



Survey and Identification of Bacteria and Fungi Associated with Two Dumpsites in Akure Metropolis

I. A. Simon-Oke^{1*} and T. Alade¹

¹*Department of Biology, Federal University of Technology, Akure, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author IASO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Authors IASO and TA managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to survey and identify bacteria and fungi associated with two major dumpsites in Akure metropolis from the months of April, June and August, 2014. Soil samples were taken from the two waste-dump sites at different intervals. A random sample of the two soils were taken into two different clean nylon bags and taken for culture on sterile and freshly prepared Nutrient Agar (NA) and Potato Dextrose Agar (PDA). All techniques were carried-out under manufacturers' instructions standard laboratory conditions. The inoculation was done in triplicate and recorded as average total viable bacteria and fungi in the soil sample. Nine (9) different bacteria and fungi were isolated, characterised and identified from the two dumpsites. The bacteria identified were *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella* sp, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris* and *P. mirabilis* while the fungi identified were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium moniliformis*, *Mucor mucedo*, *Candida albican*, *Rhizopus stolonifer*, *Neurospora* sp. and *Penicillium digitatum* of all the organisms isolated during the period of survey, *S. aureus* had the highest occurrence of 32.00 cfu and 24.25 cfu in Igbatoro and Fiwasaye dumpsite respectively while

*Corresponding author: E-mail: adepejuoke72@gmail.com;

Neurospora sp recorded highest occurrence of 5.50 sfu in Igbatoro and *A. niger* recorded highest occurrence of 4.25 sfu at Fiwasaye dumpsite. All the organisms isolated are suggestive of the public health hazard posed by the study areas.

Keywords: *Bacteria; fungi; dumpsite; survey; occurrence; identification.*

1. INTRODUCTION

Nigeria, like many other African countries still deals with the scourge of inefficient waste collection and disposal services, which often constitute both environmental and health hazard for the people. Any substance, solution, mixture or article for which no direct use is envisaged but which is transported for reprocessing, dumping, elimination by incineration or other methods of disposal could be regarded as waste [1]. In Nigeria, a large percentage of these wastes are generated from commercial, industrial, household, agricultural and educational establishments [2]. In an effort aimed at managing most of these wastes, governments at various levels have created many landfills. This is particularly important because the availability of proper waste disposal management is not only regarded as a political tool and an indicator of good government policy; it is also an important element for good health [3].

A landfill site or dump-site is a site for the disposal of waste materials by incineration or burial and it is regarded as the oldest form of waste treatment [4]. Many things are discarded everyday ranging from ordinary rubbish to old newspapers, packaging, cleaning materials, and many different kinds of junk. Methods of waste disposal pose threats to both man, animals and soil. Poisonous plants, insects, animals and other pathogens are biological hazards that may be encountered at the dumpsite [2]. The consequences resulting from improperly managed wastes include its serving as reservoir of pathogens, habitat for pests such as rats, flies and mosquitoes, reduction of usable land area of the society, obstruction of motorable roads and general nuisance and societal problems in residential areas [3]. Other negative aspects associated with unmanaged solid waste include negative impact on the value of properties surrounding it, acid rain and contamination of aquifers [4].

A waste is regarded as hazardous if it is infectious (i.e. containing viable microorganisms or toxins which are capable of causing diseases in humans or animals. Microorganisms use the

waste constituents as nutrients, thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simpler less toxic molecules. This metabolic activity can be attributed to their high growth rate, metabolism, and their collective ability to degrade a vast variety of naturally occurring organic materials [5].

Bacteria can grow in a wide range of moisture level, bacterial population of various soils is closely correlated with their moisture content. The maximum bacterial density is found in regions of fairly high moisture content and the optimum level for the activities of aerobic bacteria often is a 50%-75% of the soil moisture holding capacity. Numbers of the genera *Pseudomonas*, *Achromobacter* and *Bacillus* are found in most aerobic soils; where conditions are anaerobic and moist *Clostridium* will occur. *Actinomyces* showed a similar quantitative increase under such conditions [6].

Bennett et al. [7] reported the isolation of viable aerobic bacteria from hospital and municipal solid waste dumpsites in Benin City, Nigeria. *Aerobic bacteria Escherichia coli, Staphylococcus aureus, Klebsiella species were recorded in decreasing order of prevalence from municipal solid while Pseudomonas aeruginosa, Klebsiella sp, Bacillus subtilis, Serratia species, Staphylococcus aureus, and Escherichia coli were isolated from the hospital waste.* Also, Berkeley and Campbell [8] recorded microbial pathogens of public health significance found in waste and common sites, collected from four different dumping sites and assessed for pathogenic agents in Ede Southwest of Nigeria. The results revealed the presence of bacterial species including *Pseudomonas, Micrococcus, Actinomyces, Neisseria, Bacillus* and *Klebsiella*. Therefore, this work aimed to access the potential hazards that could result from indiscriminate dumping of wastes around residential areas.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The study areas were Fiwasaye dumpsite, located on latitude 7°15.357'N and longitude

5°13.178'E of the equator and Igbatoro dumpsite, located on latitude 7°05' 302'N and longitude 5°21'530' E of the equator, Akure, Ondo state. Fiwasaye and Igbatoro dumpsites recorded an average daily /monthly rainfall of > 1.6/48, > 2.6/60 and < 5.1/153 and 1.8/42, >2.8/67 and 11.7/183 in the months of May, July and August respectively. Vegetation consists mainly of coastal-savannah grass-lands, shrubs and some few mangroves forest.

2.2 Sterilization of Glassware and Media

Glassware such as Petri dishes, McCartney bottles, conical flasks, test tubes and pipettes were washed with detergent, rinsed in water, dried and sterilized in the autoclave at 121°C for 15 minutes. The media used were prepared according to manufacturers' specifications and sterilized at 121°C for 15 minutes [9].

2.3 Determination of Abiotic Parameters of the Waste-Dump Sites

The physical parameters observed on the waste-dump sites include; Air, temperature and relative humidity, using thermometers and hygrometers respectively for the measurement which was carried out before and after rainfall and between the hours of 10.00 and 1600 hrs.

2.4 Collection of Bacteria and Fungi Isolates from the Waste-Dump Sites

Soil samples were taken from the two waste-dump sites at different intervals for isolation and identification of likely bacteria and fungi species present. A random sample of the two soils were taken into two different clean nylon bags and taken for culture on sterile and freshly prepared Nutrient Agar (NA) and Potato Dextrose Agar (PDA). All techniques were carried-out under manufacturers' instructions standard laboratory conditions. The inoculation was done in triplicate and recorded as average total viable bacteria and fungi in the soil sample [9].

2.5 Microbiological Analyses

2.5.1 Culture media preparation

The culture media used were Nutrient agar (NA) and Potato Dextrose Agar (PDA) both manufacture by Oxoid. The Total Bacteria Count was enumerated using nutrient agar prepared by

dissolving 2.8 g of the agar to make up 100 ml distilled water in a conical flask, while the fungi was enumerated using PDA prepared by dissolving 3.9 g to make up 100 ml of distilled water in a conical flask. One percent streptomycin was added to molten PDA in order to suppress bacteria growth. They were sterilized along with the agar in the conical flasks in an autoclave (GallenKamp) at 121°C for 15 minutes [9].

2.6 Microbial Population Studies

2.6.1 Bacteria

One gram of each of the soil samples was diluted with 9 ml of sterile water to make the stock solution and the progressive serial dilution of stock solution of 10^{-4} was taken for culture on sterile Nutrient agar (NA). The culture was done using pour plate method: 1.0 ml was pipetted into Petri plates of NA and swirled gently for even distribution of microorganisms on the plate. Then the plates were allowed to set and subsequently incubated upside down for 24 hours at $35\pm 2^\circ\text{C}$. The culture was done in triplets. Colonies were counted using colony counter (Gallenkamp) and average was calculated.

2.6.2 Fungi

Fungi were identified based on references from [10].

2.6.3 Cultural characteristics

Using visible observation (macroscopic examination) and microscope at low power magnification (x40), parameters such as colony characteristics of the submerged hyphae whether rhizoid, spiral or regular and characteristic shape of mature fruiting bodies were all observed.

2.6.4 Microscopic observation

This involved transferring a small piece of mycelium free of medium using a sterile inoculation loop unto a glass slide containing a drop of cotton blue-in-lacto phenol and the mycelium was spread properly. The preparation was covered with a covered slip and observed under medium power (x100) and later at high power (x400) magnifications. The observations made were used in identifying the fungi organisms.

2.6.5 Isolation of pure culture, preservation and storage of bacterial isolates

The pure colonies (isolates) of bacteria were obtained using streak plate method by sub-culturing representative colonies of each of the different colonies present on the primary cultures on gelled plates of sterile nutrient agar and then incubated at $35\pm 2^{\circ}\text{C}$ for 24 hours. Aseptic techniques were employed in this isolation to ensure that the isolates obtained were actually pure. Bacteria were maintained by routine subculture on nutrient agar slants and stored at 4°C .

2.6.6 Isolation of fungi

Microbial (fungal) isolation was carried out on the soil samples using the standard method of serial diluted and pour plate technique. Preliminary investigations showed dilutions of 10^{-5} for fungal growth. One-milliliter of each dilution was plated in triplicate on PDA to which 0.1% streptomycin has been incorporated to inhibit bacterial growth. The plates were incubated at $28\pm 2^{\circ}\text{C}$ for 3-5

days. The pure cultures of fungal isolates were identified based on cultural and morphological characteristics according to Barnett and Hunter [10].

2.7 Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA) at $p < 0.05$ and where significant, means were separated using Tukey's test. All analyses were carried out using SPSS 17.0 software package.

3. RESULTS

3.1 Relative Humidity and Temperature of the Dumpsites

Table 1 shows the relative humidity and temperature of the two dumpsites observed from April to August 2014 in Akure metropolis. In the dumpsites, relative humidity increased as the month increased while the temperature decreased as the month increased. In the two

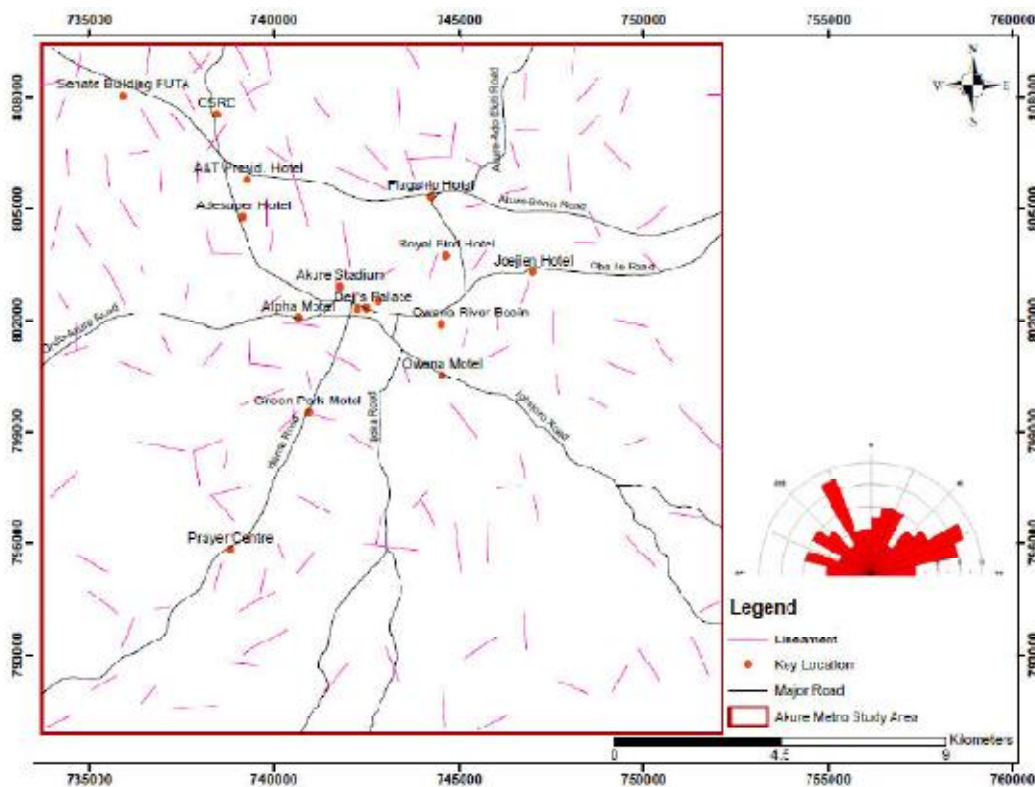


Fig. 1. Map of Akure metropolis showing the two dumpsites

dumpsites, relative humidity observed in April was significantly lower ($p < 0.05$) than those observed in June and August while the temperature observed in Fiwasaye dumpsite during April was significantly higher ($p < 0.05$) than that of June and August. However, temperature of 27.88°C observed in Igbatoro dumpsite in the month of August was significantly lower ($p < 0.05$) than those observed in April and June.

3.2 Microbial Isolates from the Two Dumpsites

A total number of nine (9) different bacteria and fungi species were isolated respectively. The bacteria species isolated from the two dumpsites include *Bacillus species*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus mirabilis*, *Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus faecalis* while the fungi isolated were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Fusarium moniliformis*, *Mucor mucedo*, *Neurospora sp*, *Penicillium digitatum* and *Rhizopus stolonifer*.

3.3 Occurrence of Bacteria Species Isolated in the Months

S. aureus has a significantly higher ($p < 0.05$) occurrence of 32.00 and 24.25 cfu in Igbatoro

and Fiwasaye dumpsite respectively when compared to other bacteria isolated in April. However, *P. mirabilis* showed the lowest occurrence. The trend observed for *S. aureus* in April and July was also observed in August. Significantly higher ($p < 0.05$) occurrence of 13.50 cfu (Igbatoro) and 9.25 cfu (Fiwasaye) was observed when compared to other bacteria isolated from the dumpsite (Table 2).

3.4 Occurrence of Fungi Species Isolated

Table 3 shows the occurrence of fungi isolated from both dumpsites. In Igbatoro dumpsite, *Neurospora sp* showed the highest occurrence of 5.50 sfu which was significantly different ($p < 0.05$) from those of other fungi except *A. flavus* (5.25 sfu). *M. mucedo* showed the least occurrence of 2.00 sfu which was significantly lower than that of *A. flavus*, *A. niger*, *C. albicans* and *Neurospora spp*. In Fiwasaye dumpsite, the occurrence of *A. niger* was significantly higher ($p < 0.05$) than those of *A. flavus*, *F. moniliforme*, *M. mucedo*, *C. albican* and *Neurospora spp*. Similarly, *C. albican* and *A. fumigatus* showed the least occurrence in Fiwasaye dumpsite. In the month of August in Igbatoro dumpsite, *A. flavus* and *A. niger* showed the highest occurrence of 4.00 cfu which was significantly higher than that of *F. moniliforme* and *M. mucedo*.

Table 1. The relative humidity and temperature of the dumpsites

Month	Relative humidity		Temperature (°C)	
	Fiwasaye	Igbatoro	Fiwasaye	Igbatoro
April	60.75±6.51 ^a	53.38±2.51 ^a	28.60±0.35 ^b	32.55±0.17 ^b
July	83.00±4.06 ^b	73.25±0.25 ^b	27.15±0.23 ^a	31.70±0.30 ^b
August	93.75±2.10 ^b	76.25±2.93 ^b	26.90±0.38 ^a	27.88±0.45 ^a

Mean± S.E having the same letter within the same column are not significantly different ($p > 0.05$) using Tukey's test

Table 2. Prevalence of bacteria (cfu) isolated in the two dumpsites

Organism	April		July		August	
	Igbatoro	Fiwasaye	Igbatoro	Fiwasaye	Igbatoro	Fiwasaye
<i>K. pneumonia</i>	5.75±1.03 ^{ab}	4.25±1.44 ^a	3.50±0.65 ^a	7.00±1.00 ^b	4.00±0.41 ^b	3.00±0.58 ^{ab}
<i>S. marsceneces</i>	2.50±0.29 ^{ab}	0.25±0.25 ^a	1.25±0.25 ^a	2.00±0.41 ^a	1.75±0.48 ^a	1.00±0.58 ^a
<i>S. aureus</i>	32.00±2.71 ^c	24.25±4.33 ^b	11.00±2.68 ^b	18.75±3.50 ^c	13.50±0.50 ^d	9.25±0.48 ^c
<i>S. faecalis</i>	3.25±0.75 ^{ab}	3.00±1.22 ^a	2.25±0.85 ^a	6.75±1.75 ^b	11.00±0.58 ^c	5.25±0.48 ^b
<i>B. subtilis</i>	5.25±0.75 ^{ab}	4.25±0.85 ^a	2.50±0.29 ^a	3.50±0.29 ^{ab}	3.25±0.25 ^{ab}	3.25±0.25 ^{ab}
<i>E. coli</i>	7.50±1.76 ^b	3.00±0.91 ^a	1.75±0.25 ^a	3.25±0.75 ^{ab}	3.75±0.48 ^{ab}	2.50±0.29 ^a
<i>Aeromonas spp</i>	4.25±0.85 ^{ab}	1.25±0.25 ^a	1.25±0.48 ^a	2.50±0.50 ^{ab}	2.75±0.48 ^{ab}	2.25±0.25 ^a
<i>P. vulgaris</i>	3.75±0.87 ^{ab}	1.00±0.41 ^a	3.00±0.71 ^a	3.50±0.65 ^{ab}	3.00±0.41 ^{ab}	2.00±0.00 ^a
<i>P. mirabilis</i>	1.00±0.41 ^a	0.25±0.25 ^a	2.00±0.71 ^a	2.75±0.48 ^{ab}	2.75±0.48 ^{ab}	1.50±0.87 ^a

Mean± S.E having the same letter within the same column are not significantly different ($p > 0.05$) using Tukey's test.

Table 3. Prevalence of fungi (sfu) isolated in the two dumpsites

Organism	April		July		August	
	Igbatoro	Fiwasaye	Igbatoro	Fiwasaye	Igbatoro	Fiwasaye
<i>A. flavus</i>	5.25±0.25 ^{cd}	3.00±0.41 ^c	3.75±0.48 ^{bc}	2.25±0.25 ^{bc}	4.00±0.00 ^b	2.25±0.25 ^c
<i>A. fumigatus</i>	2.75±0.48 ^{ab}	0.50±0.29 ^a	2.50±0.29 ^{ab}	0.50±0.29 ^a	2.50±0.29 ^{ab}	0.50±0.29 ^{ab}
<i>A. niger</i>	3.75±0.25 ^b	3.25±0.25 ^c	4.25±0.25 ^c	3.00±0.41 ^c	4.00±0.00 ^b	3.00±0.41 ^c
<i>F. moniliforme</i>	2.75±0.48 ^{ab}	1.00±0.00 ^{ab}	2.50±0.29 ^{ab}	0.75±0.25 ^a	1.50±0.29 ^a	0.00±0.00 ^a
<i>M. mucedo</i>	2.00±0.00 ^a	0.25±0.25 ^a	1.75±0.25 ^a	1.00±0.00 ^{ab}	1.50±0.29 ^a	0.25±0.25 ^a
<i>C. albican</i>	4.00±0.00 ^{bc}	0.50±0.29 ^a	3.50±0.50 ^{bc}	0.50±0.29 ^a	2.50±0.29 ^{ab}	0.25±0.25 ^a
<i>R. stolonifer</i>	3.00±0.00 ^{ab}	1.25±0.25 ^{ab}	4.00±0.00 ^{bc}	2.25±0.25 ^{bc}	3.50±0.65 ^b	2.25±0.25 ^c
<i>Neurospora sp.</i>	5.50±0.29 ^d	2.00±0.00 ^{bc}	3.25±0.48 ^{abc}	1.75±0.48	3.00±0.41 ^{ab}	1.75±0.25 ^{bc}
<i>P. notatum</i>	3.25±0.25 ^{ab}	3.00±0.41 ^c	2.75±0.25 ^{abc}	2.25±0.25 ^{bc}	2.50±0.29 ^{ab}	1.75±0.25 ^{bc}

Mean±S.E having the same letter within the same column are not significantly different ($p>0.05$) using Tukey's test

Table 4. Biochemical characteristics of bacteria isolates from the two dumpsite

Probable organism	Shape	Gram stain	Catalase	Coagulase	Spore stain	Motility	Starch hydrolysis	Nitrate reduction	Indole	Citrate	Glucose	Lactose	Maltose	Sucrose	Arabinose	Mannitol	Fructose	Xylose
<i>M. luteus</i>	Cocci	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. subtilis</i>	Rod	+	-	-	+	+	+	+	-	+	+	-	-	-	+	+	-	+
<i>E. coli</i>	Rod	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+
<i>K. Pneumonia</i>	Rod	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-
<i>P. mirabilis</i>	Rod	-	+	-	-	+	+	-	-	+	+	-	-	+	-	-	+	+
<i>P. vulgaris</i>	Rod	-	+	-	-	+	+	-	+	+	+	-	-	+	-	-	+	-
<i>S. marcescans</i>	Rod	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-
<i>S. aureus</i>	Cocci	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+	+	-
<i>S. faecalis</i>	Cocci	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-

4. DISCUSSION

The survey of different bacteria and fungi species associated with dumpsites in Akure metropolis was investigated in this study. The first sampling done in the month of April with lowest relative humidity and highest mean temperature recorded the highest number of total viable aerobic bacterial and fungi in the dumpsites. However, the occurrence of these bacteria and fungi species decreased as the months increased from April to August in Igbatoro dumpsite when compared to Fiwasaye dumpsite. This is an indication that microorganisms respond to different seasonal influence variation of climate and temperature i.e. wet and dry seasons) which may selectively favour certain physiological types of microorganisms [11].

The lower count of bacteria and fungi species found in Fiwasaye dumpsite when compared to Igbatoro dumpsite may be linked to smaller size and low human activities while Igbatoro dumpsite is bigger and use for many purposes such as farming and housing. The colonization and degradation of these ever present huge wastes in these dumpsites by various bacteria and fungi might have contributed to bad odour and higher occurrence of microbes observed [4,12].

S. aureus showed the highest occurrence of all the bacteria irrespective of the month and dumpsite. This microbe is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans [13]. The ubiquitous nature of this organism may have contributed to its highest occurrence observed in both dumpsites regardless of the months. 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 [14]. However, *K. pneumoniae* which were common in all the plates isolated was highly pathogenic and of medical important to the populace due to the various health risks (such as pneumonia, bloodstream infection, wound or surgical site infections and meningitis) associated with this organism [15].

Escherichia coli, which is one of the organisms that cause Urinary Tract Infection (UTI) and gastroenteritis in children and *Bacillus subtilis* and endospore forming bacteria were also observed. The tendency of these pathogens gaining entry into the body is high and the resultant effect will be infection, general body

malaise and in some cases death of those people working in the waste dumpsites [4]. This might be responsible for the small growths observed on the body of some of the several workers working in Igbatoro dumpsite.

In Igbatoro dumpsite, the occurrence of fungi decreased as the month progressed while that of Fiwasaye was stagnant. The reduction in the population of fungi isolated from Igbatoro dumpsite may be linked to several factors such as increase in precipitation and high decomposition of organic matter in the dumpsite. High precipitation usually increases the acidity of the soil through acid rain and leaching of the soil cations while decomposition of organic matter leads to release of carbon di oxide which may combine with water to form carbonic acid. Increase in soil acidity overtime coupled with other activities in the dumpsite such as burning of refuse dumps might have led to depletion in nutrient available for the growth of some of these microorganisms [14]. Fluctuations in rainfall pattern may have led to increase or decrease in the population of these organisms irrespective of the months.

The high occurrence of *Aspergillus species* in both dumpsites may be attributed to its high ubiquitous nature. They are widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. It is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation [16]. The growth of this organism in human tissue or within air-containing spaces of the body, such as bronchus or pulmonary cavity, is termed aspergillosis [17]. Patients exhibiting aspergillosis are generally immune-compromised, and thus susceptible to otherwise common and usually harmless microorganisms [18].

5. CONCLUSION

The occurrence of various heterotrophic bacteria and fungi varied with the month of collection and dumpsites. The occurrence of *S. aureus* and *Aspergillus niger* was the highest of all the bacteria and fungi isolated regardless of the month. These microorganisms are of public health significance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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