Microbiological Assessment of Rain Water and Air Quality of Some Areas in Port Harcourt

Abstract

Microbiological quality of Rain water and air of market, hospital and residential area was evaluated. Roof harvested and direct rain water samples were collected from the three aforementioned locations. The air quality was investigated using the plate sedimentation method. Culture-based techniques were used for 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 rain water was $1.2\pm7.1x10^6$ to $6.1\pm1.1\ x10^6$ cfu/ml, total coliform count (TCC) ranged between $8.0\pm0.00\ x10^3$ to $30.5\ \pm2.1\ x10^3$ cfu/ml, faecal coliform counts (FCC) ranged between $1.0\pm0.00\times10^3$ to $6.0\pm0.00\times10^3$ cfu/ml, fungal counts ranged from $2.0\pm0.00x10^3$ to $11.0\pm0.00x10^3$ 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, $0.01\pm0.00$to 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 ($P\leq 0.05$) in the total heterotrophic bacterial load of the roof harvested rain water and the direct rain water of the residential area, roof harvested and direct rain water of the hospital. Statistically, there was a significant difference ($P\leq 0.05$) in the total coliform counts of the water with the coliform counts of the roof harvested rain water 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 rain water 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: rain water, air quality, microoganisms

Introduction

Harvested rainwater (HRW) has been considered an effective alternative water source for drinking and various non-potable uses in a number of countries throughout the world. The most significant issue in relation to using untreated HRW for drinking or other potable uses, however, is the potential public health risks associated with microbial pathogens (Muhammad and Mooyoung, 2008). 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 (TCEQ, 2007). 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 (Sazakli *et al.*, 2007). 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 potential source of dissolved ions, alkalinity and trace metals (Ayenimo *et al.*, 2006). Diseases caused through consumption of contaminated water, and poor hygiene practices are the

leading cause of death among children worldwide, after respiratory diseases (WHO, 2012). Experience of water shortage in developing countries and communities has made residents to resort to sourcing potable water from harvested rainwater. Roof harvested rainwater is used in areas having significant rainfall but lacking conventional water supply system, and where fresh surface water or ground water is lacking (UNEP, 1983). 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 (Karim, 2010).

Adeniyi *et al*. (2005) 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 percent 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 (NHMRC, 2011), 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 judges 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 (NHMRC, 2004). Hence there is need for constant investigation and monitoring of quality of water consumed by communities in developing countries. It would prove useful in management, control and investigation of pollution cases, classification of water resources, and collection of baseline data, water quality surveillance and forecasting 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 contaminates the air due industrialization, high density of human population and their activities in urban or rural areas (Robinson & Wemedo, 2019). Rainwater could mix with microorganisms already in the atmosphere. Thus, contamination of rain water could be from the atmosphere before it touches the roof tops or ground. There is dearth of information in the microorganisms associated with rain water in Rivers State. Thus, this study is aimed at investigating the microbiological quality of rain water and air quality in major areas in Port Harcourt.

Materials and Methods

Description of study area

This study was carried out at the department of microbiology, River State University. Rain water 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

apartment 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 with 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, other food products like raw meat were also sold. Beside 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 were 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, 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

Sample collection

Water Sample

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

Air Sampling

The direct plate method was used in sampling the atmospheric air (Robinson & Wemedo, 2019). 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 (Douglas and Robinson (2018, 2019).

Enumeration and Isolation of Aeroflora

Freshly prepared nutrient agar (NA), and 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 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 freshly prepared nutrient medium until it was ascertained that there were no contaminants (Amadi *et al.*, 2014).

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 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 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.

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

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□ (Prescott *at al*., 2011). 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 24hours 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.

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 (Prescott *at al*., 2011). Fungal colonies that appeared on the SDA plate after incubation was counted and subcultured on freshly prepared SDA plates.

Characterization and Identification of Bacterial Isolates

Cultural methods of characterizations 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).

Characterization and Identification of Fungal Isolates (Macroscopy and Microscopy)

Isolates were identified using their morphological features such as colony color, shape, texture and size of 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 (Sarah *et al.*, 2016).

Physicochemical Parameters

The physicochemical parameters determined include, pH, temperature, electrical conductivity, total dissolved solid, turbidity, total suspended solid and total hydrocarbon content. The APHA method was used in determining the physicochemical parameters (APHA, 2012)

Results

Microbial Counts.

Results of the microbiological counts of the roof harvested water and direct rain water are presented in Table 2. Results showed that the total heterotrophic bacterial load of direct rain water from hospital, roof harvested rain water from hospital, direct rain water from the market, roof harvested rain water from the market, direct rain water from residential area and roof harvested rain water 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 ($P\leq 0.05$) in the total heterotrophic bacterial load of the roof harvested rain water and the direct rain water of the residential area, roof harvested and direct rain water of the hospital. Results also showed that the total coliform load of direct rain water from hospital, roof harvested rain water from hospital, direct rain water from the market, roof harvested rain water from the market, direct rain water from residential area and roof harvested rain water 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 ($P\leq 0.05$) in the total coliform counts of the water with the coliform counts of the roof harvested rain water of the market being significantly higher than the coliform counts recorded in other locations. More so, the coliform load of the roof harvested rain water of the residential area and direct

rain water from the market despite showing no significant difference were significantly higher than coliform counts of roof and direct rain water 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 rain water from hospital, roof harvested rain water from hospital, direct rain water from the market, roof harvested rain water from the market, direct rain water from residential area and roof harvested rain water 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 rain water in the market and residential areas were significantly higher ($P \leq 0.05$) 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 is 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 was 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.

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 = rain water 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 same superscript (alphabet) show no significant difference (P>0.05) down the column

The results of the cultural and biochemical characteristics of the bacteria isolated from the samples is 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 rain water and air samples. The results of the percentage occurrence of bacterial isolates associated with the rain water samples are presented in Fig. 1. While the results of the percentage distribution of the bacterial isolates across the water samples is 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 rain water from hospital and direct rain water from residential area).

The results of the percentage occurrence of the bacterial isolates associated with the outdoor air is presented in Fig. 3. Results of the percentage distribution of the bacterial isolates from the rain water of the various locations is 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 market and residential area. *Pseudomonas* sp was only isolated from hospital and market area.

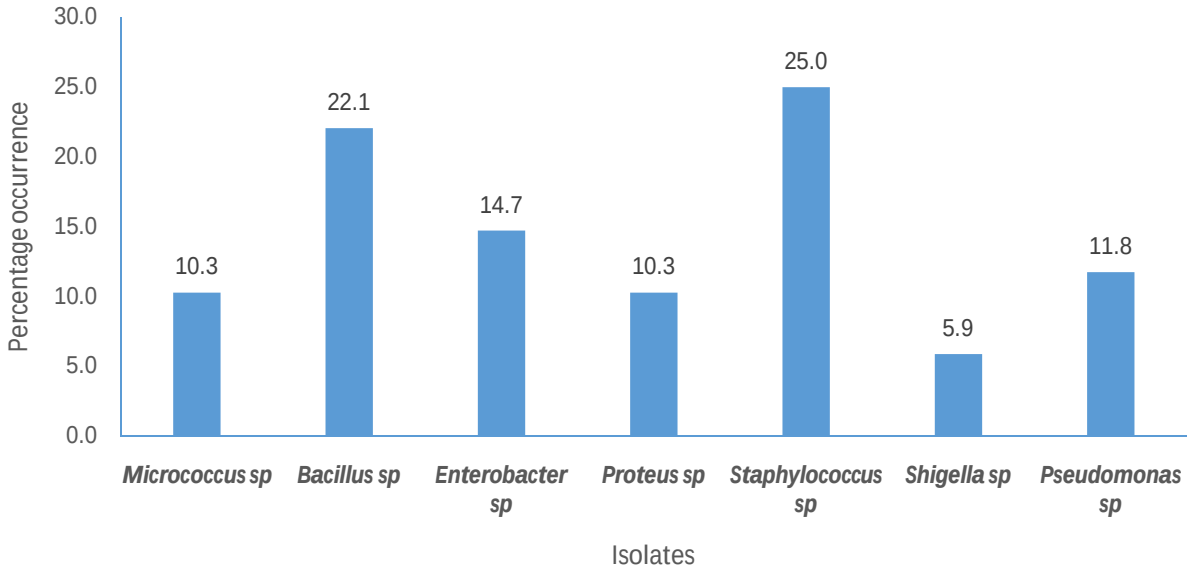

Fig 1 Percentage occurrence of Bacterial Isolates in the Water

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

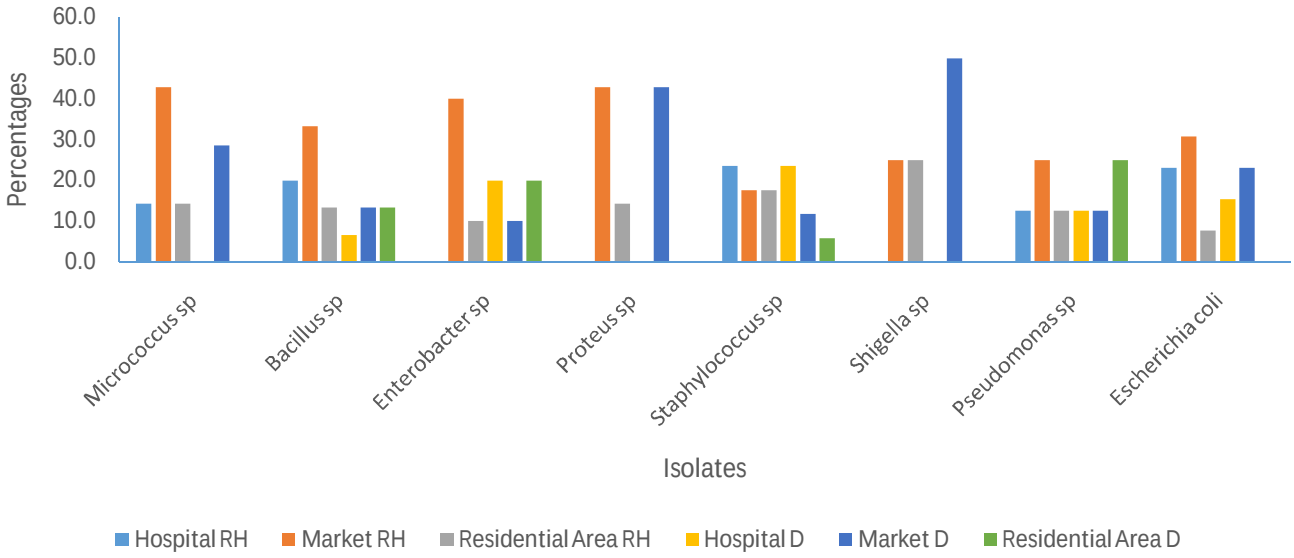

Fig 2 Percentage Abundance of Bacterial Isolates in the Water Samples across the locations

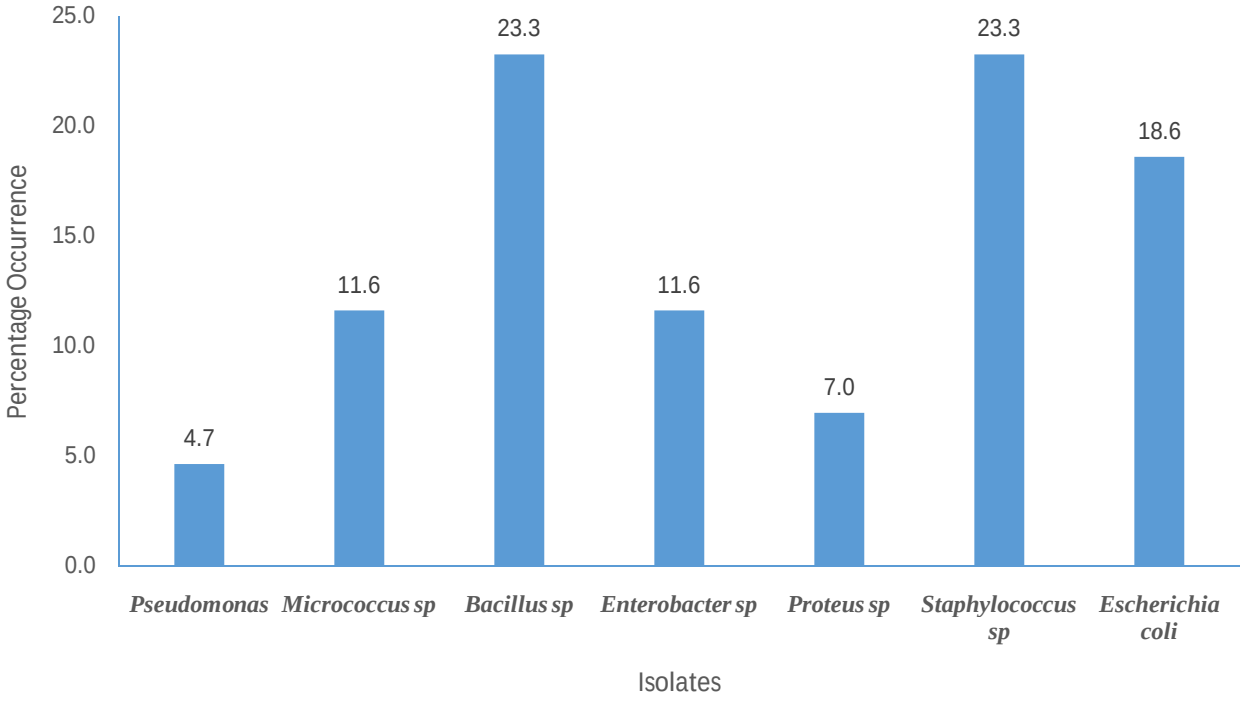

Fig 3 Percentage occurrence of Bacterial Isolates in the Air

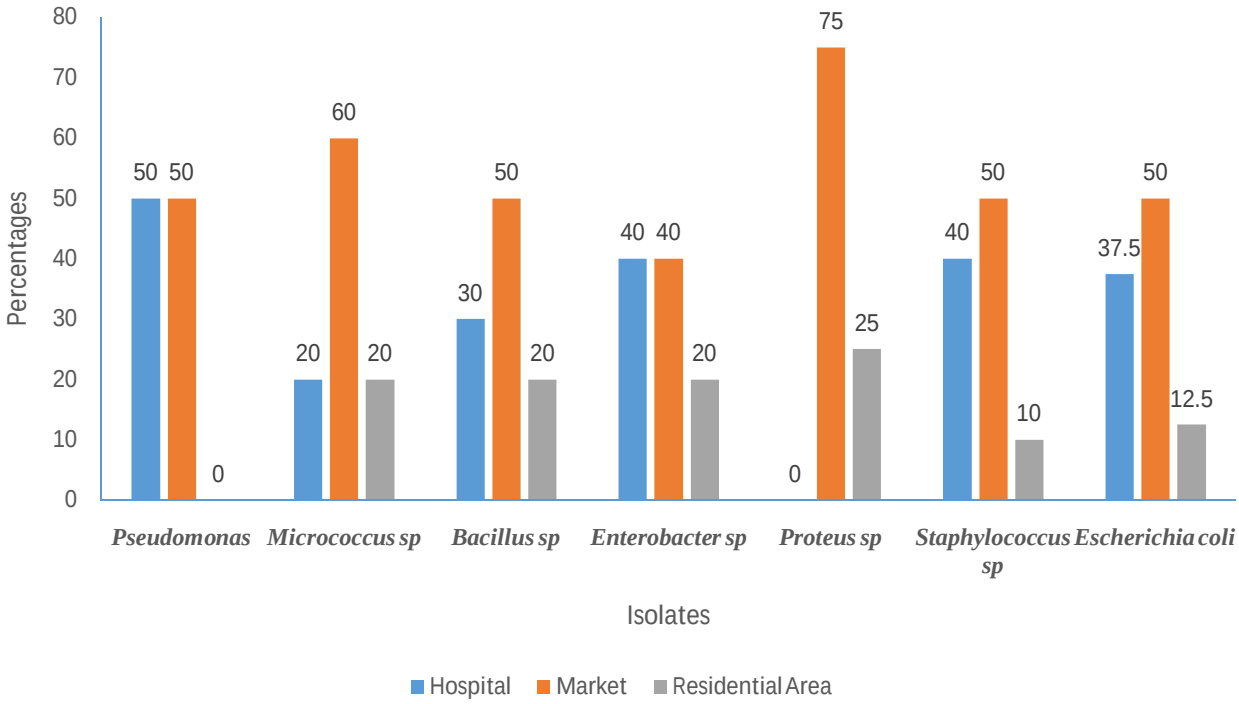

Fig 4 Percentage Abundance of Bacterial Isolates in the Air across the locations

The results of the 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 rain water samples are presented in Table 6. Results showed that *A. niger* was the only fungal isolates that occurred in all samples (i.e., both roofs harvested and direct in the three locations) while *Penicillium* sp was isolated from roof harvested rain water in hospital, market and residential areas as well as in hospital and market of direct rain water. *Candida* sp was only isolated from market of roof harvested rain water while *Mucor* sp was isolated only from the market roof harvested and direct rain water. *Rhizopus* sp was isolated in all locations of the roof

harvested rain water but was not isolated from all the direct rain water from the various locations.

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

Physicochemical Parameters of Rain water

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 rain water 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 rain water was 7.16, 28.1, 7, 0.3, 198, 24 and 0.013 mg/ml, respectively.

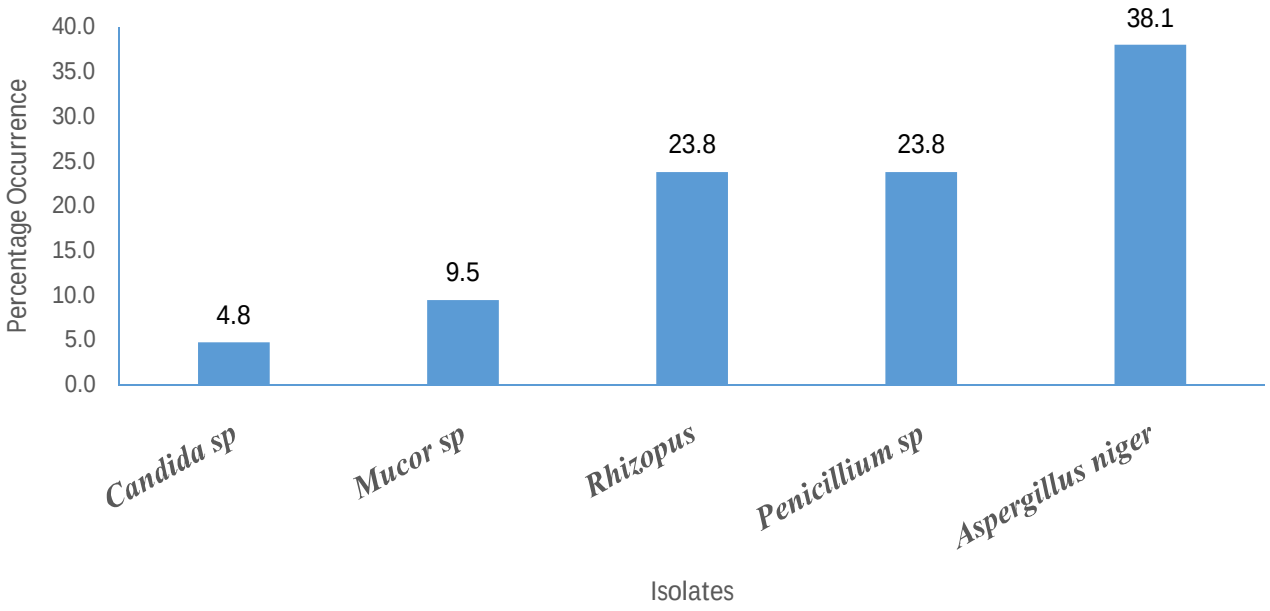

Fig 5. Percentage Occurrence of Fungal Isolates in the Water Samples

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 conidiosphore bearing conidia	*Aspergillus flavus*

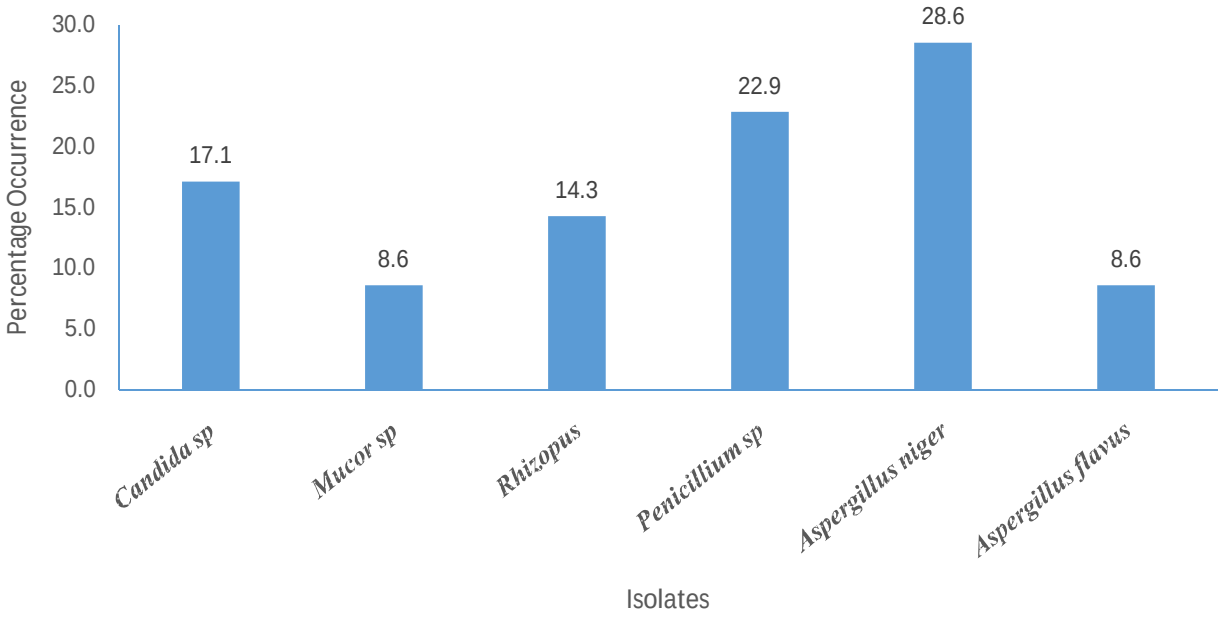

Fig 6 Percentage Occurrence of Fungal Isolates in the outdoor Air

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

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 Rain Water from study locations

Parameters	Direct Rain water	Roof harvested rain water
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

Discussion

The bacterial and fungal counts of the roof harvested water which was recorded to be higher than the direct rain water (water collected directly from the atmosphere) could be attributed to the contamination from particles or microorganisms attached to the roofs of the buildings. Unlike the direct rain water which had no contact with any known material. Comparatively, the roof

harvested rain water of the residential area had higher total heterotrophic bacterial load followed by the roof harvested rain water from the market while the roof harvested rain water 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 rain water and the direct rain water of the residential area, roof harvested and direct rain water of the hospital. The present study also showed that the bacterial and fungal load in the rain water (i.e., the roof harvested and direct) fluctuated and this could be attributed to many factors including the location of the area sampled, type of environmental condition as well as the abiotic factors in that location. According to Abbasi and Abbasi (2011), the quality of roof harvested rain water 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 dissemination of aerosols on roof tops as well as droplets from faecal sources that find its way into the atmosphere as a result of anthropogenic activities like sweeping and air currents. Brodie (2007) in a study reported that *E. coli* and other pathogens can enter roof harvested rain water 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 (Bartoszek *et al.,* 2018). Thus, this could have influenced the coliforms and other microorganisms in the water samples. The bacterial load in the rain water 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. Similar study conducted in South-Eastern Nigeria had reported high bacterial and coliform load in the range of 1.9×10^3 to 7.0×10^6 CFU/mL for total heterotrophic bacterial load and 1.0×10^2 to 8.0×10^3 CFU/mL for roof harvested rain water (Ewelike *et al.,* 2020). The WHO permissible limit for total heterotrophic bacterial load, faecal coliform and total coliform is given as 1.0×10^2 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 was 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 with heavy activities like 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 with persons coming for medical check-ups/treatment or consultations unlike the residential area which is rarely accessed by 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 (Wemedo and Robinson, 2018). 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 5.2×10^4 $CFUm^{-3}$ and 4.7×10^4 $CFUm^{-3}$ for bacteria and fungi reported by Agwaranze *et al.* (2020) of outdoor air around a hospital.

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 rain water from hospital and direct rain water from 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 rain water from market). The bacterial isolates in the rain water 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 rain water are in line with previous study (Ewelike *et al.,* 2020; Mosimanegape and Lise, 2016). *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 (Ewelike *et al.,* 2020).

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 market and residential area. *Pseudomonas* sp was only isolated from hospital and market area. The bacterial isolates in the outdoor air of the market, hospital and residential area have been reported in previous study. Agwaranze *et al.* (2020) isolated *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Bacillus* spp. and *Micrococcus* spp. Similar isolates have been reported by Ekhaise and Ogboghodo (2011), in their study on airborne microflora in the atmosphere of a hospital environment of university of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Moreover, previous report indicated that the presence of *Staphylococcus aureus* and *Escherichia coli*, in microbiological outdoor air quality of 2 major hospitals in Benin City, Nigeria. These microorganisms are known to be associated with nosocomial infections (Ekhaise and Ogboghodo, 2008). *Staphylococcus aureus* is known to cause infections of the skin, deeper tissue and organs as well as pneumonia (Agwaranze *et al.,* 2020). More so, the present study agreed with Agwaranze *et al.* (2020) who reported that *Staphylococcus* sp and *Bacillus* sp had the highest percentage occurrence among other bacterial isolates in the outdoor environment.

Fungal Isolates in Outdoor Water and Air Samples

The percentage occurrence of fungal isolates in the various rain water 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 isolates in the water

sample while *Candida* sp was the least fungal isolates in the water sample. The distribution of fungal isolates across the rain water showed that *A. niger* was the only fungal isolates that occurred in all samples (i.e., both roofs harvested and direct in the three locations) while *Penicillium* sp was isolated from roof harvested rain water in hospital, market and residential areas as well as in hospital and market of direct rain water. *Candida* sp was only isolated from market of roof harvested rain water while *Mucor* sp was isolated only from the market roof harvested and direct rain water. *Rhizopus* sp was isolated in all locations of the roof harvested rain water but was not isolated from all the direct rain water from the various locations. The presence of fungi in drinking water has been ignored especially since fungi unlike bacterial 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 (Gunhild *et al.,* 2009). The fungal isolates in the different water sample in the present study could illicit allergic reactions on the skin or may cause diseases especially to 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 a cold water has been reported (Gunhild *et al.,* 2009). 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 (Babič, *et al.,* 2017).

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.* (2016) in outdoor environment of school, motor park and college.

Physicochemical Parameters of Rain water

The mean physicochemical parameters of the direct rain and roof harvested water showed that the pH of the direct rain water and the roof harvested rain water 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. Zdeb *et al.* (2021) in their study reported pH value 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.* (2009), the intensity of precipitation causes an increase in the pH values of rain water. 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 rain water and roof harvested rain water was similar. Temperature is an essential parameter that determines the microbiological water quality since it determines the critical environmental factors that influences the taxonomic

composition of microorganisms found in rain water (Zdeb *et al.*, 2021). Interestingly, the total dissolved solids of the direct rain water was higher than the those obtained for the roof harvested rain water although the total suspended solids in the direct rain water was lower than values obtained for the roof harvested rain water. The total hydrocarbon content of the direct rain water was higher than the values recorded for the roof harvested rain water. Furthermore, the electrical conductivity and salinity of the direct rain water were higher than values recorded for the roof harvested rain water. 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 (2008) and WHO (2012). The high THC values in the direct rain water could be attributed to the presence of presence of sooth or other organic material in the atmosphere which might have mixed with the rain water.

Conclusion

This study concluded that the microbiological quality of direct rain water and roof harvested rain water collected from market, hospital and residential area is very poor and do 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 in the direct and roof harvested rain water clearly showed a potential risk of contamination if it is consumed directly or used in cleaning purposes. Furthermore, the microbial counts in the outdoor air are low and even though there is no acceptable 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 area, thus leading to poor indoor air quality. Treatment of rain water before drinking is highly recommended to avoid risk of exposure to microorganisms that could pose serious public health challenge.

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