



Original Article

Level and Health Risk Evaluation of Heavy Metals and Microorganisms in Urban Soils of Lagos, Southwest Nigeria

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ABSTRACT

Heavy metal and microbial pollution of the environment are linked to the increasing prevalence of diseases worldwide. Accordingly, this study assessed the safety of urban soils in Lagos, Nigeria, with regard to the levels of lead (Pb), nickel (Ni), cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn), and microorganisms. Soil samples were collected from Iwaya, Makoko, and Ilaje areas of the city and subjected to atomic absorption spectroscopy (AAS) and microbiological examinations using standard protocols. The mean values obtained for the heavy metals and microorganisms were compared with the World Health Organization (WHO) permissible limits. The average daily oral ingestion (ADOI), average daily dermal ingestion (ADDI), and the hazard quotient (HQ) of the heavy metals were also calculated. The AAS indicated that the soils in the three areas contained non-permissible levels of the evaluated heavy metals, except Cu and Cd. The microbiological examinations also showed that the soils contained abnormal levels of heterotrophic bacteria (HB), hydrocarbon utilizing bacteria (HUB), heterotrophic fungi (HF), and hydrocarbon utilizing fungi (HUF). The ADOI of the heavy metals in the three areas were above the recommended levels, while ADDI were normal. Moreover, the HQ of oral ingestion of each heavy metal except Cr was greater than one, while the HQ of dermal ingestion of the heavy metals was less than one. These findings suggest that the soils could predispose the residents in the areas to diseases. Thus, there is a need for regular environmental sanitation and environmental pollution control in the areas, and personal hygiene.

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1. Introduction

The burden of diseases is rising worldwide in which environmental pollution is considered a significant factor. The burden is particularly enormous in developing countries due to financial constraints, poor technology, and poor enforcement of environmental laws [1-3]. About 8.9 million people die yearly from pollution-related diseases, of which 8.4 million deaths (94%) occur in developing

countries [4, 5]. Among environmental pollutants, heavy metals are the most studied because they are toxic and non-biodegradable [6, 7]. There is no exact definition of heavy metal, but it is loosely defined as any element with a high molecular weight and a density five times greater than that of water [8, 9]. Most common heavy metals include lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg),

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manganese (Mn), nickel (Ni), zinc (Zn), chromium (Cr), cobalt (Co), copper (Cu), molybdenum (Mo), and antimony (Sb) [7, 10]. The sources of heavy metals include soil erosion, weathering, agrochemicals, mining, and waste discharges from homes and industries [7, 11]. Some heavy metals play a biological role as micronutrients but become biotoxic at certain doses [12, 13]. Heavy metals can accumulate to toxic levels in the body [6], depleting the antioxidants and generating free radicals [7, 14]. Heavy metals are implicated in several health hazards, including respiratory, genetic and haematological diseases as well as skin, sight and brain damage [15].

Another group of environmental contaminants that are implicated in the rising prevalence of diseases is microorganisms. Most microorganisms are harmless and even play an important role in the environment and biological systems [16]. However, some species of soil bacteria, fungi, protozoa, and viruses, can cause diseases at certain levels [17, 18]. Some microorganisms induce diseases in susceptible individuals, such as people with dysfunctional immune system [16, 19]. Some other microorganisms infect humans to complete their life-cycles [16, 19]. The sources of microorganisms in the environment include dumpsite wastes, septic tanks, sewage, and gardens as well as human and animal excreta [20–22].

The soil, being the reservoir for all elements, bears the greatest burden of environmental pollution [23]. Soils are polluted in a number of ways and urban soils, in particular, are often physically disturbed and chemically polluted [23, 24]. The sources of contaminants in urban soils include vehicular traffic, home and industrial wastes, and agrochemicals [25, 26]. Humans are exposed to soil contaminants through skin contact, inhalation, and ingestion [27]. Overall, these showed that urban soils bear an enormous health burden. Thus, to stem the rising prevalence of diseases worldwide, periodic monitoring of heavy metals and microorganisms in urban soils as a preventive measure is necessary. To the best of our knowledge, a monitoring study with the mentioned objectives has not been carried out in Iwaya, Makoko, and Ilaje, which are densely populated areas of Lagos, Nigeria. Accordingly, this study determined the safety of urban soils in Iwaya, Makoko, and Ilaje, with regard to the levels of selected heavy metals and microorganisms.

2. Materials and Methods

2.1. Description of the study sites

The study was conducted in Iwaya, Makoko, and Ilaje areas of Lagos, South-western Nigeria (Fig. 1). Lagos State lies between longitude 3°21'24"E and latitude 6°35'8"N

[28]. The state shares boundaries with Ogun State on the north and east as well as the Republic of Benin on the west and the Atlantic Ocean on the south. The vegetation of the state is tropic with a short dry season between December and February and a long-wet season between March and November. The temperature ranges from 28.6 °C in the wet season to 33.7 °C in the dry season [29]. Lagos is one of the rapidly urbanizing cities in the world and the most industrialized in Nigeria.

Iwaya and Makoko are located in Lagos Mainland Local Government Area of the state. Makoko is bordered on the West by Ebute Meta; in the South by Third Mainland Bridge, and East by Lagos Lagoon [28]. On the northern area of Makoko lies the Iwaya community. Iwaya is densely populated in the east of Lagos Lagoon and surrounded by several densely populated towns. Ilaje, another study site, is densely populated in Shomolu Local Government Area of the state. Generally, the study areas are characterized by heavy anthropogenic activities, filthy environments, and heavy traffics, whose health and environmental effects need to be monitored periodically.

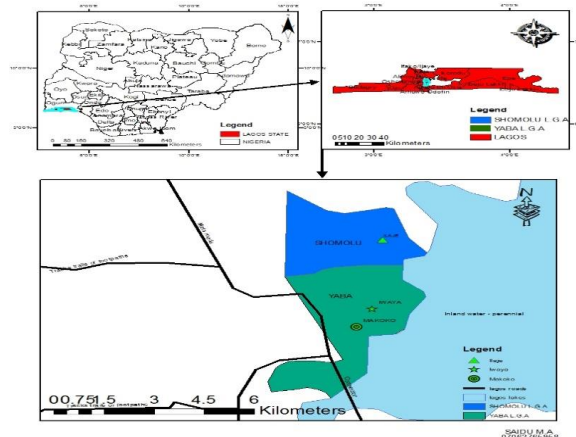


Figure 1: Location map of the study areas (ArcGIS 10.3 software).

2.2 Soil sample collection and preparation

Ninety (90) soil samples (30 from each area) were collected from Iwaya, Maroko, and Ilaje between January and June 2020. The samplings were done randomly at a depth of 0–10 cm [30] into clean and pre-sterilized polyethylene bags, labelled appropriately, and transported to the laboratory. The samples were air-dried at room temperature (29 °C) for 6 days, and later oven-dried until constant weights were attained [31]. The samples were then ground with a mortar and pestle, sieved with a 2-mm mesh sieve, and homogenized [31]. The products obtained were stored in a desiccator before further analyses.

2.3 Heavy metals analysis

The soil samples were digested and analysed for heavy metals following procedures described by Yahaya *et al.*

[32]. One (1) g each of the soil samples was transferred into a pre-washed 100-mL beaker containing analytical grade 25 mL aqua-regia mixture (70% HNO₃ and HCl ratio 3:1) and 5 mL 30% H₂O₂. The mixture was digested at 80 °C until a homogenous solution was obtained. The solution was cooled and then filtered using a Whatman No. 42 filter paper into a 50-mL volumetric flask and diluted to the mark with deionized water. The filtrate was then subjected to atomic absorption spectroscopy using a spectrophotometer (UNICAM, Model: 969) to determine the levels of Cu, Pb, Cd, Cr, Ni, and Zn.

2.4 Quality assurance and control

All glassware and plastic materials were washed with a detergent solution and rinsed with deionized water. The materials were then sterilized with 10% nitric acid and rinsed again with deionized water.

Background contamination of the samples was checked to ensure the accuracy of the data. Blank samples were analysed after five samples and all analyses were replicated three times. The reproducibility of the values was found to be at the 95% confidence level. Thus, the mean value of each heavy metal was used for further interpretation. All the acids and reagents used were of high analytical grade. The performance of the instrument was checked by analysing the standard reference material solutions concurrently to check the precision of the instrument.

2.5 Microbial analysis

The total bacteria counts were estimated using the membrane filtration technique described by Brock [33]. Ten (10) g of each soil sample was added to 95 mL of 0.1% (w/v) solution of sodium pyrophosphate to homogenize it [34]. The mixture was then passed through a sterile cellulose filter after which the filter was placed on a nutrient agar plate and incubated for about 24 hours at 35 °C. The total bacteria colonies formed on the plate were then estimated using a colony counter. The membrane filtration technique was also used to estimate the coliform numbers. However, two-step enrichment was used to grow microbes [35]. The filters containing bacteria were placed on an absorbent pad saturated with lauryl tryptose broth and incubated at 35 °C for 2 hours. The filters were then transferred to an absorbent pad saturated with M-Endo media and incubated for 22 hours at 35 °C. Sheen colonies were observed and then estimated with a colony counter. All the isolates were then subjected to morphological and biochemical tests to identify individual species following standard procedures described by Abiola and Oyetayo [36].

2.6 Health risk assessment of the heavy metals

The health risks of the soils were calculated from the average daily oral ingestion (ADOI), and average daily dermal ingestion (ADDI) of the selected heavy metals [37].

$$\text{ADOI (mg/kg/d)} = CI \times IR \times EF \times ED / ABW \times AT$$

$$\text{ADDI (mg/kg/d)} = CI \times SA \times AF \times ABS \times EF \times ED / ABW \times AT$$

In the formula above, *CI* represents the heavy metal concentration in the soil, *IR* is the ingestion rate of the soil, *EF* shows the exposure frequency, *ED* stands for exposure duration, *SA* represents the exposed surface area of skin, *AF* is the adherence factor, *ABF* shows the dermal absorption factor, *ABW* stands for the average body weight, *AT* is the average time. The standard values of these exposure factors are shown in Table 1 [38, 39].

Table 1: Standard values for calculating ADO1 and ADDI of the heavy metals

Exposure Factors	Units	Values
Concentration of metals in soil (Ci)	Mg/L	-
Ingestion rate of soil (IR)	L/day	50 for child, 24 for adult
Exposure duration (ED)	Years	6 for child, 24 for adult
Average body weight (ABW)	Kg	29 for child, 65 for adult
Average time (AT)	Days	ED x 365
Exposure frequency (Ef)	Days / Years	365
Exposure duration (ED)	Years	55
Exposed skin area (SA)	Cm ²	2800 for children, 5700 for adult
Soil to skin adherence factor	Kg/Cm ² /Day	0.000001 for children, 0.0000002 for adult
Dermal absorption factor (ABF _{Pb})	-	0.006
Dermal absorption factor (ABF _{Ni})	-	0.35
Dermal absorption factor (ABF _{Cd})	-	0.14
Dermal absorption factor (ABF _{Cr})	-	0.04
Dermal absorption factor (ABF _{Zn})	-	0.02
Dermal absorption factor (ABF _{Cu})	-	0.10

The non-carcinogenic risks of the soils were calculated from the hazard quotient (HQ) of the oral and dermal ingestion of the heavy metals [37].

$$HQ = \text{ADOI/RFD}$$

$$HQ = \text{ADDI/RFD}$$

In the formula above, *RFD* represents the reference dose of a specific substance. The standard values of the oral and dermal reference dose of each heavy metal are shown in Table 2 [40].

Table 2: Oral/dermal reference dose (RFD) of Pb, Ni, Cd, Cr, Zn, and Cu in soils

Metal	Values	
	Oral RFD	Dermal RFD
Pb	0.00014	0.00014
Ni	0.02	0.0008
Cd	0.001	0.000025
Cr	1.5	0.0195
Zn	0.300	0.3
Cu	0.04	0.04

2.7 Data analysis

Descriptive statistics were used to summarize data collected from the sampling sites as mean and standard deviation (SD).

3. Result and Discussion

3.1 Levels of the selected heavy metals in the soil samples

Table 3 reveals the levels of Pb, Ni, Cd, Cu, Cr, and Zn in the samples of urban soils obtained from Iwaya, Makoko, and Ilaje. All the mentioned heavy metals except Cd and Cu were detected in the soil samples above the World Health Organization (WHO) permissible limits. Zn had the highest concentrations in the soil samples of the three areas, of which Ilaje had the highest concentration (142.5 mg/kg), followed by Makoko (118.75 mg/kg), and Iwaya (62.5 mg/kg). Cd was not detected in the three soil samples, making it the least detected heavy metal. This result is consistent with Adeyi and Babalola [41] and Famuyiwa *et al.* [30] who reported abnormal concentrations of Pb, Ni, Cu, Zn, Cr, and Cd in urban soils in some areas of Lagos. The finding of the current study indicated that the urban soils of the three areas may predispose residents to health hazards if ingested. Through groundwater and food chain, soils can transfer pollutants to humans [42]. Pb exposure can retard growth and cause neurologic, hearing, memory and calcium metabolism imbalance, among others [43]. Ni exposure causes allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer, and dermatitis [44]. Although detected at permissible limits, long-term exposure to Cu can cause gastrointestinal problems and liver diseases [45]. High doses of Zn increase apoptosis [46], and hexavalent chromium can cause cancers [47]. The observed sources of these heavy metals in the studied areas include automobile exhausts, lubricant oils, tires and brake wears, road abrasions, industrial wastes, sewage, gardens, and dump sites.

Table 3: Heavy metal concentrations in soils from Iwaya, Makoko, and Iaje in Lagos

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	2.35 ± 0.006	1.11 ± 0.010	ND	0.13 ± 0.015	62.53 ± 0.058	0.79 ± 0.038
Makoko	2.29 ± 0.015	1.05 ± 0.012	ND	0.15 ± 0.015	118.75 ± 0.065	0.84 ± 0.006
Ilaje	2.25 ± 0.010	1.12 ± 0.015	ND	0.08 ± 0.010	142.5 ± 0.5	0.88 ± 0.015
WHO ^[48]	≤0.01	≤0.02	≤0.02	≤0.05	≤5.00	≤5.00

Values were expressed as Mean ± SD and mg/l; ND= not Detected; WHO = World Health Organization.

3.2 Health risks of the selected heavy metals

The ADOI and ADDI of Pb, Ni, Cu, Cr, and Zn by adults

Table 4: Average daily oral ingestion (ADOI) of heavy metals by adults in Iwaya, Makoko, and Ilaje Lagos

and children in Iwaya, Makoko, and Ilaje through the urban soils are presented in Tables 4-7. The ADOI of Pb and Zn by adults in the three areas as well as Cr in Iwaya were above the recommended limits (Table 4). Except for Cr in Ilaje, the ADOI of the evaluated metals by children in the three areas were above the recommended limits (Table 5). These suggest that oral ingestions of the soils might predispose both adults and children in the areas to health hazards. However, dermal ingestion of the metals may not pose any risk to adults and children in the three areas as the ADDI of the heavy metals were within the recommended limits (Tables 6 and 7). Generally, the average daily ingestions (oral and dermal) of the heavy metals by children were greater than that of adults, indicating that the soils could pose greater risks to children than adults.

The HQ of the heavy metals through oral and dermal ingestion by adults and children in the three areas are shown in Tables 8-11. Except for Cr, the HQ of the oral ingestion of the evaluated heavy metals of the three areas for both adults and children were above one, the threshold at which a substance is considered toxic (Tables 8 and 9). However, the HQ of the dermal ingestion of the heavy metals by both adults and children in the three areas was less than one (Tables 10 and 11). This again showed that the dermal ingestion of the heavy metals may not pose a significant risk compared to oral ingestion. From the Tables, HQ of both oral and dermal exposure of children in the three areas were greater than that of adults, which again indicate that the heavy metals in the soils may pose more risk to children than adults. Overall, the results indicate that the urban soils of the three areas pose health hazards to children and adults. For adults, the effects may be more in residents that live beyond the average life expectancy in Nigeria (55 years). This is because the calculation was done based on the average life expectancy in the country. This result is in line with Adedeji *et al.* [49] who reported the potential health risk of some heavy metals in residential garden soils in exposed children. Additionally, Peter and Adeniyi *et al.* [50] and Adedeji *et al.* [51] reported potential health risks of heavy metals (Cu, Zn and Cd) in urban soils for both adults and children in Lagos Mainland areas and Ijebu Ode, respectively. However, these results contradict Durowoju *et al.* [52] and Famuyiwa *et al.* [53] who reported no threat to the health of school children exposed to playground soils in certain areas of Lagos. Anthropogenic activities vary across the city which might have caused the uneven distribution of heavy metals and potential health risks.

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	0.72	0.34	0.00	0.40	19.23	0.24
Makoko	0.70	0.32	0.00	0.05	36.54	0.26
Illaje	0.69	0.34	0.00	0.02	43.85	0.27
RDI ^[54]	0.21	0.50	0.06	0.20	-	0.90

Values were expressed in mg/day; RDI = recommended daily intake.

Table 5: Average daily oral ingestion (ADOI) of heavy metals by children in Iwaya, Makoko, and Illaje Lagos

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	4.05	1.90	0.00	0.22	107.75	1.36
Makoko	3.94	1.81	0.00	0.26	204.74	1.45
Illaje	3.87	1.91	0.00	0.14	245.69	1.52
RDI ^[54]	0.21	0.50	0.06	0.20	-	0.90

Values were expressed in mg/day; RDI = recommended daily intake.

Table 6: Average daily dermal ingestion (ADDI) of heavy metals by adults in Iwaya, Makoko, and Illaje Lagos

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	0.0000002	0.0000068	0.00	0.000000091	0.000022	0.000
Makoko	0.00000024	0.0000064	0.00	0.0000001	0.00004	0.000001
Illaje	0.0000002	0.0000068	0.00	0.00000006	0.000049	0.0000015
RDI ^[54]	0.21	0.50	0.06	0.20	-	0.90

Values were expressed in mg/day; RDI = recommended daily intake.

Table 7: Average daily dermal ingestion (ADDI) of heavy metals by children in Iwaya, Makoko, and Illaje Lagos

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	0.0000013	0.000037	0.00	0.0000015	0.00025	0.0000076
Makoko	0.000027	0.00021	0.00	0.0000005	0.0002	0.000008
Illaje	0.000001	0.00003	0.00	0.0000003	0.00027	0.0000009
RDI ^[54]	0.21	0.50	0.06	0.20	-	0.90

Values were expressed in mg/day; RDI = recommended daily intake.

Table 8: Hazard quotient (HQ) of the heavy metals via oral ingestion by adults in Iwaya, Makoko, and Illaje

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	7200	17.00	0.00	0.27	64.10	6.00
Makoko	7000	16.00	0.00	0.03	121.80	6.50
Illaje	6900	17.00	0.00	0.01	146.17	6.75

Table 9: Hazard quotient (HQ) of the heavy metals via oral ingestion by children in Iwaya, Makoko, and Illaje

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	40500	94.80	0.00	0.18	359.20	9.00
Makoko	39700	90.50	0.00	0.17	682.47	36.25
Illaje	38700	95.50	0.00	0.09	818.97	38.00

Table 10: Hazard quotient (HQ) of the heavy metals via dermal ingestion by adults in Iwaya, Makoko, and Illaje

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	0.001	0.0085	0.00	0.000005	0.00005	0.00
Makoko	0.0017	0.008	0.00	0.000005	0.0001	0.000025
Illaje	0.0014	0.003	0.00	0.0000007	0.00016	0.000125

Table 11: Hazard quotient (HQ) of the heavy metals via dermal ingestion by children in Iwaya, Makoko, and Illaje

Location	Pb	Ni	Cd	Cr	Zn	Cu
Iwaya	0.009	0.046	0.00	0.000025	0.0008	0.00019
Makoko	0.193	0.262	0.00	0.00003	0.00067	0.0002
Illaje	0.0071	0.003	0.00	0.000015	0.00016	0.000003

3.3 Levels and species of microorganisms in the soil samples

The levels and species of bacteria, fungi, and coliforms detected in the urban soils of Iwaya, Makoko, and Ilaje are presented in Table 12. The soil samples of the three areas have abnormal counts of heterotrophic bacteria (HB), hydrocarbon utilizing bacteria (HUB), heterotrophic fungi (HF), and hydrocarbon utilizing fungi (HUF). There was no faecal coliform (FC) in the soil samples from the three areas. HB had the highest counts of all the microbes isolated which were as high as 2.04×10^9 in Makoko, 1.98×10^9 in Iwaya, and 1.61×10^9 in Ilaje. Morphological and biochemical characterizations of the isolates from the three studied areas revealed *Bacillus spp.* (bacteria) *Pseudomonas aeruginosa* (bacteria), *Aspergillus niger* (fungi), and *Fasarium spp.* (fungi). Makoko and Ilaje soils also contained *Clostridium spp.* (bacteria) and *Mucor spp.* (fungi). This result is consistent with Taiwo *et al.* [55] who detected abnormal bacterial and fungi counts in urban soils near electronic waste dumpsites in Alaba, Ogijo, and Ikorodu areas of Lagos. However, the absence of FC observed in this study contradicts Omotayo *et al.* [56] who detected abnormal coliform counts in urban garden soils in Lagos. These contrasting results might be due to varied anthropogenic activities and sanitary conditions across the city. The detection of bacteria and fungi species beyond permissible limits in this study again proves that the soils may pose a health threat to the residents of the three studied areas if ingested or inhaled. *Clostridium spp.* can

be contracted through the exposure of wounds to soil and cause tetanus, botulism, and gas gangrene [57]. *Clostridium spp.* can also compromise the immune function and cause diarrhoea [58]. *Bacillus spp.* in the soil can contaminate water or food and poisoning humans [58]. *Fasarium spp.* secretes mycotoxin which can cause gastrointestinal diseases [59]. *Pseudomonas aeruginosa* can cause endocarditis, osteomyelitis, pneumonia, urinary tract infections, gastrointestinal infections, meningitis, septicemia, folliculitis, ear infections, and cystic fibrosis, among others [60]. *Aspergillus niger* can induce allergy and may produce certain mycotoxins [61]. *Mucor spp.* causes mucormycosis in immunosuppressed individuals, predisposing them to allergy, gastrointestinal diseases as well as pulmonary diseases through inhalation or skin injuries [62]. Generally, the detection of HUB and HUF in the soils indicates the presence of substances containing hydrocarbons such as petrol and lubricants that may damage human health if ingested or inhaled [63]. Furthermore, the detection of HB and HF in all the soil samples suggests the presence of high concentrations of organic carbon, bio-films, and nutrients, which are necessary for the growth and survival of all microorganisms [64.]. This could partly be responsible for the abnormal microbial counts of the soil samples. The noticeable sources of microorganisms in the soils in the studied areas are nutrient-laden wastewaters from homes and small-scale industries, dumpsites, restaurants, gardens, and fishing, among others.

Table 12: Levels and species of microorganisms detected in the soils from Iwaya, Makoko, and Ilaje, Lagos

Location	THB	THF	TFC	THUB	THUF	Predominant species isolated
	($\times 10^9$)	($\times 10^4$)	($\times 10$)	($\times 10^3$)	($\times 10^2$)	
Iwaya	1.98	10	NA	57.0	3.0	<i>Bacillus spp, Pseudomonas aeruginosa, Aspergillus niger, Fasarium spp</i>
Makoko	2.04	16.00	NA	69.0	8	<i>Bacillus spp, Pseudomonas aeruginosa, Aspergillus niger, Fasarium spp Mucor spp, Clostridium spp</i>
Ilaje	1.6100	4.00	NA	45.00	1.00	<i>Bacillus spp, Pseudomonas aeruginosa, Aspergillus niger, Fasarium spp Mucor spp, Clostridium spp</i>
WHO ⁶⁵	≤ 100	≤ 50	0	≤ 100	≤ 50	

Values were expressed in CFU/g; WHO = World Health Organization.

THB = total heterotrophic bacteria; THF = total heterotrophic fungi; TFC = total faecal coliforms; THUB = total hydrocarbon utilizing bacteria; THUF = total hydrocarbon utilizing fungi.

Conclusion

Findings from the study indicated that the urban soils of Iwaya, Makoko, and Ilaje areas of Lagos contained heavy metals beyond the WHO permissible limits, particularly Pb, Ni, Cr, and Zn. The ADOI of these heavy metals by the

residents of the areas were more than the recommended limits. Furthermore, the HQ of the heavy metals through oral ingestion in the three areas was greater than one, the limit above which a substance is considered toxic. The soils from the three areas also contained abnormal counts of HB, HUB, HF, and HUF. Overall, the results showed that the urban soils in the three areas may predispose residents to diseases. While we recommend more studies to ascertain our claims, health and environmental agencies in

the areas should ensure a clean and healthy environment. Residents are also advised to practice personal hygiene and regular environmental sanitation.

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest

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