African Scientist Vol. 17, No. 3, September 30, 2016
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2016042/17304

Physicochemical, Aromatic Hydrocarbon and Bacteriological Profiles of Human Urine-Impacted-Soils Obtained From Iyaro Motor Park, Benin City, Nigeria

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(Received July 4, 2016)
(Accepted in revised form September 10, 2016)

ABSTRACT: Urine-impacted-soils were collected from different sampling points located within the vicinity of Iyaro Motor Park and analysed for physicochemical, aromatic hydrocarbon and bacteriological profiles. Standard analytical procedures which included serial dilution and pour plating, usage of appropriate meters, atomic absorption spectrophotometry and gas chromatography were employed in the evaluation of the bacteriological, physicochemical, heavy metal and poly aromatic hydrocarbon (PAH) profiles of the soils. The mean heterotrophic bacterial counts recorded for the urine contaminated soils ranged from 4.2×10^4 cfu/g to 6.1×10^4 cfu/g. *Bacillus* sp., *Proteus mirabilis, Micrococcus* sp. and *Staphylococcus* sp. were isolated from the soils. All the soils were acidic and sandy. Sodium and manganese mean values ranged from 1.93 meq/100g to 3.10 meq/100g and 29.5 mg/kg to 55.7 mg/kg respectively. The mean total PAH values for the human urine contaminated soils ranged from 229.33 µg/kg to 8265.48 µg/kg whilst the control soil has a mean total PAH value of 1062.60 µg/kg. The detrimental effect on the ascetics of the edaphic area exposed to consistent human urination is reflective of the need for increased public awareness to dissuade this low esteemed human behaviour.

Keywords: PAHs, Iyaro Motor Park, bacteriological, heavy metal, human urine, bare soils

Introduction

Soil has been described as a finite and non-renewable resource because the regeneration of soil through chemical and biological weathering of underlying rock layer requires geological time (Huber *et al.*, 2001). Dada and Aruwa (2014) reported that the organic and inorganic matter present in soil is a known determinant of soil fertility and also aid the proliferation of various micro flora which play an integral role in the maintenance of the soil's nutritional balance. The authors also stated that the topsoil has the maximal concentration of organic matter and microorganisms and it is where most of the Earth's biological soil activity occurs.

Dada and Aruwa (2014) described urine as a pale yellow fluid produced by the kidneys and it is known to contain urea, uric acid, minerals, chloride, nitrogen, sulphur, ammonia, copper, iron, phosphate, sodium, potassium, manganese, carbolic aid, calcium, salts; vitamins A, B, C, and E; enzymes, hippuric acid, creatinine and lactose, with a pH varying between 4 and 8. Other sugars are sometimes excreted in urine, if their concentration in the body becomes very high. Urea is abundant in the urine of humans and other mammals (Drangert, 2000).

Principal sources of Polycyclic Aromatic Hydrocarbons (PAHs) in soil are atmospheric deposition after local emission, long-range transport, and pollution from combustion gases emitted by industry, power plants, domestic heating and automotive exhausts (Konig *et al*., 1991). They are also generated during natural combustion like forest fires and volcanic activities (Hites *et al*., 1980). The extent of soil pollution by PAHs is known to be dependent on several factors such as the cultivation and use of the soil, its porosity, its lipophilic surface cover, and its content of humic substances (Windsor and Hites, 1978). As long as there is a carbon source for energy high population of microorganisms have been known to exist in the soil. Of this population, large number of bacteria do exists, but with a smaller biomass due to their small size (Hoormaan and Rafiq, 2010). Microorganisms have been observed to differ in their ability to tolerate disturbed and undisturbed soils. Whereas, populations of bacteria and Actinomycetes possess the ability to tolerate more soil disturbances, the reverse is the case for fungi, thus dominating in undisturbed soils (Hoormaan and Rafiq, 2010). Also, bacteria as a subset of microorganisms are generally less efficient at converting organic carbon to new cells compare to others such as fungi. According to Hoormaan and Rafiq (2010), aerobic bacteria and anaerobic bacteria assimilate approximately 5-10 and 2-5 percent of the carbon respectively, leaving behind large quantities of carbon-waste compounds. This leads to inefficiently using energy stored in the soil organic matter.

Dedeke *et al.* (2011) reported that in Nigeria, indiscriminate urine deposition in public places is rampant, and also opined that a close examination of such soil macrocosm would reveal soil patchiness, obvious discoloration and pungent ammoniacal smell. Iyaro Motor Park is a very popular vehicular location serving commuters travelling to various cities and towns within southern Nigeria. There is an observed paucity of public toilets within the park area; hence travelers and drivers alike empty their bladders indiscriminately around the premises of the park.

The aim of this research was to evaluate the physicochemical, polycyclic aromatic hydrocarbon and microbiological properties of top soils sourced from urine impacted area within the Iyaro motor park.

Materials and Methods

Collection of soil samples:

Soil samples were collected from five random sampling points sited within the vicinity of the modern bus park (Iyaro Motor Park), Benin City. Duplicate samples were collected in the month of March to June 2015. Four of the sampling points were edaphic areas within the park which were subjected to consistent human urination. The fifth sampled site which served as the control was located about 100 m from the human urine contaminated area which was covered with weed. The GPS coordinates of the motor park are 06.34662°N and 005.62367°E. The soil samples were collected at a depth of 20 cm with the aid of a soil auger. At each boring, 100 g of the respective soils were collected and dispensed into labeled polyethylene bags and transported to the laboratory for physicochemical, gas chromatographic and microbial analyses. The samples were kept in coolers containing ice packs and an average of 45 minutes was spent as transportation time prior to commencement of analyses.

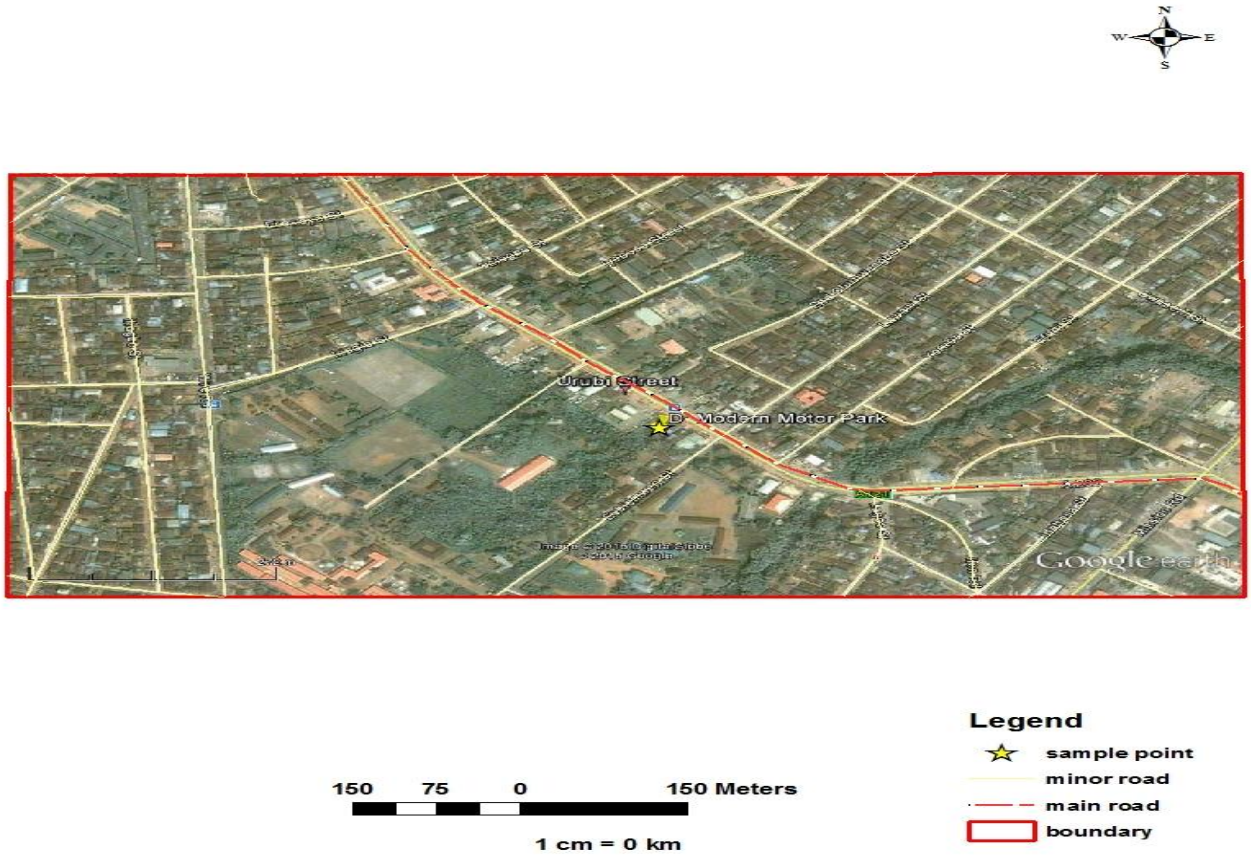

Figure 1: Map showing sampling points at Iyaro

Physicochemical and heavy metals analysis of soil samples:

Prior to physicochemical analysis, the soil samples were spread on large wooden trays and air-dried for 72 hrs. The air dried samples were also sieved using a 2mm mesh. Parameters which included: pH, exchange acidity, electrical conductivity and particle size distribution were determined using procedures described by Kalra and Maynard (1991). Exchangeable cations (Na^+, K^+ and Ca^{2+} Mg^{2+}), Total organic carbon (TOC), ammonium-nitrogen, sulphate, nitrate and available phosphorus were evaluated using procedures described by Radojevic and Bashkin (1999), Carter and Gregorich (2006). Specific heavy metals; Zinc (Zn), Iron (Fe), Lead (Pb), Copper (Cu), Vanadium (V), Nickel (Ni), Chromium (Cr) and Cadmium (Cd) were determined using a Digester (Gerhardt digester, UK.), and Atomic Absorbance Spectrophotometer (AAS) (Buck Scientific model 210 VGP USA).

Determination of the culturable heterotrophic bacterial population of the soil samples:

The culturable bacterial bio load of the soils was determined using serial dilution and pour plating method as described by Harley and Prescott (2002). One (1) ml aliquot from the seeded diluent 10^{-3} was respectively plated out in duplicates under aseptic conditions and Nutrient agar was the general purpose medium employed. Unique representative bacterial colonies were sub-cultured on freshly prepared nutrient agar plates. These plates were incubated at 35^{O}C for 24 h and the colonial characteristics of the sub-cultured bacterial colonies were recorded.

Identification of the bacterial isolates

The sub cultured bacterial isolates were subjected to an array of relevant physiological and biochemical tests which included; Gram staining, coagulase production, oxidation –fermentation (OF)

test, oxidase test, urease production and motility test. The results from these tests were collated and compared with identification schemes described by Holt *et al*. (1994) and Aneja (2003).

Evaluation of the Polycyclic Aromatic Hydrocarbon content of the soils:

This was determined with the aid of a gas chromatograph with frame ionization detector (FID). Ten grams (10g) of each soil sample was weighed into a beaker, 5 g of anhydrous sodium sulfate, 100 ml of mixed (1:1 ratio) methanol and dichloromethane (DCM) was transferred into the beaker and stirred with the magnetic stirrer for 30 mins. The solution was placed (sample and extractant) in a sonicator at 69 ^{O}C for15 mins. After extraction, the solution was allowed to cool. To one millilimetre (1ml) of the extracted samples, 60 ml of n-hexane were added in order to elute the aliphatic hydrocarbons. The eluted samples were concentrated down to 3ml using rotary evaporator. The samples were finally transferred into a Teflon lined screwed vial and labeled for analysis of PAH.

Multivariate data analysis:

The data obtained were subjected to descriptive statistical analysis such as mean, while the PAH data soils were further subjected to principal component analysis using PAST software.

Results

The physicochemical properties of both the urine contaminated top soils and the control soil are shown in Table 1. The pH varied from 4.26 for soil 3 to 5.95 for soil 1 (Table 1). Conductivity and organic carbon values ranged from 697 µS/cm for soil 4 to 1043µS/cm for soil 3 and 3.22% for soil 1 to 4.81% for soil 2 respectively. The sodium, potassium and calcium data ranged from 1.93 meq/100g for soil 3 to 3.10 meq/100g for soil 2, 0.15 meq/100g for soil 3 to 0.36 meq/100g for soil 1 and 4.57 meq/100g for soil 1 to 6.21 meq/100g for soil 3 respectively (Table 1).The pH, conductivity, organic carbon, sodium and calcium values for the control soil were; 6.24, 630µS/cm, 3.91 %, 2.75 meq/100g and 4.08 meq/100g (Table 1). Available phosphorus and ammonium -nitrogen values for the contaminated soils ranged from 9.32 mg/kg for soil 4 to 17.4 mg/kg for soil 3. All the examined soils were sandy with sand content varying from 90.9 % for soil 2 to 92.6% for soil 1 (Table 1).

The PAH profile of the soils are shown in Table 2. The total PAH values ranged from 229.33 µg/kg for soil 3 to 8265.48 µg/kg for soil 4 (Table 2). The control soil had a total PAH value of 1062.60 µg/kg (Table 2).

The heavy metal values of the soil samples are presented in Table 3. Fe readings ranged from 734.4 mg/kg for soil 4 to 1129 mg/kg for soil 2. Mn and Zn values ranged from 29.5 mg/kg for soil 4 to 55.7 mg/kg for soil 2 and 61 mg/kg for soil 4 to 194 mg/kg for soil 2 (Table 3). Copper and chromium readings ranged from 11.7 mg/kg for soil 2 to 28.6 mg/kg for soil 1 and 11.7 mg/kg for soil 3 to 23.3 mg/kg for soil 2. Lead and vanadium values varied from 15.6 mg/kg for soil 4 to 29.5 mg/kg for soil 1 and 4.89 mg/kg for soil 1 to 7.90 mg/kg for soil 3 (Table 3). Fe, Mn, Zn, Cd, Pb and V readings recorded for the control soil were; 184.1 mg/kg, 6.88 mg/kg, 17.5 mg/kg, 0.93 mg/kg, 1.47 mg/kg and 0.62 mg/kg respectively (Table 3). The heterotrophic culturable bacterial counts recorded for the urine contaminated soils ranged from 4.2 $\times 10^4$ cfu/g for soil 4 to 6.1 $\times 10^4$ cfu/g for soil 3. The control had a bacterial bio load of 1.1 $\times 10^4$ cfu/g respectively (Table 4). *Bacillus* sp., *Micrococcus* sp. and *Staphylococcus* sp. were isolated and identified from the analyzed soils whilst *Proteus mirabilis* was detected in soils 2 and 3 respectively.

Table 1: Physical and chemical mean values of the soils collected from the sampling points

Location/ Soil	pH	EC µS/cm	Org.C %	EA	Na	K	Ca	Mg	Av.P	NH_4N	NO_3	SO_2	Clay	Silt	Sand
					meq/100g of soil					mg/kg				%	
Soil 1	5.95	840	3.22	0.4	2.39	0.36	4.57	1.77	15.6	12.4	19.1	6.43	5.3	2.1	92.6
Soil 2	5.81	770	4.83	0.6	3.10	0.27	5.38	1.94	17.4	13.2	13.5	6.28	6.1	3.0	90.9
Soil 3	4.26	1043	3.70	1.2	1.93	0.15	6.21	1. 72	12.5	10.1	15.0	9.49	5.9	2.7	91.4
Soil 4	5.57	697	4.41	0.9	2.15	0.34	5.60	1.70	9.32	11.7	14.4	4.11	5.5	2.4	92.1
Control	6.24	630	3.91	0.5	2.75	0.15	4.08	1.22	5.32	6.53	8.56	3.71	4.5	3.4	93.3

Key: Over all mean values, EC: Electrical conductivity, Org. C: Organic carbon, EA: Exchange acidity, Av. P: Available phosphorus

Table 2: Available polycyclic aromatic hydrocarbons in the soil samples

PAHs	Unit	Soil 1	Soil 2	Soil 3	Soil 4	Control
Acenaphthylene	µg/kg	Nil	Nil	Nil	4851.74	Nil
Acenaphthene	µg/kg	Nil	Nil	Nil	2529.54	Nil
Phenanthrene	µg/kg	Nil	Nil	Nil	492.06	Nil
Anthracene	µg/kg	Nil	Nil	Nil	392.15	Nil
Pyrene	µg/kg	1091.12	Nil	Nil	Nil	395.14
1,2-Benzenothracene	µg/kg	1843.69	1828.98	Nil	Nil	Nil
Chrysene	µg/kg	74.00	Nil	Nil	Nil	Nil
Benzo(b)fluoranthene	µg/kg	1237.89	Nil	Nil	222.25	145.32
Benzo(k) fluoranthene	µg/kg	989.68	Nil	Nil	Nil	Nil
Benzo(a)pyrene	µg/kg	Nil	Nil	129.33	Nil	Nil
Benzo(g,h,i)pyrene	µg/kg	560.71	Nil	1	Nil	522.14
Total	µg/kg	5797.10	1828.98	229.33	8265.48	1062.60

Over all mean values

Table 3: Mean heavy metal content of the soils

Location/soil	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
					mg/kg				
Soil 1	932	47.3	128.2	28.6	19.4	22.0	29.5	5.31	4.89
Soil 2	1129	55.7	194	11.7	23.3	14.2	24.1	6.08	5.71
Soil 3	810	42.4	96.1	17.3	11.7	9.11	16.4	8.43	7.90
Soil 4	734.4	29.5	61	14.6	16.8	10.2	15.6	7.91	6.84
Control	184.1	6.88	17.5	2.90	1.12	0.93	1.47	0.74	0.62

Over all mean values

Table 4: Mean heterotrophic bacterial counts for the soils

Location/Soils	Total heterotrophic bacterial counts ($\times 10^4$ cfu/g)	Identified bacterial isolate present in the soil sample
Soil 1	5.2	*Staphylococcus* sp., *Bacillus* sp. and *Micrococcus* sp.
Soil 2	5.5	*Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and *Proteus mirabilis*
Soil 3	6.1	*Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and *Proteus mirabilis*
Soil 4	4.2	*Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp.
Control	1.1	*Bacillus* sp.

Over all mean values

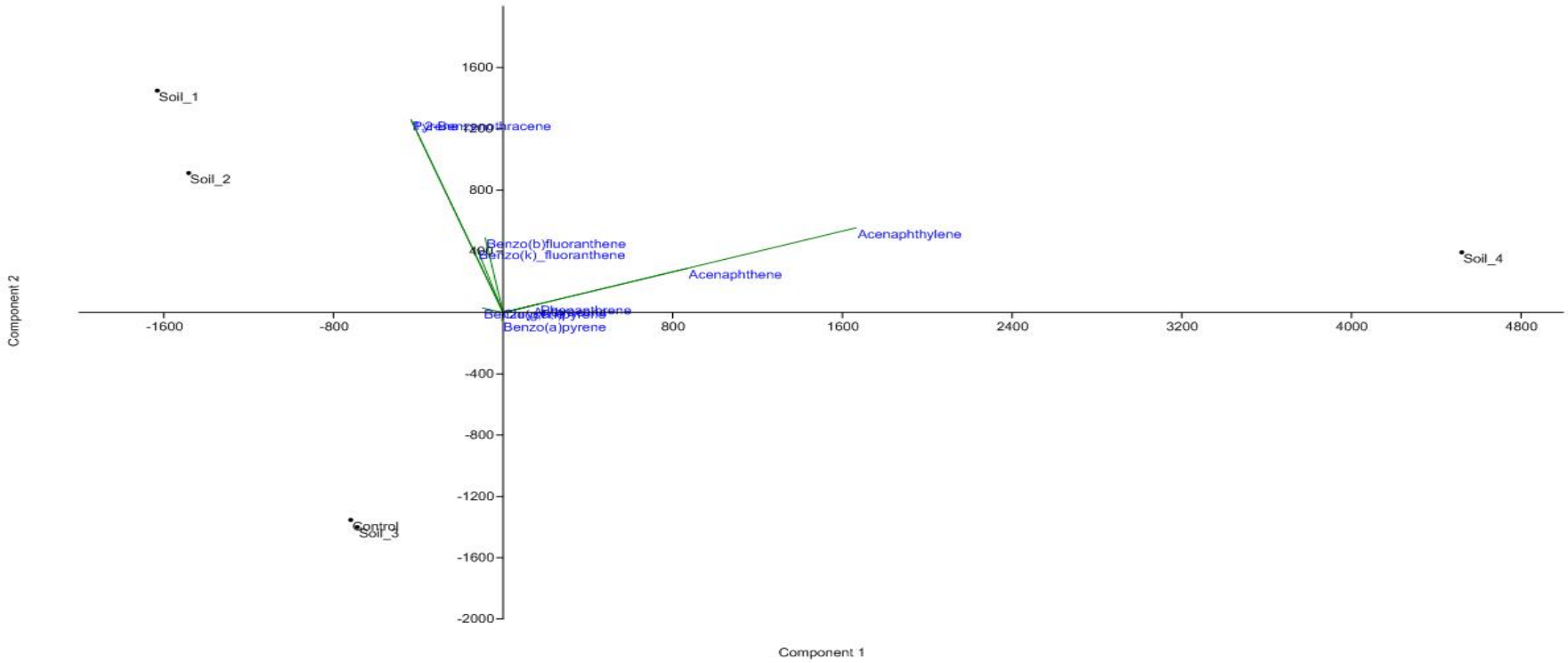

Figure 2: PCA biplot of the soils according to the PAH profiles. The first component (x_1 horizontal axis) is responsible for all of the variance in the PAH values

Discussion

All the examined soils were acidic (Table 1). Comparatively, the pH of the control soil was higher than those of the urine impacted soils. The pH values of the urine contaminated soils were lower than a range of values; 6-6.5 reported by Olayinka *et al.* (2015). The authors stated that most soil nutrients are readily available when soil pH is at 6.5, but when pH is below 6.5, elements such as manganese can reach toxic level for some sensitive plants. Expectedly, the continued urine pollution of the soils invariably boosted the available phosphorus and ammonia nitrogen content of the affected soils in comparison with the control soil (Table 1). Although the composition of urine fluctuates from one person to another and is dependent mainly on diet and physical activity (Sene *et al*., 2013), human urine is known to contain a plethora of both micro and macro elements such as nitrogen, phosphorus and copper. The positive impact of urine contamination on the nutritional and elemental status of the soil was collaborated by the higher culturable microbial biomass isolated from the polluted soils in comparison with the control (Table 4). This trend would justify the agricultural usage of human urine as a cheap form of liquid fertilizer and effectiveness has been compared successfully with commercial chemical fertilizer using many types of vegetables and crops (Mnkeni *et al*., 2008).

The microbial isolates characterized from the examined soils were similar to isolates reported by Dada and Aruwa (2014) who investigated the culturable microbial flora of about forty eight (48) human urine impacted soils collected from around several lecture theatres within Federal University of Technology, Akure. The expected isolation of soil borne Gram positive bacteria such as *Bacillus* sp. and *Micrococcus* sp. (Table 4), could be attributed to the ubiquitous distribution of these bacteria within the top soil habitat. Dada and Aruwa (2014) opined that public urinal soils could become major factors in the spread of infectious diseases especially in the absence of adequate sanitary facilities.

The heavy metal concentrations of the urine contaminated soils were comparatively higher than values recorded for the control soil (Table 3). This trend could be attributed to the composition of the underlying parent rock from where the soil was formed especially in the case of iron. The variations in the heavy metal profile of the soils could also be attributed to the impact of anthropogenic usage or practices on the soil surface. Examples of such these practices include; vehicular emissions and indiscriminate disposal of refined petroleum products and used batteries.

Human urine does not contain any known PAHs. Probable source of PAHs in the soils could be diverse ranging from pyrogenic to petrogenic origins (Olayinka *et al.*, 2015). The soils were differentiated by their PAH content according to the principal component analysis (PCA) (Fig. 2). There was no dispersion between control soil and soil 3 but soils 1, 2 and 4 were well spaced from each other (Fig. 2). The differing PAH values especially for soils 1, 2 and 4 was responsible for the observed distances between the soils (Fig. 2). The detection of PAHs in both the control and the urine impacted soils could be reflective of the ubiquitous distribution of these compounds in these soils. The trend could also be symptomatic of the possible deposition of refined petroleum products on the soils over time. However, this phenomenon is of major public health relevance as these compounds are known to possess lipophilic property, and as such, PAHs have a high potential for bio-magnification through trophic transfers (Clements *et al.*, 1994).

In view of the observed PAH values of the urine polluted soils especially soil 4 and the lead profile of the soils, it is recommended that the potential presence of PAHs and the Pb in plants growing around urine impacted soils should be investigated. The consequential detrimental effect on the ascetics of the edaphic area within the motor park being utilized as an *ad-hoc* human urinal is reflective of the need for increased public awareness to dissuade this low esteemed human behaviour.

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