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Microorganisms in Soil and Groundwater of Epe and Laje Solid Waste Dumpsites in Ondo Town, Nigeria

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ABSTRACT: The menace of open dumps is a serious concern in Nigeria because of its associated health hazards. In this work, microorganisms in soil and groundwater of Epe and Laje dumpsites which are two major dumpsites in Ondo metropolis, Nigeria were investigated using standard techniques. Bacteria isolates were later identified based on their colonial morphology, cellular morphology and their biochemical characteristics while cotton in blue lactophenol technique was used for fungal identification. Epe had higher bacteria counts (cfu/ml) in both top soil (122.0×10^6) and subsoil (72.0×10^6) when compared with bacteria counts in Laje top soil (97.0×10^6) and subsoil (52.0×10^6). Similarly, Epe also had higher fungi counts (sfu/ml) in both top (25.5×10^6) and subsoil (11.5×10^6), comparably with fungi counts in Laje top soil (17.0×10^6) and subsoil (9.5×10^6). Meanwhile, total heterotrophic bacteria counts (cfu/ml) of the ground water samples was higher in Epe (42.0×10^6) and Laje (27.0×10^6) in comparison with total heterotrophic fungi count (sfu/ml) in Epe (14.0×10^6) and Laje groundwater samples (10.5×10^6). Identified isolates included *Staphylococcus aureus, Streptococcus* spp, *Escherichia coli*, *Micrococcus luteus, Proteus* spp (bacteria) and *Mucor* spp, *Aspergillus niger* and *Fusarium* spp (fungi). Remarkably, these isolates are organisms of medical importance, suggesting serious health threats to the residents around the dumpsites.

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Wastes emanated from agricultural, industrial, residential, institutional, municipal, commercial, mining, recreation centres and other human activities in Nigeria cities generate solid waste at an alarming rate and in most cases, the volume of waste generated is often more than what the Nigerian system could absorb (Aderemi *et al.*, 2011). As a matter of fact, the rapid population increase and urbanization in Ondo metropolitan areas have caused difficulties for the state and local environmental protection agencies in providing an effective and efficient municipal solid waste management (Olarenwaju and Ilemobade, 2009). Waste generated can contaminate surface water, ground water, soil and air which poses more problems for humans, other species, and ecosystems (Obire *et al.*, 2002). In fact, the effects of the indiscriminate disposal and ineffective management of wastes result to social, economic, health and environmental consequences. Pathogenic microorganisms and harmful substances in solid waste can be introduced into the environment when the waste is not properly managed (Ogbonna and Igbanijie, 2006; Wai-Ogosu, 2004). In an effort aimed at managing most of these wastes, governments at various levels have created many landfills. Ondo town does not have a sanitary landfill and this cause waste materials to be deposited in certain "open" dump sites in some areas in the town. A dumpsite is an arena specifically used for the disposal of wastes. It is an old traditional method of waste disposal similar to landfill

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method of waste management (Ogwueleka, 2009). The waste is allowed to accumulate until they are taken away or incinerated. This method of disposal of untreated municipal solid waste according to Amadi *et al.* (2012), is not only harmful to human health but also a threat to the environment. Besides, refuse dumps provide a rich source of microorganisms most of which are pathogenic (Odeyemi *et al.,* 2011). Hence, the objective of the study was to identify the microorganisms in soil and groundwater of Epe and Laje solid waste dumpsites in Ondo Town, Nigeria.

MATERIALS AND METHODS

Study area: The study areas included Epe and Laje dumpsites situated in Ondo West Local Government of Ondo state (latitude $7^{01}1000^{11}$N and longitude $4^{0}8417^{11}$E of the Greenwich meridian).

Sampling locations: Epe dumpsite ($7^{0}\ 6^{1}42^{11}$N and $4^{0}\ 47^{1}8^{11}$E) is an open land along Ife road in which wastes collected from various part of the town are dumped and the components of the dumpsite include metal, plastics, used papers, industrial waste, nylons, broken bottles and organic materials (food debris). Meanwhile, Laje dumpsite ($7^{0}\ 4^{1}\ 32^{11}$N and $4^{0}\ 49^{1}\ 2^{11}$E) is also an open land along Ondo State University of Medical Sciences Teaching Hospital in which wastes collected from various part of the town such as plastic, used paper, nylon, broken bottles, saline bags, used injections, hand gloves and bandages are dumped. However, Igba ($7^{0}\ 7^{1}36^{11}$N and $4^{0}\ 44^{1}8^{11}$E) which is a residential area and free from wastes (5.76km and 10.65km away from Epe and Laje dumpsites respectively) served as control site. Maps of locations of the respective dumpsites are shown in Fig 1.

Collection of samples Top and subsoil (10-15 cm depth) samples were collected from four different points using a quadrant from each of the dumpsites with the aid of stainless steel auger sampler and hand trowel which were mixed together to form a composite sample. This was later transferred to sampling bowl disinfected with alcohol while water samples were collected from the well, 400-800meters away from the dumpsites into sampling bottles which were labelled appropriately.

Physicochemical analyses: The pH of the soil and the ground water was determined using pH meter (SETRA PHS 25 model) which was calibrated before each sampling with standard buffer solution (pH: 4, 7 and 10) while mercury thermometer was used to determine temperature.

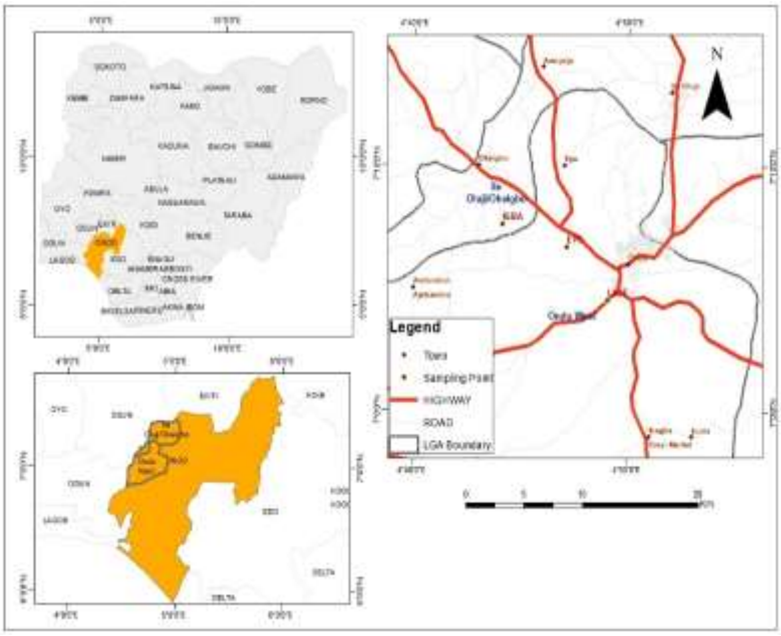

Fig.1: Maps of locations showing Epe and Laje dumpsites

Microbial Analysis: Pour-plate technique was used for counting and isolation of bacteria present in each soil sample and underground water obtained from the selected dumpsites One gram of soil sample and 1ml of underground water was separately and serially diluted with sterile distilled water to primary dilutions ($\times 10^{1}$). One millilitre of dilution factor ($\times 10^{1}$) was then pipetted into a sterilized Petri-dish aseptically. This was followed by the pouring of already prepared sterilized molten nutrient agar medium. The Petri dish was later swirled gently to allow the contents to mix together, then allowed to solidify and incubated at 37 °C for 24 hours. After 24 hours, bacteria count was carried out with a colony counter (Gallenkamp model). Visible number of colonies between 30 and 300 were multiplied by the reciprocal of the dilution factors and recorded as colony forming units per ml (cfu/ml) of soil/water. Sub culturing of any observable isolate was done several times until a pure culture was obtained. The same procedure was repeated for fungi count and isolation except that the agar used was acidified potato dextrose containing streptomycin (1 mg/100 ml) and counting was done after 72 hours at 28 °C incubation period and colonies recorded as spore forming units per ml (sfu/ml) of soil/water.

Identification of microbial isolates: The bacteria were identified based on their colonial morphology, cellular morphology and their biochemical characteristics such as colour, shape, Gram and spore stain, catalase test, fermentation of sugar, imdole and coagulase (Williams and Hakam, 2016). For fungal identification, a drop of lactophenol solution was put on a slide, the test fungal isolate was placed on the slide and stained with the lactophenol and was then covered with a cover slip. Excess liquid was drained off with a filter paper and examined under a binocular microscope.

Data analysis: All data obtained were subjected to one way analysis of variance (ANOVA) at P<0.05 significant, means were separated using Duncan multiple range test and T-test of SPSS version 22.0 software package.

RESULTS AND DISCUSSION

Physicochemical parameters: The physicochemical parameters in both soil and groundwater samples are presented in Table 1. The soil temperature (°C) of Laje dumpsite (27.00±0.10) was significantly different (p<0.05) from soil temperatures of Epe (28.06±0.06) and the control site (28.09±0.10). This is consistent with the works of Obire et al., (2002) who reported a temperature range of 27-28 °C in a waste-dump site. Similarly, the groundwater temperature (°C) of Laje (20.60±0.06) was also significantly different (p<0.05) from Epe (21.40±0.10) but not significantly different from the control site (20.20±0.10). This agreed with the previous works of Hassan *et al*. (2010) who found that the water temperature values were ranged between 20-25 °C in domestic sewage. Generally, it was observed that the soil temperatures of the respective dumpsites differed significantly from their groundwater temperatures. In the same vein, the pH values of Laje soil (5.80±0.02) was significantly different from that of Epe (6.06±0.05) and the control site (6.03±0.05).). Interestingly, the pH is within the NESREA limit of 6.50-8.50 and more so, similar pH values have been reported for soil samples in previous studies (Velsivasakthivel and Nandini, 2014; Argudin *et al.*, 2010). Likewise, the pH values of Laje groundwater (8.90 ±0.06) was significantly different from Epe (10.90±0.10) but not significantly different from the control site (8.90±0.12). It should be noted also that the soil pH of the respective dumpsites differed significantly from their groundwater pH.

Microbial Counts: The total heterotrophic bacteria count of the respective dumpsites is shown in Fig. 2. Epe had higher bacteria counts (cfu/ml) in both top soil (122.0 $\times 10^6$) and subsoil (72.0 $\times 10^6$) when compared with bacteria counts in Laje top soil (97.0 $\times 10^6$) and subsoil (52.0 $\times 10^6$). In the same vein, the total heterotrophic fungi counts of dumpsites is shown in Fig. 3. Epe had higher fungi counts (sfu/ml) in both top (25.5$\times 10^6$) and subsoil (11.5$\times 10^6$), comparably with fungi counts in Laje top soil (17.0$\times 10^6$) and subsoil (9.5$\times 10^{6)}$). Similarly, total heterotrophic bacteria counts (cfu/ml) of the underground water samples from the selected dumpsites as shown in Fig. 4 was higher in Epe (42.0 x10^6) and Laje (27.0x10^6) in comparison with total heterotrophic fungi count (sfu/ml) in Epe (14.0x10^6) and Laje (10.5x10^6) groundwater samples. The higher bacteria and fungi counts observed in top, subsoil and groundwater samples from Epe dumpsite when compared with Laje cannot but be linked to the smaller size and low human activities that characterise Laje dumpsite.

Table 1: Temperature and pH values of soil and ground water samples of the dumpsites

Soil / dumpsites	Temperature (°C)	pH
Laje	27.00±0.10^a	5.80±0.02^a
Epe	28.06±0.06^b	6.06±0.05^b
Igba (control)	28.09±0.10^b	6.03±0.05^b
Groundwater / dumpsites		
Laje	20.60±0.06^c	8.90 ±0.06^c
Epe	21.40±0.10^d	10.90±0.10^d
Igba (control)	20.20±0.10^c	8.90±0.12^c

Mean ± Standard Error represent three replicates. Mean having the same alphabet within the same column are not significantly different (p > 0.05) using Duncan's New Multiple Range Test

Meanwhile, Epe dumpsite is bigger and used for many purposes such as farming, housing and industrialisation. In fact, it is a major coalition point for refuse dump in Ondo town and its environs and the age of its refuse dump which was between 10-15 years is far higher than Laje dumpsite whose refuse age was between 3-5 years. Again, the type of waste in Epe dumpsite which principally are industrial wastes and organic materials (food debris), unlike Laje dumpsites whose wastes are predominantly non-biodegradable materials such as plastic, nylon, broken bottles, saline bags, used injections, hand gloves and bandages, equally accounts for its higher microbial count.

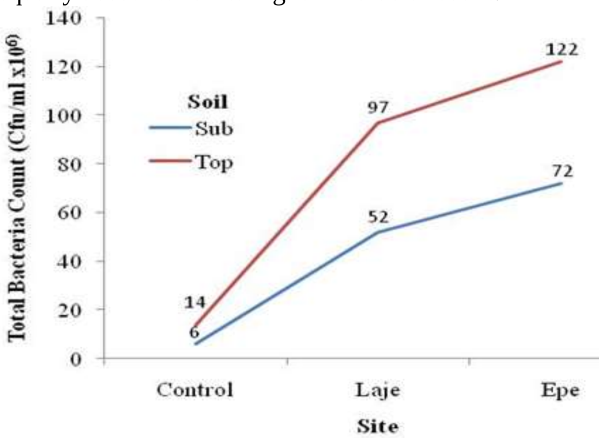

Fig 2: Total heterotrophic bacteria counts in Epe and Laje dumpsites

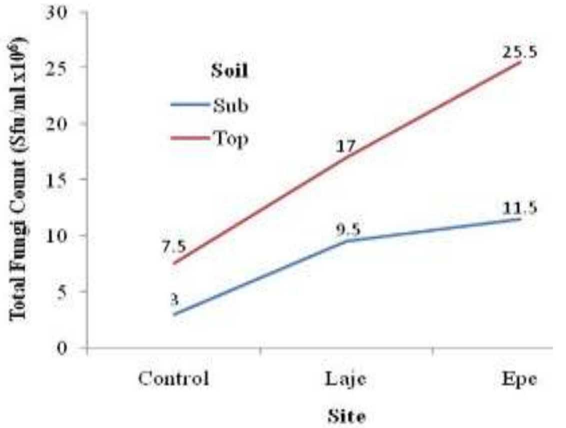

Fig. 3: Total heterotrophic fungi counts in Laje and Epe dumpsites

The colonization and degradation of these ever present huge wastes in Epe dumpsites by various bacteria and fungi might have contributed to its higher microbial counts (Aboagye-Larbi *et al.*, 2014; Wachukwu *et al.*, 2010). When waste is dumped on land, microorganisms such as bacteria and fungi proliferate using the components of the waste materials as source of nutrients for growth as well as degrading the organic materials in the waste (Wachukwu *et al.*, 2010). Furthermore, temperature plays an important role in the physicochemical and physiological behaviour of biotic components of an ecosystem (Sawant *et al.*, 2010), and is one of the most important physical factors that influence species distribution on earth. Hence the higher soil temperature and pH of Epe over Laje dumpsites must have also contributed to its higher microbial count, because low pH restricts microbial growth. Overall, variation in the microbial loads observed in this present study with respect to each dumpsite could be supported by the results of many researchers (Aboagye-Larbi *et al.*, 2014; Obire *et al.*, 2002) who observed varying microbial counts at different dumpsites. This variation may be due to the sizes of dumpsites or the proximity of the dumpsites to the commercial and residential areas.

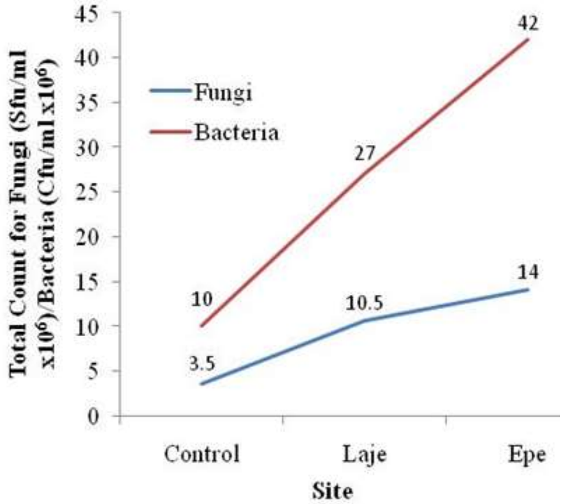

Fig. 4: Total heterotrophic fungi and bacteria counts of ground water from Laje and Epe

Occurrence of microbial isolates in the dumpsites: The morphological and biochemical characteristics of bacteria isolates associated with the respective dumpsites are described in Table 2 while occurrence of each microbial isolate in the dumpsites is shown in Table 3. The identified isolates that occurred in the two dumpsites were *Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli*, *Micrococcus luteus*, *Proteus* spp (bacteria) and *Mucor* spp, *Aspergillus niger* and *Fusarium* spp (fungi). Similar organisms have been previously identified from the works of other researchers from different waste dumpsites in Nigeria (Ebe *et al.*, 2015; Williams and Hakam, 2014; Onyido *et al.*, 2011; Babayemi *et al.*, 2009; Obire *et al.*, 2002). They reported isolation of *Bacillus* spp., *Escherichia coli*, *Klebsiella* spp, *Proteus* spp, *Pseudomonas* spp, *Staphylococcus aureus*, *Streptococcus* spp, *Aspergillus niger*, *Fusarium* spp, *Mucor* spp, *Penicilluim* spp and *Saccharomyces* spp from their sampled dumpsites.

The occurrence of *Staphylococcus aureus* in Epe and Laje dumpsites attest to the fact that the organism is commonly found in the environment (soil, water and air), nose and skin of humans (Argudin *et al.*, 2010). Unfortunately, high numbers of this bacterium is known to cause staphylococcal food poisoning (a form of gastroenteritis with rapid onset of symptoms), infections in the nostril and pimples on the human faces (Ewekeye *et al.*, 2012).

Table 2: Morphological and biochemical characteristics of bacteria isolates

Morphological/ Biochemical characteristics	Probable isolates				
	Staphylococcus aureus	*Streptococcus* spp	*Escherichia coli*	*Micrococcus luteus*	*Proteus* spp
Shape	Cocci	Cocci	Rod	Cocci	Rod
Gram stain	+	-	_	+	-
Motility	-	-	+	-	+
Spore stain	-	-	-	-	-
Catalase	+	-	-	+	+
Coagulase	+	_	-	-	-
Starch hydrolysis	+	-	+	-	+
Indole	-	-	+	-	-
Sugar fermentation					
Glucose	+	+	+	-	+
Sucrose	+	-	+	-	-
Lactose Maltose	+	-	+	-	-
	+	-	+	-	-

+ = positive; - = negative

Also, the presence of *Streptococcus* spp, *Escherichia coli*, *Proteus* spp and *Micrococcus luteus* in the two dumpsites probably showed that these isolates had strong ability to decompose solid waste and utilize it as their sole source of carbon and energy (required for growth). Microorganisms use waste constituents as nutrients, thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simpler less toxic molecules. Adversely, these isolates are highly pathogenic and of medical important to the populace due to the various health risks associated with them (Ebe *et al*., 2015). Besides, the occurrence of *Aspergillus* species in both dumpsites may be attributed to its ubiquitous nature.

Table 3. Occurrence of microorganisms in the dumpsites

Bacteria isolates	Dumpsites	
	Laje	Epe
Staphylococcus aureus	+	+
Streptococcus spp	+	+
Escherichia coli	+	+
Micrococcus luteus	+	+
Proteus spp	+	+
Fungi isolates		
Mucor spp	+	+
Aspergillus niger	+	+
Fusarium spp	+	+

+ = present; - = absent

They are geographically widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates (Velsivasakthivel and Nandini, 2014). They secrete mycotoxins that are poisonous to health when contacted (Onuegbu, 2002).

Conclusion: Higher microbial counts were observed in Epe comparably with Laje dumpsite and the identified isolates (both bacteria and fungi) are organisms of medical importance, suggesting serious health threats to the residents. Proper management of solid waste through sanitary landfill or processing waste into bio products is hereby recommended to avoid the spread of its associated pathogens to the public.

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