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Effect of Different Storage Vessels on the Microbiological and Physicochemical Properties of Rainwater Harvested from Different Rooftops in Owerri, Nigeria

Nwachukwu M. I. [a*], Ihenetu F. C. [a] and Obasi C. C. [b]

[a] *Department of Microbiology, Imo State University, Owerri, Nigeria.*
[b] *Department of Public Health, Imo State University, Owerri, Nigeria.*

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

This study evaluated the effect of different storage vessels on the microbiological and physicochemical properties of rainwater harvested from different rooftops in Owerri. Different storage vessels were assembled and used to stored rainwater delivered by using different catchments. The catchments used were corrugated iron sheet roof, asbestos roof, stone coated roof, aluminum roof, and thatched roof. Rainwater was also collected directly from rainfall, in the open environment and stored in the vessels. The storage vessels were plastic contain, metal tank, earthen ware pot, and block/cemented tank. The study was designed in such a way that each of the storage vessels received water from each of the catchments for storage. In all, a total of 24 storage vessels were used. Each of the catchment delivered rainwater to a peculiar plastic container, metal

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

samples were allowed to stay in the vessels for some days before they were taken to the laboratory for analysis using standard methods. From the results observed, the storage vessels made more microbial impact on the stored rainwater samples that physicochemical impact. Six bacterial and mould organisms were isolated from the stored rainwater samples. However, the molecular identification study for the bacterial organisms revealed plasmid possessed *E. coli, P. aeruginosa,* and *Enterobacter sp.,* which made them resistant to the antibacterial drugs. From the observation of the present study, storage vessels may impact more on bacterial contamination than physicochemical or metal contamination. This study has shown the effect of different storage vessels on the microbiological and physicochemical properties of rainwater harvested from different rooftops in Owerri, Nigeria.

Keywords: Catchments; storage vessels; rainwater; rooftops; molecular identification.

1. INTRODUCTION

Apart from surface and ground water sources, rainfall also forms a substantial source of water to the living world [1,2]. The harvest of rainwater during rainfall and the role rainwater plays as a natural source of water cannot be overstated [3]. It has been reported that rainwater contains mixed electrolytes with varying amounts of major and minor ions [4,5]. 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 of rainwater [6,7]. Rainwater constituents are influenced by natural and anthropogenic sources [8]. Different authors have noted that the chemical characteristics of rainwater in the urban areas are from the local pollution while the chemical characteristics of rainwater of rural areas indicate the degree of impact of anthropogenic and natural source [9,10].

In the continents of the world, where rainwater is considered as a drinking source of water, it is normally harvested using rainwater harvesting technology for further uses. According to Ojo [11], rainwater harvesting technology defines the technology used to collect, convey and stored rainfall from relatively clean surfaces for later use. Helmreich and Horn [12] noted that harvesting of rainwater involves the collection and storage of rainwater from rain that falls on a roof surface for later use. Nizam *et al*. [13] noted that rainwater harvesting system is a method of collecting rainwater from man-made surfaces such as rooftops and constructed area which can be used for household, agriculture, commercial, and other various sectors. The harvesting of rainwater from rain bridges the gap and provides the necessary avenue to cope with shortage of water in most developing and underdeveloped nations of the world. Tobin *et al*. [14] and Tobin [15] noted that the process of rainwater harvest from rain involves catchment area or catchment surfaces, conveying system and collection devices.

The use of rooftops as catchment surfaces has gained acceptability in places where rainwater is considered as a very important source of water. Rain that falls on a catchment surface during rainfall is delivered through a conveying system to a collection device. Collection devices are normally storage vessels. Different storage vessels are used to store harvested rainwater. Among such vessels are tank, which could be made of plastic or metal, block/concrete cemented tank, and earthen ware pot. According to Chalchisa *et al*. [16], safe drinking water is water with microbial, chemical, and physical characteristics that meet the requirements as stipulated by WHO guidelines. Nigeria has a drinking water guideline stimulated as Nigerian Standard for Drinking Water Quality (NSDWQ) [17]. Poor hygienic condition of storage vessels contribute to water degradation between sources and point of use [18-20]. Kausar *et al*. [5] noted that significant deterioration of water quality has been detected during the storage of water in different vessels at homes in both rural and urban areas. Existing studies on storage vessels and water quality [5,21] have not really been towards the direction of finding out how the vessels can affect the quality of stored rainwater. This study looked into that area with the view of finding out how storage vessels such as plastic container, metal tank, block/concrete cemented tank, and earthen ware pot can relatively affect the quality of rainwater during storage.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Owerri, the capital territory of Imo State. Owerri is located within coordinates of 5° 28' 34.7160" N and 7° 1' 33.0708" E. It is about 500-600 km east of Lagos. It consists of three local government areas which include Owerri Municipal, Owerri North, and Owerri West. Two rivers; Otamiri river in the east and Nwaorie river in the south transverse Owerri. It is regard as the heartbeat of southeastern region because it is centrally located within the region. Owerri is located in one of the most densely populated areas of Nigeria. It is a great city of social life, hospitality, and religion. The inhabitants of the area are mainly civil servants, farmers, and traders; and are predominantly Christian Igbo people.

2.2 Collection and Storage of Rainwater Samples

Rainwater was collected from different rooftops into the selected vessels for storage. Rainwater samples were collected from corrugated iron sheet roof, asbestos roof, stone coated roof, aluminum roof, and thatched roof into plastic container, metal tank, earthen ware pot, and block/concrete cement tank for storage. The storage vessels were placed in such a way that each of them received rainwater during rainfall from each of the roofs. Each of the storage vessels also received rainwater directly from rainfall (an open environment). Each storage vessel was placed on a stand of about 2 ft high mounted beneath a rooftop to collect rainwater into the vessel. The stands were aseptically treated and all the vessels were properly sterilized. The sampling was carried out at the peak of the rainy season (June-September 2021). Rainwater was collected after allowing the raindrops to wash the rooftops for 20 mins. The stored rainwater from the vessels was aseptically collected using sterile sampling bottles and transported in a cooler of ice to the laboratory for analysis after 48 hrs.

2.3 Physicochemical Analysis of the Samples

The standard methods as described by APHA [22] were used for the determination of physicochemical parameters of the rainwater samples. The parameters analyzed were appearance, odour, colour, total suspended solids (TSS), total dissolved solids (TDS), pH, temperature, conductivity, turbidity, phosphate (PO_4^-), sulpahte (SO_2^-), nitrate (NO_3^-), and chloride (Cl^-).

2.4 Metals Analysis of the Samples

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

2.5 Characterization and Identification of Isolates

The methods described by Vandepitte *et al*. [23] and Cheesbrough [24] were used for bacterial isolates. Bacterial isolates were identified morphologically using cellular characteristics, mobility tests, gram staining, and biochemical properties. Biochemical tests carried out include catalase test, coagulase test, oxidase test, citrate utilization test, indole test, urease test, methyl red test, Voges-prokauer test, H_2S production, glucose tests, sucrose, and lactose utilization test. The fungal isolates were identified morphologically using cellular characteristics and microscopic identification methods.

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

2.6 Molecular Identification of Bacterial Isolates

All the bacterial isolates were subjected to molecular identification following the methods of DNA extraction, DNA quantification, 16S rRNA amplification, and sequencing. The obtained sequences were subjected to phylogenetic analysis for evolutionary distances.

DNA extraction was carried out using the boiling method. Five ml of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000 rpm for 3 min. The cells were resuspended in 500 µl of normal saline and heated at 95℃ for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml micro-centrifuge tube and stored at -20℃ for other downstream reactions.

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two µl of the extracted DNA was located in the lower pedestal; the upper pedestal was brought down to make contact with the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

16S rRNA region of the rRNA gene of the isolates was amplified using the 27F; 5`AGAGTTTGATCMTGGCTCAG-3` and 1492R; 5`-CGGTTACCTTGTTACGACTT-3` primers on a ABI 9700 Applied Biosystem thermal cycle at a final volume of 40 µl for 35 cycles. The PCR mix included; the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of µM and the extracted DNA as the template. The PCR conditions were as follows: initial denaturation, 95℃ for 5 minutes; denaturation, 95℃ for 30 seconds; annealing, 52℃ for 30 seconds; extension, 72℃ for 30 seconds for 35 cycles and final extension, 72℃ for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transillumiator.

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inquba Biotechnological. Pretoria, South Africa. The sequencing was done at a final volume of 10 µl, the components include 0.25 µl BigDye terminator v1.1/v3.1, 2.25 µl of 5□BigDye sequencing buffer, 10 µM Primer PCR primer, and 2-10 ng PCR template per 100 bp, The sequencing conditions were as follows 32 cycles of 96℃ for 10s, 55℃ for 5s and 60℃ for 4 minutes.

Obtained sequences were edited through phylogenetic analysis using the bioinformation algorithm trace edit. Similar sequences were downloaded from National Center for Biotechnology Information (NCBI) database using BLASTN. The sequences were aligned using MAFFT. The evolutionary history was inferred using Neighbor-Joining method in MEGA 6.0 [25]. The bootstrap consensus tree inferred from 500 replicates [26] was taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method [27].

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.

3. RESULTS AND DISCUSSION

The results of the physicochemical characteristics of rainwater stored in the different vessels were presented using Tables 1 -4. From the results, all the stored rainwater samples had clear appearance and were odourless. It therefore means that none of the storage vessels affected the appearance or the odour of the rainwater stored in them. The rainwater collected from thatched roof catchment and stored in a plastic container had the highest colour value of 13.35 units while the rainfall collected directly from the rainfall (open environment) and stored in a plastic container had the lowest colour of 10.65 units. The rainwater samples collected from the catchments made of corrugated iron sheet, asbestos, stone coated, and aluminum and stored in plastic containers had colour values of 12.25 units, 12.65 units, 13.14 units, and 11.85 units respectively. It could be that the catchments impacted on the colour of the stored rainwater samples. The colour values of the rainwater samples collected and stored in the plastic containers were lower than the WHO standard and NSDWQ of 15 units (Table 1). The colour values of the water stored in metal tanks showed that the rainwater collected from thatched roof catchment and stored in metal tank had the highest colour of 16.50 units while the one collected directly from rainfall, in the open environment had the lowest colour of 13.00 units. The rainwater collected from corrugated iron sheet roof, asbestos roof, stone coated roof, and aluminum roof catchments and stored in metal tanks had colour values of 15.65 units, 14.20 units, 15.20 units, 15.00 units respectively. It could be that the metal tanks had different impacts on the collected and stored rainwater samples (Table 2). The rainwater sample collected directly from rainfall (Open environment) and stored in metal tank had the lowest colour value of 13.00 units (Table 2). The rainwater samples collected from corrugated iron sheet roof, stone coated roof, and thatched roof catchments and stored in metal tanks had colour values higher the standards of WHO and NSDWQ of 15 units. The colour values for the rainwater samples collected from different catchments and stored in earthen ware pot were 12.00 units for Corrugated iron sheet roof, 13.04 unit for Asbestos roof, 11.64 units for Stone coated roof, 10.20 units for stone coated roof, and 11.05 units for thatched roof (Table 3). The rainwater sample collected directly from the rainfall and stored in earthen ware pot had the lowest colour value against the rainwater samples collected from different catchments and stored in earthen ware pot (Table 3). The observed colour values of the collected rainwater samples which were stored in the earthen ware pots were lower than the standard of WHO and NSDWQ. The colour value of rainwater samples collected from different catchments and stored in block/cemented tanks was ranged from 120.25 to 14.50 units. The rainwater collected from thatched roof catchment and stored in block/cemented tank had the highest colour while the rainwater collected from corrugated iron sheet roof and stored in block/cement tank was the lowest. The observed values were lower than the colour value of the rainwater collected directly from rainfall in an open environment and stored in block/cemented tank (Table 4). The amount of carbon dioxide dissolved in water determines the pH of water [28]. The pH of rainwater collected from different catchments and stored in plastic containers (Table 1) ranged from 2.78 to 6.96. Only the rainwater collected from aluminum roof catchment and stored in plastic container had pH that falls within the WHO standard and NSDWQ. The pH of rainwater collected from different catchments and stored in metal tanks (Table 2) ranged from 5.35 to 5.68. The pH of rainwater samples collected from different catchments and stored in earthen ware pot (Table 3) ranged from 2.58 to 5.95. The pH of rainwater samples collected from different catchments and stored in earthen ware pots (Table 3) ranged from 2.58 to 5.95. The pH of rainwater samples collected from different catchments and stored in block/cemented tanks (Table 4) ranged from 5.64 to 6.60. Only the rainwater collected from asbestos roof catchment and stored in block/cemented tank had pH that falls within the WHO standard and NSDWQ. All the observed pH values for the rainwater samples which were stored in the different vessels were on the alkaline side of pH. The effects of using alkaline water domestically and in the body system have been reported by Duru *et al*. [20]. The temperature of rainwater samples collected from different catchments and stored in the plastic containers ranged from 20.90 to 29.30 ℃, and were within the WHO standard and NSDWQ. The temperature of the rainwater samples collected and stored in metal tanks ranged from 28.50 to 29.50 ℃. The temperature of all the rainwater samples collected and stored in metal tanks were within the standard of WHO and NSDWQ. The temperature of the rainwater samples collected and stored in earthen ware pots ranged from 26.80 to 30.90 ℃. Rainwater collected from corrugated iron sheet roof catchment and stored in the earthen ware pot had temperature value higher than the standard of WHO and NSDWQ. The temperature of the rainwater samples collected and stored in block/cemented tanks ranged from 27.20 to 30.00 ℃. All the rainwater samples collected from different catchments and stored in block/cemented tanks were within the standards of WHO and NSDWQ. TDS of rainwater collected and stored in the plastic containers ranged from 3.62 to 16.52 mg/L. The TDS of rainwater collected directly from rainfall was the highest but all the collected and stored rainwater samples had TDS values that were lower than the standard of WHO and NSDWQ. The TDS for rainwater samples collected from different catchments and stored in metal tanks ranged from 3.49 to 17.11 mg/L. The observed values were lower than the standard of WHO and NSDWQ. TDS values of rainwater collected and stored in earthen ware pots and block/cement tanks had were lower than the WHO standard and NSDWQ. TSS ranged from 4.03 to 14.20 mg/L for the rainwater samples collected and stored in plastic containers; 4.00 to 14.00 mg/L for the rainwater samples collected and stored in metal tanks; 4.00 to 13.50 mg/L for the rainwater samples collected and stored in earthen ware pots; and 3.40 to 12.80 mg/L for the rainwater samples collected from different catchments and stored in block/cemented tanks. The stored rainwater6 samples had TDS values lower than the WHO standard and NSDWQ. Turbidity is known to affect the transparency of water as result of the presence of suspended particles or particulates of matter [29]. Algae, plankton, silt, and chemicals are among the materials that can affect the clarity of water [29]. Turbid water can create aesthetic and nuisance problems. The turbidity of the rainwater collected and stored in plastic containers ranged from 2.61 to 5.82 NTU. The rainwater collected from thatched roof catchment and stored in the plastic container had turbidity higher than the WHO standard and NSDWQ. All the rainwater samples collected and stored using metal tanks had turbidity values higher than the standard for WHO and NSDWQ. The turbidity for rainwater collected and stored in earthen ware pots ranged from 4.00 to 40.00 NTU. The turbidity of the rainwater samples collected using corrugated iron sheet roof and asbestos roof catchments were high than the standard for WHO and NSDWQ. The turbidity of rainwater collected and stored in block/cemented tanks ranged from 3.00 to 6.00 NTU. All the stored rainwater samples in block/cemented tanks had turbidity lower than the standard for WHO and NSDWQ. The conductivity of the rainwater samples measured the relative capacities of the collected and stored rainwater samples to allow the passage of current through it [28]. The conductivity of rainwater collected and stored in plastic containers ranged from 7.31 to 32.80 us/cm; 9.63 to 118.63 us/cm for rainwater collected and stored in metal tanks; 14.20 to 54.60 us/cm for rainwater collected and stored in earthenware pots; and 13.90 to 35.60 us/cm for rainwater collected and stored in block/cement tanks. All the observed conductivity values for the collected and stored rainwater samples were lower than the standard for WHO and NSDWQ. It is being argue that PO_4^{3-} cannot be found in rainwater naturally. However, the anthropogenic activity of man may have influenced the presence in rainwater in recent times. The PO_4^{3-} of rainwater collected and stored in plastic containers from different catchments ranged from 0.20 to 1.71mg/L; 0.90 to 1.90 mg/L for rainwater collected from different catchments and stored in metal tanks; 0.60 to 2.00 mg/L in rainwater collected and stored in earthen ware pots; and 0.50 to 2.11 mg/L for rainwater collected and stored in block/cemented tanks. The SO_4^{2-} found in rainwater could be from gasses and dissolved impurities of the atmosphere. The SO_4^{2-} of rainwater collected and stored in plastic containers ranged from 10.80 to 16.64 mg/L; 12.15 to 17.02 mg/L in rainwater samples collected from different catchments and stored in metal tanks; 10.80 to 16.32 mg/L in rainwater samples collected and stored in earthen ware pots; and 10.64 to16.40 mg/L in rainwater samples collected and stored in block/cemented tanks NO_3^- is among the disease causing parameters of water. It is associated with blue baby syndrome in infants [28]. The NO_3^- in rainwater samples collected from different catchments and stored using plastic containers ranged from 3.50 to 7.19 mg/L; 5.21 to 13.98 mg/L in rainwater samples collected and stored in metal tanks; 3.47 to 7.40 mg/L in rainwater samples collected and stored in earthen ware pots; and 3.67 to 6.89 mg/L in rainwater samples collected and stored in block/cemented container. Cl^- is among the parameters of water associated with health and should not exceed a certain limit. The Cl^- in rainwater collected and stored in plastic containers ranged from 17.70 to 29.82 mg/L; 18.15 to 28.95 mg/L in rainwater samples collected and stored in metal tanks; 18.98 to 29.65 mg/L in rainwater samples collected and stored earthen ware pots; and 16.63 to 29.28 mg/L in rainwater samples collected from different catchments and stored in block/cemented tanks. The observed PO_4^{3-} , SO_4^{2-} , NO^{3-} , and Cl^- of the collected and stored rainwater samples were lower than the standard of WHO and NSDWQ.

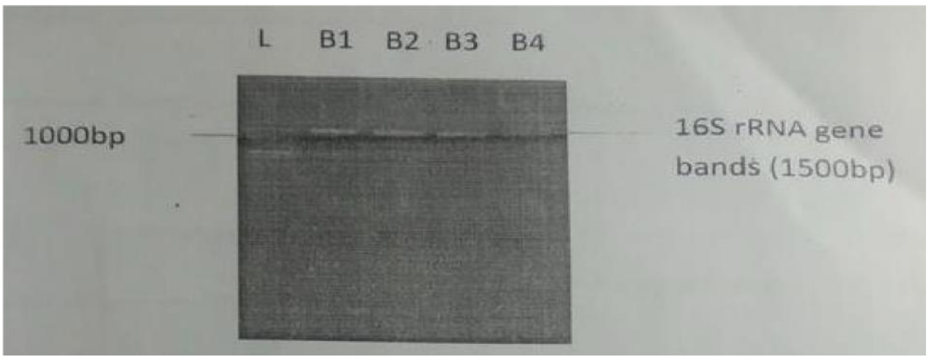

Plate 1. Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates
Lane B1-B4 represent the 16 rRNA gene bands (1500bp).Lane L represents the 100 bp molecular ladder

Table 1. Physicochemical characteristics of rainwater stored in plastic containers

	Parameters												
Rainfall Catchment	**Appearance**	**Odour**	**Colour (units)**	**pH**	**Temp. (℃)**	**TDS (mg/L)**	**TSS (mg/L**	**Turbidity (NTU)**	**Conductivity (us/cm)**	**PO_4^{3-} (mg/L)**	**SO_4^{2-} (mg/L)**	**NO_3^- (mg/L)**	**Cl^- (mg/L)**
Corrugated iron sheet roof	Clear	Odourless	12.25	5.45	28.25	6.64	7.32	3.00	13.43	0.90	16.64	7.19	25.20
Asbestos roof	Clear	Odourless	12.65	5.54	28.21	6.64	8.14	3.40	27.50	1.60	11.05	4.16	23.20
Stone coated roof	Clear	Odurless	13.14	5.69	20.90	13.93	6.00	3.60	12.85	1.71	10.80	6.28	28.64
Aluminum roof	Clear	Odorless	11.85	6.96	29.30	6.40	4.03	2.61	7.31	0.80	14.70	4.96	29.82
Thatched roof	Clear	Odurless	13.35	2.78	28.91	3.62	14.20	5.82	19.50	0.70	14.10	3.50	19.26
Open environment	Clear	Odurless	10.65	5.45	28.42	16.52	5.00	3.04	32.80	0.20	14.60	5.20	17.70
WHO Standard			15	6.5-8.5	20-30	250	50	5	1000	5	250	40	250
NSDWQ			15	6.5-8.5	20-30	100	50	5	1000	3	250	50	250

Results are presented as mean of triplicate determinations. Temp.=Temperature; TDS= Total dissolved solids; TSS- Total suspended solids

Table 2. Physicochemical characteristics of rainwater stored in metal tank

	Parameters												
Rainfall Catchment	**Appearance**	**Odour**	**Colour (unit)**	**pH**	**Temp. (℃)**	**TDS (mg/L)**	**TSS (mg/L**	**Turbidity (NTU)**	**Conductivity (us/cm)**	**PO_4^{3-} (mg/L)**	**SO_4^{2-} (mg/L)**	**NO_3^- (mg/L)**	**Cl^- (mg/L)**
Corrugated iron sheet roof	Clear	Odourless	15.65	5.40	28.50	3.49	6.04	6.40	116.86	1.25	17.02	8.25	23.45
Asbestos roof	Clear	Odourless	14.20	5.43	28.50	17.11	7.50	5.50	34.10	1.70	12.15	5.65	21.64
Stone coated roof	Clear	Odourless	15.20	5.68	29.30	8.40	6.20	5.60	116.24	1.90	12.65	7.65	27.39
Aluminum roof	Clear	odourless	15.00	5.50	28.10	4.96	4.20	5.40	9.63	0.90	15.70	5.21	28.95
Thatched roof	Clear	16,50	16.50	5.65	29.50	9.63	14.00	6.80	118.26	0.91	15.37	13.98	18.15
Open environment	Clear	Odourless	13.00	5.35	28.50	9.36	4.00	6.20	118.63	1.20	15.28	6.10	18.25
WHO Standard			15	6.5-8.5	20-30	250	50	5	1000	5	250	40	250
NSDWQ			15	6.5-8.50	20-30	250	50	5	1000	5	100	40	250

Results are presented as mean of triplicate determinations. Temp.=Temperature; TDS= Total dissolved solids; TSS- Total suspended solids

Table 3. Physicochemical characteristics of rainwater stored in earthen ware pots

	Parameters												
Rainfall Catchment	**Appearance**	**Odour**	**Colour (unit)**	**pH**	**Temp. (℃)**	**TDS (mg/L)**	**TSS (mg/L**	**Turbidity (NTU)**	**Conductivity (us/cm)**	**PO_4^{3-} (mg/L)**	**SO_4^{2-} (mg/L)**	**NO_3^- (mg/L)**	**Cl^- (mg/L)**
Corrugated iron sheet roof	Clear	Odourless	12.00	5.80	30.90	28.10	6.00	40.00	54.60	1.33	16.32	7.40	24.82
Asbestos roof	Clear	Odourless	13.02	5.95	26.80	12.60	7.20	8.20	14.20	1.51	11.22	4.26	22.85
Stone coated roof	Clear	Odourless	11.64	5.59	29.30	27.30	5.80	3.80	54.50	2.00	10.80	6.19	27.90
Stone coated roof	Clear	Odurless	10.20	5.57	28.90	20.10	5.40	3.20	40.10	1.00	14.75	4.80	29.65
Thatched roof	Clear	Odurless	11.05	2.58	28.60	10.30	13.50	6.50	30.50	1.20	14.30	3.47	18.98
Open environment	Clear	Odurless	10.01	5.65	27.90	18.25	4.00	4.00	35.60	0.60	14.80	5.25	250
WHO Standard			15	6.5-8.5	20-30	250	50	5	1000	5	250	40	250
NSDWQ			15	6.5-8.5	20-30	100	50	5	1000	5	100	50	250

Results are presented as mean of triplicate determinations. Temp.=Temperature; TDS= Total dissolved solids; TSS- Total suspended solids

Table 4. Physicochemical characteristics of rainwater stored in block/cemented tanks

	Parameters												
Rainfall Catchment	**Appearance**	**Odour**	**Colour (unit)**	**pH**	**Temp. (℃)**	**TDS (mg/L)**	**TSS (mg/L**	**Turbidity (NTU)**	**Conductivity (us/cm)**	**PO_4^{3-} (mg/L)**	**SO_4^{2-} (mg/L)**	**NO_3^- (mg/L)**	**Cl^- (mg/L)**
Corrugated iron sheet roof	Clear	Odourless	12.65	5.64	29.20	8.25	5.80	3.50	13.90	1.38	16.40	6.89	24.05
Asbestos roof	Clear	Odourless	12.80	6.60	29.00	11.72	7.00	3.00	16.80	1.62	11.55	4.65	21.74
Stone coated roof	Clear	Odourless	13.00	6.20	30.00	28.20	5.40	4.00	35.60	2.11	10.64	6.56	28.25
Aluminum roof	Clear	Odourless	13.72	5.68	29.30	15.20	5.40	3.80	35.20	1.10	14.26	4.62	29.28
Thatched roof	Clear	Odurless	14.50	5.93	27.20	11.25	12.80	6.00	34.20	0.90	13.95	3.67	18.67
Open environment	Clear	Odurless	12.25	5.70	29.00	16.40	3.40	4.50	25.50	0.50	14.70	5.62	16.63
WHO Standard			15	6.5-8.5	20-30	250	50	5	1000	5	250	40	250
NSDWQ			15	6.5-8.5	20-30	250	50	5	1000	5	100	50	250

Results are presented as mean of triplicate determinations. Temp.=Temperature; TDS= Total dissolved solids; TSS- Total suspended solids.

The metals found in the rainwater samples collected from different catchments and stored in the different storage vessels were presented in Tables 5 to 8. Calcium in rainwater samples collected and stored in plastic containers ranged from 21.10 to 32. 25 mg/L; 21.25 to 33.10 mg/L in rainwater samples collected and stored in metal tanks; 22.03 to 72.60 mg/L in rainwater samples collected using different catchments and stored in earthen ware pots; and 24.55 to 35.48 mg/L in rainwater samples collected in block/cemented tanks. All the calcium content of the stored rainwater samples were lower than the WHO standard and NSDWQ. Potassium in the stored rainwater samples using plastic containers ranged from 3 .10 to 8.25 mg/L ; 3.30 to 8.96 mg/L in rainwater samples stored in metal tanks ; 3.62 to 7.98 mg/L in rainwater samples collected and stored using earthen ware pots; and 2.97 to 8.15 mg/L in rainwater samples collected and stored using block/cemented tanks. The impact of magnesium in water has been reported by different authors. In recent years, evidences have linked the metals found in water to some negative health effects. Several epidemiological studies have revealed the risk of cardiovascular diseases, reproductive failures, growth problems, and other problems associated to hardness to magnesium and calcium contents of water [30]. The magnesium found in rainwater samples stored in plastic tanks ranged from 11.02 to 18.20 mg/L ; 13.10 to 19.50 mg/L in rainwater samples collected and stored in metal containers; 11.50 to 18.10 mg/L in rainwater samples collected and stored in earthen ware pots; and 10.90 to 19.35 mg/L in rainwater samples collected and stored in block/cemented tanks. The hardness of water is determined by its calcium and magnesium content. The observed dissolved calcium and magnesium values of the collected and stored rainwater samples, classify the stored rainwater samples as soft water. Low iron in water is not harmful but it can encourage the growth of bacterial organisms. Elevated iron content in water can bring about an overload in iron, which could result in diseases such as stomach problems, nausea, diabetes, and hemochromatosis [30]. The iron content of rainwater samples collected and stored using plastic containers ranged from 0.03 to 0.19 mg/L; 0.16 to 0.35 mg/L for rainwater samples collected and stored in metal tanks; 0.03 to 0.20 mg/L in rainwater samples collected and stored in earthen ware pots; and 0.04 to 0.021 mg/L in rainwater samples collected and stored block/cemented tanks. Only the rainwater collected from corrugated iron sheet roof catchment and stored in metal tanks had iron content that was higher than the standard for WHO and NSDWQ. Elevated zinc in water can affect the taste of water and also bring about vomiting, nausea, and irritation of the intestinal tracts [31]. The zinc content of rainwater samples collected using different roof catchments and stored using plastic containers ranged from 0.15to 2.65 mg/L; 0.08 to 2.81 mg/L in rainwater collected and stored in metal tanks; 0.12 to 2.67 mg/L in rainwater samples collected and stored in earthen ware pots; and 0.06 to 2,58 mg/L in block/cemented tanks. All the collected and stored rainwater samples had zinc contents that were lower than the WHO standard and NSDWQ. Aluminum in drinking water could be a concern because long exposure aluminum has been associated with some degenerative disorders such as bone disease, Alzheimer's disease, and kidney disease. Infants exposed to aluminum are prone to the inhibition of brain development/function and bone disease. The aluminum content of the rainwater samples collected and stored in plastic containers ranged from 0.01 to 0.19 mg/L; 0.02 to 0.70 mg/L in

Table 5. Metals found in rainwater stored in plastic containers

	Metals (mg/L)					
Rainfall catchment	**Calcium**	**Potassium**	**Magnesium**	**Iron**	**Zinc**	**Aluminum**
Corrugated iron sheet roof	24.30	3.78	18.20	0.09	2.65	0.09
Asbestos roof	27.30	5.21	16.40	0.06	0.19	0.03
Stone coated roof	28.25	6.25	14.49	0.19	0.15	0.06
Aluminum roof	22.75	4.64	11.62	0.07	0.69	0.19
Thatched roof	32.25	8.25	11.02	0.05	0.19	0.06
Open environment	21.10	3.10	12.18	0.03	0.25	0.01
WHO Standard	75	-	-	0.3	5	0.20
NSDWQ	75	-	-	0.3	5	0.20

Results are presented as mean of triplicate determinations

Table 6. Metals found in rainwater stored in metal tanks

	Metals (mg/L)					
Rainfall catchment	**Calcium**	**Potassium**	**Magnesium**	**Iron**	**Zinc**	**Aluminum**
Corrugated iron sheet roof	26.15	4.25	19.50	0.35	2.81	0.70
Asbestos roof	27.16	6.15	17.26	0.26	0.38	0.08
Stone coated roof	27.95	6.35	15.69	0.29	0.92	0.08
Aluminum roof	23.85	4.89	13.10	0.16	0.82	0.22
Thatched roof	33.10	8.96	13.26	0.19	0.08	0.07
Open environment	21.25	3.30	13.20	0.18	0.32	0.02
WHO Standard	75	-	-	0.3	5	0.2
NSDWQ	75	-	-	0.3	5	0.2

Results are presented as mean of triplicate determinations

Table 7. Metals found in rainwater stored in earthen ware pots

	Metals (mg/L)					
Rainfall catchment	**Calcium**	**Potassium**	**Magnesium**	**Iron**	**Zinc**	**Aluminum**
Corrugated iron sheet roof	25.20	3.62	18.10	0.08	2.67	0.08
Asbestos roof	30.25	5.40	16.36	0.07	0.17	0.04
Stone coated roof	28.65	5.96	15.37	0.20	0.12	0.06
Aluminum roof	72.60	4.62	11.50	0.09	0.74	0.18
Thatched roof	34.62	7.98	12.10	0.07	0.12	0.05
Open environment	22.03	3.65	13.10	0.03	0.28	0.01
WHO Standard	75	-	-	0.3	5	0.2
NSDWQ	75	-	-	0.3	5	0.2

Table 8. Metals found in rainwater stored in block/cemented tanks

				Metals (mg/L)		
Rainfall catchment	**Calcium**	**Potassium**	**Magnesium**	**Iron**	**Zinc**	**Aluminum**
Corrugated iron sheet roof	26.80	3.72	19.35	0.09	2.58	0.08
Asbestos roof	28.50	5.30	16.54	0.08	0.19	0.08
Stone coated roof	28.70	6.22	14.95	0.21	0.14	0.03
Aluminum roof	24.55	3.98	10.90	0.08	0.75	0.06
Thatched roof	35.48	8.15	11.83	0.08	0.06	0.18
Open environment	24.65	2.97	12.21	0.04	0.21	0.05
WHO Standard	75	-	-	0.3	5	0.2
NSDWQ	75	-	-	0.3	5	0.2

rainwater samples stored in metal tank; 0.01 to 0.18 mg/L in rainwater samples collected and stored in earthen ware pots; and 0.03 to 0.18 mg/L in rainwater samples collected from different catchments and stored in block/cemented tanks. The aluminum content of rainwater samples collected from corrugated iron sheet roof and aluminum roof catchments were higher than the WHO standard and NSDWQ.

Bacterial isolates from the rainwater samples in the storage vessels (Table 9) revealed the presence of *Pseudomonas aeruginosa, Escherichia coli, Klebsilla pneumoni, Entrobacter sp., Proteus mirabilis,* and *Vibro sp.* Apart from *E.coli* which is an indicator organism of fecal contamination, other organisms are disease causing pathogens. Moulds such as *Aspergillus sp.,* and *Penicillum sp. were* isolated from the stored rainwater samples (Table 10). The microbial organisms isolated from the collected and stored rainwater samples become very important when their health effects are considered [32-34]. The percentage occurrence of bacterial isolates associated with rainwater stored in the storage vessels as presented in Table 11 revealed that *Pseudomonas aeruginosa* had the highest occurrence rate of 1.7% in rainwater collected using stone coated roof catchment and stored in metal container.

Table 9. Bacterial isolates from therainwater samples in the storage vessels

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

Table 10. Identification of mould isolates from the storage vessels

Cultural and morphological characteristics	Microscopic characteristics	Possible mould isolates
Yellow or yellowish green colonies with distinct margin	Conidiophores arise from a footcell. Club shaped vesicles at top of the conidiophores. Conidia are found in chains	*Aspergillus sp.*
Fast-growing colonies in green colour with dense Conida	Branched conidiophores with chains of conidia looks like a brush	*Penicillum sp.*

Table 11. Percentage occurrence of bacterial isolates associated with rainwater stored in the storage vessels

		Bacterial isolates						
Rainfall catchment	**Storage containers**	***Pseudomonas aeruginosa***	***Escherichia coli***	***Klebsilla pneumonia***	***Entrobacter sp.***	***Proteus mirabilis***	**Vibro sp**	**Total**
	Plastic container	0(0.0)	0(0.0)	3(5.2)	0(0.0)	1(1.7)	1(1.7)	5(8.6)
	Metal tank	0(0.0)	0(0.0)	2(3.4)	0(0.0)	1(1.7)	0(0.0)	3(5.2)
Corrugated iron sheet roof	Earthen ware pot	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Block/cemented tank	0(0.0)	0(0.0)	0(0.0)	1(1.7)	2(3.4)	1(1.7)	4(6.9)
	Plastic container	0(0.0)	0(0.0)	0(0.0)	2(3.4)	0(0.0)	1(1.7)	3(5.2)
	Metal tank	0(0.0)	2(3.4)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	3(5.2)
Asbestos roof	Earthen ware pot	0(0.0)	0(0.0)	2(3.4)	0(0.0)	0(0.0)	0(0.0)	2(3.4)
	Block/cemented tank	0(0.0)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	1(1.7)	2(3.4)
	Plastic container	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(3.4)	0(0.0)	2(3.4)
	Metal tank	1(1.7)	2(3.4)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	4(6.9)
Stone coated roof	Earthen ware pot	0(0.0)	1(1.7)	0(0.0)	2(3.4)	1(1.7)	0(0.0)	4(4.9)
	Block/cemented tank	0(0.0)	2(3.4)	1(1.7)	0(0.0)	0(0.0)	1(1.7)	4(4.9)
	Plastic container	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(3.4)	0(0.0)	2(3.4)
	Metal tank	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	1(1.7)
Aluminum roof	Earthen ware pot	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Block/cemented tank	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1,7)	0(0.0)	1(0.0)
	Plastic container	0(0.0)	0(0.0)]	1(1.7)	0(0.0)	0(0.0)	0(0.0)	2(3.4)
	Metal tank	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(5.2)
	Earthen ware pot	0(0.0)	0(0.0)	1(1.7)	2(3.4)	0(0.0)	0(0.0)	4(4.9)
Thatched roof	Block/cemented tank	0(0.0)	]0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	2(3.4)
	Plastic container	0(0.0)	0(0.0)	2(3.4)	0(0.0)	1(1.7)	0(0.0)	2(3.4)
	Metal tank	0(0.0)	2(3.4)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	1(1.7)
Open environment	Earthen ware pot	0(0.0)	0(0.0)	3(1.7)	0(0.0)	0(0.0)	1(1.7)	3(5.2)
	Block/cemented tank	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	1(1.7)
	Total	1(1.7)9(15.5) 14(24.1)	8(13.8) 13(22.4) 13(22.4)	58(100)				

Escherichia coli had the highest occurrence rate of 3.4% in rainwater collected and stored in metal tanks, followed by its occurrence in earthen ware pot (1.7%). *Klebsilla pneumonia* had the highest occurrence rate of 5.2% in rainwater sample collected using corrugated iron sheet roof catchment and stored in plastic container. *Entrobacter sp* had the highest occurrence rate of 3.4% in rainwater samples collected from catchments such as asbestos roof and stored in the plastic container, stone coated roof and stored n earthen ware pot, and thatched roof and stored in earthen ware pot. *Proteus mirabilis* had the highest occurrence rate of 3.4 % in rainwater samples stored in plastic containers, followed by 1.7 % occurrence rate in rainwater samples stored in block/cemented tanks. *Vibro sp* had 1.7% highest occurrence rate in rainwater stored in plastic containers, metal tanks, and block/cemented tanks. In terms of total sum of occurrence rate for the stored bacterial organisms, *Klebsilla pneumonia, was the highest with 24.1%, then followed by Vibro sp* and *Proteus mirabilis* with the 22.4% each. *Aspergillus sp.* had an occurrence rate of 7.3 % in rainwater collected and stored in plastic container and metal tanks. *Penicillum sp.* had the highest occurrence rate 5.5% in rainwater stored in metal tank and plastic container, followed by 3,3% occurrence in rainwater stored in earthen pot. *Aspergillus sp.* had a sum total occurrence rate of60.0% against 40.0% overall occurrence of *Penicillum sp.*

Table 12. Percentage occurrence of mould isolates associated with rainwater stored in the storage vessels

		Mould isolate		
Rainfall catchment	**Storage containers**	***Aspergillus sp.***	***Penicillum sp.***	**Total**
Corrugated iron sheet roof	Plastic container	0(0.0)	0(0.0)	0(0.0)
	Metal tank	2(3.6)	0(0.0)	2(3.6)
	Earthen ware pot	0(0.0)	0(0.0)	0(0.0)
	Block/cemented tank	0(0.0)	0(0.0)	0(0.0)
Asbestos roof	Plastic container	3(5.5)	0(0.0)	3(5.5)
	Metal tank	3(5.5)	0(0.0)	3(5.5)
	Earthen ware pot	1(1.8)	0(0.0)	0(1.8)
	Block/cemented tank	0(0.0)	0(0.0)	0(0.0)
Stone coated roof	Plastic container	4(7.3)	1(1.8)	5(9.1)
	Metal tank	4(7.3)	3(5.5)	7(12.7)
	Earthen ware pot	3(5.5)	2(3.6)	5(9.1)
	Block/cemented tank	2(3.6)	2(3.6)	4(7.3)
Aluminum roof	Plastic container	1(1.8)	0(0.0)	1(1.8)
	Metal tank	0(0.0)	1(1.8)	1(1.8)
	Earthen ware pot	1(1.8)	0(0.0)	1(1.8)
	Block/cemented tank	0(0.0)	1(1.8)	1.(1.8)
Thatched roof	Plastic container	2(3.6)	3(5.5)	5(9.1)
	Metal tank	2(3.6)	3(5.5)	5(9.1)
	Earthen ware pot	1(1.8)	1(1.8)	2(3.6)
	Block/cemented tank	3(5.5)	2(3.6)	5(9.1)
Open environment	Plastic container	0(0.0)	0(0.0)	0(0.0)
	Metal tank	0(0.0)	1(1.8)	1(1.8)
	Earthen ware pot	0(0.0)	2(3.6)	2(3.6)
	Block/cemented tank	1(1.8)	0(0.0)	1(1.8)
	Total 33(60.0)	22(40.0)	55(100)	

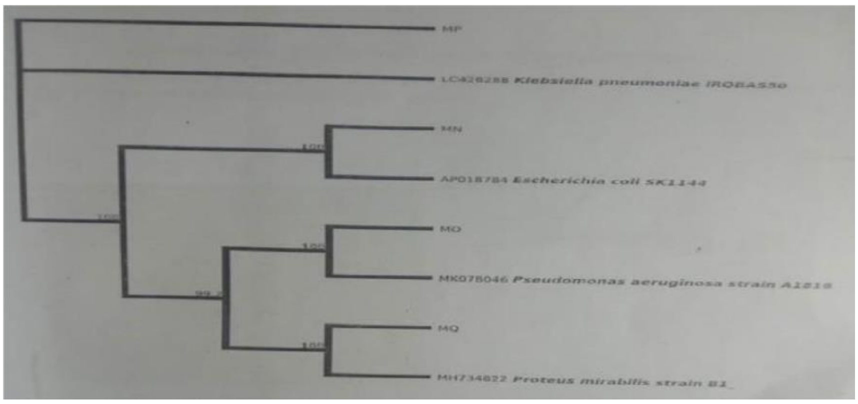

Plate 2. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

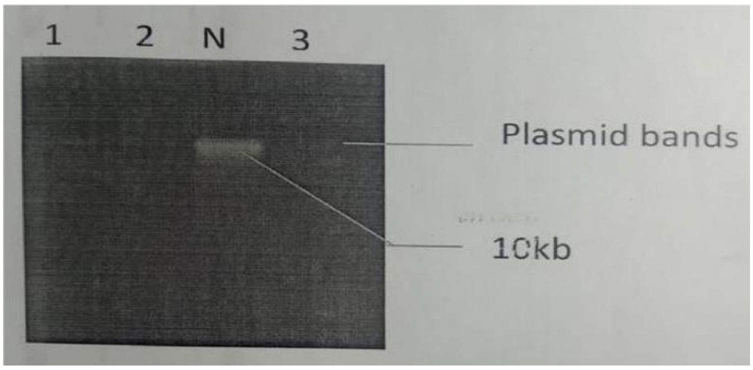

Plate 3. Agarose gel electrophoresis for the plasmid of the bacterial isolates. Lane N represents a 1 kb molecular ladder

The results of molecular identification study for the bacterial isolates with the 16S rRNA sequence from the isolates produced the exact match with similar sequences from NCBI non-redundant nucleotide database. The phylogenetic placement of the 16S rRNA of the isolates was in agreement with evolutionary distances after computation with jukes-Cantor methods. After the profiling (Plate 2), *E.coli, P. aeruginosa,* and *Enterobacter sp.* possessed plasmids and this made them resistant to the antibacterial drugs used.

4. CONCLUSION

The stored rainwater samples in the vessels were not affected much in terms of physicochemical parameters. Most of the stored rainwater samples in the storage vessels were collected using different catchments, but the investigated physicochemical parameters were not affected against the known standards except in few cases. However, the storage vessels exhibited more microbial impact than physiochemical impact on the stored rainwater samples. The molecular studies of the isolated bacterial organism revealed a plasmid possessed *E.coli, P. aeruginosa,* and *Enterobacter sp.* that are resistant to the antibacterial drugs. This study has shown the effect of different storage vessels on rainwater harvested from different rooftops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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