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# Microbiological Assessment of Rainwater and Air Quality of Some Areas in Port Harcourt

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The microbiological quality of Rainwater and air of the market, hospital, and residential area was evaluated. Roof-harvested and direct rainwater samples were collected from the three aforementioned locations. The air quality was investigated using the plate sedimentation method. Culture-based techniques were used for the enumeration and isolation of microorganisms in water samples, while the identities were confirmed using morphological, microscopic, and biochemical tests. The range of the total heterotrophic bacterial counts (THBC) of rainwater was 1.2±7.1x$10^6$ to 6.1±1.1 x$10^6$ cfu/ml, total coliform count (TCC) ranged between 8.0±0.00 x$10^3$ to 30.5 ±2.1 x$10^3$ cfu/ml, faecal coliform counts (FCC) ranged between 1.0±0.00×$10^3$ to 6.0±0.00×$10^3$ cfu/ml, fungal counts ranged from 2.0±0.00x$10^3$ to 11.0±0.00x$10^3$ cfu/ml. The THB, TCC, and FCC of the air samples, ranged between 0.04±0.02 to 0.13±0.04 cfu/min-$m^2$, 0.01±0.00to 0.03±0.00 cfu/min-$m^{2,}$ and 0.01±0.00 to 0.02±0.00 cfu/min-$m^2$, respectively. There was a significant difference (P≤ 0.05) in

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the total heterotrophic bacterial load of the roof-harvested rainwater and the direct rainwater of the residential area, roof-harvested and direct rainwater of the hospital. Statistically, there was a significant difference (P≤ 0.05) in the total coliform counts of the water with the coliform counts of the roof-harvested rainwater of the market being significantly higher than the coliform counts recorded in other locations. Seven bacterial genera belonging to *Micrococcus* sp, *Staphylococcus, Escherichia coli, Proteus* sp, *Pseudomonas* sp, *Enterobacter* sp, and *Bacillus* sp were isolated. The fungal isolates were *Candida* sp, *Mucor* sp, *Rhizopus* sp, *Penicillium* sp, *Aspergillus niger,* and *Aspergillus flavus*. The results showed that rainwater is not fit for consumption due to its components and can cause harm to individuals if consumed without treatment. proper treatment is recommended before consumption. The results also showed that the microbiological quality of the air samples was influenced by their environment.

*Keywords: Rainwater; air quality; microorganisms.*

## 1. INTRODUCTION

Harvested rainwater (HRW) has been considered an effective alternative water source for drinking and various non-potable uses in several countries throughout the world. The most significant issue concerning using untreated HRW for drinking or other potable uses, however, is the potential public health risks associated with microbial pathogens [1]. Rainwater harvesting can be classified into two broad categories: land-based and roof based. Land-based rainwater harvesting occurs when rainwater runoff from the land is collected in ponds and small impoundments before it has a chance to reach a river or stream. Roof-based harvesting, on the other hand, involves collecting the rainwater that falls on a roof before the water even reaches the ground [2]. Roofs represent an important percentage of the large impermeable areas covered by cities and communities, hence offering a significant possibility for rainwater collection. Factors such as type of roof material; dry period and surrounding environmental conditions; faecal droppings by birds; lizards, rodents and cats, which can access rainwater catchments areas, may transfer pathogenic microbes that are harmful to health and influence rainwater quality [3]. The typical roofing materials that are commonly used in Nigeria today include ceramic tiles, metal sheets, galvanized iron, anodized aluminium, and asbestos. All these materials are a potential source of dissolved ions, alkalinity and trace metals [4]. Diseases caused by the consumption of contaminated water, and poor hygiene practices are the leading cause of death among children worldwide, after respiratory diseases [5]. The experience of water shortage in developing countries and communities has made residents resort to sourcing potable water from harvested rainwater. Roof-harvested rainwater is used in areas having significant rainfall but lacking conventional water supply systems, and where fresh surface water or groundwater is lacking [6]. While studies, such as rooftop rainwater harvesting study in Bangladesh, show that ingesting untreated rainwater can pose a significant health burden, outbreaks of waterborne diseases attributed to rainwater use are frequently not reported [7].

Adeniyi et al. [8] analysed trace metals in bulk freefall and roof-intercepted rainwater in Ile-Ife, Southwest Nigeria. The samples of bulk freefall and roof-intercepted rainwater were collected over five roof types. They observed that the mass concentrations and per cent detection of the trace metals were generally higher in roof-intercepted samples than in the free-fall with an enrichment factor within the range of 1 and 5, and the potability of bulk rainwater sources did not fall completely within the allowable guidelines of most international organizations showing rainwater sources are non-complimentary with set drinking guideline in terms of bacteriological quality. According to the Australian Drinking Water Guidelines [9], monitoring includes “regular sampling and testing to assess if water quality is meeting guideline values and any regulatory requirements or agreed levels of service”. The aesthetic qualities of appearance, taste and odour are generally the characteristics by which the public judge water quality. However, the absence of any unpleasant qualities does not guarantee water safety. Therefore, the safety of water, in public health terms, is determined by its microbial, physical, chemical and radiological quality [10]. Hence there is need for constant investigation and monitoring of the quality of water consumed by communities in developing countries. It would prove useful in the management, control and investigation of pollution cases, classification of water resources,

collection of baseline data, water quality surveillance and forecasting of water quality. Air can be considered one of the least hospitable environments for microbes because it holds fewer nutrients and thus supports relatively fewer organisms. In a previous study, it was reported that biological sources such as bacteria, fungi, pollen, viruses and mites contaminate the air due to industrialization, the high density of the human population and their activities in urban or rural areas [11]. Rainwater could mix with microorganisms already in the atmosphere. Thus, contamination of rainwater could be from the atmosphere before it touches the rooftops or ground. There is a dearth of information on the microorganisms associated with rainwater in Rivers State. Thus, this study is aimed at investigating the microbiological quality of rainwater and air quality in major areas in Port Harcourt.

## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Area

This study was carried out at the department of microbiology, River State University. Rainwater and air samples were obtained from a residential area, a market area and a hospital area. The residential area was located at Bisi Ejekwu Street; behind the Mile III police station with GPS coordinates of 4.48'22" N, 6.59'21" E. The site was opposite a church and adjacent to an area where building materials were sold, moving forward was a school and surrounding apartments making the influx and outflow of humans at a high rate, therefore, increasing the microbial population. The roofs were made of aluminium some of which had been stained with bird droppings and some of which had been rusting. The market area was located at Port Harcourt Mile III market $4^0$48'17.1" N, $6^0$59'33.3" E. The site was surrounded by little shops which sold different ranges of products including raw meat, dried fish, clothes, fruits and many other household products. The market was enclosed and could barely contain the influx and outflow of people signifying that the microbial load would be at a high rate. The roof was made of old and rusting aluminium, the roofs were dirty in that old nylon and food remnants were stuck on the roof, in some parts the roofs were tacky and almost pulling off. Opposite was a bus stop where buses dropped off and picked up their various passengers. The hospital area was located at Mile II, 53 Ojoto Road, Diobu 500101, Port Harcourt La Rosa clinic and diagnostic centre $4^0$.79'21.5" N, 6.99'36" E. The site is opposite a market where a trailer load of banana fruits were sold and carried, and other food products like raw meat were also sold. Besides the site was a generator with leaking diesel and a small shop where people filled their gas cylinders, on the other hand, was a shop where gym equipment was sold. An ambulance and other cars were parked in front of the hospital, the influx of people into the hospital was not much as visitors were not frequent, and the environment was serene and had an open space. The GPS coordinates are presented in Table 1.

**Table 1. GPS coordinates of the sample locations**

| Sample Location | Coordinates |
|---|---|
| Residential Area | 4.48'22"N, 6.59'21"E |
| Market Area | $4^0$48'17.1"N, $6^0$59'33.3E |
| Hospital Area | $4^0$.79'21.5" N, 6.99'36"E |

### 2.2 Sample Collection

#### 2.2.1 Water sample

The rainwater was collected both directly from open-air space and from the roof catchments (roof-harvested rainwater). For the collection of the direct rainwater, A table was placed one (1) meter above the ground in an open space and a sterilized beaker was placed on the table to enable the rain to get in. The beaker was placed away from rain splashes to ensure that only direct rainwater got into it. The beaker used was sterilized in the autoclave at 121℃ for 15 minutes at 15psi. For the collection of the roof-harvested rainwater, a beaker which had already been sterilized was placed under the roof catchments to allow the rain to get in and it was covered with foil. The samples were covered and transported to the microbiology laboratory for assessment.

#### 2.2.2 Air sampling

The direct plate method was used in sampling the atmospheric air [11]. In this method, Petri dishes containing sterile media were exposed to the atmosphere of the sampled stations. This was to allow microbial flora in the atmosphere to settle on the exposed plates. Plates were kept one meter above the ground. The plates were exposed for 15 minutes at each sampled site [12,13].

#### 2.2.3 Enumeration and isolation of aeroflora

Freshly prepared nutrient agar (NA), Eosin Methylene Blue (EMB) Agar and Sabouraud

Dextrose agar (SDA) plates in duplicates were exposed to the atmosphere of the different sampling sites for about 15 minutes to allow air microflora within the pen to settle on the surface of the medium by gravity. The plates were kept about 1m above ground level to eliminate possible contamination and aid the quick settling of microbial particles. These plates were transported to the Microbiology laboratory, Rivers State University, Port Harcourt and incubated for 24-48 hours at 37$^{o}$C. Counts were made for plates that showed significant growth at the end of incubation. Discrete colonies on the different media plates were picked and inoculated onto freshly prepared nutrient agar plates. Pure cultures of the isolates were obtained by streaking the isolates on a freshly prepared nutrient medium until it was ascertained that there were no contaminants [14].

#### 2.2.4 Enumeration of total heterotrophic bacteria (THB) in water samples

After a 10-fold serial dilution was carried out, an aliquot (0.1 ml) from $10^{-4}$ dilution was inoculated onto the surface of dried nutrient agar in duplicates. Using a flamed glass spreader, the aliquot was spread evenly on the plate. Bacteria isolates were incubated at 37$^{0}$C for 24 hours. After incubation, bacterial colonies that appeared on the incubated Nutrient agar plates were counted and the mean was calculated and expressed as CFU/ml for the samples. Discrete colonies were then sub-cultured on freshly prepared nutrient agar plates for the isolation of pure cultures.

$$\mathrm{CFU/ml} = \frac{\text{Number of colonies}}{\text{Dilution} \times \text{Volume plated (0.1)}} \quad \text{(Equation 1)}$$

#### 2.2.5 Total coliform count ((TCC)

Total Coliform Count were enumerated on Eosin methylene blue agar by inoculating aliquot of $10^{-2}$ dilution on dried EMB plates and incubated at 37℃ [15]. Bacterial colonies that appeared on the EMB agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from $10^{-2}$ dilutions and incubated at 37$^{0}$C for 24 hours were counted and the mean expressed as CFU/ml for the samples. Discrete colonies on the EMB agar plates were then sub-cultured onto freshly prepared nutrient agar plates for the isolation of pure cultures.

#### 2.2.6 Total fungal counts (THF)

Total Heterotrophic Fungal Count was enumerated on Sabouraud Dextrose agar (SDA) plates supplemented with tetracycline by inoculating aliquot of $10^{-2}$ dilution on dried SDA plates and incubated at 22$^{O}$C for 3-7 days [15]. Fungal colonies that appeared on the SDA plate after incubation was counted and sub-cultured on freshly prepared SDA plates.

#### 2.2.7 Characterization and identification of bacterial isolates

Cultural methods of characterization employed were colour, shape, texture, odour, and microscopy under an oil immersion light microscope. Biochemical tests adopted include motility, catalase test, citrate utilization, oxidase, Methyl-Red, Voges Proskauer, indole and sugar fermentation tests (glucose, lactose, sucrose and mannitol).

#### 2.2.8 Characterization and identification of fungal isolates (macroscopy and microscopy)

Isolates were identified using their morphological features such as colony colour, shape, texture and size of the colony followed by microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue and reference made to fungal identification manual [16].

### 2.3 Physicochemical Parameters

The physicochemical parameters determined include pH, temperature, electrical conductivity, total dissolved solids, turbidity, total suspended solids and total hydrocarbon content. The APHA method was used in determining the physicochemical parameters [17].

## 3. RESULTS

### 3.1 Microbial Counts

Results of the microbiological counts of the roof-harvested water and direct rainwater are presented in Table 2. Results showed that the total heterotrophic bacterial load of direct rainwater from the hospital, roof-harvested rainwater from the hospital, direct rainwater from the market, roof-harvested rainwater from the market, direct-rainwater from the residential area and roof-harvested rainwater from the residential area was $1.2\pm0.7\times10^{6}$, $1.6\pm0.6\times10^{6}$, $3.3\pm0.2\times10^{6}$, $3.8\pm0.2\times10^{6}$, $1.3\pm0.6\times10^{6}$ and $6.1\pm1.1\times10^{6}$ cfu/ml, respectively. There was a

significant difference (P≤ 0.05) in the total heterotrophic bacterial load of the roof-harvested rainwater and the direct rainwater of the residential area, roof-harvested and direct rainwater of the hospital. Results also showed that the total coliform load of direct rainwater from the hospital, roof-harvested rainwater from the hospital, direct rainwater from the market, roof-harvested rainwater from the market, direct rainwater from the residential area and roof-harvested rainwater from the residential area was $8.0x10^3$, $2.4x10^4$, $3.8x10^3$, $7.7x10^4$, $1.6x10^4$ and $3.1x10^4$ cfu/ml, respectively. Statistically, there was a significant difference (P≤ 0.05) in the total coliform counts of the water with the coliform counts of the roof-harvested rainwater of the market being significantly higher than the coliform counts recorded in other locations. More so, the coliform load of the roof-harvested rainwater from the residential area and direct rainwater from the market despite showing no significant difference were significantly higher than coliform counts of the roof and direct rainwater from the hospital. Faecal coliforms were also detected in the water but despite the disparity in counts, there was no significant difference. The fungal load of direct rainwater from the hospital, roof-harvested rainwater from the hospital, direct rainwater from the market, roof-harvested rainwater from the market, direct rainwater from the residential area and roof-harvested rainwater from the residential area was $4.0x10^3$, $6.0x10^3$, $2.0x10^3$, $1.1x10^4$, $3.0x10^3$ and $6.5x10^3$ cfu/ml, respectively. The fungal load from roof-harvested rainwater in the market and residential areas were significantly higher (P≤ 0.05) than the fungal load recorded in other samples.

The results of the microbiological counts of the outdoor air of the Hospital, Mile III market and the Residential area are presented in Table 3. Results showed that the total heterotrophic bacterial (THB) of the hospital, Mile III market and residential area was 0.04±0.02, 0.13±0.04 and 0.07±0.04 CFU/min-$m^2$, respectively. The total coliform counts (TCC) of the hospital, mile III market and residential area were 0.02±0.00, 0.03±0.00 and 0.01±0.00 CFU/min-$m^2$ while the total fungal count (FC) was 0.01±0.00, 0.02±0.00 and 0.01±0.00, respectively.

The results of the cultural and biochemical characteristics of the bacteria isolated from the samples are presented in Table 4. Results showed that seven bacterial genera belonging to *Micrococcus* sp, *Staphylococcus* sp*, Escherichia coli, Proteus* sp, *Pseudomonas* sp, *Enterobacter* sp and *Bacillus* sp were isolated from the rainwater and air samples. The results of the percentage occurrence of bacterial isolates associated with the rainwater samples are presented in Fig. 1. While the results of the percentage distribution of the bacterial isolates across the water samples are presented in Fig. 2. The results showed that *Bacillus* sp, *Staphylococcus* sp and *Pseudomonas* sp were all isolated from the water samples while *E. coli* and *Enterobacter* sp were isolated from five samples (i.e., roof-harvested market sample, roof-harvested residential sample, direct rainwater from the hospital and direct rainwater from the residential area).

**Table 2. Microbial counts (CFU/ml) of water samples**

| Sample | THB ($x10^6$) | TCC ($x10^3$) | Faecal coliform ($x10^3$) | Fungi ($x10^3$) |
|---|---|---|---|---|
| Hospital Direct | 1.6±5.7[a] | 8.0±0.00[a] | 2.0±0.00[a] | 4.0±1.4[a] |
| Hospital RH | 1.2±7.1[a] | 24.0±4.2[bc] | 1.0±0.00[a] | 6.0±4.2[ab] |
| Market Direct | 3.3±1.5[ab] | 3.8±7.1[d] | 6.0±0.00[a] | 2.0±0.00[a] |
| Market RH | 3.8±2.0[ab] | 77.5±7.8[e] | 4.0±0.00[a] | 11.0±0.00[b] |
| Residential Area Direct | 1.3±6.4[a] | 16.5±4.9[ab] | 2.0±0.00[a] | 3.0±0.00[a] |
| Residential Area RH | 6.1±1.1[b] | 30.5±2.1[cd] | 3.0±0.00[a] | 6.5±2.1[b] |

**Means with same superscript (alphabet) show no significant difference (P>0.05) down the column*
*Keys: FC = fungal count, THB = Total heterotrophic bacteria, TCC = total coliform counts, RH = roof harvested, Direct = rainwater with no contact of roof.*

**Table 3. Microbial load (Cfu/min-$m^2$) of the study locations**

| Sample | THB | TCC | FC |
|---|---|---|---|
| Hospital | 0.04±0.02[a] | 0.02±0.00[a] | 0.01±0.00[a] |
| Market | 0.13±0.04[a] | 0.03±0.00[a] | 0.02±0.00[a] |
| Residential Area | 0.07±0.04[a] | 0.01±0.00[a] | 0.01±0.00[a] |

**Means with the same superscript (alphabet) show no significant difference (P>0.05) down the column*

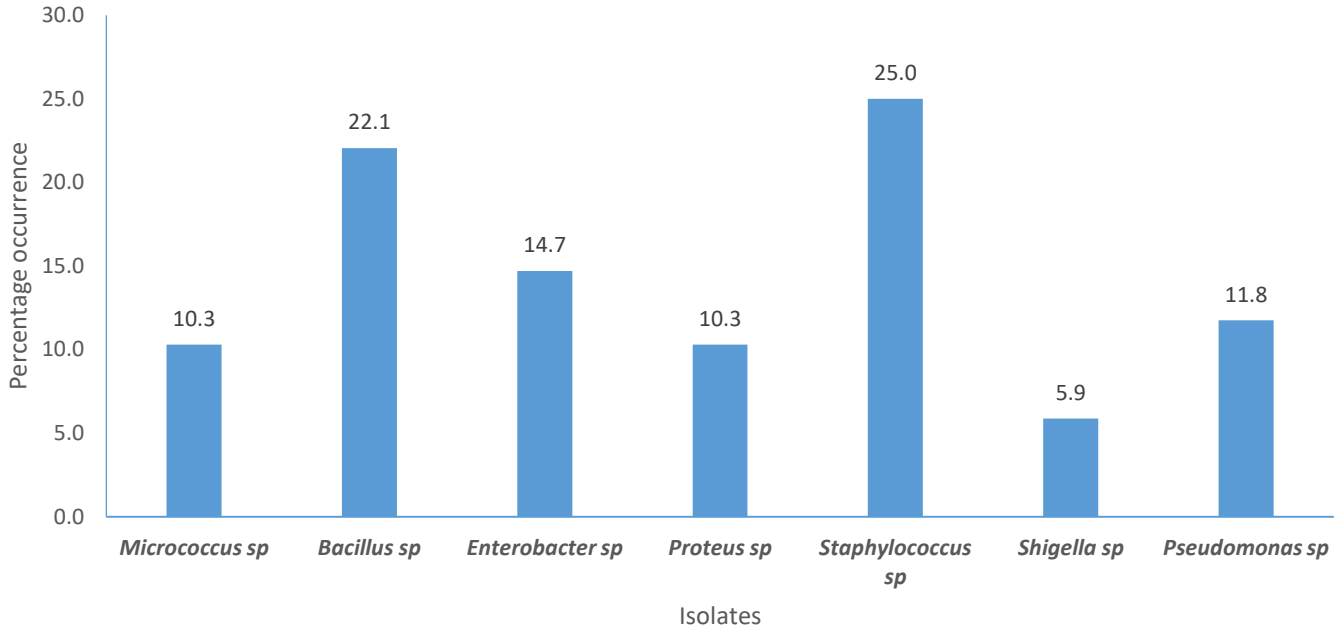


**Fig. 1. Percentage occurrence of bacterial isolates in the water**

**Table 4. Cultural and biochemical characteristics of bacterial isolates**

| Isolate | Shape | Elevation | Opacity | Edge | Colour | Gram reaction and cell morphology | Catalase | Oxidase | Citrate | Indole | Methyl Red | Voges Proskauer | Sucrose | Motility | Glucose | Mannitol | Lactose | Probable Identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a. | Starlike | Flat | Opaque | Smooth | Milky | -ve rod | + | - | + | - | - | + | + | + | A | A | - | *Proteus* sp |
| b. | Circular | Convex | Transparent | Smooth | Yellow | +cocci | + | - | + | - | - | - | A | - | - | - | - | *Micrococcus* sp |
| c. | Circular | Convex | Transparent | Smooth | Golden yellow | +ve cocci | + | - | + | - | + | + | A | - | A/G | A | A | *Staphylococcus aureus* |
| d. | Circular | Convex | Translucent | Smooth | Metallic | –ve rods | + | - | - | + | + | - | - | + | A/G | A | A/G | *Escherichia coli* |
| e. | Circular | Convex | Opaque | Smooth | Cream | –ve rods | + | - | + | - | - | - | - | + | A | A | A | *Proteus* sp |
| f. | Circular | Convex | Opaque | Smooth | Light green | –ve rods | + | - | + | - | - | - | A | + | A/G | A | A | *Pseudomonas sp* |
| g. | Circular | Convex | Opaque | Mucoid | Deep pink | -ve rod | + | - | + | - | - | - | A | + | A | A | A | *Enterobacter sp* |
| h. | circular | Flat | Opaque | Rough | Cream | +ve rod | + | - | + | + | + | - | - | + | A | - | - | *Bacillus* cereus |
| i. | Circular | Flat | Opaque | Rough | White | +ve rod | + | + | + | - | - | + | - | + | A | A | A | *Bacillus* sp |
| j. | Circular | Flat | Opaque | Rough | White | +ve rod | + | + | + | - | - | + | - | + | A | - | A | *Bacillus* sp |
| k. | Circular | Flat | Opaque | Rough | White | +ve rod | + | + | + | - | - | + | - | + | A | A | A | *Bacillus* sp |
| l. | Circular | Flat | Opaque | Rough | White | +ve rod | + | + | + | - | - | + | - | + | A | - | A | *Bacillus* sp |

*KEY: A- Acid, G- Gas, + Positive, - Negative*

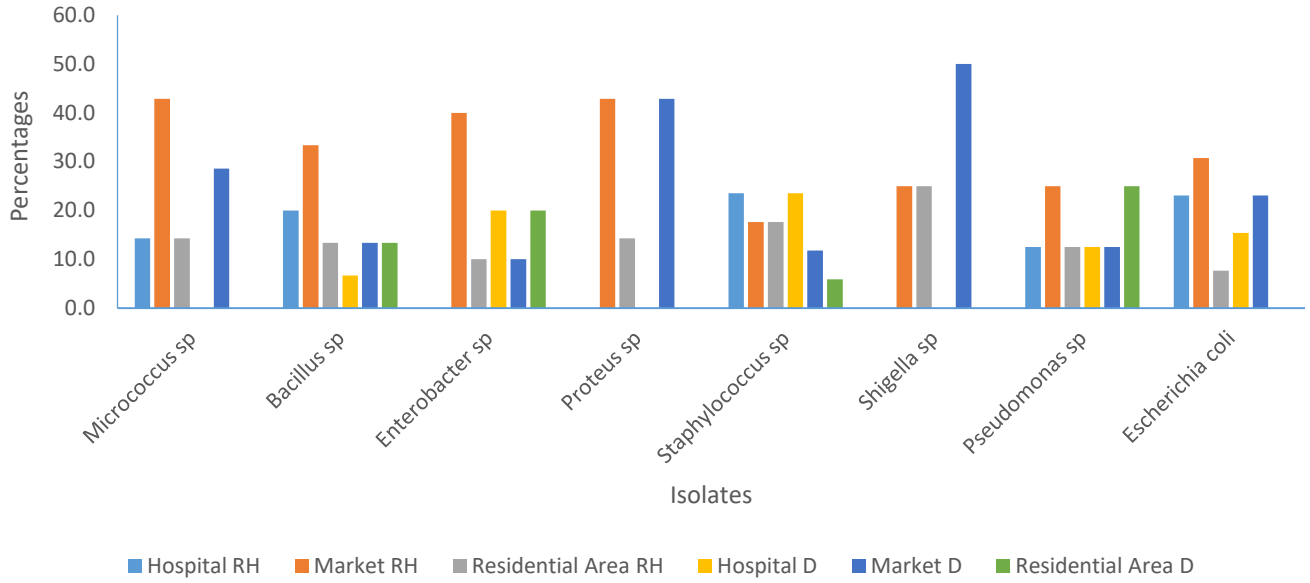


**Fig. 2. Percentage abundance of bacterial isolates in the water samples across the locations**

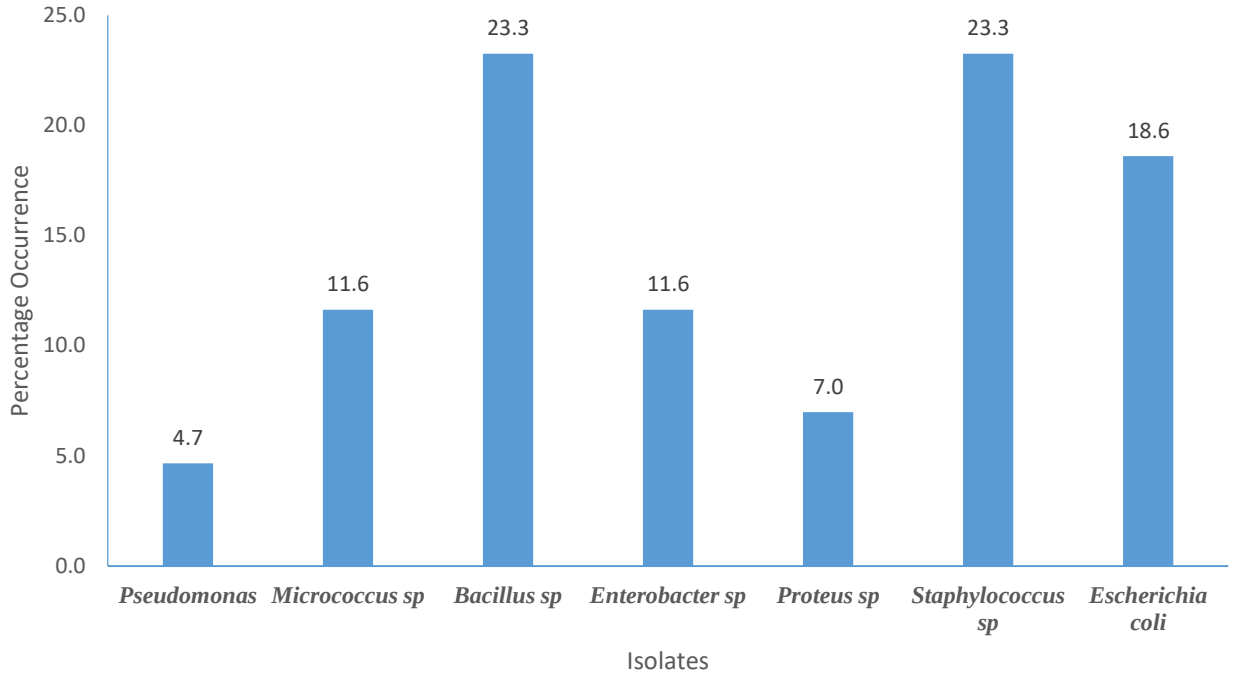


**Fig. 3. Percentage occurrence of Bacterial Isolates in the Air**

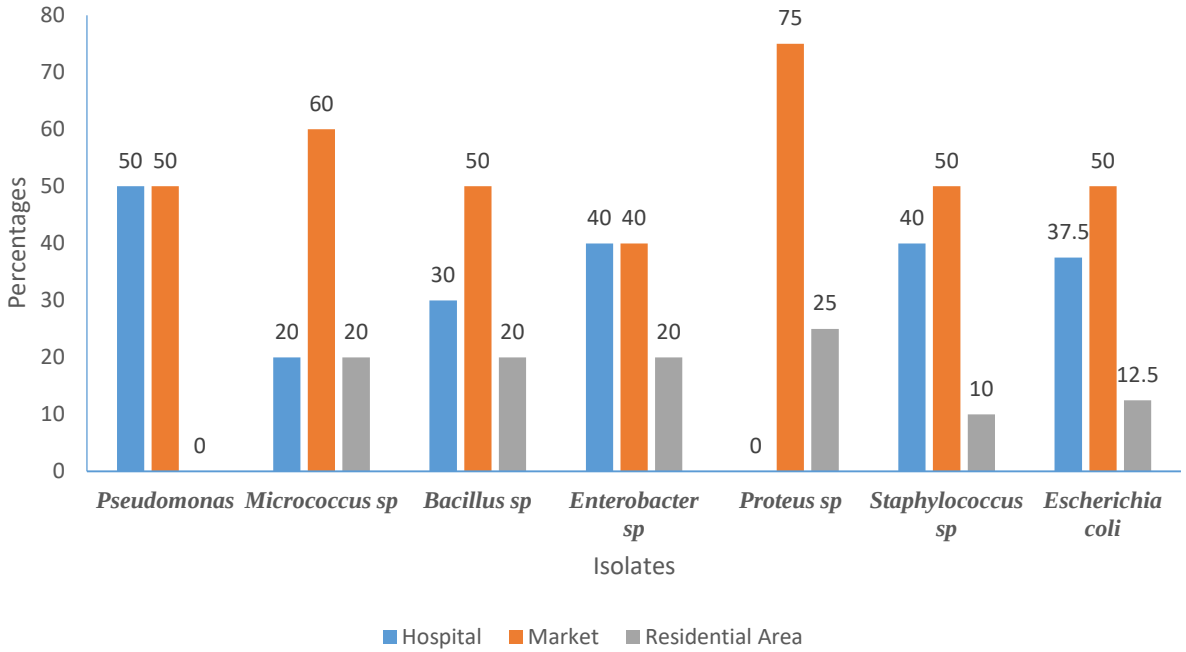


**Fig. 4. Percentage abundance of bacterial isolates in the air across the locations**

The results of the percentage occurrence of the bacterial isolates associated with the outdoor air are presented in Fig. 3. Results of the percentage distribution of the bacterial isolates from the rainwater of the various locations are presented in Fig. 4. Results showed that *Micrococcus* sp, *Staphylococcus* sp*, Escherichia coli, Enterobacter* sp and *Bacillus* sp were isolated from all locations while *Proteus* sp was isolated from the market and residential area. *Pseudomonas* sp was only isolated from the hospital and market area.

The results of the cultural characteristics of the fungal isolates and their probable identity are presented in Table 5. The results showed that the morphological and microscopic characteristics of the fungal isolates matched the identities of *Candida* sp, *Mucor* sp, *Rhizopus* sp, *Penicillium* sp, *Aspergillus niger* and *Aspergillus flavus.* The results of the distribution of fungal isolates across the rainwater samples are presented in Table 6. Results showed that *A. niger* was the only fungal isolate that occurred in all samples (i.e., both roofs harvested and direct in the three locations) while *Penicillium* sp was isolated from roof-harvested rainwater in hospital, market and residential areas as well as in hospital and market of direct rainwater. *Candida* sp was only isolated from the market of roof-harvested rainwater while *Mucor* sp was isolated only from the market roof-harvested and direct rainwater. *Rhizopus* sp was isolated in all locations of the roof-harvested rainwater but was not isolated from all the direct rainwater from the various locations.

Results of the percentage occurrence of fungal isolates in the various rainwater and air samples of the different locations are presented in Figs. 5 and 6, respectively. The results showing the distribution of fungal Isolates in the air samples across the location are presented in Table 7. Results showed that the distribution of the fungal isolates was not uniform across the samples.

## 3.2 Physicochemical Parameters of Rainwater

Results of the mean physicochemical parameters are presented in Table 8. Results showed that the pH, Temperature, Total dissolved solids (mg/l), Total suspended solids (mg/l), Total hydrocarbon content (mg/l), Electrical conductivity (mg/l) and Salinity for direct rainwater was 7.3, 28.1, 29, 0.2, 232, 64 and 0.036 mg/ml, respectively. The pH, Temperature, Total dissolved solids (mg/l), Total suspended solids (mg/l), Total hydrocarbon content (mg/l), Electrical conductivity (mg/l) and Salinity for roof-harvested rainwater was 7.16, 28.1, 7, 0.3, 198, 24 and 0.013 mg/ml, respectively.

**Table 5. Macroscopic and microscopic identification of fungal isolates**

| Isolates | Macroscopy | Microscopy | Probable Identity |
|---|---|---|---|
| A | Cream large round | Oval budding blasto conidia | *Candida* sp |
| B | Fluffy white cottony, white reverse | Aeseptate hyphae bearing sporangiospores | *Mucor* sp |
| C | Fluffy white to grey cottony, yellow reverse | Aeseptate hyphae bearing sporangiospores | *Rhizopus* |
| D | Green powdery surface surrounded by white lawn, brown reverse | Septate hyphae with septate conidiophores bearing conidia | *Penicillium* sp |
| E | Black spores surrounded by white lawn-like growth | Aeseptate conidiophores bearing conidia | *Aspergillus sp* |
| F | Light green lawn surrounded by white lawn-like growth | Septate hyphae with aeseptate conidiophores bearing conidia | *Aspergillus flavus* |

**Table 6. Distribution of air fungal isolates across the study location**

| Isolate | Hospital | Market | Residential Area |
|---|---|---|---|
| *Candida* sp | - | + | + |
| *Mucor* sp | - | + | + |
| *Rhizopus* | + | + | + |
| *Penicillium* sp | + | + | + |
| *Aspergillus niger* | + | + | + |
| *Aspergillus flavus* | - | + | + |

*Keys: + = isolated; - = not isolated*

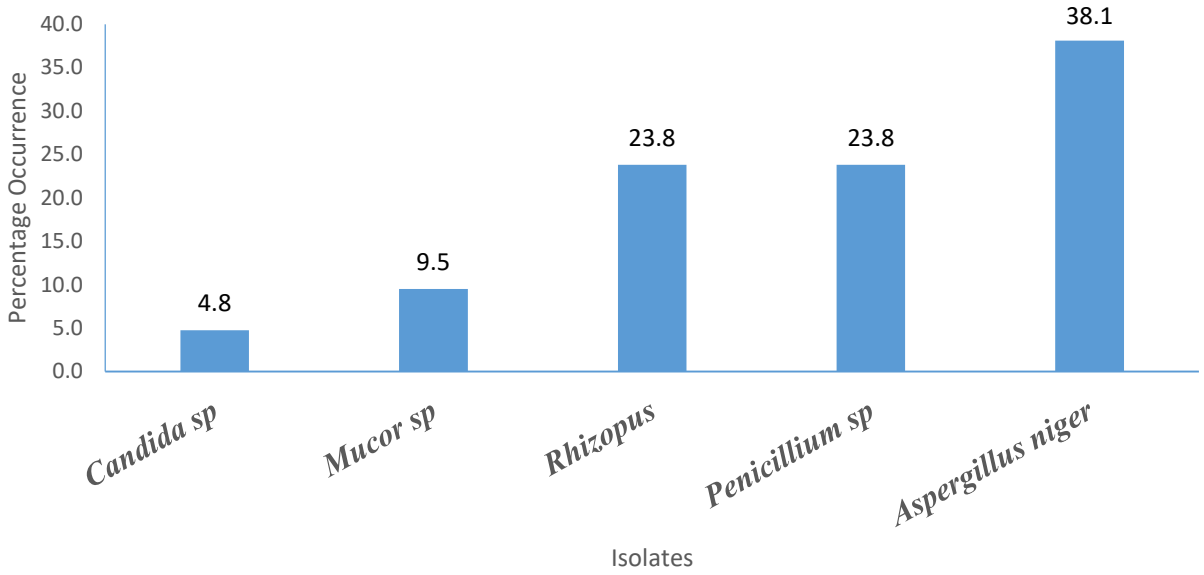


**Fig. 5. Percentage occurrence of fungal isolates in the water samples**

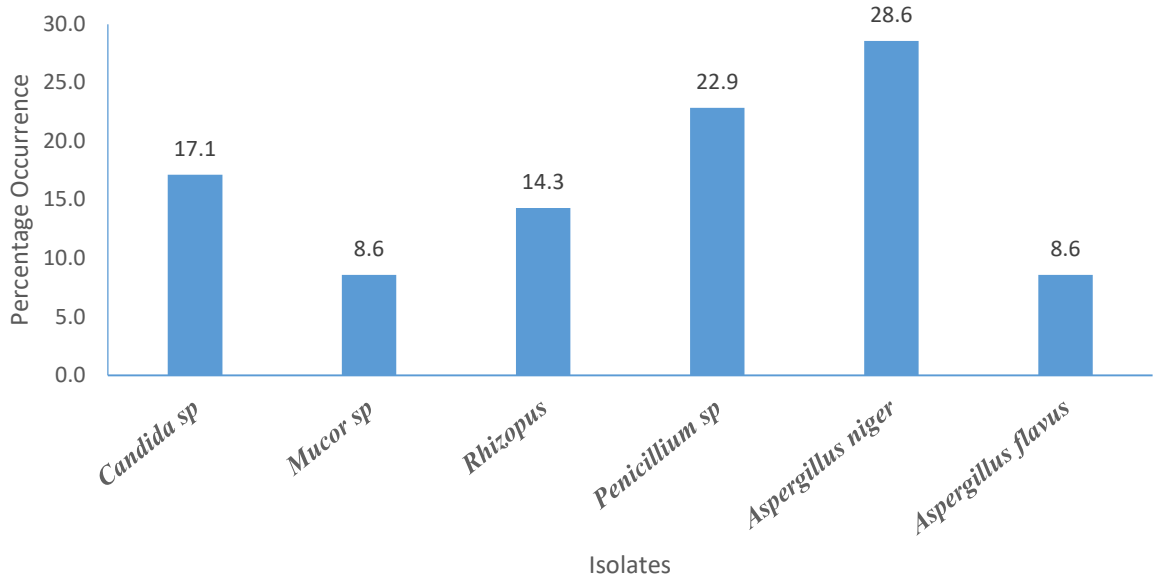


**Fig. 6. Percentage occurrence of fungal isolates in the outdoor air**

**Table 7. Distribution of fungal isolates across the study location**

| Isolate | Roof Harvested | | | Direct Rain | | |
|---|---|---|---|---|---|---|
| | Hospital | Market | Residential Area | Hospital | Market | Residential Area |
| *Candida* sp | - | + | - | - | - | - |
| *Mucor* sp | - | + | - | - | + | - |
| *Rhizopus* | + | + | + | - | - | - |
| *Penicillium* sp | + | + | + | + | + | - |
| *Aspergillus niger* | + | + | + | + | + | + |

*Keys: + = isolated; - = not isolated*

**Table 8. Mean physicochemical parameters of rainwater from study locations**

| Parameters | Direct Rainwater | Roof-harvested rainwater |
|---|---|---|
| pH | 7.3 | 7.16 |
| Temperature | 28.1 | 28.1 |
| Total dissolved solids (mg/l) | 29 | 7 |
| Total suspended solids (mg/l) | 0.2 | 0.3 |
| Total hydrocarbon content (mg/l) | 232 | 198 |
| Electrical conductivity (mg/l) | 64 | 24 |
| Salinity | 0.036 | 0.013 |

## 4. DISCUSSION

The bacterial and fungal counts of the roof-harvested water which was recorded to be higher than the direct rainwater (water collected directly from the atmosphere) could be attributed to the contamination from particles or microorganisms attached to the roofs of the buildings. Unlike direct rainwater which had no contact with any known material. Comparatively, the roof-harvested rainwater from the residential area had a higher total heterotrophic bacterial load followed by the roof-harvested rainwater from the market while the roof-harvested rainwater of the market on the other hand had higher coliform and fungal load compared to those obtained in the residential and hospital samples. There was a significant difference ($P \leq 0.05$) in the total heterotrophic bacterial load of the roof-harvested rainwater and the direct rainwater of the residential area, roof-harvested and direct rainwater of the hospital. The present study also showed that the bacterial and fungal load in the rainwater (i.e., the roof-harvested and direct) fluctuated and this could be attributed to many factors including the location of the area sampled, the type of environmental condition as well as the abiotic factors in that location. According to Abbasi and Abbasi [18], the quality of roof-harvested rainwater could vary according to geographic and catchment locations, climatic conditions, organic material in the gutter, the presence of animal faeces, and the roof condition. More so, the presence of faecal coliforms in the water samples could be attributed to the dissemination of aerosols on rooftops as well as droplets from faecal sources that find their way into the atmosphere as a result of anthropogenic activities like sweeping and air currents. Brodie [19] in a study reported that *E. coli* and other pathogens can enter roof-harvested rainwater through aerosol deposition, tree litter, and animal faecal matter. Rainwater may be contaminated with microorganisms already at the stage of precipitation formation, during the runoff from the surface from which it is collected or at rainwater harvesting systems [20]. Thus, this could have influenced the coliforms and other microorganisms in the water samples. The bacterial load in the rainwater sources across the location are generally high and the presence of faecal coliforms in the samples showed that the water is highly polluted with faecal matter. A similar study conducted in South-Eastern Nigeria had reported high bacterial and coliform load in the range of $1.9\times10^3$ to $7.0\times10^6$ CFU/mL for total heterotrophic bacterial load and $1.0\times10^2$ to $8.0\times10^3$ CFU/mL for roof-harvested rainwater [21]. The WHO permissible limit for total heterotrophic bacterial load, faecal coliform and total coliform is given as $1.0\times10^2$ CFU/mL, 0 CFU/100ml and ≤ 3 CFU/ml. thus, with this specification, the water samples in the present study are said to have exceeded limits and therefore not fit for drinking.

The results of the outdoor air environment showed that the bacterial and fungal counts of the market were higher than the bacterial counts of the hospital and the residential area. The location with the second high bacterial and fungal load was the hospital while the residential area had low counts in bacterial and fungal population. The high bacterial and fungal load detected in these locations could be attributed to the high influx and outflow of individuals, the different activities taking place in these areas as well as other anthropogenic activities. The market for example is characterized by heavy activities like the interaction between buyers and sellers, vehicular movement, pushing of trucks regular inflow of people of all works of life, etc while the hospital is characterized by persons coming for medical check-ups/treatment or consultations, unlike the residential area which is rarely accessed by a high population of persons as compared to the market and the hospital. In a previous study, it was reported that the microbial load of an environment is to a large extent characterized by the number of persons using that environment as well as the different activities being carried out in that environment [22]. Thus, this statement agreed with the present study. More so, despite the high bacterial load in the outdoor air of the market and hospital, results showed no significant difference in the bacterial and fungal load of the three locations. The microbiological load in the present study is lower than the $5.2\times10^4$ $CFUm^{-3}$ and $4.7\times10^4$ $CFUm^{-3}$ for bacteria and fungi reported by Agwaranze et al. [23] of outdoor air around a hospital.

### 4.1 Bacterial Isolates of Water and Air Samples

The frequency of occurrence of bacterial isolates in the water samples was *Micrococcus* sp (10.3%), *Bacillus* sp (22.1%), *Enterobacter* sp (14.7%), *Proteus* sp (10.3%), *Staphylococcus* sp (25.0%), *Shigella* sp (5.9%) and *Pseudomonas* sp (11.8%). Findings showed that *Staphylococcus* sp had the highest frequency of occurrence followed by *Bacillus* sp while *Shigella*

sp had the least occurrence. While the percentage distribution of the bacterial isolates across the water samples showed that *Bacillus* sp, *Staphylococcus* sp and *Pseudomonas* sp were all isolated from the water samples while *E. coli* and *Enterobacter* sp were isolated from five samples (i.e., roof-harvested market sample, roof-harvested residential sample, direct rainwater from the hospital and direct rainwater from the residential area). *Shigella* sp and *Proteus* sp were only isolated from three samples (i.e., roof-harvested market sample, roof-harvested residential sample and direct rainwater from the market). The bacterial isolates in the rainwater samples are similar to the bacterial isolates in the air samples and this could support our assertion that the microorganisms contained in droplets as well as in the atmosphere had influenced the microorganisms in the water. The bacterial isolates in the roof-harvested rainwater are in line with a previous study [21,24]. *Escherichia coli* is excreted in large numbers by man and animals and its presence in water confirms that faecal matter has entered the water source and that the source is liable to contamination with dangerous intestinal pathogens [21].

The percentage occurrence of bacterial isolates in the outdoor environment was *Micrococcus* sp (11.6%), *Staphylococcus* sp (23.3%), *Escherichia coli* (18.6%), *Proteus* sp (7.0%), *Pseudomonas* sp (4.7%), *Enterobacter* sp (11.6%) and *Bacillus* sp (23.3%). *Staphylococcus* sp and *Bacillus* sp were the most abundant bacterial isolates while *Proteus* sp was the least abundant. The percentage distribution of the bacterial isolates in the outdoor air showed that *Micrococcus* sp, *Staphylococcus* sp, *Escherichia coli, Enterobacter* sp and *Bacillus* sp were isolated from all locations while *Proteus* sp was isolated from the market and residential area. *Pseudomonas* sp was only isolated from the hospital and market area. The bacterial isolates in the outdoor air of the market, hospital and residential area have been reported in the previous study. Agwaranze et al. [23] isolated *Staphylococcus aureus*, *Staphylococcus epidermidis, Escherichia coli, Bacillus* spp. and *Micrococcus* spp. Similar isolates have been reported by Ekhaise and Ogboghodo [25], in their study on airborne microflora in the atmosphere of a hospital environment at the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Moreover, a previous report indicated the presence of *Staphylococcus aureus* and *Escherichia coli*, in the microbiological outdoor air quality of 2 major hospitals in Benin City, Nigeria. These microorganisms are known to be associated with nosocomial infections [26]. *Staphylococcus aureus* is known to cause infections of the skin, deeper tissue and organs as well as pneumonia [23]. More so, the present study agreed with Agwaranze et al. [23] who reported that *Staphylococcus* sp and *Bacillus* sp had the highest percentage occurrence among other bacterial isolates in the outdoor environment.

## 4.2 Fungal Isolates in Outdoor Water and Air Samples

The percentage occurrence of fungal isolates in the various rainwater sample is given as *Candida* sp (4.8%), *Mucor* sp (9.5%), *Rhizopus* sp (23.8%), *Penicillium* sp (23.8%), and *Aspergillus niger* (38.1%). *A. niger* was the most predominant fungal isolate in the water sample while *Candida* sp was the least fungal isolate in the water sample. The distribution of fungal isolates across the rainwater showed that *A. niger* was the only fungal isolate that occurred in all samples (i.e., both roofs harvested and direct in the three locations) while *Penicillium* sp was isolated from roof-harvested rainwater in the hospital, market and residential areas as well as in hospital and market of direct rainwater. *Candida* sp was only isolated from the market of roof-harvested rainwater while *Mucor* sp was isolated only from the market of roof-harvested and direct rainwater. *Rhizopus* sp was isolated in all locations of the roof-harvested rainwater but was not isolated from all the direct rainwater from the various locations. The presence of fungi in drinking water has been ignored especially since fungi unlike bacteria and viruses, the consumption of water contaminated with fungi has not caused any diseases but could be regarded as a chronic problem in drinking water distribution systems [27]. The fungal isolates in the different water samples in the present study could illicit allergic reactions on the skin or may cause diseases, especially in immune-compromised individuals especially since most of these fungi are known to produce toxins. Many species in both genus *Penicillium* and *Aspergillus* are known to produce mycotoxins and the detection of aflatoxins produced by *A. flavus* in water from cold water has been reported [27]. Although the WHO and the European Union drinking water directive does not address fungi explicitly either. However, the directive states that wholesome drinking water should be “free from any microorganisms and parasites and from

any substances which, in numbers or concentrations, constitute a potential danger to human health. Thus, this definition implies that the presence of pathogenic or allergenic fungi in the drinking water is not acceptable either [28].

The fungal isolates associated with the outdoor air include *A. niger, A. flavus, Mucor, Penicillium, Rhizopus* sp and *Candida* sp. The percentage occurrence is given as *Candida* sp (17.1%), *Mucor* sp (8.6%), *Rhizopus* sp 14.3%), *Penicillium* sp 22.9%), *Aspergillus niger* (28.6%) and *Aspergillus flavus* (8.6%). The most predominant fungal isolates in the air were *Aspergillus niger* followed by *Penicillium* sp while *Mucor* sp and *A. flavus* had the least percentage occurrence. The distribution of fungal Isolates in the air samples across the location showed that *Rhizopus, Penicillium* and *A. niger* were isolated from the outdoor air of the hospital, market and residential area. Thus, these fungal isolates were the predominant fungal isolates. *Candida* sp, *Mucor* sp and *A. flavus* were only isolated from three locations: Market and residential areas. The fungal isolates in the outdoor air agreed with fungal isolates reported by Kirti et al. [29] in the outdoor environment of the school, motor park and college.

### 4.3 Physicochemical Parameters of Rainwater

The mean physicochemical parameters of the direct rain and roof-harvested water showed that the pH of the direct rainwater and the roof-harvested rainwater are all slightly neutral. The pH could be dependent on many factors including the continued heavy rainfall, soot and other particles in the atmosphere and roofing sheets. The current pH is within the 6.5-8.5 pH limits for drinking water [5]. Zdeb et al. [30] in their study reported pH values within the range of slight acidity and slight alkaline (6.0-7.3) and attributed the pH fluctuations to the sampling season and type of roofing material used. According to Despins et al. [31], the intensity of precipitation causes an increase in the pH values of rainwater. Thus, this could be the reason why the pH in the present study increased due to the continuous rain in the month of June 2022. The temperature of both direct rainwater and roof-harvested rainwater was similar. Temperature is an essential parameter that determines microbiological water quality since it determines the critical environmental factors that influence the taxonomic composition of microorganisms found in rainwater [30]. Interestingly, the total dissolved solids of the direct rainwater were higher than those obtained for the roof-harvested rainwater although the total suspended solids in the direct rainwater were lower than the values obtained for the roof-harvested rainwater. The total hydrocarbon content of the direct rainwater was higher than the values recorded for the roof-harvested rainwater. Furthermore, the electrical conductivity and salinity of the direct rainwater were higher than the values recorded for the roof-harvested rainwater. The EC value refers to the ability of the water to conduct electricity. The EC values in the present study are very low and fall under the permissible limit of 500 and 1000 µs/cm standard of the NSDWQ [32] and WHO [5]. The high THC values in the direct rainwater could be attributed to the presence of soot or other organic material in the atmosphere which might have mixed with the rainwater.

## 5. CONCLUSION

This study concluded that the microbiological quality of direct rainwater and roof-harvested rainwater collected from the market, hospital and residential area is very poor and does not satisfy the guideline for water quality. Thus, it is not fit for human consumption and domestic purposes as well as bathing unless treated. The presence of known human pathogenic microorganisms and faecal indicators in the direct and roof-harvested rainwater clearly showed a potential risk of contamination if it is consumed directly or used for cleaning purposes. Furthermore, the microbial counts in the outdoor air are low and even though there is no accepted standard for the volume of microbial load in the outdoor air, previous works references were higher than those obtained in the present study. More so, the bacterial and fungal isolates associated with the outdoor air could influence the microbial quality of indoor air especially the hospitals and residential areas, thus leading to poor indoor air quality. Treatment of rainwater before drinking is highly recommended to avoid the risk of exposure to microorganisms that could pose serious public health challenges.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Muhammad TA, Mooyoung H. Probable sources of microbial contamination of stored rainwater and its remediation; 2008.

2. Texas Commission on Environmental Quality, TCEQ. Rainwater harvesting: guidance for public water systems TCEQ, P.O. box. Austin. 2017;13087.
3. Sazakli E, Alexopoulos A, Leotsinidis M. Rainwater harvesting, quality assessment and utilization in Kefalonia Island, Greece. Water Res. 2007;41(9): 2039-47.
4. Ayenimo JG, Adekunle AS, Makinde WO, Ogunlesi GO. Heavy metal fractionation in roof run-off in Ile-Ife. Niger Environ Sci Technol J. 2006;3:221-7.
5. World Health Organization.Global annual assessment of sanitation and drinking-water GLAAS): Targetin resources for better results; 2012.
6. UNEP. United Nations environmental programme. Rain and storm water harvesting in rural areas, Nairobi. Water Resources Series Five Journal. 1983;92-113.
7. Karim MR. Microbial contamination and associated health burden of rainwater harvesting in Bangladesh. Water Sci Technol. 2010;61(8):2129-35.
8. Adeniyi IF, Olabanji IO. Trace metals in bulk freefall and roof intercepted rainwater at Ile-Ife, Southwestern Nigeria. J Chem Ecol. 2005;21(3):149-66.
9. NHMRC, NRMMC. Australian drinking water guidelines: Paper 6. National water quality management strategy. National Health and Commonwealth of Australia, Canberra: Medical Research Council, National Resource Management Ministerial Council; 2011.
10. NHMRC. Australian drinking water guidelines. Available. National Health and Medical Research Council and Canberra: Natural Resource Management Ministerial Council; 2004.
11. Robinson VK, Wemedo SA. Molecular characterization of indoor air microorganisms of a model primary health care in Port Harcourt, Rivers State, Nigeria. Asian J Biotechnol Genet Eng. 2019;2(1):1-9.
12. Douglas SI, Robinson VK. Indoor microbiological air quality in some wards of a Tertiary Health Institution in Port Harcourt, Nigeria. J Pharm Biol Sci. 2019;14:44-50.
13. Douglas SI, Robinson VK. Fungal pollution of indoor air of some health facilities in Rivers State. Int J Trop Dis Health. 2018; 32(2):1-7.
14. Amadi EN, Kiin-Kabari DB, Kpormon LB, Robinson VKK. Microbial flora and nutritional composition of adult Palm – Wine Beetle (*Rhychophorus phoenicus*). Int J Curr Microbiol Appl Sci. 2014;3(11): 189-92.
15. Prescott LM, Harley J, klein DA. Microbiology 8th.ed. New York: McGraw-Hill. 2011;809-11.
16. Sarah K, Catriona H, Helen A, David E. Descriptions of medical fungi. 3rd Ed; 2016.
17. American Public Health Association. 23rd ed. APHA. Washington, DC: APHA; 2012. Standard methods for the examination of water and wastewater.
18. Abbasi T, Abbasi SA. Sources of pollution in rooftop rainwater harvesting systems and their control. Environ Sci Technol. 2011;41(23):2097-167.
19. Brodie EL, DeSantis TZ, Parker JP, Zubietta IX, Piceno YM, Andersen GL. Urban aerosols harbor diverse and dynamic bacterial populations. Proc Natl Acad Sci U S A. 2007;104(1):299-304.
20. Bartoszek L, Miąsik M, Koszelnik P. Trophic degradation predispositions and intensity in a high-flow, silted reservoir. *Peer-reviewed* Journal. PeerJ. 2018;8: e9374.
21. Ewelike NC, Ogbonnaya PC, Ekwebelem VC, Onyema JU, Chuku PE, Anoka EO. Microbiological quality of harvested rainwater used for drinking and domestic purposes in Southeastern Nigeria. Singapore J Sci Res. 2020;10:47-51.
22. Wemedo SA, Robinson VK. Evaluation of indoor air for bacteria organisms and their antimicrobial susceptibility profiles in a government health institution. Adv Microbiol. 2018;11(3):1-7.
23. Agwaranze DI, Ogodo AC, Ezeonu ES, Brow C, Nwaneri CB, Husie LM. Microbiological assessment of indoor and outdoor air quality in a general hospital in North-East Nigeria. Res J Microbiol. 2020;15:9-14.
24. Jongman M, Korsten L. Microbial quality and suitability of roof-harvested rainwater in rural villages for crop irrigation and domestic use. J Water Health. 2016;14(6): 961-71.
25. Ekhaise FO, Ogboghodo BI. Microbiological indoor and outdoor air quality of two major hospitals in Benin City, Nigeria. Sierra Leone J Biomed Res. 2011;3:169-74.

26. Ekhaise FO, Ighosewe OU, Ajakpori OD. Hospital indoor airborne microflora in private and government owned hospitals in Benin City, Nigeria. World J Med Sci. 2008;3:34-8.
27. Hageskal G, Lima N, Skaar I. The study of fungi in drinking water. Mycol Res. 2009;113(2):165-72.
28. Babič MN, Gunde-Cimerman N, Vargha M. Fungal contaminants in drinking water regulation? a tale of ecology, exposure, purification and clinical relevance. Int J Environ Res Public Health. 2017;14(6):636.
29. Bomala K, Saramanda G, Reddy B. T and Jyothi K. Int J Curr Res. Microbiological indoor and outdoor air quality of selected places in Visakhapatnam city, India. 2016;8(04):29059-62.
30. Zdeb M, Zamorska J, Papciak D, Skwarczyńska-Wojsa A. Investigation of microbiological quality changes of roof-harvested rainwater stored in the tanks. Resources. 2021;10(10):103.
31. Despins C, Farahbakhsh K, Leidl C. Assessment of rainwater quality from rainwater harvesting systemsin Ontario, Canada. J Water Supply Res Technol Aqua. 2009;58(2):117-34.
32. Nigeria standard for drinking water quality. Niger Ind Stand Approve Stand Organ Niger Gov Counc. 2008;20(13):15-9.



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