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Some Physico-Chemical and Bacteriological Characteristics of Soil Samples around Calabar Metropolis, Cross River State, Nigeria

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Abstract : - Physico-chemical and bacteriological parameters of soil samples around Calabar Metropolis, Cross River State, Nigeria were examined to determine the pollution status of the soil quality. Results of the physico-chemical analysis showed that the soil samples had pH range of 4.4 – 5.2. Tinapa soil has the highest value of Copper (39.63mg/kg) and Nickel (11.36mg/kg) and Anantigha has the highest value of Zinc (14.59mg/kg), Iron Fe (78.19mg/kg) and Manganese (47.42mg/kg). The results revealed a high total count of $23.5x10^{6}$ cfu/g in Anantigha and $24.5x10^{-3}$ cfu/g in Tinapa for bacteria and fungi respectively. Some bacteria isolates found during the study includes, Escherichia coli, Bacillus subtilus, Clostridium sp, Arthrobacter sp, Streptomyces sp, Nocardia sp, Pseudomonas sp and Micrococcus sp., and Fungal isolates includes, Actinomycete sp, Verticullium sp, Aspergillus sp, Mucor sp, Nigospora sp and Paecilomyces sp. From the result, soil sample from Anantigha have comparatively the highest Total Bacterial Counts compared to the other two locations. The health implications of this work is that Anantigha and Tinapa areas being low lying were likely, because of the presence of Escherichia coli, to experience gastro-intestinal diseases such as dysentery and cholera than the Ediba environments.

Keywords: *- Physico-chemical, Microbial, Soil Samples, Calabar Metropolis, Nigeria*

I. INTRODUCTION

Soil is the part of the earth crust where geology and biology relate. Soil surface provides home for plants, animals and microbial life. However, soil contamination by heavy metals and toxic elements due to parent or point sources often occurs on a limited area and is easy to identify (He *et al*., 2001).The soil environment may be divided into layers called horizons. It comprises of four major layers, the top soil, sub soil, parent material and bedrock (Asikong and Udofia, 2005). The top soil may act as carriers of pollutants and other contaminants. Several studies have indicated that industrial discharges and municipal wastes are the major sources of environmental pollution. A major path way of soil contamination is through atmospheric deposition of heavy metals from point source such as metaliferous metal smelting and industrial activities (Singh, 2001). Other sources of contamination affecting soil include inputs such as, fertilizers, pesticides, sewage sludge and microbial activities. Several studies have indicated that vegetables, particularly leafy crops, grown in heavy metal contaminated soil have higher concentrations of heavy metal than those grown in uncontaminated soil (Dowdy and Larson, 1995). It has been reported that movement of these chemical in plant and water bodies depends on the extent of soil contamination and may endanger human health through consumption of sea food and vegetable crops or directly through skin contact and inhalation of dust particles (Ayodele and Gaya, 2003). Leafy vegetables occupy a very important place in the human diet, but unfortunately constitute a group of foods which contributes maximally to nitrate and other anions as well as heavy metals consumption. The excessive application of nitrogen and other inorganic fertilizers and organic manures to these vegetables can accumulate high levels of nitrate and other anions as well as heavy metals (Akan *et al*., 2010). This study is aimed at assessing the physico-chemical parameters of soil in the three different locations that represent the ecological area of Calabar Metropolis in view of determining the pollution status.

II. MATERIALS AND METHODS

2.1 DESCRIPTION OF STUDY AREA

The study areas comprises of Tinapa (5º3'12"N, 8º19'5"E), Ediba (4º58'44"N, 8º20'43"E) and Anantigha (4º55'3"N, 8º19'5"E) areas located within Calabar Metropolis in Cross River States, Nigeria. Calabar can be described as being nearly level to gently undulating slopes which provide a very stable physiographic environment for relatively uniform parent materials.

2.2 SAMPLE COLLECTION

Soil samples were taken in the surface soil at a distance away from the flow of traffic at a depth of 0-15cm with a trowel. The depth of 0-15cm was used because it is believed that pollution decreases with increase in soil depth. The soil samples were collected into labeled sterile polyethylene bags and taken in ice-packed cooler to the laboratory for bio-component and physico-chemical analysis.

2.3 PREPARATION OF SAMPLES FOR ANALYSIS

Samples collected in each location were divided into two parts, one part for physico-chemical analysis while the other for microbial analysis. Each sample meant for physico-chemical analysis was air dried for five days, and then sieved to ensure homogeneity using a 2mm size sieve.

2.3.1 PHYSICO-CHEMICAL ANALYSIS

Physico-chemical analysis was carried out in Soil Science Department laboratory, University of Calabar. The particles size analysis was determined using hydrometric method (Bouyoucos, 1962). 100g of each soil sample was analyzed, in which 50ml of calgon was used as a dispersing agent. 200ml of water was added, stirred and kept for 24 hours so that the dispersion in circulating the soil particle can be completed. Then it was pulled into a measuring cylinder of 1000ml for the particles to settle for reading. Soil pH was determined in water and 0.1 M KCl solution at 1:2.5 Soils. Organic carbon content was found by the modified $K_2Cr_2O_7$ digestion of Walkley-Black method (Nelson and Sommers, 1996). Effective cation exchange capacity was determined by adding the 1 *M* KCl extractable acidity to cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) exchanged by neutral 1 *M* $NH_4C_2H_3O_2$ as described by (Thomas, 1982). The exchangeable acidity was determined by titration and the cation exchange capacity (CEC) was obtained by summation of exchangeable cations and exchange acidity. The available phosphorus was determined by the Bray P-1 method. 3g of each soil sample was measured into a100ml conical flask.15g of the extractor (0.03 NH_4 and 0.025 HCl) was added for 1min and then filtered. Ascorbic acid solution was added to 2ml of the filtrate and the available phosphorus was determined by the blue calorimetric method as also described by (Bray and Kutz, 1945). The total nitrogen was determined on 5g of each soil sample using Micro-kjel Dahl digestion procedure, using Sodium Sulphate ($NaSO_4$), Copper Sulphate ($CuSO_4$) and Concentration Sulphuric acid (H_2SO_4) as also described by (Bremner, 1965).

2.3.2 HEAVY METALS ANALYSIS

All reagents used were of analytical grade and from which standard solutions were prepared. Glassware were thoroughly washed with detergent and rinsed with distilled water. The digestion method previously described by Francek *et al*., (1994) was adopted for the extraction of trace metals in this study. One gram each of air-dried soil sample was crushed to fine powder in an agate mortar and digested in 10 ml of 1:1 concentrated HNO3. The mixture was evaporated to near dryness on a hot plate and then cooled. This procedure was repeated with a 15 ml solution of 1:1 concentrated HCl. The extracts were filtered with Number forty (40) Whatman filter paper and then made up to 100 ml volume with 2% HNO3. Solutions of the sample and blanks were run using Atomic Absorption Spectrometer (AAS) (200A Model).

2.3.4 MICROBIAL ANALYSIS

2.3.4 1 SERIAL DILUTION

Ten-fold Serial dilutions of the soil samples were made as described by (Collins and Lyne, 1976; Harrigan and McCance, 1976).

2.3.4.2 INOCULATION AND INCUBATION

One milliliter of appropriate ten-fold serial dilutions of the soil sample were Inoculated onto Nutrient agar (Oxoid CM 314), Reinforced Clostridial Agar Oxoid CM 149, 151), Malt Extract Agar (Oxoid) and Sabouraud Dextrose Agar plates in triplicates using pour plate methods as described by (Collins and Lyne, 1976; Harrigan and McCance, 1976) and spread plates methods by (Demain and Davies, 1999). Soil plate techniques as described by (Eka and Fogathy, 1972; Cruickshank et al., 1976) were also used for the isolation of Actinomycetes using the Starch Nitrate Agar. Inoculated plates were incubated at 28+2oC for 18-24 hours and

48-72 hours for the enumeration of total heterotrophic bacteria, fungi and Actinomycetes respectively. Visible discrete colonies in incubated plates were counted and expressed as colony forming units per gram (cfu/g) of soil samples.

2.3.4.3 PURIFICATION AND MAINTENANCE OF ISOLATES

The isolates obtained were purified by repeated sub-culturing on fresh agar medium and incubated under normal condition for growth. Pure colonies isolated were inoculated on agar slants using Maconey bottles to serve as stock cultures, incubated at 37ºC and were stored in the refrigerator at 6ºC ± 2ºC for future research.

2.3.4.4 CHARACTERIZATION AND IDENTIFICATION OF MICROBIAL ISOLATES

Pure cultures of microbial isolates were identified based on cultural parameters, microscopic techniques and biochemical tests including carbohydrate utilization (Cruickshank *et al*., 1976). Identification of the bacterial isolates was accomplished by comparing the characteristics of the cultures with that of known taxa as in (Holt *et al*., 1994). Characterization and identification of fungal isolates was carried out as described by (Domsch *et al*., 1980; Barnet and Hunter, 1987). Actinomycetes were characterized and identified as described by (Eka and Fogathy, 1972).

III. RESULTS

4.1 PHYSICO-CHEMICAL PARAMETERS

The particle size of the soils varies from the sand, clay and a silt fraction around Calabar Metropolis is shown in Table 1 and variation in percentage distribution of particle size of the soil within the sampling locations is shown in Figure 1. The results of the determination of heavy metals in the soil samples from different sampling locations around Calabar Metropolis are shown in Table 2 and Variation in Heavy metals concentration of different sampling locations around Calabar Metropolis is shown in Figure 2. The values and percentages of the physico-chemical parameters of soils recorded from sampling locations around Calabar Metropolis during the Study period is shown in Table 3. Table 4 shows the Total Bacteria Count (TBC), Isolates and their Biochemical Characteristics found in soil samples from the Sampling location around Calabar Metropolis after been view under the microscope.

IV. DISCUSSION

The study revealed high percentage content of sand and low clay and silt contents at the various study areas. Anantigha (89.6%) has the highest percentage of sand particle sizes when compared with Ediba (72.6%) and Tinapa (65.6%). Aggressive weather conditions might equally be responsible for the soil texture observed as reported by (Jungerius and Levellt, 1964). The values obtained for cations calcium, magnesium, sodium and potassium except exchangeable acidity revealed increase in values in the studied sample collected at Anantigha, compared with those of Ediba and Tinapa. These increase in the cations especially calcium and magnesium in this sample could have been caused by the plants effluent. The result for the samples analyzed all indicated soil pH that were acidic with a range of pH 4.4-5.2. Soil pH controls the available phosphorus. In acid soil, available Phosphorous concentration increases as pH increases, while in alkaline soil, available Phosphorous concentration decreases as pH increasing (Holford and Mattingly, 1975). The result also shows Heavy metals of Calabar Metropolis during the study. Anantigha has the highest value of Zinc (14.59mg/kg), Iron (78.19mg/kg) and Manganese (47.42mg/kg). Although some heavy metals such as Copper, Zinc, Manganese and Iron are essential in plant nutrition, many of them do not play any significant role in plant physiology. The uptake of these heavy metals by plants especially leafy vegetables is an avenue of their entry into human food chain with harmful effects on health (Barnet and Hunter, 1987). Tinapa soil has the highest value of a Heavy Metal, Copper (39.63mg/kg) and Nickel (11.36mg/kg). It is probable that the nickel is by-products from the industries situated in the Exporting Processing Zone (EPZ) industrial area, some few kilometres from away. The nickel level is above the permissible level set by world health organisation (WHO) which is 10mg/kg. Excess level of nickel in the soil has been reported to be toxic and can affect soil fauna such as earthworm which are adjuncts to the microflora inorganic matter decomposition. The lungs, nasal cavity and tissues are the target organs affected by respiratory carcinogen when human are exposed to Nickel poisoning (Ayodele and Gaya, 2003). From the result, soil sample from Anantigha have comparatively the highest Total Bacterial Counts compared to the other two locations. Nine bacterial organisms were isolated in all from the Anantigha, Ediba and Tinapa; *Escherichia coli*, *Azomomas* sp, *Bacillus subtilus*, *Clostridium* sp, *Arthrobacter* sp, *Streptomyces* sp, *Nocardia* sp, *Pseudomonas* sp *and Micrococcus* sp.This result also revealed that *Micrococcus* sp and *Bacillus subtilus* were found in all the three sampling locations. Total Bacterial Count of the sample collected Anantigha was significantly higher than that of Tinapa. Also, there was significant difference between count result of sample collected at Tinapa and Ediba. Sample collected at Tinapa recorded the least average bacterial count value of 5 x

10^6cfu/g and the highest average fungal count value of 24.5 x 10^{-4}cfu/g compared to samples collected at Anantigha and Ediba. This higher fungal count could be attributed to the more robust nature of fungi which enables them to withstand the more acidic environment of the location than bacteria. This ability of fungi to thrive better than bacteria in acid soils had been reported (Rangaswami and Bargyaraj, 1993). Eight fungal organisms were isolated in all from the Anantigha, Ediba and Tinapa; *Actinomycete* sp, *Verticullium* sp, *Aspergillus* sp, *Diplosporium* sp, *Mucor* sp, *Hormodendrium* sp, *Nigospora* sp and *Paecilomyces* sp. The result from the table revealed that *Actinomycete* sp, *Hormodendrium* sp and *Paecilomyces* sp were found in all the three locations. Microbial biomass can reflect soil quality (Brookes, 2001). The health implications of this work is that Anantigha and Tinapa areas being low lying were likely, because of the presence of *Escherichia coli*, to experience gastro-intestinal diseases such as dysentery and cholera than the Ediba environments.

V. CONCLUSION

The results of the study have revealed the values or percentages of physico-chemical parameters, and some bacterial and fungal isolates that can be found in soil within Calabar, Cross River state. Long-term development prospects of countries all over the world appear to have been threatened by severe environmental degradation, particularly in the developing countries. Land pollution is a major cause of environmental degradation. Most times, water pollution occurs when pollutants from adjoining uplands flow into water bodies through run–off or when carried by wind into the water bodies. Calabar is surrounded by important rivers and streams that serve for both domestic and industrial purposes to the inhabitants of the city. The sampling locations are subjected to different levels of Urban Developments that could lead to the release of some pollutants that could impact the soil. It is quite obvious that when the soil is polluted, the nearby water body would be affected. This is true because when it rains, the pollutant would be washed into the surface water (river, stream, etc) while some peculate into the underground water. Chemicals applied on land can evaporate into the atmosphere and increase atmospheric pollution. All these contribute to environmental degradation which invariably results to hampering long-term development. The research is focused on environmental degradation via land pollution which forms a link to both water and atmospheric pollution. While it notes that a careful management of land as a resource is essential for meeting a major demand created by accelerated urbanization, industrialization and agricultural development. It is also important to note that loss of revenue and declining health-care as some of the economic implications could be accrued to land pollution.

Table (1): Particle Size Distribution of Soils in Encountered within the Sampling Locations

Sampling locations	SAND (%)	CLAY (%)	SILT (%)
Anantigha	89.6	2.7	7.4
Ediba	72.6	10.7	16.4
Tinapa	65.6	22.7	11.7

Table (2): Heavy Metals Concentration of Soil Samples within the Sampling Locations

Sampling location	Copper (mg/kg)	Zinc (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Nickel (mg/kg)
Anantigha	35.31	14.59	78.19	47.42	5.33
Ediba	35.55	11.51	75.12	45.61	8.14
Tinapa	39.63	11.34	70.15	39.15	11.36

Table (3): The Physico-Chemical Parameters of Soils around Calabar Metropolis

Sampling location	pH	Org.C (%)	T.N (%)	Avail.P (mg/kg)	(mol/kg)						
					Ca	Mg	K	Na	H	Al	ECEC
Anantigha	5.2	1.4	0.12	6.00	4.6	1.2	0.09	0.05	0.4	0.04	6.38
Ediba	4.5	2.3	0.19	12.75	3.2	1.0	0.07	0.04	1.1	1.1	6.51
Tinapa	4.4	1.3	0.11	13.25	1.4	1.0	0.05	0.04	0.9	0.7	4.09

AN = Anantigha, ED = Ediba, TI = Tinapa, Org.C = Organic Carbon, Avail.P = Available Phosphorus, Ca = Calcium, Mg = Magnesium, K = Potassium, Na = Sodium, H = Hydrogen, Al = Aluminum, T.N = Total

Nitrogen, ECEC = Effective Cation Exchange Capacity

Table (4): Total Bacterial Counts Isolates and their Biochemical Characteristics found Around Calabar Metropolis

			FERMENTATION				BIOCHEMICAL TEST							
Sampling locations	**Isolate colony count**	**Gram Reactions**	**Lactose**	**Mannitol**	**Glucose**	**Sucrose**	**Citrate**	**Methyl Red**	**Voges Proskauer**	**Catalase**	**Oxidase**	**Coagulate**	**Motility**	**Probable Isolates**
ANANTIGHA	23.5 X10^6 cfu/g	Gram –ve rods singly	AG	AG	AG	A	-ve	-ve	-ve	-ve	+ve	-ve	-ve	*Escherichia coli*
		Gram +ve rods in pairs	–	A	–	–	-ve	+ve	-ve	-ve	-ve	+ve	+ve	*Clostridium* sp.
		Gram +ve cocci	A	–	A	A	+ve	-ve	-ve	+ve	+ve	-ve	-ve	*Micrococcus* sp.
		Gram variable singly or pairs	AG	AG	AG	AG	+ve	-ve	-ve	+ve	+ve	-ve	-ve	*Azomomas* sp.
		Gram +ve hyphae	–	–	A	–	-ve	+ve	-ve	+ve	-ve	+ve	-ve	*Streptomyces* sp.
		Gram +ve rods in chains	A	A	–	A	+ve	+ve	+ve	+ve	+ve	-ve	-ve	*Bacillus subtilus*
EDIBA	18 X10^6 cfu/g	Gram +ve cocci	A	–	A	A	+ve	-ve	-ve	+ve	+ve	-ve	-ve	*Micrococcus* sp.
		Gram variable	A	–	–	–	-ve	-ve	+ve	+ve	+ve	+ve	-ve	*Nocardia* sp.
		Gram +ve rods in chains	A	A	–	A	+ve	+ve	+ve	+ve	+ve	-ve	-ve	*Bacillus subtilus*
		Gram –ve rods	–	A	A	–	-ve	+ve	-ve	+ve	-ve	-ve	+ve	*Pseudomonas* sp.
		Gram +ve rods or coccids or variable	–	–	A	–	-ve	-ve	-ve	+ve	-ve	-ve	+ve	*Arthrobacter* sp.
TINAPA	5 X10^6 cfu/g	Gram +ve rods in chains	A	A	–	A	+ve	+ve	+ve	+ve	+ve	-ve	-ve	*Bacillus subtilus*
		Gram +ve cocci	A	–	A	A	+ve	-ve	-ve	+ve	+ve	-ve	-ve	*Micrococcus* sp.
		Gram +ve hyphae	–	–	A	–	-ve	+ve	-ve	+ve	-ve	+ve	-ve	*Streptomyces* sp.
		Gram –ve rods singly	AG	AG	AG	A	-ve	-ve	-ve	-ve	+ve	-ve	-ve	*Escherichia coli*
		Gram variable	A	–	–	–	-ve	-ve	+ve	+ve	+ve	+ve	-ve	*Nocardia* sp.

A = Acid, AG = Acid and Gas, +ve = Positive, -ve = Negative, − = No Reaction, *spp* = species, cfu/g = colony forming unit per gram

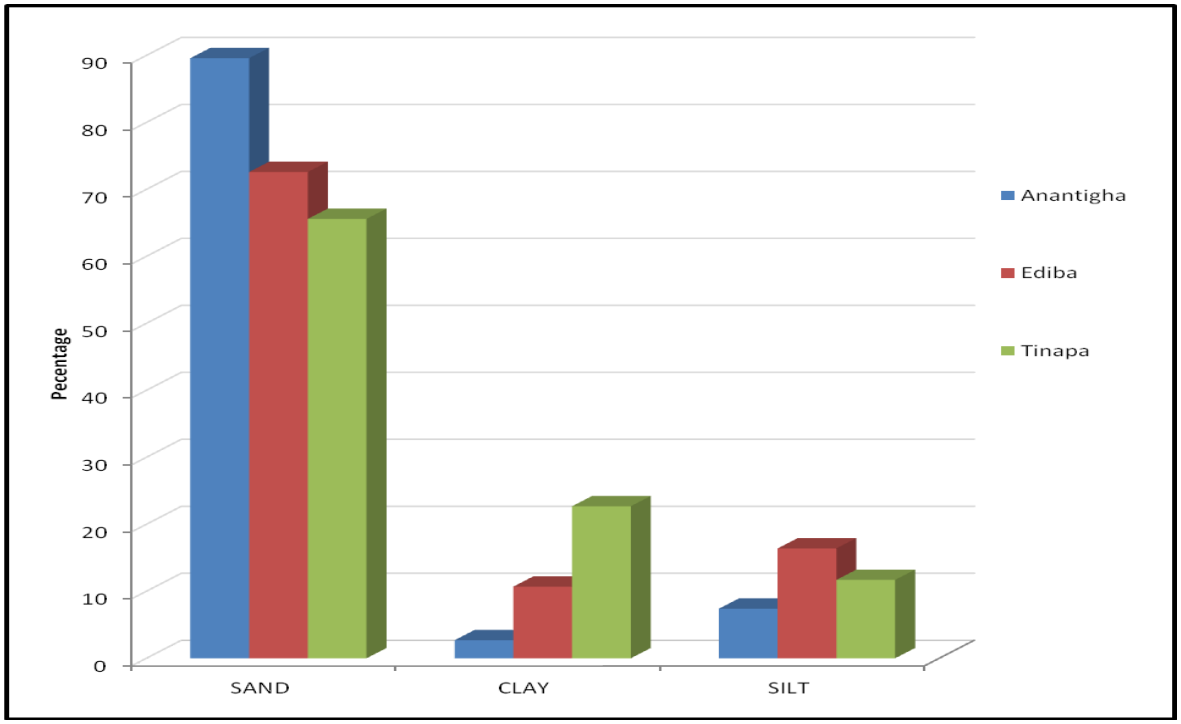

Figure (1): Variation in Percentage Distribution of Particle Size of the Soil within the Sampling Locations

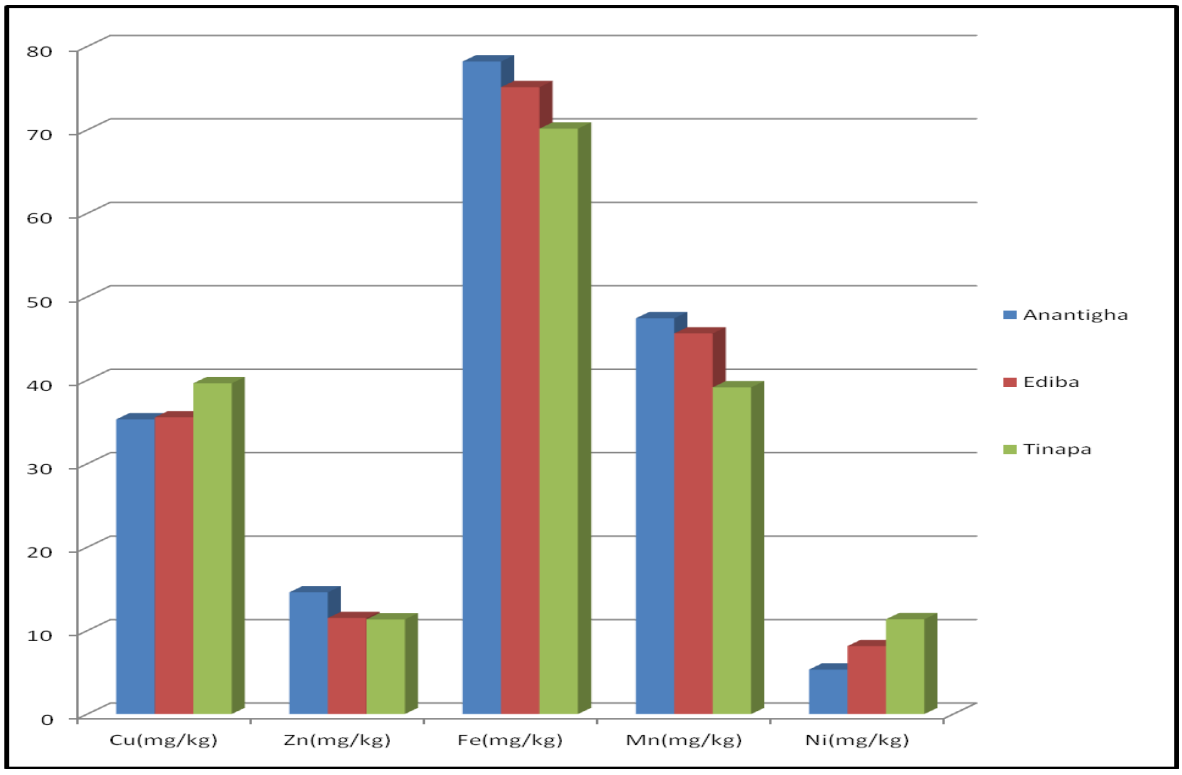

Figure (2): Variation in Heavy Metals Concentration of Different Sampling Locations around Calabar Metropolis during the Study Period

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