

Microbial Assessment of Air In The Vicinity Of Some Dump Sites in Delta State

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Abstract: - The microbial loads of air in the vicinity of various dumpsites in Delta State, Nigeria, were conducted for a period of 6 months (July - December, 2012) using standard pour plate and spread plate microbiological techniques. Results showed that all the tested parameters decreased with distance away from the dumpsites and the microbial loads were higher than regulatory limits. The heterotrophic bacteria count ranged from 1.41×10^8 to 29.2×10^{14} cfu/ml, while fungal counts ranged from 1.12×10^8 to 1.39×10^{14} cfu/ml. Bacterial counts were higher in the months of July, August and September, while fungi were more in the months of October to December. The bacterial genera isolated were *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Bacillus*, *Pseudomonas*, *Escherichia coli*, and *Klebsiella* species, while the fungal isolates were *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor*, *Curvularia*, *Rhizopus* and *Cladosporium* sp. Statistical analysis showed that there were significant differences ($P < 0.05$) between the microbial load of the various dumpsites, periods and distance of sampling sites. This study showed that potential airborne pathogens not only abound in the vicinity of waste dumpsites but also decreased with increasing distance from the dump sites. It is therefore recommended that dumpsites be located at a minimum 1km distance away from residential quarters.

Keywords: - Microbial loads, dumpsites, heterotrophic bacteria count, residential quarters

I. INTRODUCTION

Indiscriminate dumping of waste has become a common practice in Nigeria and other African countries. In fact, virtually every available space close to residential areas, roads, farms, markets, hospital and churches in many Nigerian communities now serve as waste dump sites [1]. The lack of proper refuse disposal and waste treatment facilities in these areas, therefore, makes the populace to be at risk of infections from pathogens that are airborne in the dumpsite areas. The World Health Organization estimates that about two million people die prematurely every year as a result of air pollution, while many more suffer from breathing ailments, heart disease, lung infections and even cancer [2].

Over the years, studies have been extensively carried out on the microbial qualities of air in hospital environments [3-11]. In order to develop appropriate air quality management plans, however, it is necessary first to have reliable information about the state of airborne bacteria and fungi especially in the vicinity of waste dumpsites. Therefore, this study was undertaken to determine the microbial load of air in the vicinity of some waste dumpsites in selected towns (Warri, Agbarho and Agbor,) in Delta State, Nigeria.

II. MATERIALS AND METHODS

2.1 Study Area

The study area is located within Nigeria Delta in the Southern portion of Nigeria (Fig. 1). Warri is between latitude $5^{\circ} 32' N$ and $5^{\circ} 40' N$ and longitude $5^{\circ} 42' E$ and $5^{\circ} 50' E$, while Agbor is situated between latitude $6.00^{\circ} N$ and longitude $6^{\circ} 05' E$ and $6^{\circ} 25' N$ and longitude $6^{\circ} 05' E$ and $6^{\circ} 25' E$. Agbarho is between latitude $5^{\circ} 34' N$ and $5^{\circ} 40' N$ and longitude $5^{\circ} 42' E$ and $5^{\circ} 50' E$. All the communities are in Delta State, Nigeria.

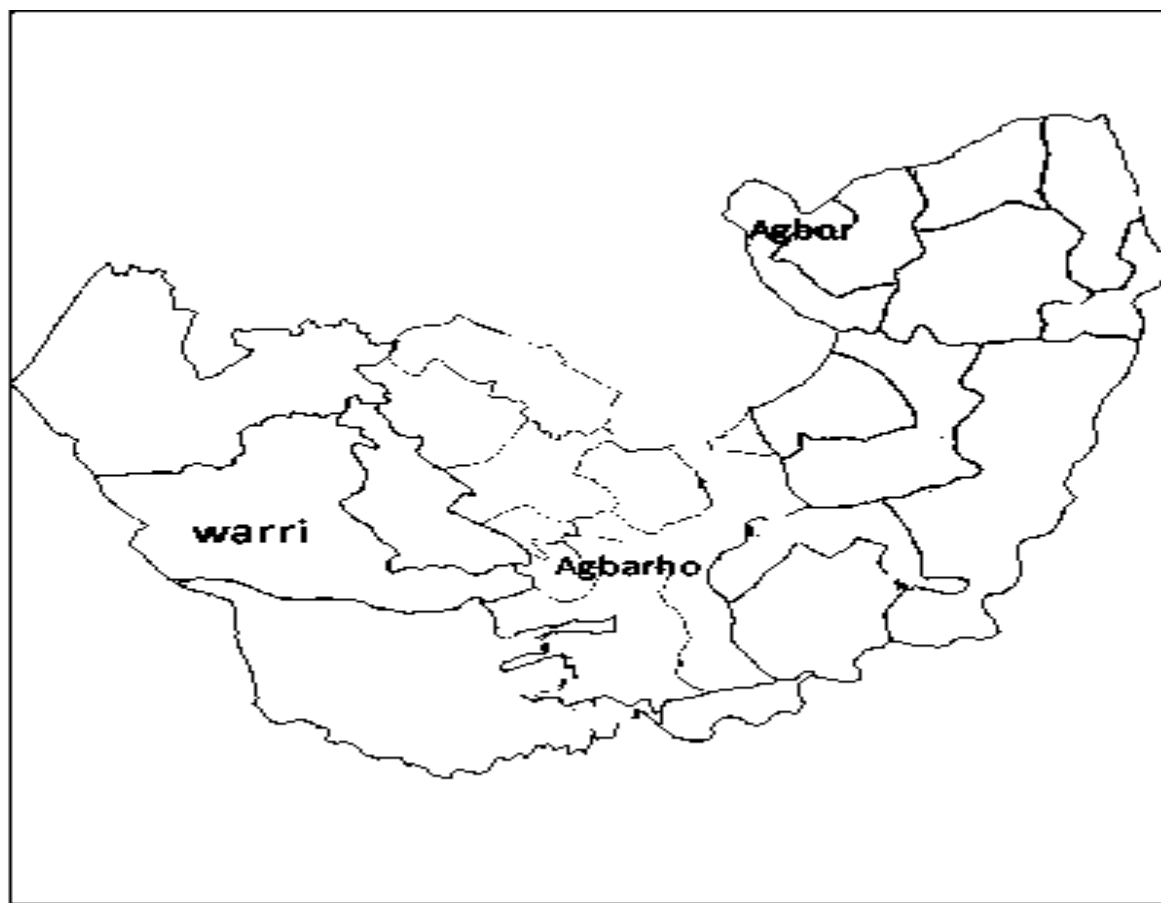


Fig. 1: Map of Delta State Showing Warri, Agbarho and Agbor.

2.2 Sample Collection

Air samples were collected monthly for period of 6 months (July – December) from the various dumpsites in Agbor, Warri and Agbarho. At each sampling area, wide mouth bottles containing sterile peptone water was exposed for 15 minutes in the dumpsite and at distances of 100m, 200m, 300m, 400m and 500m away from the dumpsite. In all ninety air samples were collected during the period and the sample bottles were corked and taken to the laboratory for analysis.

2.3 Culturing and Enumeration of Bacteria in Air Samples

Each sterile peptone water exposed to air was thoroughly shaken to obtain a homogenous sample. An aliquot (1.0 ml) was diluted serially to 10^{-15} dilution [12]. From the dilution of 10^{-8} , 10^{-12} and 10^{-14} of each sample, 0.1 ml aliquot was transferred aseptically onto freshly prepared Nutrient agar plates. The inoculated plates were inverted and incubated at 37°C for 24 hrs after which the plates were examined for growth. The discrete colonies which developed were counted and the average counts for duplicate cultures were recorded as aerobic heterotrophic bacteria in the sample.

2.4 Isolation, characterization and identification of bacteria in the air samples

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types, which appeared on the cultured plates onto freshly prepared Nutrient agar plates and MacConkey agar plates and incubated at 37°C for 24 hrs. Discrete bacteria colonies which developed were sub-cultured onto Nutrient agar slopes and incubated at 28°C for 24 hrs. These served as pure stock cultures for subsequent characterization and identification via physiological and biochemical tests [13].

2.5 Culturing and enumeration of fungi in air samples

Each air sample was shaken thoroughly to obtain a homogenous sample. Aliquot (1.0 ml) of it was diluted serially to 10^{-15} dilution. From the dilution of 10^{-8} , 10^{-12} and 10^{-14} of each air sample, 0.1 ml aliquot was transferred aseptically onto freshly prepared Potato dextrose agar plates containing 0.2 ml of 0.5 % Ampicillin to inhibit the growth of bacteria [14]. The inoculum was spread with a sterile bent glass rod. The inoculated plates were inverted and incubated at (room temperature) $28 \pm 2^{\circ}\text{C}$ for 5 days. The colonies which developed were counted and the average counts for duplicate cultures were recorded as viable fungal counts in the sample.

2.6 Isolation, characterization and identification of fungi in air samples.

Pure cultures of fungi were obtained by subculturing discrete colonies onto freshly prepared Potato Dextrose Agar plates and inoculated at room temperature (28 ± 2 °C) for 5 days. The fungal isolates which developed were further sub cultured onto agar slopes and incubated at room temperature. The isolates which developed were pure cultures which were stored in the refrigerator (4 °C) as stock cultures for subsequent characterization via macroscopic and microscopic examination. The identification of fungal isolates was done by comparing the result of their cultural and morphological characteristics with those of known taxa [14].

2.7 Statistical Analysis

Data obtained was analysed using Microsoft 2003 excel programme. Analysis of variance ANOVA (F-test) was done to determine the statistical difference in microbial count between the sampling stations while seasonal variation in bacterial and fungal counts for the air samples at 95 % confidence level was determined using student's t - test.

III. RESULTS

3.1 Bacterial Load for Air Samples

The bacterial load for the air samples from the dumpsites in Warri, Agbarho and Agbor are presented in Figs 4.1, 4.2 and 4.3 respectively. It was observed that the bacterial counts decreased with distance from the dumpsite and were higher in the months of July, August and September, but decreased slightly in the months of October to December. The bacterial counts in the air samples from the dumpsite in July - September ranged from 15.47 to 14.22 \log_{10} cfu/ml while the bacterial counts in the air samples from the dumpsite in October-December ranged from 14.37 to 12.37 \log_{10} cfu/ml. Similarly the bacterial counts in the air samples taken from the various distances ranged from 13.58 to 8.44 \log_{10} cfu/ml in July - September while the value ranged from 14.37 to 7.44 \log_{10} cfu/ml in October-December. Statistical analysis showed that there were significant differences ($P < 0.05$) between the bacteria load of the various sampling stations and seasonal variations

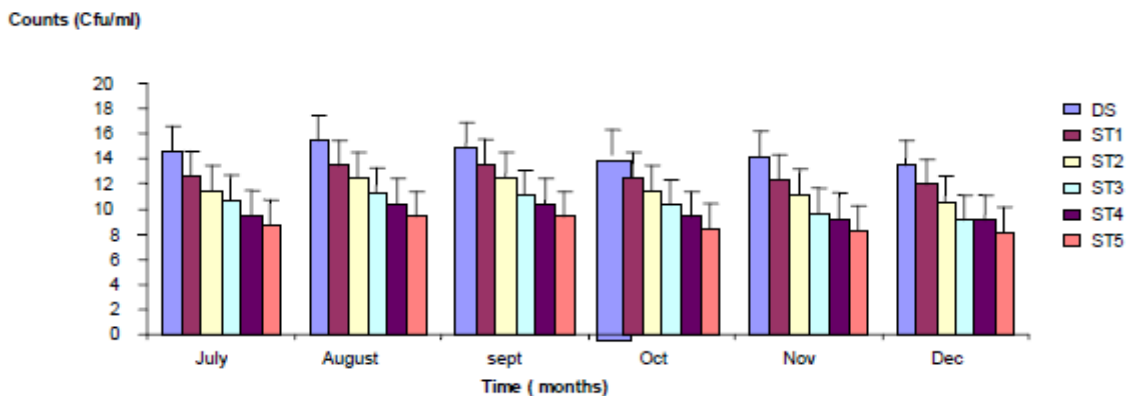


Fig 2: Bacterial Counts for Warri Air Samples

Key: DS = dumpsite, ST1 = station 1 (100m from dumpsite), ST2 = station 2 (200m away from dumpsite), ST3 = station 3 (300m away from dumpsite), ST4 = station 4 (400m away from dumpsite), ST5 = station 5 (500m away from dumpsite).

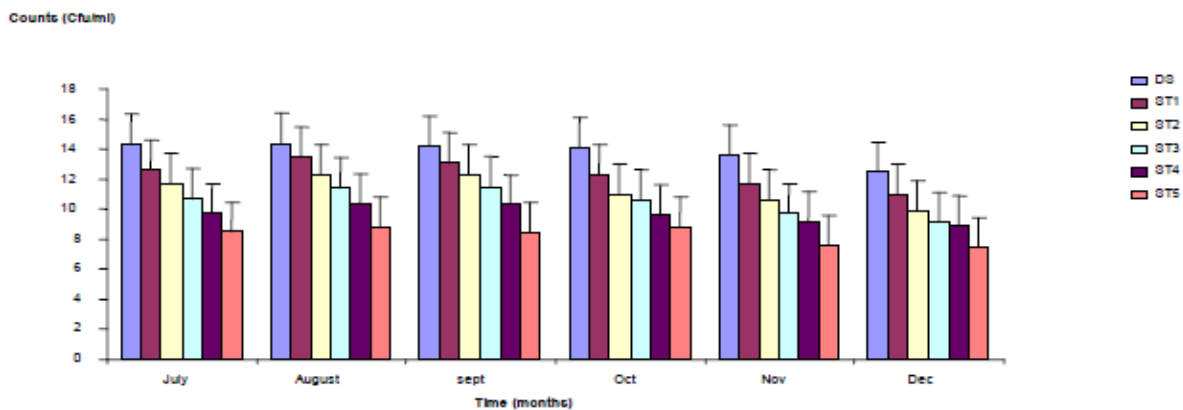


Fig 3: Bacterial Counts for Agbarho Air Samples.

Key: DS = dumpsite, ST1 = station 1 (100m from dumpsite), ST2 = station 2 (200m away from dumpsite), ST3 = station 3 (300m away from dumpsite), ST4 = station 4 (400m away from dumpsite), ST5 = station 5 (500m away from dumpsite).

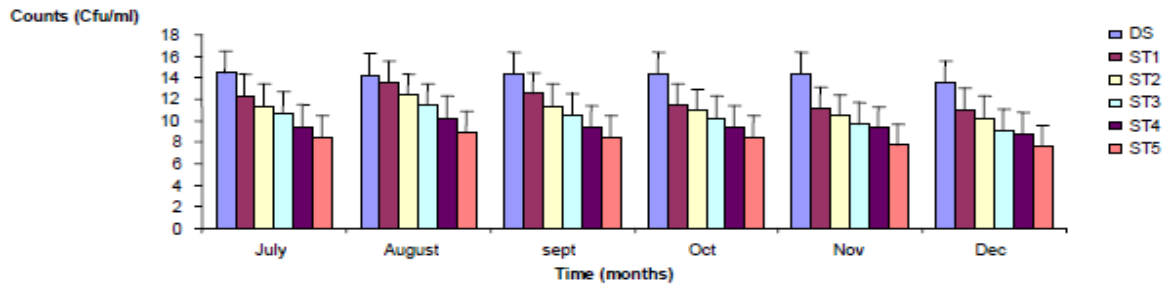


Fig. 4: Bacterial counts for Agbor Air Samples.

Key: DS = dumpsite, ST1 = station 1 (100m from dumpsite), ST2 = station 2 (200m away from dumpsite), ST3 = station 3 (300m away from dumpsite), ST4 = station 4 (400m away from dumpsite), ST5 = station 5 (500m away from dumpsite).

3.2 Fungal Count for Air Samples

The fungal counts of the air samples from the dumpsites in Warri, Agbarho and Agbor are presented in Figs 5, 6 and 7. It was observed that the fungal counts decreased with distance from the dumpsite and the fungal counts increased in the months of November and December. The fungal counts in the ambient air of the dumpsite in July - September ranged from 12.58 to 10.58 log₁₀ cfu/ml and the fungal load in the air samples from the dumpsite in October-December ranged from 13.67 to 12.57 log₁₀ cfu/ml. Similarly the total fungal counts in the air samples taken from the various distances ranged from 11.78 to 8.04 log₁₀ cfu/ml in July - September while the value ranged from 13.49 to 8.48 log₁₀ cfu/ml in October-December. Statistical analysis showed that there were significant differences (P<0.05) between the fungal loads of the various sampling stations and during the different seasons.

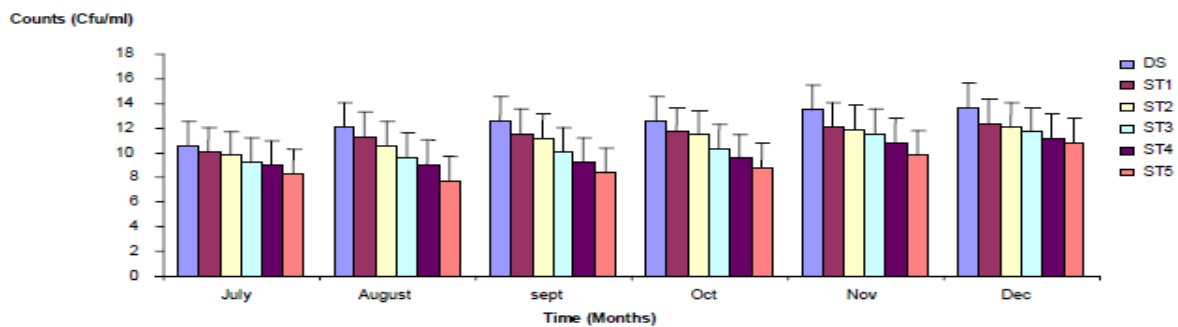


Fig 5: Fungal Counts for Warri Air Samples

Key: DS = dumpsite, ST1 = station 1 (100m from dumpsite), ST2 = station 2 (200m away from dumpsite), ST3 = station 3 (300m away from dumpsite), ST4 = station 4 (400m away from dumpsite), ST5 = station 5 (500m away from dumpsite).

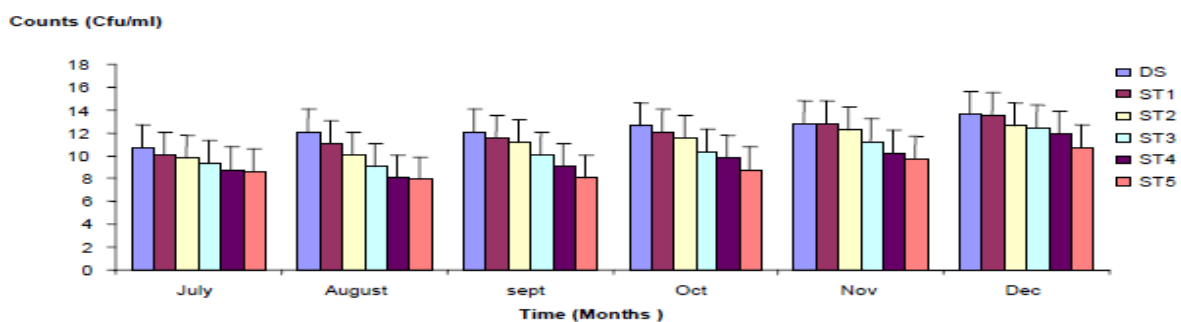


Fig 6: Fungal Counts for Agbarho Air Samples

Key: DS = dumpsite, ST1 = station 1 (100m from dumpsite), ST2 = station 2 (200m away from dumpsite), ST3 = station 3 (300m away from dumpsite), ST4 = station 4 (400m away from dumpsite), ST5 = station 5 (500m away from dumpsite).

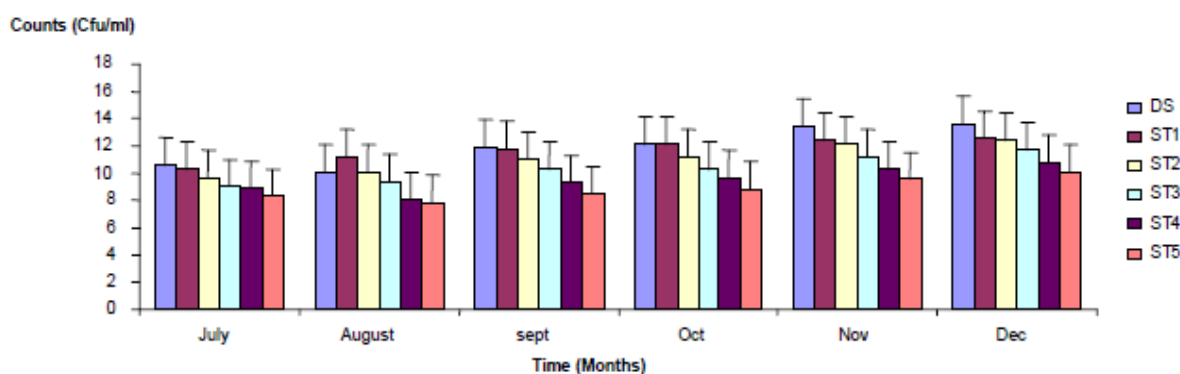


Fig 7: Fungal Counts for Agbor Air Sample

Key: DS = dumpsite, ST1 = station 1 (100m from dumpsite), ST2 = station 2 (200m away from dumpsite), ST3 = station 3 (300m away from dumpsite), ST4 = station 4 (400m away from dumpsite), ST5 = station 5 (500m away from dumpsite).

3.3 Bacterial Isolates from Air Samples

The bacterial genera isolated from the air sample were *Staphylococcus* sp, *Streptococcus* sp, *Micrococcus* sp, *Bacillus* sp, *Pseudomonas* sp, *Escherichia coli*, and *Klebsiella* sp were isolated from the air samples (Table 1)

Table 1: Identification and characterization of bacteria from air samples

Isolate	A	B	C	D	E	F	G
Cultural characteristics	Creamy converse colonies with smooth edges	Cocci clusters	Round clusters dented	white flat & smooth colonies	Dented and raised colonies	Pink convex Smooth colonies	Flat yellow swarming colonies
Pigmentation	Cream	Cream	Yellow	Cream	Green	Pink	cream
Cellular morphology	Cocci	Cocci	Cocci	Rods	Rods	Rods	Rods
Gram Stain	+	+	+	+	-	-	-
Aerobic growth	+	+	+	+	+	+	+
Anaerobic growth	+	+	-	-	-	-	+
Motility Test	-	-	-	+	+	+	+
Catalase Test	+	-	+	+	+	+	+
Oxidase Test	-	-	+	-	+	-	-
Urease Test	+	-	+	-	+	-	+
Endospore Test	-	-	-	+	-	-	-
Indole Test	+	-	-	-	-	+	-

Citrate Test	+	+	+	+	+	+	-
Glucose Fermentation test	+	-	+	+	+	+	+
Lactose Fermentation test	-	-	-	-	-	+	+
H ₂ S production test	-	-	-	-	-	-	-
Organism identified	<i>Staphylococcus</i> sp	<i>Streptococcus</i> sp	<i>Micrococcus</i> sp	<i>Bacillus</i> sp	<i>Pseudomonas</i> sp	<i>E. coli.</i>	<i>Klebsiella</i> sp

Key: + =positive, - =negative

3.4 Distribution of Bacterial Isolates in Air Samples

The distribution of the bacterial isolates obtained from the air samples in wet and dry seasons are presented in Tables 2 and 3 respectively. It was observed that *Streptococcus* sp, *Escherichia coli*, *Staphylococcus* sp, *Pseudomonas* sp, *Micrococcus* sp, *Klebsiella* sp and *Bacillus* sp were more abundant in the air in the dumpsite and decreased with distance away from the dumpsites in the wet season while *Micrococcus* sp and *Bacillus* sp were abundant in both wet and dry seasons.

Table 2: Distribution of air bacterial isolates in wet season

Organism	Dumpsite	ST1	ST 2	ST3	ST 4	ST 5
<i>Streptococcus</i> sp	+++	+++	++	++	++	++
<i>Bacillus</i> sp	+++	+++	++	++	++	++
<i>Escherichia coli</i>	+++	+++	++	++	++	++
<i>Micrococcus</i> sp	+++	+++	++	++	++	++
<i>Staphylococcus</i> sp	+++	+++	++	++	++	++
<i>Pseudomonas</i> sp.	+++	+++	++	++	++	++
<i>Klebsiella</i> sp	+++	+++	++	++	++	++

Key: - Absent, + Rare, ++ Intermediate, +++ large in number

Table 3: Distribution of air bacterial isolates in dry season

Organism	Dumpsite	ST1	ST 2	ST3	ST 4	ST 5
<i>Streptococcus</i> sp	++	++	++	++	+	+
<i>Bacillus</i> sp.	+++	+++	+++	+++	++	++
<i>Escherichia coli</i>	++	++	++	++	+	+
<i>Micrococcus</i> sp	+++	+++	+++	+++	++	+
<i>Staphylococcus</i> sp	++	++	++	++	+	+
<i>Pseudomonas</i> sp.	++	++	++	+	+	+
<i>Klebsiella</i> sp	++	++	++	+	+	-

Key: - Absent, + Rare, ++Intermediate, +++large in number

3.5 Fungal Isolates from Air Samples

The fungal isolates from the air, samples are presented in Table 4. The fungal genera isolated were *Alternaria* sp, *Aspergillus* sp, *Cladosporium* sp, *Curvularia* sp, *Mucor* sp, *Penicillium* sp and *Rhizopus* sp.

Table 4: Identification of fungal isolates from air samples

	A	B	C	D	E	F	G
Colonial Morphology	Grayish-green or black colonies with gray edges rapidly swarming over entire plate. Aerial mycelium not very dense, appearing grayish to white.	White colonies became greenish-blue, black/ brown as culture matures	Small, greenish/black powdery colonies	Brown conidiophore, simple or sometimes branched, bearing spores apically	Resembles the colonies of Rhizoids	Mature cultures usually greenish or blue-green	Rapidly growing white, aerial, cottony and fuzzy coloured mycelium swarming over entire plate
Microscopic Appearance	Multi-celled pear-shaped conidia attached to single conidiophores arising from a septate mycelium conidia	Single-celled conidia in chains developed at the end of the sterigma with septate mycelium.	Conidia developed at the end of conidiophores with septate brownish mycelium	Produced dark conidia with lighter ends and enlarged centers	Oval spores, non-septate mycelium giving to single sporangiophores with globular sporangium containing a columella	Single-celled conidia in chains at the end of the sterigma with the conidiophores arising from a septate mycelium.	Oval spores, colorless/ brown non-septate mycelium gives rise to straight sponangiophores that terminate with black sporangia containing columella
Organism identified	<i>Alternaria sp</i>	<i>Aspergillus sp</i>	<i>Cladosporium sp</i>	<i>Curvularia sp</i>	<i>Mucor sp</i>	<i>Penicillium sp</i>	<i>Rhizopus sp</i>

3.6 Distribution of Fungal Isolates in Air Samples

The distribution of the fungal isolates obtained from the air samples in wet and dry seasons are presented in Tables 5 and 6 respectively. It was observed that all the fungal isolates were abundant in the dry season while *Aspergillus sp* and *Mucor sp* were abundant in both wet and dry seasons.

Table 5: Distribution of air fungal isolates in wet season

Organism	Dumpsite	ST1	ST2	ST3	ST4	ST 5
<i>Aspergillus sp</i>	+++	++	++	++	++	++
<i>Cladosporium sp</i>	++	+	+	+	+	+
<i>Curvularia sp</i>	++	+	+	+	+	+
<i>Mucor sp</i>	+++	++	++	++	++	++
<i>Rhizopus sp</i>	++	+	+	+	+	+
<i>Penicillium sp</i>	++	+	+	+	+	-
<i>Alternaria sp</i>	++	+	+	+	+	+

Key: - Absent, + Rare, ++Intermediate, +++large in number

Table 6: Distribution of air fungal isolates in dry season

Organism	Dumpsite	ST1	ST2	ST3	ST4	ST 5
<i>Aspergillus sp</i>	+++	++	++	++	++	++
<i>Cladosporium sp</i>	+++	++	++	++	++	++
<i>Curvularia sp</i>	+++	++	++	++	++	++

<i>Mucor</i> sp	+++	++	++	++	++	++
<i>Rhizopus</i> sp	+++	++	++	++	++	++
<i>Penicillium</i> sp	+++	++	++	++	++	++
<i>Alternaria</i> sp	+++	++	++	++	++	++

Key: – Absent, + Rare, ++Intermediate, +++large in number

IV. DISCUSSION

The microbial loads of the air samples taken from the dumpsites were higher than the normal atmospheric concentration of the microorganisms as the reported average level of the microbes in the ambient air is 3.0 log₁₀cfu/ml [15]. This is an indication of the extent of microbial pollution of waste dump sites in the study sites

The microorganisms isolated from the air samples taken from the various locations (Warri, Agbarho and Agbor), and distances sampled were similar in composition and distribution with those isolated from the air samples taken from the dumpsite. Generally the microbial load decreased with distances away from the dumpsite. The bacterial load for the air samples from Warri, Agbarho and Agbor presented in Figs 2- 4 showed that the bacteria counts decreased with distance from the dumpsite. It was equally observed that the bacterial counts were higher in the months of July August and September (wet season) and decreased slightly in the months of October, November and December (dry season). The decreasing bacterial counts with distance away from the dumpsite could be due to increased microbial activity in the dumpsite as a result of putrefaction and increased decomposition of organic matter in the vicinity of the dumpsite. It could also be as a result of household and industrial wastes deposited in the waste dumpsites as well as contaminants generated naturally that were propelled through the air, such as particles of dust and soil microbial spores in the air within the dumpsites. These results agree with the report of McCarthy [16], who listed these amongst others as possible sources of air contaminants.

The observed increased trend in the bacterial counts in wet season could be as a result of increased rainfall in the months of July, August and September and decreased rainfall in the months of October, November and December leading to reduced water activity. These results agree with the reports of Obire *et al.* [17], who stated that seasonal variations favour physiological types.

The fungal load for the air samples from Warri, Agbarho and Agbor as presented in Figs 5-7 showed that the fungal counts decreased with distance away from the dumpsites. It was equally observed that the fungal counts were lower in the months of July August and September (wet season) and increased slightly in the months of October, November and December (dry season). The decreasing fungal count with distance away from the dumpsite could be due to same reasons propounded for bacterial counts above. These results agreed with the report of McCarthy [16], who reported similar suggestions

The observed trend in the fungal counts could be due to spore formation due to increased rainfall in the months of July, August and September and decreased rainfall in the months of October, November and December. These findings also agree with the reports of earlier researchers [17] who opined that seasonal variations favour physiological types.

The bacterial and fungal genera encountered in this study had been reported by previous workers [18-20], as the possible microbial isolates from the air. The isolated bacteria were more abundant in wet season than in the dry season. This could possibly be due to the atmospheric particles to which the microbes are attached being deposited by the process of rainfall. The increased water activity therein provides favourable conditions for bacteria to thrive and multiply. *Bacillus* sp and *Micrococcus* sp were more abundant in the air sample both in wet and dry seasons. The presence and prevalence of some of these species of bacteria in the dumpsite could be as a result of the presence of damp organic materials, materials impregnated with water, food and food products and spores of microorganisms propelled through the air. These results agree with the report of Osha [21] and Sola [22], who reported these as possible sources of air microflora. These bacteria, according to Douwes [23] can cause different forms of bacterial pneumonia, influenza and gastrointestinal diseases.

The general trend of the isolated fungi showed that they were more abundant in dry season than in the wet season. This could be as a result of various climatic and local topographic factors including reduced rainfall leading to reduced water activity which favour germination of spores. It could also be as a result of the wind action and harsh weather conditions experienced during the dry season. *Cladosporium* sp, *Penicillium* sp, *Mucor* sp, *Alternaria* sp, *Aspergillus* sp, *Rhizopus* sp and *Curvularia* sp were observed to be predominant in the air samples during the dry season while *Aspergillus* sp and *Mucor* sp species were predominant in both the wet and

dry season. These results agree with the report of Prescott *et al.* [24], who listed these amongst the most common allergenic moulds associated with man and live stocks. Obire *et al.* [17] also identified *Aspergillus* sp as one of the most common fungi.

The present study has revealed that the dumpsite had impact on both the microbial load and quality of such environment. Also, the microbial load of air in dumpsites decreases with increase in distance from the dump sites. Airborne bacteria at dumpsites are more prevalent in wet seasons than in the dry seasons, the reverse being the case for airborne fungi. It is therefore recommended that dumpsites should be located at about 1 km away from residential areas to avoid contamination of the air. Additionally, educational programmes should be organized for public awareness of impact of indiscriminate dumping of refuse.

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