytotoxic drugs, equipment contaminated with cytotoxic substance. (5) Chemical waste comprising discarded solids, solutions and gaseous chemical, disinfecting products, fixes and photographic developers etc. (6) Radioactive waste arising from radioactive nuclides for *in vitro* analysis of body tissues and nuclear medicine, (7) Waste containing heavy metals from batteries and mercury from broken thermometers or manometers, fluorescents light tubes. Hospital wastes are reported to be loaded with microbes (ICRC, 2011). Reports indicate that, more often than not, biomedical wastes generated from hospitals in Nigeria are

deposited and burnt in poor uncontrolled dumpsites, poured in drains, or flushed into sewage tanks within the hospital premises (Inyang *et al.,* 2012). Hospital wastes may be hazardous, toxic or lethal owing to its level of heavy metal content and microbial load. Hospital waste with pathogenic bacterial load may portend great danger for disease transmission and the overall contamination of the environment (Veda *et al.,* 2007; Babanyara *et al*. 2012). Improper handling of these waste could be detrimental to the health of the host community (Aljabre, 2002). The indiscriminate dumping and sometimes burning of hospital wastes may lead to the accumulation of heavy metals in soil, plants and water aquifers which can lead to serious negative impact on human and animal consumers (Kawu and Shaibu, 2007). Researches so far carried out on hospital waste in Nigeria have been on its environmental impact and identified a zero to low-level management of wastes emanating from hospital activities, despite of the attendant risk connected with them (Ngwuluka *et al.,* 2009).

Nigeria is a signatory to the Stockholm Convention and has some relevant laws on the books (UNDP, 2020), for proper hospital waste management. However, it is a common practice in Nigeria that hospital wastes are burnt in open dumps within the hospital premises while effluents are disposed into drains. This practice is unethical but the most endangering aspect is the practice of leasing out small portions of land within the hospital facility for farming activities. Some farmers have resorted to growing crops at distances less than one meter from the hospital dumps while their livestock feed on free-range on the dump with its attendant risk. Farmers children scavenge for plastics and metals from the dumpsite. Hospital wastes could pose a great challenge for both humans and the natural environment as pathogenic substances may become accessible to plants, farmers, scavenger and animals (Lekwot *et al.,* 2012). The growing level of farming activities around hospital waste dump sites calls for concern as this could lead to an outbreak of diseases in humans/animals and accumulation of metals in edible parts of plants. On the other hand, disposal of biomedical wastes of heavy metal origin may lead to the accumulation of heavy metals in soil and surface water and could be inimical to deep feeding plants (Anikwe and Nwobodo, 2001). Though a good number of metals are indicated in plant and animal nutrition when present in trace level, nevertheless, they pose great danger when accumulated in large amounts (Tuzen, 2003). Researches so far carried out on hospital waste in Nigeria have been on its environmental impact and identified a zero to low-level management of wastes emanating from hospital activities, despite of the attendant risk connected with them (Ngwuluka *et al.,* 2009). There has been a dearth of knowledge on the implication of hospital waste for agricultural activities. Therefore, this research seeks to assess the microbial status and the heavy metal concentrations of plants and soil under cultivation within selected hospital facilities in Calabar metropolis. Findings from this study will help enlighten farmers and the general public on the inherent dangers and implications of carrying on agricultural activities using hospital wastes or within hospitals which many researchers have not explored.

## MATERIALS AND METHODS

***Location of study*:** The research was located within the Calabar metropolis. Calabar lies between latitudes 04º 57' 32.15" N and longitudes 08º 19' 37.02" E. The study locations include: the control, Hospital 1, Hospital 2, Hospital 3, Hospital 4 and Hospital 5 as shown in Figure 1. Four hospitals with open dumps and one with an incinerator were identified within Calabar metropolis. Farming activities were ongoing within the hospital premises as at the time of sample collection. Crops were cultivated within 1 m distance around the open dumps and incinerator within the hospital facility. Common crops cultivated in the study area included cassava, okra, waterleaf, maize, potato, and fluted pumpkin. This research project was conducted from March 2018 to September 2019.

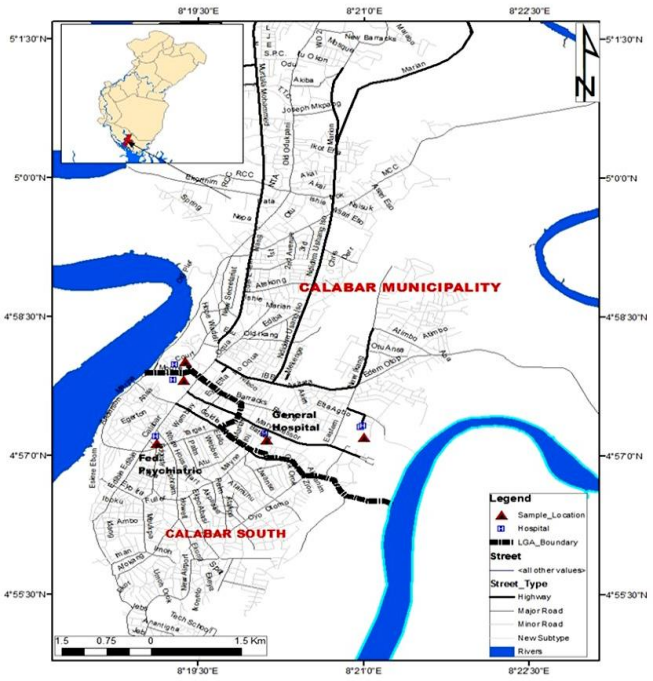


**Figure 1. Study Area Map**

***Climate and Geology of the Study Area*:** Calabar has a tropical climate comprising mainly of rainfall and excessive cloud cover. Annual rainfall in the area ranged between 2290 mm to 2680 mm. This large amount of rainfall is distributed following a bimodal pattern (June to July and September to October) with a clear dry season of about 3–4 months. Relative humidity (82 to 87%) and Ambient temperature (21

to 24ºC and 27 to 30ºC) are high throughout the year. The area is characterized by the tropical maritime wind (FAN, 2013). Calabar is located within the rainforest ecological zone with ample areas of virgin vegetation. The soils are developed from a coastal plain sand parent material (John *et al.,* 2018; Akpan-Idiok *et al.,* 2012) which are characterized by low natural fertility.

***Sampling procedures*:** Soil samples were obtained using an auger from 4 points in cropped areas within each site at two depths; 0-15 cm (surface) and 15- 30 cm (sub-surface) giving eight samples per location and a total of forty-eight (48) soil samples from the six study sites. The samples were carefully placed into well-labeled polythene bags and then taken to the laboratory for physical and chemical analyses. Soil samples collected for microbial analysis were gotten using sterile specimen vessels, placed in an ice chest and transported to the microbiology laboratory for immediate analysis.

***Chemical Analyses*:** Soil pH was determined potentiometrically in a 1:2.5 soil to water ratio. The soil organic matter (SOM) was calculated from the soil organic carbon (SOC) obtained by the Walkley and Black wet oxidation method as outlined by Udo *et al*. (2009) by multiplying with a standard, 1.72.

***Microbial Analyses*:** Soil extract media was used as a diluent for the recovery and enumeration of soil bacteria as outlined by (Alef, 1995; Zuberer, 1994). Lauryl tryptose broth with methylumbelliferone glucuronide (LTB-MUG) was used for the detection of *E. coli* as outlined by Turco (1994). Fungi were isolated using malt extract agar, potato dextrose agar and Czapek Dox agar following the procedures of Parkinson (1994).

Soil samples obtained from the study sites were serially diluted to $x10^{10}$ folds as outlined by Alef (1995). Aliquots from dilution of $10^{-6}$ and $10^{-3}$ were used for culturing bacteria and fungi colonies accordingly. The pour plate method was employed for culturing of the total heterotrophic bacterial (THB) while the spread plate technique was used for the growth of total heterotrophic fungi (THF). Platings were carried out in triplicates. Incubation of the culture was done at 30 °C (bacteria) to 45 °C (fungi) for 24 and 48 h in the same order. Pure cultures were obtained by subculturing colonies into specific agar slants. Biochemical tests were carried out on pure cultures (Alef, 1995; Zuberer, 1994).

***Characterization:*** This was done by assessing spore formation and motility. Other confirmatory tests carried on include: catalase, indole, urease, oxidase and coagulase production; sugar fermentation, Citrate and glucose (oxidative/fermentation) utilization; hydrolysis of starch; methyl red and Voges Proskauer reaction as indicated in Table 5b were performed following Alef (1995) and Zuberer (1994). Bacterial characteristics were observed by viewing under an objective lens (X40) and identified following Bergey's Manual (1994). Blue to the violet color indicated Gram-positive (+ve) bacteria while gram-negative (-ve) were coloured red. Macroscopic and microscopic examination of fungal isolates (Table 5c) were carried out following the wet mounts technique (Alef, 1995).

***Heavy metal Analysis*:** Composite soil samples collected for the research were air-dried and replicated, 2.5 g of each sample were accurately weighed under laboratory conditions, ground and passed through a 2 mm mesh of sieves. Samples were then analyzed with XRF Delta Premium Spectrometer 2019 using the procedures of Tejnecky *et al*. (2015). These metals zinc (Zn), lead (Pb), copper (Cu), chromium (Cr), and iron (Fe) were measured in triplicates using the machine.

***Quality Assurance*:** The quality assurance and control purpose, the standard reference material for a portable device (XRF 2711a and NIST 2711a, the National Institute of Standards and Technology), was applied in the analysis to ensure quality compliance. The reference material was occasionally measured alongside with soil samples to ensure that the analysis remained accurate until completion. The detection limits for the heavy metals tested were < 10 mg / kg (Ni), < 10 mg / kg (Cu), < 5 mg / kg (Sr), < 20 mg / kg (Ba), < 5 mg / kg (Ti), < 10 mg / kg (Fe) and < 10 mg / kg (Cr).

***Statistical Data Analysis*:** The data collected were subjected to discrete statistics using SPSS and R software.

***Soil metal contamination/pollution index (MPI) quantification*:** Determination of the metal contamination /pollution index (MPI), in soil was calculated as defined by Lacatusu (2000) as in the formula given below while values obtained were interpreted following the guidelines given in Table 1.

$$\text{MPI} = \frac{\text{Metal in soil}}{\text{Concentration in soil (control)}}$$

***Geoaccumulation Index (Igeo) Assessment*:** The index of geo-accumulation (*I*geo) is used to evaluate the contamination by comparing the level of heavy metal at the background sediment with levels in the contaminated sediment (Muller, 1969). The index is then calculated using the equation:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5 * B_n}\right)$$

Interpretatively, Class 0 = Igeo <0, means practically uncontaminated.

The ratio of the heavy metal concentration in the edible parts of the plant (leaves, stem, root) and the "exchangeable" or potential availability of metals in rhizosphere soils which is referred to as Bio-concentration Factor (BCF) was determined using the formula:

$$\text{BCF} = \text{Cp/Cs}$$

Interpretatively, Cp = concentration of heavy metal in the edible part of vegetable (mg $kg^{-1}$)

Cs = concentration of heavy metal in soil (mg $kg^{-1}$).

**Table 1. Interval of contamination/pollution index of heavy metals in soil and its significance.**

| MPI | Significance | Remark |
|---|---|---|
| < 0.1 | Very slight contamination | No negative effect on soil, plant and environment |
| 0.10 – 0.25 | Slight contamination | " |
| 0.26 – 0.5 | Moderate contamination | " |
| 0.5 – 0.75 | Severe contamination | " |
| 0.76 – 1.00 | Very severe contamination | " |
| 1.1 – 2.0 | Slight pollution | Will pose negative effect on soil, plant and environment |
| 2.1 – 4.0 | Moderate pollution | " |
| 4.1 – 8.0 | Severe pollution | " |
| 8.1 – 16.0 | Very severe pollution | " |
| > 16.0 | Excessive pollution | " |

Adapted from Lacatusu (2000)

## RESULTS AND DISCUSSION

Particle size analysis from the different locations indicated that sand particles dominated the study area with sand fraction > 70%, silt values ranged between 7% and 13%, while clay fraction across the study area was less than 14% for the surface and subsurface soils in the control and hospital sites. The soils were mostly loamy sand except for Hospital 5 where the soils were sandy loam (Table 2).

The pH for the control soil was strongly acid in the surface (5.2) and subsurface (5.1), while the hospital sites soils were slightly acid to near neutral in both surfaces (6.6 – 6.9 and 6.5 – 6.7). The acidic pH values obtained for the control site is typical of the soils in Calabar and in line with values reported by earlier researchers for the same area (Ediene and Umoetok, 2017). The slightly to near neutral pH observed at the hospital site could probably be attributed to the different components of hospital effluent released into the soil ecosystem.

The hospital soils differed in organic matter content from the control soil with a standard deviation (SD) of 8.72 and 6.94 and a CV of 63% and 62% (Table 3) for the surface and subsurface soils, respectively. The high organic matter recorded at the different hospital sites may not be unconnected with the indiscriminate disposal and burning of hospital wastes.

***Microbial properties of the soils studied*:** The soil microbial community has a fundamental role in remediating contaminated soils and mineralization of nutrients. The

**Table 2. Textural class, mean pH and organic matter contents of the soils from the hospital sites**

| Sampling location | Depth (cm) | Sand % | Silt % | Clay % | Texture | pH | Organic matter g $kg^{-1}$ |
|---|---|---|---|---|---|---|---|
| Control ($04^0$ 57' 13' N, $08^0$ | 0.15 | 82.0 | 7.7 | 10.3 | Loamy sand | 5.2 | 1.72 |
| 20'25' E) | 15-30 | 80.0 | 11.0 | 9.0 | Loamy sand | 5.1 | 1.50 |
| Hospital sites | | | | | | | |
| Hospital 1 ($04^o$ 56' 56"N, | 0-15 | 80.0 | 12.0 | 8.0 | Loamy sand | 6.6 | 14.96 |
| $08^o$ 19' 03" E) | 15-30 | 77.0 | 13.0 | 10.0 | Loamy sand | 6.5 | 12.38 |
| Hospital 2 ($04^o$ 57' 08" N | 0-15 | 75.0 | 12.0 | 13.0 | Loamy sand | 6.7 | 24.42 |
| $08^o$ 21' 02" E) | 15-30 | 74.0 | 12.0 | 14.0 | Loamy sand | 6.5 | 17.54 |
| Hospital 3 ($04^o$ 57' 08" N, | 0-15 | 78.0 | 12.0 | 10.0 | Loamy sand | 6.6 | 19.26 |
| $08^o$ 20' 09" E) | 15-30 | 75.0 | 13.0 | 12.0 | Loamy sand | 6.6 | 17.72 |
| Hospital 4 ( $04^o$ 57' 55"N | 0-15 | 83.0 | 10.0 | 7.0 | Loamy sand | 6.6 | 17.20 |
| $08^o$ 19' 08" E) | 15-30 | 81.0 | 10.0 | 9.0 | Loamy sand | 6.6 | 12.04 |
| Hospital 5 ( $04^o$ 57' 48" N, | 0-15 | 77.0 | 10.0 | 13.0 | Sandy loam | 6.9 | 17.20 |
| $08^o$ 19' 18" E) | 15-30 | 76.0 | 10.0 | 12.0 | Sandy loam | 6.7 | 15.48 |

**Table 3. Measures of dispersion for pH and organic matter contents of soils from the control and hospital soils in Calabar metropolis.**

| Parameter | | Min. | Max. | Mean | SD | Variance | CV (%) |
|---|---|---|---|---|---|---|---|
| pH | 0-15 | 5.10 | 6.90 | 6.20 | 0.750 | 39.46 | 12 |
| | 15-30 | 5.10 | 6.70 | 6.15 | 0.072 | 38.36 | 11 |
| Organic matter | 0-15 | 1.72 | 24.42 | 13.79 | 8.720 | 227.56 | 63 |
| | 15-30 | 1.50 | 17.72 | 11.18 | 6.940 | 116.34 | 62 |

biochemical land morphological characterization, bacterial and fungal counts and the probable diversity identified from the control and hospital sites are presented in Table 4 a,b, and c. The bacterial isolates from the control location include; *Bacillus spp., Mycobacterium spp., Pseudomonas spp.* and *Clostridium spp.* while the isolates from the hospital sites are: *Bacillus spp., Salmonella spp., E. coli, Streptococcus spp, Nocardia spp., klebsiella spp., Staphylococcus spp., pseudomonas spp., Klebsiella spp., Corynebacterium spp., Mycobacterium spp., Enterococcus spp., Vibrio spp,* and *clostridium spp.* Fifteen species of bacteria were isolated across the various hospital planting locations while four were identified from the control. The four bacterial isolates identified from the control were also observed to occur at the different hospital soils indicating that they are native to the soils under investigation. From the results obtained, it is obvious that the hospital sites had more bacterial population and diversity than the control site. Among the various hospital sites, Hospital 5, had the highest percentage count (36%) followed by Hospital 4 and Hospital 1 with 19% and 18.4%, respectively. The results for fungal isolates indicate that *Aspergillus spp., Penicillium spp., Microsporum spp., Rhizopus spp.* and *Mucor spp.* were identified for the control location while *Aspergillus spp., Mucor spp., Paecilomyces spp., Phoma spp., Alternaria spp., Scopulariopsis spp., Microsphaeropsis spp., Rhizopus spp., Syncephalastrum spp., Fusarium spp., Penicillium spp., Verticillium spp.*, and *Drechslera spp.* were isolated from the hospital sites. The hospital sites had higher fungal counts and diversity than the control. The results revealed the enrichment of bacterial and fungal communities, most of which are pathogenic (*Bacillus spp., Salmonella spp., E. coli, Staphylococcus spp., Klebsiella spp., Mycobacterium spp, Vibrio spp, Mucor spp., Paecilomyces spp., Phoma spp., Alternaria spp., Scopulariopsis spp., Microsphaeropsis spp., Rhizopus spp., Syncephalastrum spp., Fusarium spp., Penicillium spp., Verticillium spp.* and *Drechslera spp.* etc.) at the hospital sites than the control. A similar trend of higher microbial communities has been documented from medical waste sites (Babanyara et al. 2012; Abah and Ohimain, 2011; Ngwuluka *et al.*, 2009). The occurrence of non-soil living organisms in a the large population could be attributed to invading organism responding to substrate available in the hospital effluent/waste, or from laboratory cultures and specimens containing these living organisms. Although non-soil microbes are expected to have a short survival duration or regrowth in the soil (ICRC, 2011), the continuous hospital activities and indiscriminate discharge of medical effluent/waste may account for their continuous presence in soil. The slightly acid to near neutral pH (6.5 - 6.9) observed at the hospital site is favourable for a wide range of the organism. A favourable pH and availability of substrate are key to microbial survival, activities, growth, and proliferation. Biomedical wastes have high attendant risk associated with them due to the high pathogenic microbial load (Babanyara *et al.*, 2012). Farming activities in hospital locations receiving such waste/effluent could predispose farmers to disease contamination. Undue exposure of farmers to medical waste could culminate in infections, typhoid, dermatitis, astma, cholera, mutagenicity and other viral infections (Askarian, 2004). Though Ngwuluka *et al*. (2009), is of the opinion that the effect on exposure to hospital contaminants in some cases may be felt immediately until some years after. It could also contaminate the food chain with harmful microbes mostly as leafy vegetables are grown there. This could be detrimental to grazing animals and humans as well.

***Heavy metal contamination of soil and accumulation in plant tissue at the study site*:** Toxicity of heavy metals could be best described as the ability of a metal to cause undesirable changes in soil ecosystems and plants. The toxicity of a particular heavy metal is dependent on its bio-availability and its absorbed quantity. The toxic effect on soil flora and fauna is aggravated by the metal's persistent nature in soil. Results for metal contents in soil and plants at the hospital sites are shown in Tables 6 and 7, respectively while the geoaccumulation Index and enrichment factor are presented in Fig. 2. The result indicates that the control site had means of heavy metals 16.80, 5.45, 900.44 and 1.35 mg $kg^{-1}$ while the hospital sites had metal ranges of 26.30–39.0, 6.05–6.95, 2244–14764, 3.0 - 8.0 and 1.20–2.20 mg $kg^{-1}$ for Zn, Cu, Fe Cr, and Pb, respectively. The metal, Cr was not detected in the control soil. The contents of metals obtained from soils at the hospital sites were much higher than the control, giving a trend: Fe > Zn > Cu > Cr > Pb. The high concentration of Fe in the hospital site may have been contributed from expired or leftover drugs/medications, rusted hospital equipment, etc. dumped on soil for open burning or subsequent evacuation. Results obtained for the control site were similar to those obtained by Ediene and Umoetok (2017) for a control site in Calabar.

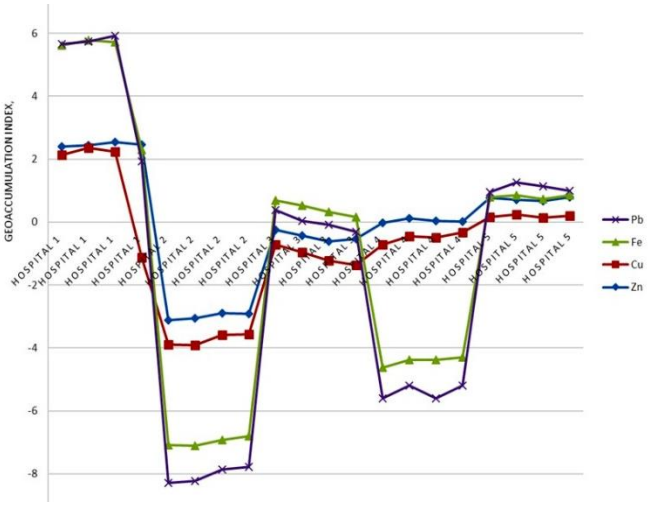


**Figure 2. Geoaccumulation plot**

**Table 4a. Bacteria and fungi species diversity and counts from hospital sites in Calabar metropolis**.

| Locations | Probable bacterial isolates | Counts | Occurrence % | Fungal isolates | Cfu/g | Percentage (%) Occurrence |
|---|---|---|---|---|---|---|
| Control ($04^0$ 57' 13 N $008^0$ 20 25 E) | *Bacillus spp.* | $1x10^6$ | | *Aspergillus spp.* | $1x10^3$ | |
| | | | | *Mucor spp.* | $1x10^3$ | |
| | *Mycobacterium spp.* | $1x10^6$ | 0.6 | *Pennicillium spp.* | $2x10^3$ | |
| | | | | *Mycosporium spp.* | $1x10^3$ | |
| | *Pseudomonas spp.* | $2x10_6$ | | *Rhizopus spp.* | $2x10^3$ | 2. |
| | *Clostridium spp.* | $2x10^6$ | | | | |
| Total | | $6x10^6$ | | | $7x10^3$ | |
| Hospital sites | | | | | | |
| Hospital 1($04^0$ 56' 56 N $008^0$ 19' 03 E) | *Bacillus spp.* | $40x10^6$ | | *Aspergillus spp.* | $9x10^3$ | |
| | *Salmonella spp.* | $40x10^6$ | | *Mucor spp.* | $5x10^3$ | |
| | *Streptococcus spp.* | $10x10^6$ | | *Paecilomyces spp.* | $6x10^3$ | 19. |
| | *Mycobacterium spp* | $20x10^6$ | | *Phoma spp.* | $4x10^3$ | |
| | *Klebsiella spp.* | $10x10^6$ | | *Alternaria spp.* | $8x10^3$ | |
| | *Nocardia spp.* | $10x10^6$ | 18.4 | *Scopulariopsis spp.* | $10x10^3$ | |
| | *Staphylococcus spp.* | $10x10^6$ | | *Microsphaeropsis* | $8x10^3$ | |
| | *Vibrio spp* | $5x10^6$ | | *Drechslera spp.* | 5x10 | |
| | *Pseudomonas spp.* | $32x10^6$ | | *Verticillium spp* | $4x10^3$ | |
| Total | | $177x10^6$ | | | $59x10^3$ | |
| Hospital 2 ($04^0$ 57' 08 N $008^0$ 21' 02 E) | *Klebsiella spp.* | $20x10^6$ | | *Phoma spp.* | $3x10^3$ | |
| | | | | *Penicillium spp.* | $10x10^3$ | |
| | *Staphylococcus spp.* | $10x10^6$ | | *Mucor spp.* | $11x10^3$ | |
| | *Salmonella spp.* | $7x10^6$ | | *Paecilomyces spp.* | $5x10^3$ | |
| | *Enterococcus spp.* | $20x10^6$ | 11 | *Syncephalastrum spp.* | $4x10^{3\backslash}$ | 17 |
| | *Mycobacterium spp* | $15x10^6$ | | *Fusarium spp.* | $10x10^3$ | |
| | *Pseudomonas spp.* | $11x10^6$ | | *Aspergillus spp.* | $15x10^3$ | |
| | *E.coli* | $10x10^6$ | | *Scopularioposis spp.* | $5x10^3$ | |
| | *Mycobacterium spp.* | $5x10^6$ | | *Drechslera spp* | $5x10^3$ | |
| | *Vibrio spp* | $10x10^6$ | | | | |
| Total | | $103x10^6$ | | | $53x10^3$ | |
| Hospital 3 ($04^0$ 57' 08 N $008^0$ 20' 09 E) | *E. coli* | $8x10^6$ | | *Verticillium spp.* | $2x10^3$ | |
| | | | | *Scopularioposis spp.* | $2x10^3$ | |
| | *Bacillus spp.* | $40x10^6$ | | *Paecilomyces spp.* | $10x10^3$ | |
| | *Corynebacterium spp.* | $30x10^6$ | | *Phoma spp.* | $5x10^3$ | |
| | *Clostridium spp.* | $10x10^6$ | 15 | *Pennincillium spp.* | $15x10^3$ | 21 |
| | *Mycobacterium spp* | $20x10^6$ | | *Mucor* | $5x10^3$ | |
| | *Klebsiella spp.* | $10x10^6$ | | *Fusarium spp.* | $15x10^3$ | |
| | *Salmonella spp.* | $5x10^6$ | | *Aspergillus spp* | $5x10^3$ | |
| | *Vibrio spp* | $10x10^6$ | | *Drechslera spp* | $5x10^3$ | |
| Total | | $143x10^6$ | | | $64x10^3$ | |
| Hospital 4 ($04^0$ 57' 55 N $008^0$ 19' 08 E) | *Streptococcus spp* | $40x10^6$ | | *Aspergillus spp* | 1x10 | |
| | *Vibrio spp* | $10x10^6$ | | *Verticillium spp* | $7x10^3$ | |
| | | | | *Drechslera spp* | $1x10^3$ | |
| | *Mycobacterium spp* | $20x10^6$ | | *Fusarium spp* | $3x10^3$ | |
| | *Klebsiella spp* | $30x10^6$ | 19 | *Phoma spp* | $2x10^3$ | |
| | *Actinobacter spp* | $20x10^6$ | | *Paecilomyces spp* | $8x10^3$ | |
| | *Corynebacterium spp* | $10x10^6$ | | *Mucor spp* | $5x10^3$ | |
| | *E.coli* | $20x10^6$ | | *Rhizopus spp* | $5x10^3$ | 20 |
| | *Clostridium spp* | $10x10^6$ | | *Alternaria spp* | $1x10^3$ | |
| | *Bacillus spp* | $20x10^6$ | | *Syncephalastrum spp* | $2x10^3$ | |
| | | | | *Scopularioposis spp* | $2x10^3$ | |
| Total | | $180x10^6$ | | | $61x10^3$ | |
| Hospital 5 ($04^0$ 57' 48 N $008^0$ 19' 18 E) | *Streptococcus spp* | $40x10^6$ | | *Pennincillium spp* | $10x10^3$ | |
| | | | | *Fusarium spp* | 5x10 | |
| | *Pseudomonas spp* | $40x10^6$ | | *Phoma spp* | $6x10^3$ | |
| | *Corynebacterium spp* | $30x10^6$ | | *Verticillium spp* | 5x10 | |
| | *Mycobacterium spp* | $30x10^6$ | | *Paecilomyces spp* | $10x10^3$ | |
| | *Klebsiella spp* | $40x10^6$ | 36 | *Mucor spp* | $3x10^3$ | 21 |
| | *Actinobacter spp* | $30x10^6$ | | *Rhizopus spp* | $1x10^3$ | |
| | *E. coli* | $20x10^6$ | | *Scopularioposis spp* | $1x10^3$ | |
| | *Vibrio spp* | $6x10^6$ | | *Aspergillus spp* | $17x10^3$ | |
| | *Clostridium spp* | $10x10^6$ | | *Drechslera spp* | $5x10^3$ | |
| | *Bacillus spp* | $20x10^6$ | | | | |
| Total | | $347x10^6$ | | | $63x10^3$ | 100 |

**Table 4b. Biochemical tests and identification of bacterial isolates**

| | ***Clostridium spp.*** | ***Bacillus spp.*** | ***Pseudomonas spp.*** | ***Micrococcus spp.*** | ***Corynebacterium spp.*** | ***vibrio spp.*** | ***E.coli*** | ***Streptococcus spp.*** | ***Salmonella spp.*** | ***Staphylococcus spp.*** | ***Nocardia spp.*** | ***Enterococcus spp.*** | ***Mycobacterium spp.*** | ***Klebsiella spp*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Morphology | Rod | Rod | Rod | coccus | Rod/ clubbed | Rods | Rod | coccus | Rods | coccus | Rod/ coccus | coccus | Rod | Rod |
| Arrangement | Pair/ chains | Chain/ pairs | single | Tetrads/ cluster | Irregular | irregular | single | Pairs/ chains | Irregular | cluster | Single /pairs | Pair/ chains | Mycelial-like | Single |
| Grams stain | + | + | - | + | + | - | - | + | - | + | +/- | + | + | - |
| Pigmentation | Brown /yellow | - | Blue/green | Yellow | Yellow | - | - | - | - | - | Yellow | - | Red | - |
| Motility | + | + | + | - | - | + | + | - | + | - | | - | - | - |
| Endospore | + | + | | - | - | | | - | | - | - | - | - | |
| Starch | | + | - | - | - | | - | d | | [-] | | + | [-] | + |
| Catalase | - | + | + | + | + | | + | - | + | + | + | d | + | + |
| Oxidase | - | - | + | + | | + | - | - | - | - | | - | d | - |
| Indole | - | - | - | - | - | | [+] | - | - | - | - | - | - | - |
| VP | | + | - | d | | + | - | + | - | + | | + | d | - |
| Citrate | - | [+] | + | + | | + | - | - | + | [-] | | [-] | - | + |
| Urease | - | - | [+] | d | d | - | - | - | - | = | + | - | - | + |
| Gelatinase | + | + | [+] | [+] | - | + | | - | - | [+] | | d | - | - |
| Nitrate red | d | + | - | [-] | | + | + | - | + | + | + | - | [+] | + |
| Glucose | d | + | - | [-] | + | + | + | + | + | + | = | + | + | + |
| Lactose | + | [-] | - | - | - | - | + | + | _ | + | + | + | - | + |
| Sucrose | - | + | - | [-] | d | + | d | + | - | + | | + | + | + |
| Mannose | - | + | | [-] | + | + | + | + | + | + | + | + | [-] | + |
| Mannitol | -. | | [+] | - | | + | | - | + | - | + | + | | + |
| MR | - | | - | | + | d | + | | + | | | | | + |
| O/F | OA | OA | OA | OA | FA | FA | FA | FA | FA | FA | OA | FA | OA | FA |

Legend :FA= Facultative anaerobe; OA = Obligate aerobe; + = 90% or more positive; - = 90% or more negative; d = <75% positive; [-] = 76-89% negative; [+]= 76-89% positive

**Table 4c. Morphological identification of fungal isolates.**

| Growth rate | Media used | Morphological description (Growth on agar and microscopy) | Colour | Isolate |
|---|---|---|---|---|
| Rapid | Czapek Dox agar | Granular and flat on agar with radial grooves. Microscopically, conidial heads radiate and subsequently splitts into loose columns. Conidiophore stipes were hyaline, coarse and rough. Conidia were sub-globose shapped and 3.5-5 µm in diameter. Brownish sclerotia were present. | Pale green, yellow to dark yellow-green withage. | *Aspergillus spp* |
| Rapid | Potato dextrose agar | Cottony aerial mycelium was observed. Microscopically, conidia were formed from slender phialides. Two celled microconidia were formed. Microconidia were hyaline, small, pyriform, fusiform, straight or curved in shape. Chlamydospores were present | White to cream anverse with yellow reverse. | *Fusarium spp* |
| Rapid | Potato dextrose agar | Microscopically, conidia are formed from slender phialides. Macroconidia present. Macroconidia are glass-like, multi-celled, fusiform to sickle-shapedwith elongated apical cells. | white to purple anverse, with dark blue or dark purple reverse. | *Fusarium spp* |
| Rapid | Potato dextrose agar | Colonies are flat and spread out on agar with a dense suede-like surface. Microscopically, conidia not produced. Thick-walled intercalary chlamydospores were observed. Pectinate and racquet hyphae noted | Greyish to white in colour with yellow-brown reverse. | *Microsporum spp* |
| Rapid | Potato dextrose agar | Colonies exude a, slightly aromatic smell on agar. Microscopically, translucent, erect slightly flattened with a diffluent membrane, unbranched, dark brown sporangiaphores were observed. Subglobose columellae with small collarettes present. Sporangiospores are spherical with smooth-walled | Greyish-brown | *Mucor spp* |
| Rapid | Potato dextrose agar | Microscopically, sporangiophores are erect, and branched, with large terminal, globose, multispored and have well developed subtending columellae. A conspicuous collarette was observed at the bottom of the columella. Sporangiospores are translucent, grey ellipsoidal, with very smooth walls. | Dark-grey, | *Mucor spp* |
| Rapid | Malt extract agar | Colonies are powdery and suede-like on agar. Microscopically, phialides are enlarged at their bases and gradually become narrow culminating in a slender neck observed. Phialides exits in a group of two as verticils. Smooth conidiophore stipes, and chlamydospores were noted. | Green-gold/yellow-brown with yellow reverse. | *Paecilomyces spp* |
| Rapid | Potato dextrose agar | Dense conidiophores were observed. Single-celled chained conidia (with new conidia form at the base of the chain), produced from phialide. Phialide produce branched metulae, forming a penicillus. Conidiophores are simply branched, glassy in nature, rough-walled, flask-shaped and consist of a cylindrical basal lanceolate. Conidia are in chains, divergent, ellipsoidal, and fusiform. Sclerotia was observed. | Green | *Penicillium spp* |

| Growth rate | Media used | Morphological description (Growth on agar and microscopy) | Colour | Isolate |
|---|---|---|---|---|
| Rapid | Potato dexrose agar | Microscopically, colonies show single-celled chained conidia arrangeded in basipetal order from a annellide. Annellides are organized into a different penicillus. Conidia are , rough, pear-shaped with the rounded portion situated away from the point of attachment and translucent. | White/grey/light brown | *Scopulariopsis spp.* |
| Rapid | Potato dextrose agar | Cottony to fluffy on agar. Microscopically, colonies produced sympodially branching, erect and stolon-like sporangiophores with terminal vesicles bearing finger-like merosporangia. Mmerosporangia is thin, smooth-walled, evanescent and contain 6-8 globose. | white / light grey become dark grey with age. | *Syncephalastrum spp* |
| Rapid | Potato dextrose agar | Colonies are suede-like on agar. Microscopically, hyphomycete, verticillateiy branched conidiophores producing concentric circles of slender awl-shaped phialides developed in different directions were observed. | white /pale yellow anverse yellow/ brown reverse. | *Verticillium spp* |
| Rapid | Potato dextrose agar | Dense cottony growth on agar. Microscopically, Stolons and pigmented rhizoids were observed. Sporangiophores are apophysate, formed in clusters directly from nodes above a rhizoid, columellate and multispored. Sporangiospores are spherical, single-celled, brown and narrow . | White to dark grey/ brown | *Rhizopus spp* |
| Rapid | Potato dextrose agar | Floccose on agar. Microscopically, blastocatenate chains of dictyoconidia produced sympodially from obclavate dictyoconidia, with short cylindrical beaks. | Black/grey | *Alternaria spp* |
| Rapid | Malt agar | Suede-like powder spreading on agar. Microscopically, Large, globose, membranous, ostiolate pycnidia observed. Abundant conidia produced in pycnidia on strait thread-like phialides noted. Conidia are one-celled, glassy, and produced in slimy clusters from the apex ostiole. | Greyish-brown | *Phoma spp* |
| Rapid | Malt extract agar | Microscopically, pale brown subcylindrical, straight, transversely septate, smooth-walled, poroconidia formed in a sympodially elongating, geniculate conidiophore observed. The hilum is not protuberant. | Brown to blackish brown anverse with a black reverse. | *Drechslera* |
| Slow | Potato dextrose agar | Dense aerial mycelium present on agar. Microscopically, Hyphae are septate, irregularly shaped, pigmented, and with swollen segments. Pycnidia are subspherical, with a pseudoparenchymatous wall composed of very densely packed textura angularis. Conidia are brown, thick, cylindrical and smooth-walled. | Green to dark brown | *Microsphaeropsis spp* |

**Table 5. Metal Contamination/Pollution Index (MPI) of hospital soils in Calabar Metropolis**

| Locations | Zn (mg kg$^{-1}$) | | Cu (mg kg$^{-1}$) | | Fe (mg kg$^{-1}$) | | Cr (mg kg$^{-1}$) | | Pb (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 16.80 | | 5.45 | | 900.44 | | ND | | 1.35 | |
| Hospitals Location | Mean Zn in soil | MPI | Mean Cu in soil | MPI | Mean Fe in soil | MPI | Mean Cr in soil | MPI | Mean Pb in soil | MPI |
| Hospital 1 | 139.00 | **8.27** | 6.95 | **1.27** | 14764.00 | **16.39** | 8.00 | **8.00** | 1.95 | **1.44** |
| Hospital 2 | 26.30 | **1.46** | 6.20 | **1.13** | 2344.00 | **2.60** | ND | **---** | 1.40 | **1.03** |
| Hospital 3 | 28.65 | **1.75** | 6.05 | **1.11** | 9894.00 | **10.98** | 3.00 | **3.00** | 1.60 | **1.19** |
| Hospital 4 | 44.13 | **2.62** | 6.28 | **1.15** | 979.30 | **1.09** | ND | **---** | 1.20 | **0.88** |
| Hospital 5 | 110.00 | **6.54** | 6.47 | **1.18** | 2244.00 | **2.49** | 3.00 | **3.00** | 2.20 | **1.63** |

**Table 6. Mean heavy metals accumulation in plants from hospital sites in Calabar Metropolis**

| Location | Plant species/ Part analyzed | Zn | BCF | Cu | BCF | Fe | BCF | Cr | Pb |
|---|---|---|---|---|---|---|---|---|---|
| | | → | | → | mg kg$^{-1}$ | ← | | ← | |
| Control | Water leaf | 2.00 | 0.11 | 0.80 | 0.14 | 2.42 | 0.002 | ND | ND |
| | Cassava leaves | ND | - - | 1.80 | 0.33 | ND | - - | ND | ND |
| Hospital 1 | Cocoyam tuber | 5.20 | 0.03 | 11.00 | 1.58 | 28.40 | 0.001 | ND | ND |
| Hospital 2 | Cassava leaves | ND | - - | 2.00 | 0.32 | ND | - - | ND | ND |
| Hospital 3 | Potato leaves | ND | - - | 5.00 | 0.82 | 4.00 | 0.0004 | ND | ND |
| | Water leaf | 17.30 | 06 | 6.00 | 0.99 | 11.50 | 0.001 | ND | ND |
| Hospital 4 | Water leaf | 17.40 | 0.39 | 2.00 | 022 | 13.20 | 0.013 | ND | ND |
| | Pumpkin leaf | 18.80 | 0.42 | 4.00 | 0.6 | 11.00 | 0.011 | ND | ND |
| Hospital 5 | Cassava leaves | 14.30 | 0.13 | 9.00 | 1.43 | ND | - - | ND | ND |
| | Water leaf | 4.00 | 0.03 | 5.00 | 0.77 | 13.40 | 0.005 | ND | ND |

ND = Not Dictated, BCF = Bio-concentration Factor

Generally, the metal contents in the control and hospital site were within soil ranges as documented by Husein *et al*. (2019) for uncontaminated soils. However, the metal Pollution/ contamination index indicates that Hospital 1, was slightly polluted with Cu (1.27 mg $kg^{-1}$) and Pb (1.44 mg $kg^{-1}$), severely polluted with Cr (8.00 mg $kg^{-1}$), very severely polluted with Zn (8.27 mg $kg^{-1}$) and excessively polluted with Fe (16.39 mg $kg^{-1}$). Hospital 2 location, was slightly polluted with Zn (1.46 mg $kg^{-1}$), Cu (1.13 mg $kg^{-1}$) and Pb (1.03 mg $kg^{-1}$), moderately polluted with Fe (2.60 mg $kg^{-1}$), while Cr was below the detectable limit. Hospital 3 location was slightly polluted with Zn (1.75 mg $kg^{-1}$), Cu (1.11 mg $kg^{-1}$) and Pb (1.99 mg $kg^{-1}$), moderately polluted with Cr (3.00 mg $kg^{-1}$), and very severely polluted with Fe (9894.00 mg $kg^{-1}$). Hospital location 4 was slightly polluted with Cu (1.15 mg $kg^{-1}$), Fe (1.09 mg $kg^{-1}$) and Pb (0.88 mg $kg^{-1}$), moderately polluted with Zn (2.62 mg $kg^{-1}$) while Cr was not dictated. Hospital 5 location was slightly polluted with Cu (1.18 mg $kg^{-1}$) and Pb (1.63 mg $kg^{-1}$), moderately polluted with Fe (2244.00 mg $kg^{-1}$) and Cr (3.00 mg $kg^{-1}$), and severely polluted with Zn (6.54 mg $kg^{-1}$). This trend could also be seen in the geo-accumulation index (Fig. 2). High metal contents in soil could affect the number, diversity, and activities of soil microbial communities. It could also slow down the growth and reproduction rate of fast indigenous microbes, thus hampering their primary roles and allowing slower growing microbes with lower diversity and higher resistance to heavy metal pollution to prevail (Ediene and Iren, 2017; Husein *et al.*, 2019). Based on Lacatusu (2000) remarks, the slight pollution to very severe metal pollution observed for the different hospital sites could pose negative effects on soil, plant, and environment at large.

The heavy metals presented in this study occurs in the physiological, biochemical and metabolic processes of plants, soil organisms. They often time function as a cofactor for some enzymes. However, the majority have no known biological functions in plants and soil microflora. They appear toxic when they occur in excessive amounts (Fashola *et al.*, 2106). Edible plant samples cultivated within the different hospital locations at the time of sampling include waterleaf, cassava, cocoyam, cassava, potato, and pumpkin. Results of the analysis of these plants revealed that Cr and Pb were not phyto-available in all the plants analyzed across the various locations. Waterleaf at the control site accumulated 2.0 mg

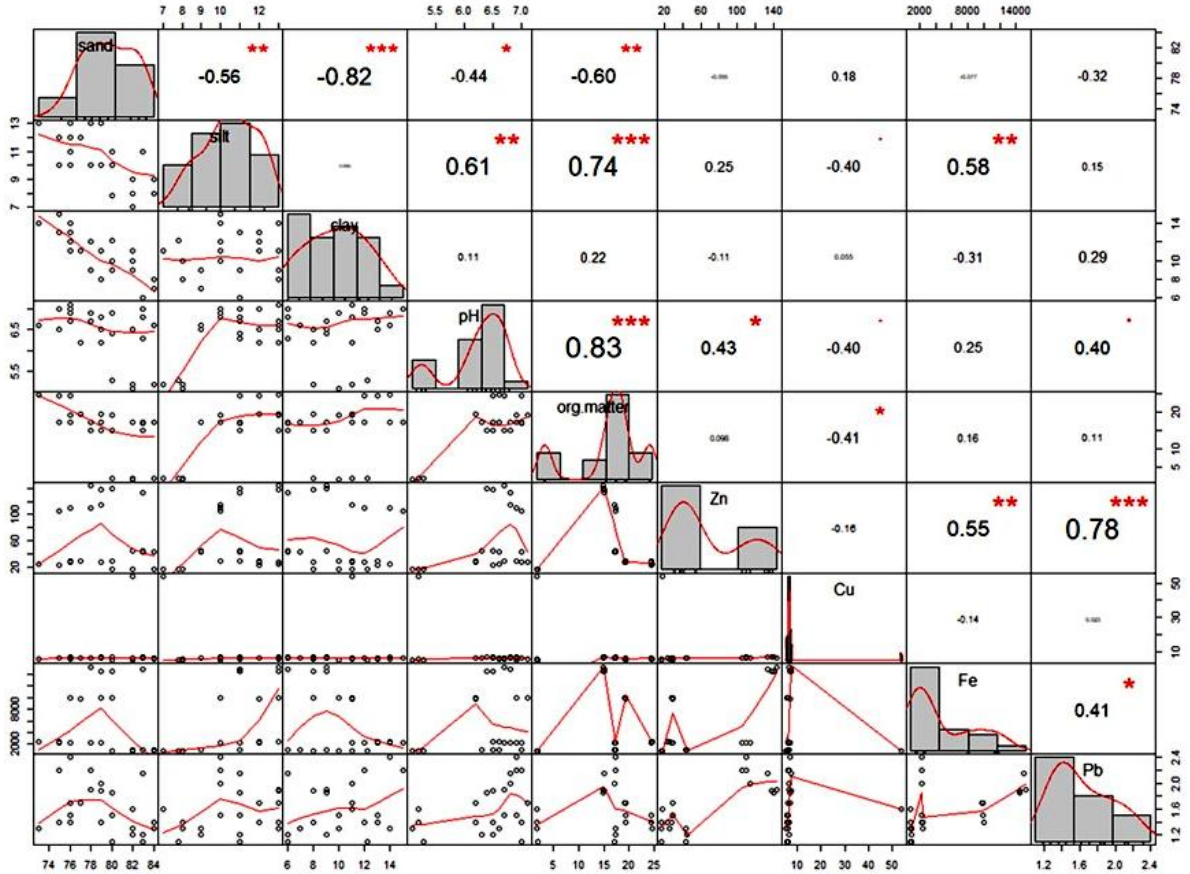


**Figure 3. Correlation matrix plot**

$kg^{-1}$, of Zn with a BCF of 0.11 mg $kg^{-1}$ while waterleaf at the hospital sites had Zn content ranging between 4.0 and 17.4 mg $kg^{-1}$ with waterleaf at hospital 4 having the highest BCF (0.39 mg $kg^{-1}$) for Zn. Zinc was not detected in cassava plants from the control site, however, the cassava plants grown at Hospital 5 accumulate 14.30 mg $kg^{-1}$ in the leaves. Similarly, the pumpkin leaves analyzed from Hospital 4 location was observed to bio-accumulate 18.80 mg $kg^{-1}$ of Zn. Zinc contents of plants from the hospital sites were higher than those of the control. However, the vegetables did not surpass the FAO/WHO Zn accumulation limits of 99.4 mg $kg^{-1}$. Results of plant analysis for Cu content indicated that waterleaf and cassava plant from the control site had uptake values of 0.8 and 1.8 mg $kg^{-1}$, respectively while water leaf from the hospital sites had mean Cu contents between 2.0 mg $kg^{-1}$ and 6.00 mg $kg^{-1}$. Cassava leaves from the control site had Cu content of 1.80 mg $kg^{-1}$ with a BCF of 0.33 while cassava from the hospital sites had mean content 9.00 mg $kg^{-1}$ with BCF of 1.43. The concentrations of Cu in the vegetable were much lower than 99.4 mg $kg^{-1}$ standard of FAO/WHO. The accumulation of Fe in the vegetative parts of waterleaf from the control site was 2.42 mg $kg^{-1}$ with a BCF of 0.002. While those from the hospital site accumulated between 11.0 mg $kg^{-1}$ and 13.4 mg $kg^{-1}$. Pumpkin from the hospital site accumulated 11.0 mg $kg^{-1}$. The results obtained for plants from the hospital sites were higher than the control. However, they were far below the 425 mg $kg^{-1}$ allowed by FAO/WHO. The reason for the higher content of Zn, Cu, and Fe in plants from the hospital sites over the control could be attributed to the higher quantities of these elements in the hospital soils. Generally, the concentrations of metals in plant tissues were lower than in soil.

***Correlation analysis between soil properties and some selected heavy metals*:** The result of the correlation analysis between some soil properties and some selected heavy metals is presented in Fig. 3. The sand content was negatively correlated with silt (r = –0.56), clay (r = –0.82) and organic matter (r = –0.60). Silt content yielded a moderate positive correlation with pH (r = 0.61) and Fe (r = 0.58), while it produced a strong positive correlation with organic matter (r = 0.74). Soil pH produced a strong positive correlation with soil organic matter (r = 0.83). Zinc gave a moderate positive correlation with Fe (r = 0.55) and a strong positive correlation with Pb (r = 0.78). The positive correlations presented by some of the heavy metals suggest that each of the paired elements in the studied soil had common sources of contamination which is traceable to the hospital wastes accumulation. The result corroborates with the report of Adama *et al*. (2016).

***Conclusion*:** This research discovered the possibility of food chain contamination from heavy metals and pathogenic organism at hospital sites. This contamination is traceable to hospital effluents, and solid wastes loaded with pathogenic microbes and heavy metals from ashes of burnt hospital waste. Plants grown on soil within the hospital, absorb these metals and pathogens. Animals grazing on these plants, ingest the metals and pathogens. Farmer in turn eat these crops and animals grown/reared in hospital sites, thus, culminating in a vicious cycle of contamination. Based on the results obtained, it is evident that hospital soils are heavily loaded with pathogenic microbes, which could pose a direct danger to the health of people engaged in farming or animals grazing in such areas. These pathogenic organisms abound in effluents discharge from medical activities. They could hitherto contaminate the food chain when such crops are not properly washed or cooked as some of these microbes can withstand high temperatures. Another source of concern is heavy metal concentration from hospital soils. These heavy metals arise from dust of the hospital waste burnt in open dumps or incinerators at the hospital sites. The result portrays and increasing trend in metals at the hospital sites when compared with the control. The BCF showed higher values in hospital sites than the control, implying that if such metals are increased in the soil as a result of the continuous discharge of hospital effluent, we are likely to have increased uptake by the plant. It is important hospitals in Nigeria adhere to world best practices in medical waste disposal. It is strongly recommended that agricultural activities should not be carried out at hospital sites for safety sake.

***Conflict of Interest:*** The authors have declared no conflict of interest.

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