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Assessment of Physicochemical and Microbiological Characteristics of Rainwater Harvested from Different Rooftops in Owerri, Imo State, Nigeria

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Abstract

Assessment of physicochemical and microbiological characteristics of rainwater harvested from different rooftops in Owerri, Imo State was carried out using standard methods. Rainwater samples collected from different rooftops were analyzed for quality and compared to the standard for World Health Organization (WHO) and the National Standard for Drinking Water Quality (NSDWQ). Rainwater was also collected directly from the rainfall and analyzed. The result of the physicochemical parameters showed that only the rainwater harvested from asbestos rooftop had the pH value that falls within WHO standard and NSDWQ. The turbidity value of rainwater harvested from the thatched rooftop was higher than the WHO standard and NSDWQ. Organisms isolated from the rainwater samples harvested from the rooftops include *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsilla pneumonia*, *Entrobacter* sp., *Proteus mirabilis*, *Aspergillus* sp., *Penicillum* sp., and *Vibro* sp. However, after molecular studies, *K. pneumoniae, P. aeroginosa*, *E. coli,* and *P. mirabilis* were the four bacterial species identified. The rainwater samples had high levels of microbial loads against WHO standard and NSDWQ. Rainwater sample from thatched rooftop had the highest total heterotrophic count (THC) but all the studied rainwater samples had THC higher than WHO standard and NSDWQ. The rainwater harvested directly from rainfall had low values for the physicochemical parameters and microbial loads considered in this study, when compared

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to the rainwater samples harvested from the rooftops. The bacterial and fungal isolates of rainwater samples from the rooftops have been associated with different diseases. There is a need to treat harvested rainwater properly before domestic or potable use. This study has assessed the physicochemical and microbiological characteristics of rainwater harvested from different rooftops in Owerri, Imo State, Nigeria.

Introduction

There is a recent growing public concern in the quality of water from natural sources due to environmental pollution. The threat posed by environmental pollution on surface and ground water sources has elevated the search for other reliable sources of domestic water supply.[1] Rainfall forms a substantial source of water to the living world after surface and groundwater sources.[2] The harvest of rainwater from rainfall is seasonal and the role it plays as a natural source of water cannot be overemphasised.[3] Rainwater contains mixed electrolytes with varying amounts of major and minor ions.[4-5] It has been reported that sodium, nitrate, ammonia, nitrite, nitrogen, potassium, calcium, magnesium, bicarbonate, sulfate, and chloride are among the major ions while iodine, bromine, boron, iron, alumina, and silica are among the minor ions found in rainwater.[6-7] Oceans, fresh water, landmasses, saline lakes, volcanic eruptions and industries have been implicated as the sources of rainwater constituents.[8] Sanusi *et al.*,[9] and Al-Momani[10] noted that the local pollution sources contribute to the chemical characteristics of rainwater in the urban areas while the level of impact of anthropogenic activity and natural sources reveal the chemical characteristics of rainwater in the rural areas. Rainwater is considered a drinking source of water in the continents of Africa, Asia, and Australia.[11] However, the air pollution inherent from increasing population, urbanization, and industrial growth impact on rainwater and reduce its potable nature.[12] Helmreich and Horn,[13] Rajni and Rajasekhar,[14] and Zdeb *et al.*,[15] described the entry of microbial pathogens such as protozoa, viruses, and bacteria as the greatest contamination of rainwater. Rajni and Rajasekhar[14] and Zdeb *et al.*[15] further described organisms such as thermotolerant strains of coliforms, *E. coli, Vibrio*, *Salmonella, Cryptosporidium, Giardia*, *Campylobacter*, *Shigella*, and *Pseudomonas* as indicator organisms for rainwater contamination.

According to Abdullah *et al.,*[6] the harvesting of rainwater (HRW) from rainfall has been in practice in many regions of the world that experience dryness since 4.500 B.C. With the increasing problem of inadequate supply of pipe borne water, and scarcity of domestic water sources, there is an increasing reliance on HRW to meet the human demands for water. According to Helmreich and Horn,[13] HRW involves collecting and storing rainwater from rain that falls on a roof surface for later use. Nizam *et al.*,[16] further noted that the system for harvesting of rainwater constitutes the method of rainwater collection from constructed areas and rooftops, which can be used for household, agricultural, and commercial purpose. HRW provides the avenue to manage the shortage of water in most developing and underdeveloped countries.[17] HRW involves catchment area (Catchment surfaces), conveying system and collection devices.[18-19] The use of house rooftops as catchment surfaces for HRW has been globally accepted because of their less perceived contamination effects on harvested rainwater compared to other catchments used for HRW.[20-21] However, it has been reported that different materials used in the production of different house rooftops impact differently on harvested rainwater.[21] The roof materials degrade with time and contribute both degraded matters and dissolved chemicals to the rooftop runoff. Washed dirt blown by the wind, leaves, insects, waste products from birds and feaces from animals can contaminate rainwater harvested from rooftops and ultimately pose a risk to human health.[21] Harvested rainwater is stored using different vessels or containers. Duru *et al.*[22] reported the effect of different storage vessels on water quality.

In 2006, Evan and his team reported that over one billion people would have no access to safe water and one billion people would be associated with death from water related diarrhea disease yearly.[23] World Bank reported that an estimate of 12% of the global population have access to the larger chuck (80%) of water supplies available, while about one billion, one hundred million people of the world are either left with no access to water supplies or inadequate water supplies.[24] It has been reported that shortage of water may affect every two out of three individuals in 2025.[25] The report also noted that young people in developing countries could be at risk due to the water shortage when compared to their counterparts in the developed world.[25] According to World Bank, an individual needs between 20 to 50 litres of safe water for hygiene and daily metabolic activities. Ojeh and Semaka[24] noted that safe water of about 20 litres is needed by a person to meet up with the daily hygiene, metabolic and domestic needs. The national water requirements in Nigeria are twenty-three litres/person/day for urban area and sixty litres/person/day for rural area. Access to potable water, and safe water for domestic household activities reveals the status of health of a nation`s population as well as the country`s capacity.[12] Due to inadequate pipe-borne water supply, most communities in Nigeria virtually depend on water from natural sources.[17,26] Most households in Nigerian communities are faced with problem of availability of adequate and clean water.[19] Okovido *et al.*[17] noted that many Nigerian rural communities still trek long distances in seach of water for daily household actitivites and other domestic uses. Kabir[27] report on water, sanitation, and hygiene noted that only 18 million Nigerians which represents less than 10 percent of the country`s population have access to pipe-borne water supply. A large proportion of people in the so-called big cities of Nigeria have no access to drinking water and these people resort to any available source of water. It has been reported that in the absence of pipe-borne water supply, boreholes, and protected wells, many Nigerians living in urban and rural communities resort to other available sources of water.[28] HRW for immediate and later usage is synonymous with most Nigerian communities in the rainy season.[26] With the perceived perception that rainwater is pure, most communities in Nigeria even subject water collected from rainfall to drinking without treatment.Obviously, there is a fundamental need to search for water that poses no risk to human health and is suitable for drinking, food preparation, and personal hygiene. Despite the existing research studies on rainwater harvested from rooftops, there are still gaps to bridge in the research involving harvested rainwater from roof surfaces. Based on the economic importance of rainwater to humans and developing nations, there is the need for a detailed study on the quality of rainwater harvested from rooftops and the need to narrow the gaps created by previous studies.

The present study assessed the physicochemical and microbiological characteristics of rainwater harvested from different rooftops in Owerri.

Materials and Methods

Study Area

This study was conducted in Owerri. Owerri is the capital territory of Imo State. Owerri capital territory is within coordinates 7° 1' 33.0708'' E and 5° 28' 34.7160'' N. Two rivers; Otamiri river located in the east and Nwaorie river found in the south transverse Owerri. Owerri is located in one of the most densely populated areas of Nigeria. It is a great city of social life, hospitality, and religion,

Fig. 1: Map of Imo State showing Owerri and few other towns.

Selection of Different Rooftops

Houses with the required rooftops for the present study were randomly selected and the consents of the owners of those houses were obtained. The different rooftops considered were corrugated iron sheet roof, thatched roof, aluminum roof, asbestos roof, and stone coated roof.

Collection of Rainwater Samples

The method described by Achadu *et al.*,[29] and Emerole *et al.*[30] was employed in the collection of rainwater samples. The sampling was carried out at the peak of the rainy season (June-September 2021), and in triplicates for each of the rooftops considered. Rainwater samples were collected after allowing the raindrops to wash the rooftops for 20 mins. A stool of 2 ft high with the top swabbed with absolute ethanol was mounted beneath the rooftop and sterile containers were placed on it for rainwater from the rooftop to drip in. The rainwater collected in the containers was transferred into a 1 L sterile sample bottle and then ttaken to the laboratory for analysis in a pack of ice. This was done for all the rooftops of interest. A sterile basin placed on a 2 ft high stool left in the open without any interference from the environment was used for direct rain drops. This rainwater sample served as the control for this study. Care was taken to avoid accidental contamination of the samples during sampling. Sample containers were thoroughly washed using a clean soapy solution and soaked in bleach overnight prior to sample collection followed by proper rinsing with sterile water to remove every form of microbial contamination.

Physicochemical Analysis of the Samples

The standard methods as described by American Public Health Associaiton (APHA)[31] were used for the determination of physicochemical parameters of the rainwater samples. The parameters analyzed were appearance, odour, total suspended solids (TSS), colour, pH, total dissolved solids (TDS), turbidity, temperature, conductivity, phosphate (PO_4-), sulpahte (SO_2-), nitrate (NO_3-), and chloride (Cl-).

Metal Analysis of the Samples

Metals such as calcium, iron, zinc, copper, aluminum, potassium, and manganese were analyzed in the harvested rainwater samples using the Atomic Absorption Spectrophotometer (AAS) (Bulk Scientific AAS JEWAY 6310).

Characterization and Identification of Isolates

The methods described by Vandepitte*et al.*,[32] and Cheesbrough[33] were used for bacterial isolates. Bacterial isolates were identified using morphological, cellular characteristics, mobility tests, gram staining, and biochemical properties. Oxidase test, coagulase test, catalase test, methyl red test, urease test, citrate utilization test, indole test, Voges-prokauer test, glucose tests, H2S production, sucrose, and lactose utilization test were among the biochemical tests used to ascertain the biochemical properties of the isolates. Microscopic and morphological identification methods were used for the fungal isolates.

Microbiological Analysis of the Samples

The samples were analyzed using the membrane filtration method. Analyses were carried out for total heterotrophic count (THC), total coliform count (TCC), total viable count (TVC), and total fungal count (TFC). THC, TCC, TVC, and TFC were estimated with the methods as described by Onyeagba.[34]

Antibacterial Susceptibility Test on Bacteria Isolates

The disc diffusion method as described by Jorgensen and Turnidge[35] was adopted for the antibacterial susceptibility test. The preparation of McFarland solution and the bacteria inoculum standardization were carried out following the methods described by Uzoigwe and Agwa[36]

Molecular Studies of the Bacterial Isolates

The methods of DNA extraction, quantification of DNA, amplification (16S rRNA), and sequencing were used for the molecular studies of the bacterial isolates. The obtained sequences were subjected to phylogenetic analysis for evolutionary distances. The boiling method was used for DNA extraction by following the procedure as stated. Five millitre of broth culture of bacterial isolates (in Luria Bertani (LB)), which has stayed over night was taken and spun for 3 minutes at 14000 rpm. The cells were kept at a temperature of 95° for 20 minutes, and suspended in normal saline of 500 µl. The suspension obtained was cooled using ice and then spun at 14000 rpm for 3 minutes after which the DNA of the supernatant was trasfered into a micro-centrifuge tube of 1.5 ml and was stored for downstream reactions at the temperature of 20°.

The DNA (genomic DNA) extracted got quantified with 1000 spectrophotometer (Nanodrop). The icon of the Nanodrop was double clicked on to launche the equipment software which was initialized with sterile distilled water (2 µl) and normal saline was then used as blank. The upper pedestal of the Nanodrop was lowered to make contact with 2 ul of the extracted DNA found on the lower pedestal and a click on the button measured the concentration of the DNA.The amplification of 16S rRNA region was done using the appropriate primers. 1% of agrose gel at 130V which lasted for 30 minutes and a blue visualised light tramsillumiator were used to rsolve the product. The sequencing was carried out on a 3510 ABI (Inquba Biotechnological) using the Big Dye terminator kit. The bioinformation algorithm of phylogenetic analysis was deployed to edit the sequences obtained. The sequences obtained were compared with similar ones found in the database of the National Center for Biotechnology Information (NCBI) with the help of BLASTIN while MAFFI was use dfir their their aligment . The methd of Neigbor-Joining[37] was used to infer the evolutionary history and were computed with the methd of Jukes-Cantor method.[39]

E.coli, Pseudomonas, and Enterobacter showing multiple drug resistance were selected and screened using plasmid profile to determine how they gain resistance to the antimicrobial drugs used.

Table 1: Physicochemical analysis of rainwater harvested from different rooftops.

Rooftops	Appear.	Odour	Colour	pH	Temp.	TSS	TDS	Cond.	Turb.	PO_{43}-	SO_{42}-	NO_3-	Cl-
Corrugated iron rooftop	Clear	Odourless	10.25	6.16	28.80	8.0	5.36	70.51	3.2	0.9	16.65	7.23	25.16
Asbestos rooftop	Clear	Odourless	11.42	7.16	28.00	8.5	14.8	29.20	3.8	1.5	11.23	4.28	23.28
Stone-coated rooftop	Clear	Odourless	12.15	5.47	28.6	6.0	6.71	14.29	3.8	1.7	10.9	6.32	28.62
Aluminum rooftop	Clear	Odourless	11.32	5.33	29.2	4.0	3.83	47.00	2.8	0.8	14.56	4.98	30.42
Thatched rooftop	Clear	Odourless	12.85	5.20	27.8	16	12.25	18.2	6.2	0.6	14.25	3.69	19.25
Open environment	Clear	Odourless	10	5.26	28.8	5.0	6.14	18.31	3.0	0.2	14.62	5.20	17.70
WHO standard			15	6.5-8.5	20-30	50	250	1000	5.0	5	250	40	250
NSDWQ			15	6.5-8.5	20-30	50	100	1000	5.0	5	100	50	250

Appear= Appearance; Colour (PTCO); TSS=Total suspended solis (mg/L TDS =Total dissolved solids ((mg/L); Condi.=Conductivity (µs/cm); Turb.=turbidity (NTU); Temp.= Temperature (°C); PO_4- (mg/L), SO_4- (mg/L), NO_4 (mg/dl); Cl- (mg/L); WHO=World Health Organization; and NSDWQ= Nigerian Standard for Drinking Water Quality.

Results and Discussion

Physicochemical analysis of rainwater harvested from different rooftops (Table 1) showed that all the water samples collected from the rooftops were clear in appearance and odourless. Colour in water becomes important due to the relationship it shares with the support of the growth of aquatic plants. It comes from iron complexes, bacterial action on dissolved manganese particles, as well as organic and humic materials. The rainwater samples had varying colour values that ranged from 10.00 to 12.85 PtCo. Rainwater harvested from the open environment which served as the experimental control had the lowest colour of 10.00 Pt-Co while rainwater harvested from the thatched rooftop had the highest colour impact of 12.85 Pt-Co. All the rainwater samples had colour values lower than the WHO[40] standard and NSDWQ[41] for drinking water. The observed varying colours of the rainwater samples in this study may have come from the different rooftops used. Rainwater with low pH could be due to polluted air as a result of land combustion and industries.[5] The oxides of carbon, sulphur, and nitrogen, form the constituent pollutants of polluted air. They untimately result in acid rain. Alvarez *et al.*,[42] and Imo *et al.*,[3] noted that dissolved carbon dioxide controls the pH of natural precipitation. This is made possible due to the interaction of carbon dioxide and droplets of water. The pH of rainwater changes on addition of acid or alkaline components. pH of harvested rainwater samples of the present study ranged from 5.20 to 7.16. Rainwater harvested from thatched rooftop had the lowest pH value of 5.20, while the one harvested from asbestos rooftop had the highest pH value of 7.16. Okpoebo *et al.*,[43] affirmed the slightly acidic nature of rainwater. Only the rainwater harvested from the asbestos rooftop had the pH value within the WHO standard and NSDWQ limit of 6.5-8.5. The rooftops may have impacted on the pH of the harvested rainwater samples. Due to the direct relationship of pH to other chemical constituents of water, it could impact on human health.[40,44] Akubugwo and Duru[45] reported the implication of consuming water of alkaline or acid pH. The pH range of the harvested rainwater samples in the present study is lower than the pH range (4.78–5.85) recognized in Potatian City where rainwater is known to be corrosive because it has aggressive CO_2[46] In a related study, Adeyeye *et al.*,[47] reported a pH range of 8.039.17 for rainwater samples harvested from rooftops of painted pan, aluminum, galvanized zinc, and coated pan. Ojo[44] observed a pH range of 6.13-6.25 for rainwater harvested from corrugated iron roof, aluminum roof, and asbestos roof in the Federal University of Technology Akure (FUTA). Factors of the air affect the temperature of raindrops. The harvested rainwater samples had temperature values that ranged from 27.80 to 29.20°C. The rainwater sample harvested from the thatched rooftop had the lowest temperature value while the rainwater harvested from the aluminum rooftop had the highest temperature. The observed difference in temperature of the harvested rainwater samples may have come from the different rooftops due to the rate at which they absorb or radiate heat from the sunlight. The observed temperature values were within the WHO[40] standard and NSDWQ[41] limit of 20-30°C. The total suspended solids (TSS) of the present study ranged from 4.0 to 16.0 mg/L. Rainwater collected from the aluminum rooftop had the lowest TSS value while the one harvested from the thatched rooftop had the highest TSS. Total dissolved solids (TDS) constitute all organic and inorganic matter as well as salts present in water. TDS constitutes cations such as magnesium, calcium, and potassium; and anions such as nitrate, chloride sulfate, carbonate, and bicarbonate.[48] Some of these ions are toxic whereas some are beneficial to life.[49] Dissolved solids present in water are among the causes of sediments and turbidity in drinking water and can result in disease conditions when left unfiltered.[49] Calcium and magnesium constituents of TDS form the hardness of wate.[49] TDS of rainwater harvested from rooftops were between 3.83 and 14.80 mg/L. TDS of rainwater harvested from the asbestos rooftop was the highest followed by that of the thatched rooftop while the TDS of rainwater harvested from the aluminum rooftop was the lowest. Conductivity shares a relationship with dissolved solids in water. The conductivity values of the rainwater samples ranged from 14.29 to70.51 µs/cm. Rainwater harvested from stone-coated rooftop had the lowest conductivity while that of corrugated iron rooftop had the highest conductivity value. The conductivity values of harvested rainwater samples were lower than WHO[40] and NSDWQ[41] limits of 1000 mg/l Turbidity in rainwater could be a result of organic or inorganic substances. High turbidity levels in water could be an indication of physical, chemical, or biological pollution. The turbidity of the harvested rainwater ranged from 2.8 to 6.2 NTU. Turbidity of rainwater harvested from thatched rooftop was the highest while the rainwater harvested from aluminum rooftop had the lowest turbidity value. All the observed turbidity values were lower than WHO[40] standard and NSDWQ[41] limit of 5 NTU. Phosphate comes into the environment in diverse forms but it usually comes in the form of anion PO_4^{3-} in natural waters. PO_4^{3-} in the harvested rainwater samples ranged from 0.2 to 1.7 mg/L. Rainwater harvested from open environment had the lowest while the rainwater harvested from stone-coated rooftop had the highest. Phosphate is not toxic to humans unless present at a very high level. At such a level, digestive problems may set in. SO_4^{2-} is known in the open atmosphere for its role in the formation of acidic rain. SO_4^{2-} content of rainwater harvested from stone-coated rooftop was the lowest while rainwater harvested from corrugated iron rooftop had the highest SO_4^{2-} content. NO_3^- ranged from 3.69 to 7/23 mg/L. Harvested rainwater sample from thatched rooftop had the lowest NO_3^- while the one from corrugated iron rooftop produced the highest NO_3^-. The harvested rainwater samples had Cl- that ranged from 17.70 to 30.42 mg/L. Rainwater harvested from open environment had the lowest Cl- while that of aluminum rooftop had the highest Cl- content. The observed values of PO_4^{3-}, SO_4^{2-}, NO_3^-, and Cl- were lower than WHO[40] standard and NSDWQ[41] limit.

Table 2: Metals analyzed in rainwater samples (mg/L) from different rooftops.

Rooftops	Potassium	Calcium	Magnesium	Zinc	Aluminum	Iron
Corrugated iron rooftop	3.89	24.25	18.26	2.65	0.09	0.08
Asbestos	5.20	27.25	16.42	0.18	0.03	0.06
Stone-coated rooftop	6.25	28.20	14.58	0.16	0.07	0.08
Aluminum rooftop	4.63	22.68	11.62	0.75	0.21	0.06
Thatched rooftop	8.26	32.40	10.68	0.68	0.05	0.04
Open environment	3.26	20.20	12.20	0.25	0.01	0.02
WHO standard	12	75	50	5	0.2	0.3
NSDWQ	-	75	-	5	0.2	0.3

- = Not available.

The chemistry of water ingested in the human body has been ascribed as being very important in disease conditions of the body.[43] Metallic rooftops and fittings are among the sources of metal contamination to harvested rainwater. Rainwater has the capacity to dissolve metals as well other impurities of the catchment material or those of the storage vessel.[43] Metals analyzed in the harvested rainwater samples from different rooftops, as presented in Table 2 showed that potassium in rainwater harvested from different rooftops ranged from 3.26 to 6.25 mg/L. The rainwater sample from an open environment had the lowest potassium, while the one from the stone-coated rooftop had the highest potassium content. Calcium in the harvested rainwater sample ranged from 20.20 to 32.40 mg/L. Rainwater from an open environment had the lowest calcium content while the rainwater from thatched roof had the highest calcium content. Magnesium in the rainwater samples ranged from 10.68 to 18.26 mg/L. The rainwater harvested from an open environment had the lowest magnesium content, while the magnesium found in rainwater from the corrugated iron rooftop was the highest. Zinc is very essential and it is known for its astringency at high levels in water.[47] Zinc in the harvested rainwater samples ranged from 0.18 to 2.65 mg/L. Rainwater harvested from corrugated iron roof had the highest zinc level while that of open environment was the lowest. Kingett[50] also reported an increased concentration of zinc in rainwater harvested from galvanized iron roofs. He attributed such phenomena to weathering. Van Metre and Mahler[51] found galvanized metal roofs to be a source of particulate zinc and cadmium. This observation is in line with the present study where the rainwater harvested from corrugated iron rooftop was the highest in zinc, followed by the one harvested from an aluminum rooftop. Hence, the two metal rooftops may have impacted on the raindrops on them. According to Temijenovic[52] aluminum is a readily available metal to human and possesses neurotoxic potential. Water has been recognized as one of the pathways through which humans ingest aluminum.[52] Aluminum has been associated with alzheimer's disease of the elderly, which is associated with specificity in the aberrations of the neural system.[47] Aluminum ranged from 0.01 to 0.21 mg/L. Rainwater harvested from aluminum rooftop had the highest aluminum content while the one from open environment had the lowest aluminum content. Iron ranged from 0.02 to 0.08 mg/L. Rainwater from an open environment was had the lowest iron content while the ones from the corrugated iron rooftop and stone-coated rooftops were the highest in iron content. All the observed metals were lower than their respective WHO[40] standard and NSDWQ.[41]

Table 3: Bacterial isolates from rainwater samples collected from different rooptops.

Gram Reaction	Cellular Morphology	Motility	Catalase	Coagulase	Oxidase	Citrate	Indole	Urease	Methyl Red	Voges Proskeaur	H_2S	Glucose	Sucrase	Lactose	Probable organism
-ve	Rod	+	-	-	+	+	-	+	+	-	+	A	A	A	*Pseudomonas aeruginosa*
-ve	Rod	+	+	-	-	-	+	-	+	-	-	A/G	A/G	A/G	*Escherichia coli*
-ve	Rod	+	+	-	-	+	-	+	+	-	+	A/G	A/G	A/G	*Klebsilla pneumonia*
-ve	Rod	+	+	-	-	+	-	-	-	+	-	A/G	A	-	*Entrobacter sp.*
-ve	Rod	+	+	-	-	+	-	+	+	-	+	A/G	-	-	*Proteus mirabilis*
-ve	Rod	+	+	-	+	+	+	-	+	-	-	A	A	A	*Vibro sp.*

Key:-ve = Gram negative; + = positive; - = negative; A= Acid; G=Gas; sp= species.

Rainwater is not only contaminated by chemical and physical substances, microorganisms such as parasites, bacteria, viruses, and some stench of macroorganisms such as fungi can as well contaminate rainwate.[53] It has been reported that both indicators and pathogenic microorganisms are found in rainwater.[14-15] The bacterial isolates from the harvested rainwater samples as present in Table 3 were *Pseudomonas aeruginosa, Escherichia coli, Klebsilla pneumonia, Entrobacter* sp., *Proteus mirabilis, and Vibro sp.Enterobacter* sp. had the highest occurrence of 11.1% in rainwater harvested from asbestos rooftop (Table 4). *Pseudomonas aeruginosa* was observed in the rainwater samples from stone coated and thatched rooftops at an occurrence rate of 5.5% each. *Escherichia coliwas* observed in rainwater from asbestos and thatched rooftops at 5.5% occurrence rate each. *Klebsiella pneumoniae* was observed in rainwater samples from open environment and aluminum rooftop at 5.5% each. *Proteus mirabilis* had 5.5% occurrence in rainwater from corrugated iron rooftop and 11.1% in rainwater from the thatched rooftop. *Vibro sp*. 5.5% occurrence each for rainwater from open environment, corrugated rooftop, stone coated rooftop, and aluminum rooftop; but 11,1% occurrence in rainwater from the thatched rooftop. In all, rainwater harvested from thatched roof had the highest occurrence of bacterial isolates with 33.3%, followed by rainwater from corrugated iron and asbestos rooftop with percentage occurrence of 16.7% each, while rainwater samples from open environments, stone coated roof, and aluminum rooftop had 11.1% percentage occurrence of bacterial isolates each. *Vibro sp*. occurred most with 33.3%, followed by *Escherichia coli* and *Proteus mirabilis* with the percentage occurrence of 16.7% each. *Aspergillus* sp. and *Penicillum* sp. were the observed mould isolates in this study (Table 5). *Aspergillus* sp. had the. had the highest occurrence percentage of 62.5% against *Penicillum*sp with 37.5% percentage occurrence. The rainwater sample from stone coated rooftop had 50.0% percentage occurrence of mould isolates, followed by rainwater from thatched rooftop with the percentage occurrence of 27.5% for mould isolates (Table 6). Table of microbial load enumeration of harvested water samples (Table 7) showed that THC load of 30 CFU/ml for thatched rooftop, 20 CFU/100 ml for corrugated iron rooftop, 15 CFU/.100 ml for stone coated rooftop,13 CFU/100 ml for aluminum rooftop, 12 CFU/100 ml for asbestos rooftop, and 6 CFU/100 ml for open environment. Rainwater from the thatched rooftop was the highest in THC microbial load while the one from an open environment was the least though all the observed THC loads of the observed rainwater samples were lower than the WHO[40] standard and NSDWO[41] limit of 3 CFU/100 ml and 5 CFU/100 ml, respectively. Rainwater samples from the corrugated iron rooftop, stone coated rooftop, and thatched rooftop had TCC load of 1 CFU/100 ml each, while the TFC load of 1 CFU/100 ml was enumerated in rainwater harvested from the stone coated rooftop and thatched rooftop.

Table 4: Percentage occurrence bacterial isolates from rainwater samples of different rooftops.

Bacterial isolates	Different Rooftops						
	Open enviro -nment	Corrugated iron roof	Asbestos roof	Stone Coated roof	Aluminum roof	Thatched roof	Total
Enterobacter sp.	0(0.0)	0(0.0)	2(11.1)	0(0.0)	0(0.0)	0(0.0)	2(11.1)
Pseudomonas aeruginosa	0(0.0)	0(0.0)	0(0.0)	1(5.5)	0(0.0)	1(5.5)	2(11.1)
Escherichia coli	0(0.0)	1(0.0)	1(5.5)	0(0.0)	0(0.0)	1(5.5)	3(16.7)
Klebsiella pneumoniae	1(5.5)	0(0.0)	0(0.1)	0(0.0)	1(5.5)	0(0.0)	2(11.1)
Proteus mirabilis	0(0.0)	1(5.5)	0(0.0)	0(0.0)	0(0.0)	2(11.1)	3(16.7)
Vibro sp.	1(5.5)	1(5.5)	0(0.0)	1(5.5)	1(5.5)	2(11.1)	6(33.3)
Total	2(11.1)	3(16.7)	3 (16.7)	2(11.1)	2(11.1)	6(33.3)	18(100.0)

Table 5: Identification of mould isolates.

Cultural and morphological characteristics	Microscopic characteristics	Possible mould isolates
Yellowish green or yellow colonies with margin that is distinct.	Footcell produces canidiophores. The top of conidiophores bears club shaped vesicle. Conidia are in chains	*Aspergillus sp.*
Green fast growing clonies with conidia which are dense.	Branched conidiophoes with brush looking chains of conidia	*Penicillum sp.*

Table 6: Percentage occurrence mould isolates from rainwater samples of different rooftops.

Bacterial isolates	Different Rooftops						
	Open enviro -nment	Corrugated iron roof	Asbestos roof	Stone Coated roof	Aluminum roof	Thatched roof	Total
Aspergillus sp.	0(0.0)	0(0.0)	1(12.5)	2(25.0)	0(0.0)	2(25.0)	5(62.5)
Penicillum sp,	0(0.0)	0(0.0)	0(0.0)	2(25.0)	0(0.0)	1(12.5)	3(37.5)
Total	0(0.0)	0(0.0)	1(12.5)	4(50.0)	0(0.0)	3(27.5)	8(100,0)

The 16S rRNA sequence from four of the six bacterial isolates subjected to molecular identification produced the exact match of similar sequences during the megablasy search from NCBI data for non-rudundant nuceotie (nr/nt). There was a percentage of 99% similarity of the 16S rRNA of the solates to other species while the phylogenetic placement of the 16S rRNA of the isolates agree with the computed evolutionary distances. Species of bacteria isolated after the molecular identification include *K. pneumoniae, P. aeroginosa, E. coli,* and *P. mirabilis*

Table 7: Microbial load enumeration of harvested rainwater samples from different rooftops.

Samples	THC (CFU /100 ml)	TCC (CFU /100 ml)	TVC (CFU /100 ml)	TFC (CFU /100 ml)
Corrugated iron roof	20	1	0	0
Asbestos roof	12	0	0	0
Stone coated roof	15	1	0	1
Aluminumroof	13	0	0	0
Thatched roof	30	1	0	1
Open environment	6	0	0	0
WHO standard	3	0	0	0
NASDWQ	10	0	0	0

THC= Total heterotrophic count; TCC= Total coliform count; TVC=Total viable count; TFC= Total fungal count. NSDWQ= *National Standard for Drinking Water Quality.*

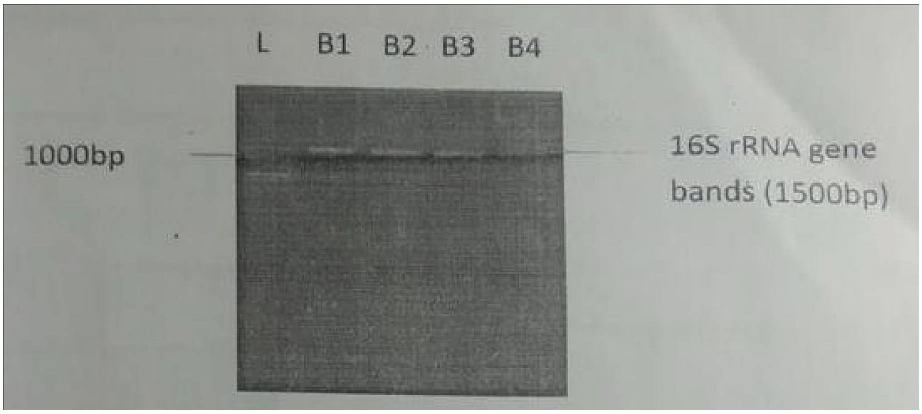

Plate 1: Gel electrophoresis (using Agarose method) of the gene (16S rRNA gene) of some bacteria isolated.

Lane B1-B4 represent the 16 rRNA gene bands (1500bp).
Lane L represents the 100 bp molecular ladder.

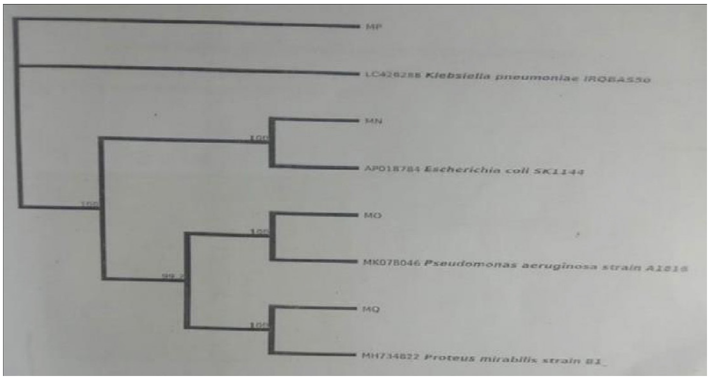

Fig. 2: The evolutionary distance represented with phylogenetic tree for the bacteria isolated.

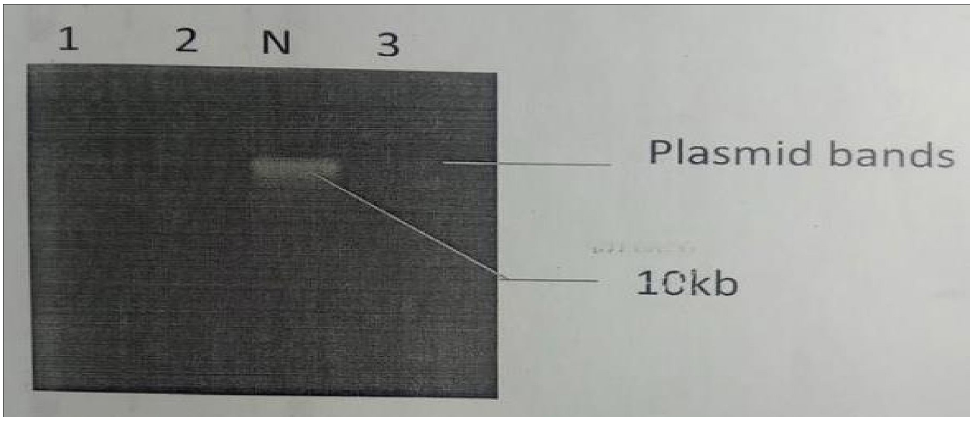

Plate 2: Gel electrophoresis (using Agarose method) of plasmid for bacteria isolated. Lane N represents a 1 kb molecular ladder.

After the profiling (Plate 2), *E.coli*, *P. aeruginosa*, and *Enterobacter sp*. possessed plasmids and this made them resistant to the antibacterial drugs used.

The organisms observed in this study become important when their effects are considered on human health. Most of the bacterial isolates are associated with gastrointestinal disorders in immunocompromised people and children[34]

Most of the bacterial isolates have been reported to cause various gastrointestinal disorders in immunocompromised people and children.[34] K. *pneumoniae* is a non-motile, gram negative, and encapsulated bacterium associated with pneumonia in diabetes mellitus patients and people with alcohol use disorder.[54] P. aeruginosa is an opportunistic organism, which can cause infections in the blood, lungs, and other parts of the body.[34] Some strains of *E.coli* have been associated with diseases stomach cramps, bloody diarrhea, and vomiting. P. *mirabilis* has been associated with most Proteus infections in humans.[55] Infections such as soft tissue infections, urinary tract infections (UTI), osteomyelitis, endocarditis, respiratory infections, as well as some nosocomial infections have been associated with *Enterobacter* sp. Among the diseases associated with the harmful effect of *Penicillum*spare keratitis, otomycosis, pneumonia, allergic pulmonary, urinary tract infections, and peritonitis. *Aspergillus* sp. is known to affect people with the comprised immune system as well as lung diseases.[34] The presence of microorganisms in the rainwater harvested from different roofs has been reported by Edem *et al.*[56]

Conclusion

The physicochemical parameters of the harvested rainwater samples were within the standard of WHO and NSDWQ. The harvested rainwater samples were high on THC, TCC, and TFC microbial loads when compared to the standard stipulated by WHO and NSDWQ. Some of the bacterial and fungal species isolated from the harvested rainwater samples are identified as causative organisms to human diseases. From the findings of this study, rainwater harvested from corrugated iron sheet roof, thatched roof, aluminum roof, asbestos roof, and stone coated roof should be properly treated before demestic or potable use. This study has assessed the physicochemical and microbiological characteristics of rainwater harvested from different rooftops in Owerri, Imo State, Nigeria.

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Conflict of Interest

The authors do not have any conflict of interest. We declare that we all saw the article and approved it should be sent to Current World of Environment.

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