Post Impact Assessment of Urbanization on Microbial Abundance and Diversity of Soils in Port Harcourt Area

Ruhuoma Wosu Kinika and Lucky Obukowho Odokuma

Department of Microbiology, Faculty of Science, University of Port Harcourt, East-West Road, P. M. B. 5323, Choba, 500004 Port Harcourt, Nigeria.
unipas013@gmail.com

Abstract: The impact of urbanization on microbial abundance and diversity of soils in Port Harcourt area was investigated. The Rau and Wooten tool for existing impact quantification and ranking was used. The study sites investigated were categorized into four: highly industrialized (Trans-Amadi), moderately industrialized (Waterlines), low industrialized (Mile 1) and very low industrialized (Choba) areas. Emohua, a relatively non-industrialized area was taken as control. Soil samples were taken at the depth of 0-15 cm using a soil auger. Impact quantification, evaluation and ranking were determined using the Rau and Wooten tool. The results showed variation in microbial parameter with the highest negative impact recorded in highly industrialized area (Trans Amadi); the least impact in low (Mile 1) and very low (Choba) industrialized areas. The physicochemistry of the soil showed similar impacts. Urbanization showed a reduction in the microbial population (Total heterotrophic bacteria, total fungi, hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi) in diversity and abundance and an increase in some physicochemical parameters. Bacteria isolated are *Staphylococcus, Serratia, Micrococcus, Escherichia coli, Pseudomonas* and *Bacillus* while fungal genera characterized includes *Mucor, Aspergillus, Candida, Penicillium, Rhizopus, Trichosporon* and *Sacharomyces*. Mitigation actions are recommended to ameliorate the impact of urbanization on soil microbiota, especially in the highly and moderately industrialized areas. Primary treatment plants are necessary for proper wastes management as this, will not only reduce its risks on human health but also to the entire ecosystem.
[Wosu Kinika R. and L.O. Odokuma. **Post Impact Assessment of Urbanization on Microbial Abundance and Diversity of Soils in Port Harcourt Area.** *Rep Opinion* 2017;9(4):48-56]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 5. doi:10.7537/marsroj090417.05.

Key words: Urbanization, impact quantification, Primary treatment plants, microbial abundance and diversity, wastes management, Mitigation actions, Port Harcourt area, soil microbiota.

1. Introduction

The existence of man will continue to register its effects and impacts on the environment. The health of an organism can often be associated with a specific type of intensity of pollution and its presence can then be used to indicate polluted conditions relative to unexpected conditions in soil environment. Port Harcourt and its environments are known industrial hub and houses majority of these industries operating in the Niger Delta region of Nigeria.

Some of the industries located in the area are Shell Petroleum Development Company of Nigeria Ltd, Mobil, Chevron Nigeria Ltd, Total Nigeria Limited, Nigeria Agip Oil Company, Texaco Nigeria Limited and their subsidiaries. Others include Nigerian Bottling Company Ltd, Pabod Breweries Ltd and Rivers Vegetable Company Ltd (RIVOC) (UNEP, 2011); during which activities resulted to the influx of people from different spheres of life, thereby transforming it into one growing urban centres. A healthy soil has several physical, chemical and biological properties: it needs to incorporate adequate biodegradable organic matter, have a good structure, and be home to diverse group of organisms.

Agricultural soil's health is linked to human health, as poor soils yield fewer crops with decreased nutritional value. The anthropogenic influences, stemming from increased urbanization, industrialization, oil exploration activities, sabotage and illegal refining of petroleum hydrocarbons (Nwaichi *et al*., 2010) in Port Harcourt areas of Rivers State, on biogeochemical cyclying of matter could impede on soil quality evaluation (Akpahwe and Solomon, 2012).

1.1 Urbanization and its impacts on microbial diversity in soils environment

Soil, like any other natural resource, is not just of public health and economic importance, but it is home for life. Many case studies from heavily concentrated industrial soils around the world, particularly (but by no means exclusively) in developing countries, indicate the possible environmental and health impacts of high levels of soil contamination resulting from the activities of humans and industries.

Urbanization refers to the population shift from rural to urban areas, "the gradual increase in the proportion of people living in urban areas", and the ways in which each society adapts to the change

(Henry and Heinke, 1996). It is predominantly the process by which towns and cities are formed and become larger as more people begin living and working in central areas (FEPA, 1991). The post impact of urbanization in a broader sense could leads to indiscriminate discharges of industrial effluents and anthropogenic wastes into receiving media (Mbakwem-Aniebo *et al.*, 2014).

The improper disposal of domestic wastes, particularly in urban centres of Port Harcourt has been reported to pose serious hazard to both human and ecosystem (Alex and Solomon, 2016). Microbial populations are a quick and effective indication of changing environmental parameters in the form of shifts in microbial diversity and abundance (species richness and evenness).

Changes in the relative abundance of particular groups of microorganisms indicate that the environment has been altered (Rosello-Mora and Amann, 2001; Tate, 2000; Lenart-Boron and Boron, 2012). Microorganisms are able to do this because they are much more sensitive to selective pressures (in the form of a changing environment) and are able to adapt quickly (EPA, 2012). In as little as 20 minutes, some microbes are capable of 4 simultaneous mutations in every gene and doubling in cell count. Consequently, a microbial population can produce a next generation in only a few hours as compared to weeks, months or years for higher organisms.

Different microbial groups inhabit various niches within an ecosystem and therefore differ in their sensitivity to nutritional and environmental change (Giller *et al.,* 1998). In both field and laboratory experiments, structural changes in different microbial communities under stress were observed (Frostegard *et al.*, 1993b; Pennanen *et al*., 1996; Moffet *et al*., 2003; Abaye *et al*., 2005).

Urbanization and human activities usually results to contamination of ecological media, reduction of microbial biomass and distortion of microbial community structure (Doelman, 1986; Wosu-Kinika and Odokuma, 2016). Impact evaluation and quantification scheme is showed in Table 1 while the criteria and weighting scale used in evaluating this significance are based on Rau and Wooten's scheme (Rau and Wooten, 1980) and is expressed thus:

$$IM = \frac{\text{Control result/standard value} - \text{Obtained result}}{\text{Control result/tandard value}} \times 100$$

Where,
IM = Impact magnitude

Pollutant exposure may lead to the establishment of tolerant microbial populations, which are often represented by several Gram-positive genera such as *Bacillus, Arthrobacter* and *Corynebacterium* or Gram-negative bacteria such as *Pseudomonas, Ralstonia* or *Burkholderia* (Okerentugba and Ezeronye, 2003; Piotrowaka – Seget *et al*., 2005).

Community structure is an important aspect of the microbial biomass as the microbial community structure is the parameter controlling microbial activity (Ramsey *et al*., 2005) and influences ecosystem functioning (Gadd, 2008; Kiikkila, 2003). Contamination can seriously affect soil's ability to perform some of its key functions in the ecosystem (Brevik *et al*., 2013; Onyema *et al.,* 2013). Ecosystem system functioning is incomplete without soil microorganism, as they affect the chemical, biological and physical characteristic of soil.

Because microbial community regulates decomposition processes and nutrient cycling, it is of keen interest to understand how its structure is affected by urbanization and industrial pollution stress.

Table 1: Impact Evaluation and Weighing Scheme (Rau and Wooten, 1980).

Impact magnitude (%)	Impact evaluation	Impact classification	Definition of impact classification (IC)
0 – 20	1	Negligible	No significant impact of the parameter on the soil. It does not require remediation at all.
21− 40	2	Low	No significant impact of the parameter on the soil. It does not require remediation at all.
41− 60	3	Medium	Significant impact of the parameter on the soil. It requires remediation technique.
61− 80	4	High	Severe significant impact of the parameter on the soil. It requires remediation technique to restore soil to its original state.
> 80	5	Severe	Persistent severe significant environmental impact or damage by the parameter to the soil. It requires remediation technique to restore soil to its original state. Remediation is more extensive.

Key: Impact evaluation (IE), Impact classification (IC), Impact magnitude (IM)

The objectives of our study therefore, are to assess the impact of urbanization on the microbial abundance and diversity and physicochemical changes in the soil in Port Harcourt area of Rivers State. It will further proffer sustainable solutions on the way forward on how to ameliorate the problems.

2. Materials And Methods

2.1 Samples collection

Soil samples were collected from five different areas within Port Harcourt and its environments. These study areas includes Trans-Amadi area, Mile 1 area, Waterline area, Emuoha area, and Choba area of Port Harcourt, Rivers State, Nigeria. At each site, three replicate bulk samples were taken, consisting of 30 randomly selected sub-samples from surface soil (0-15 cm depth) using soil auger into polythene bags. The samples were transported to the laboratory for microbiological, gas chromatographic and physicochemical analyses within 24-48h.

2.2 Experimental design

Five treatments setup showing sample station, study station and study area description are as presented in Table 2.

Table 2: Description of experimental treatment protocol

S/No.	Sample station	Study station	Study area description
1.	TMA	Trans-Amadi	High Industrial area
2.	WLA	Waterlines	Moderate Industrial area
3.	MIA	Mile 1	Low Industrial area
4.	CBA	Choba	Very low Industrial area
5.	EMA	Emuoha	None Industrial area (control)

2.3 Sample processing

Soil slurry was prepared by adding 1g of the soil samples aseptically to each test tube containing nine milliliters (9ml) of sterile saline (0.85% w/v) as diluents. The test tubes were vigorously shaken to dislodge the microorganisms that might adhere to the soil particles. The content of the tubes were diluted (10-fold). From each dilution of 10^{-3} to 10^{-6}, 0.1ml was plated on sterile Nutrient agar, Saboraud dextrose agar and Mineral salt agar for microbial enumeration.

2.4 Microbiological analyses

2.4.1 Enumeration of total heterotrophic bacterial and fungal counts

Aliquots (0.1ml) of the serially diluted soil samples were spread-plated out in duplicates on nutrient agar plates following the method previously described by Chikere *et al*. (2009). The plates were incubated at 35^0C for 24 to 48h for total culturable heterotrophic bacteria count. Also, for total heterotrophic fungi count, the same procedures were followed but 1ml of lactic acid was added in the Saboraud dextrose agar medium to inhibit the growth of bacteria.

2.4.2 Enumeration of hydrocarbon utilizing bacterial and fungal counts

Hydrocarbon utilizing bacterial count (HUBC) was enumerated using a modified mineral salt medium of Mills *et al*. (1978). It contained: $MgSO_4.7H_2O$, 0.40g; KCl, 0.28g; KH_2PO_4, 0.80g; Na_2HPO_4, 1.20g; NH_4NO_3, 0.40g, NaCl, 15g; agar No. 2, 20g in 1 liter of de-ionized water. The pH was adjusted to 7.1 and media autoclaved at 121^0C for 15 min. Soil slurry was prepared and used for 10-fold dilution by mixing 1g of wet soil with 9ml of sterile saline suspension.

Crude oil was added by soaking a 9cm Whatman No. 1 filter paper with 10 ml of fresh Bonny light crude oil. The flooded filter paper was then placed on the lid of the agar plate and incubated for 7 days at 25±8 ^{0}C in an inverted position following the method of Abu and Ogiji (1996).

For hydrocarbon utilizing fungal count, the same procedure was followed except that 1ml of lactic acid was added in the modified mineral salt medium to inhibit the growth of bacteria. The filter papers placed on the lid of the agar plate served as a source of energy and carbon and supplied the hydrocarbons by vapour-phase transfer to inverted inoculums.

2.5 Physicochemical analysis

The American Public Health Association (APHA) method was adopted following standard analytical procedures (AOAC, 1990; FEPA, 1991; APHA, 1998). The parameters analyzed were phosphate content, nitrate content, total organic carbon content and potassium content of the soil.

2.6 Identification of isolates

Bacterial isolates were examined for colonial morphology and biochemical characteristics. Test employed include Gram staining, motility test, catalase test, citrate utilization, indole test, voges proskauer test, oxdase test and sugar fermentation test. Confirmatory identities of the bacteria were made using the *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994; Cheesbrough (2004).

2.7 Statistical analysis

The statistical tool of Two ways analysis of variance (ANOVA) was used to analyze the data obtained from the study station to determine the level of significance.

3. Results And Discusion

The results obtained from the impact evaluation and weighing scheme using the method of Rau and Wooten (1980) for the different physiological groups of microorganisms are presented in Tables 3 while the percentage occurrence of bacterial and fungal population in the various study station are presented in Table 4.

Results for impact evaluation for physicochemical properties of total organic carbon, phosphate, nitrate, potassium and oil and grease are presented in Tables 5. Table 3 indicated the percentage of total heterotrophic bacteria (THB) population for Mile 1 area (MIA), Trans-Amadi area (TMA), Emuoha area (EMA), Waterlines area (WLA) and Choba area (CBA), giving values of 39.6 %, 80.4 %, 18.5 %, 60.2 % and 18.1 % respectively for the study areas.

These results in Table 3 showed that CBA and EMA with values of 18.1 % and 18.5 % had negligible (0−20 %) impacts on the soil environment; indicating that there was no significant impact of the parameter on the soil while TMA study station recorded high 80.4 (61−80 %) impact; showing that there was severe significant impact of the parameter on the soil; this requires remediation to restore soil to its original state.

More so, MIA study area had 39.6 (21−40 %), thus indicating that there was no significant impact of the parameter on the soil while WLA study area with values of 60.2 (41−60 %), implies that there was significant impact of the parameter on the soil. The Table also shows the impact classification of total fungi (TF) population in the various study station in the course of the study. Hence, values of 40.1%, 79.5%, 14.6%, 59.7% and 19.3% were obtained for MIA, TMA, EMA, WLA and CBA respectively, indicating low, high, negligible, medium and negligible impacts for the various study areas.

The table further indicated that the percentage HUB enumerated during the study period showed values of 24.8% (MIA), 78.3 % (TMA), 13.9 % (EMA), 56.7 % (WLA) and13.5% (CBA), thus representing low, medium and high impact classification for MIA, WLA and TMA respectively with EMA and CBA having negligible impact. Again, this shows that there was no significant impact of the parameter on the soil.

Furthermore, the hydrocarbon utilizing fungi (HUF) for the various study sites are showed in Table 3. The MIA, TMA, EMA, WLA and CBA recorded low, high, negligible, medium and negligible impact classification during the study period.

The results indicated an impact evaluation (IE) of 2, 4, 1, 3 and 1 respectively. In this study where the amount of colony-forming bacteria present was determined, the results show that total culturable heterotrophic bacterial counts were higher in the urbanized area than the non-urbanized area. This result was in tandem with the higher population of the areas recorded during the study periods, confirming that these organisms require nutrients from human and industrial activities as their source of carbon.

The microorganisms in the polluted soil have efficient ability in utilizing the pollutants as source of carbon (Okpokwasili, 2006; Odokuma, 2012). This is in agreement with those of previous researchers (Odokuma and Dickson, 2003). Table 3 also indicated impact evaluation for hydrocarbon utilizing fungal population. The species of HUF identified were *Aspergillus, Mucor, Rhizopus* and *Cladisporium* while the HUB include *Bacillus, Pseudomonas, Escherichia, Micrococcus* and *Serratia;* which are common inhabitants of Niger Delta.

The increase in the bacteria population is attributed to the stimulatory effect of additional carbon and energy sources in the form of crude oil and allied fluids (Odokuma *et al.*, 2015) which leads to an enrichment of the oil microbial population. The microbial diversity and abundance and some physicochemical parameters contributes to soil quality because of the increase in soil stress resulting from effluent discharge and emissions that might affect the soil health and make it more hospitable to different physiological groups of soil microbiota (soil microorganisms).

Table 3: Impact evaluation for microbial diversity and abundance in soil environment

Study station	Microbiological parameters				Impact assessment of microbiological population		
	THB (%)	TF (%)	HUB (%)	HUF (%)	IM (%)	IE	IC
TMA	80.4	79.5	78.3	68.8	61−80	4	High
WLA	60.2	59.7	56.7	49.3	41−60	3	Medium
MIA	39.6	40.1	24.8	24.5	21−40	2	Low
EMA	18.5	14.6	13.9	12.9	0−20	1	Negligible
CBA	18.1	19.3	13.5	11.9	0−20	1	Negligible

Key: %: percentage, THB: Total heterotrophic bacteria, TF: total fungi, HUB: hydrocarbon utilizing bacteria, HUF: hydrocarbon utilizing fungi, IM: impact magnitude, IE: impact evaluation, IC: impact classification.

The percentage occurrence of all isolates in the various study stations (Table 4) showed that *Bacillus* sp. and *Escherichia coli* recorded 42.9% while *Citrobacter*, *Micrococcus* and *Pseudomonas* genera had 28.6%, *Serratia* gave 14.3% and *Staphylococcus* recorded 71.4% while the percentage of the single isolates were 85.7 (TMA), 42.9 for CBA, EMA, WLA and WLA respectively.

Results of diversity and abundance of fungal population of all isolates indicated the following: *Aspergillus* 3(60%), *Candida, Pencillium* and *Saccharomycetes* genera had 1(20%) while *Mucor* sp. had 2(40%). Single isolate recorded 20% for TMA and EMA, 40% in WLA, MIA and CBA in the study.

The total heterotrophic bacteria and fungi of the soils revealed higher counts in the dry season than in the wet season. This may be due to the more favourable physiochemical conditions such as nutrients (NO_3, PO_4^{2-} and SO_4^{2-}), pH and temperature contributed by allochthonous materials of the soil samples during the period (Han and Gu, 2010; Gadd, 2008). Statistically, there was significant difference (P<0.05) in microbial diversity and abundance in the study areas.

Heavy metals receive particular concern considering their strong toxicity even at low concentrations (Bong *et al*., 2010: Appenroth, 2010). Results of physicochemical analyses (total organic carbon, phosphate, nitrate and potassium are presented in Tables 5. The table showed that the percentage TOC in mile 1 area, Trans-Amadi area, Emuoha area, Waterlines area and Choba study areas were 32.8, 48.4, 18.7, 40.9 and 16.2, which fell into impact magnitudes of 21-40%, 41-60%, 0-20%, 41-60% and 0-20% respectively.

Based on the impact evaluation tool of Rau and Wooten (1980), the study areas can be classified into low (Mile 1 area), medium (Trans-Amadi and Waterlines area) and negligible (Choba and Emuoha area).

Table 4: Percentage occurrence of bacterial and fungal population in the study stations

Microbial isolate	Study station					Occurrence/ Frequency	% occurrence of all isolates
	TMA	WLA	MIA	CBA	EMA		
Bacteria							
Bacillus	+	-	-	+	+	3	42.9
Citrobacter	+	+	-	-	-	2	28.6
E. coli	+	-	+	-	+	3	42.9
Micrococcus	+	-	+	-	-	2	28.6
Pseudomonas	-	+	-	+	-	2	28.6
Serratia	+	-	-	-	-	1	14.3
Staphylococus	+	+	+	+	+	5	71.4
No. of Organism	6	3	3	3	3		
% of single isolate	85.7	42.9	42.9	42.9	42.9		
Fungi							
Aspergillus	+	+	-	+	-	3	60
Candida	-	-	+	-	-	1	20
Mucor	-	+	+	-	-	2	40
Penicillum	-	-	-	-	+	1	20
Saccharomyces	-	-	-	+	-	1	20
% of single isolate	1	2	2	2	1		
	20	40	40	40	20		

On the other hand, Table 5 shows the impact evaluation and classification for phosphate content of the soil in study areas MIA, TMA, EMA, WLA and CBA respectively. From the Rau and Wooten tool (1980), The Mile 1 area (MIA) is classified as having low impact on the soil environment while Emuoha (EMA) and Choba (CBA) study areas had negligible impact assessment on the soil. The TMA and Waterlines study areas (WLA) recorded medium impact classification respectively.

Table 5 further showed the impact magnitude, evaluation and classification for nitrate content in soil environment of the different study areas. The results indicated that MIA with 21.1% nitrate content had low impact classification, showing that there was no significant impact of the parameter on the soil. The TMA and WLA study areas with values of 40.3% and

42.2% showed medium impact which implies that there was significant impact of the parameter on the soil while EMA and CBA were negligible, with values of 18.2% and 14.3% respectively. This showed that there was no significant impact of the parameter on the soil.

Again, this conforms to Rau and Wooten (1980) classification. Soil with heavy metal can cause serious health effect with various symptoms depending on the nature and quantity of the metal ingested. They produce their toxicity by forming complexes with proteins, in which carboxylic acid (-COOH), amino (-NH_2), and thiol (-SH) group are involved (Abay *et al*., 2005; Apprenroth, 2010; Atlas and Bartha, 1973). Soil is a living resource, but once contamination exceeds a certain threshold, the soil may be considered 'functionally dead' and as such, lacking essential nutrients. Population explosion, increasing petroleum exploration, refining and other allied industrial activities in the Niger Delta has led to the wide scale contamination of most of its creeks, swamps, rivers and stream with hydrocarbons including oil and grease and dispersant products.

The contamination of these habitats constitutes public health and socio-economic hazards (Okeretugba and Ezeronye, 2003). The contaminants have been shown to be present in concentrations which may be toxic individually to different soil organisms ((Atlas and Bartha, 1973; Mbakwem-Aniebo *et al.,* 2014).

Oil and grease had values of 20.5%, 40.6%, 17.5%, 56.3% and 17.3%, giving impact classification of low, medium, negligible, medium and negligible respectively for station MIA, TMA, EMA, WLA and CBA. The potassium content of the different study areas recorded values of 23.1% for MIA, representing low impact while values of 42.7% and 44.3% obtained for TMA and WLA indicated medium impact on the soil environment.

The EMA and CBA study station with values of 20.1% and 18.6% represented negligible impacts on the soil. Urbanized societies and areas of high population densities and industrial activities have been reported by other workers to significantly contribute to contamination of almost all components of the soil environment (Chaillan *et al*., 2004).

Table 5: Impact evaluation for physicochemical parameters of the soil environment

Study station	Physicochemical parameters					Impact assessment of physicochemical parameters		
	TOC (%)	P (%)	N (%)	K (%)	O/G (%)	IM (%)	IE	IC
TMA	48.4	40.8	40.3	42.7	40.6	41−60	4	Medium
WLA	40.9	42.4	42.2	44.3	56.3	41−60	3	Medium
MIA	32.8	22.1	21.1	23.1	20.5	21−40	2	Low
EMA	18.7	19.5	18.2	20.1	17.5	0−20	1	Negligible
CBA	16.2	18.7	14.3	18.6	17.3	0−20	1	Negligible

***Key:** TOC: total organic carbon, P: phosphate, N: nitrate, K: potassium, O/G: oil and grease.

Excessive production of organic materials could leads to the buildup of 'sludge' and the mineralization process consumes all dissolved oxygen from the water column, which causes fish kills (Osibanjo *et al*., 2011; Henry and Heinke, 1996). Urbanization may leads to pollution by organic contaminants and is practically irreversible (European commission, 2012).

Pollution of the soil ecosystem poses a serious threat to soil organisms and ultimately the entire ecosystem (Zar, 1999; Okpokwasili, 1998; Solomon *et al.*, 2016). The continued discharge of improperly treated effluent into soils in Port Harcourt and its environs could further compound and worsening environmental problem of soil and cause ecological imbalance of micro-flora and fauna in the soil (Solomon *et al*., 2016).

The microbial diversity and abundance assessed in this study were generally lower in density per kg of soil than those of Chikere and Okpokwasili (2002), but similar to those reported from various Niger Delta soil ecosystems (Odokuma and Dickson, 2003). The variability of microbes in the study station may be attributed to the difference in human activities.

Open and indiscriminate dumping of solid wastes in drainages and river banks has been reported to be one of the most critical problems facing the city of Ibadan (Omoleke, 2004; Wosu-Kinika *et al.*, 2016; Wosu-Kinika *et al.*, 2017).

4 Conclusion

The microbial abundance and diversity and some physicochemical parameters contributes to soil qualities because of the increase in soil stress, due to emission and effluent discharge that might affect the soil and make it more hospitable to soil microbiota.

Urbanization showed a reduction in the microbial population (Total heterotrophic bacteria, total fungi, hydrocarbon utilizing bacteria and

hydrocarbon utilizing fungi) in diversity and abundance and an increase in some physicochemical parameters.

Mitigation actions such as proper waste management methods should be put in place to ameliorate the negative impact of urbanization on microbial diversity and abundance in soils in Port Harcourt area, especially in the highly industrialized areas.

Furthermore, primary treatment plants should be made available in urban centers for proper wastes disposal and reduce its threat to human health and the ecosystem.

Correspondence to:
Ruhuoma Wosu Kinika,
Department of Microbiology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria
Tel.: +2348-060-123-331
Email: unipas013@gmail.com

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4/22/2017