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# BACTERIOPLANKTON COMPOSITION AND DIVERSITY OF AWBA DAM RESERVOIR, SOUTHWESTERN NIGERIA

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**Abstract:** Bacterioplankton community composition in the Awba dam reservoir, a man-made lake located in the University of Ibadan, the first university on sub-Saharan region of West Africa was characterized using 16S rRNA gene clone library construction. Some physico-chemical characteristics of the reservoir were also evaluated. The mean nitrate concentration of the Awba reservoir exceeded the set limits. Bacterial 16S rRNA gene sequences from Awba stream and reservoir exhibited broad phylogenetic diversity, including sequences representing the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria, Actinobacteria, Bacteriodetes, Lentisphaerae, Firmicutes, Verrucomicrobia* and *Cyanobacteria.* Proteobacteria was the dominant phylum in the study area and members of *Betaproteobacteria* subdivision were the most abundant in all sampling sites. The Shannon Weiner (2.44) and Evenness (0.95) diversity indices of the reservoir was higher than lotic site. A remarkable portion of the operational taxonomic unit could not be classified into any known taxonomic unit. The study had contributed to the growing data set on the composition of bacteria communities in tropical freshwater ecosystem.

**Keywords**: Awba dam reservoir, bacterial community composition, 16S rRNA clone library, diversity indices, tropical freshwater lake

## INTRODUCTION

Man –made lakes (reservoirs) created by damming of rivers lead to accumulation of the freshwater that is used for drinking, irrigation and generation of hydroelectric power. These reservoirs, like natural lakes and rivers, also act as important sinks for organic material originating not only from their natural catchment but also from human activities. The resident microbiota actually consumes major proportions of such inflows (Pernthaler 2013), making them essential factors directly affecting stored water properties and quality. In a lacustrine ecosystem, microorganisms play a pivotal role in driving the biogeochemical processes (Debroas *et al*.,2009). The photosynthetic and chemolithotrophic microorganisms are the main contributor of the primary productivity. The chemoorganotrophic and chemoheterotrophic microorganisms are actively involved in the decomposition of organic matters (Fang *et al*., 2015).

Furthermore, appreciable amounts of microorganisms are the foodstuff for filter-feeding organisms and form the foundation of the aquatic food web (Wang *et al*., 2004). Bacterioplankton are considered as a good indicator of water quality because they are sensitive and respond rapidly to changes in the hydrology and water quality (Li *et al.,* 2016). The pelagic microbial communities in rivers adapt to changes in the concentration and composition of organic matter (Kirchman *et al*., 2004) and nutrients (Cebron *et al*., 2004). Due to their ecological and public health implications, the understanding of the diversity, composition, and structure of microbial communities is necessary for us to predict and/or experimentally determine their ecosystem functions (Liu *et al*., 2014). Freshwater planktonic microbiota studies have attracted much attention in a variety of habitats, especially in the cold temperate regions using culture-independent methods for mapping bacterial community composition (BCC) (including Lindström *et al*., 2005; Xing *et al*., 2009; Zhengui *et al*., 2012 and Li *et al*., 2016) and typical genotype clusters emerge from these studies. However, studies on the bacterioplankton community composition of freshwater in tropical systems which included Peduzzi and Schiemer 2004 in Sri Lanka; Petrucio *et al*., 2006 in Brazil; De Wever *et al*., 2008 in Zambia; Amado *et al*. 2013 in Amazonian rivers and lake; Anissi *et al*., 2014 in Morroco; Souffreau *et al*., 2015 in South America and Avila *et al*., 2016 in Brazil are limited especially in West Africa. De Wever *et al*. (2008) point out the need for more studies on microbial communities of freshwater ecosystem from tropical region to find out if differences occur between it and the clusters of bacteria from temperate region or not. Information on the microbial communities of freshwater in Nigeria are limited to those produced by culture-dependent method including Ogbuagu *et al*., 2012 ; Atobatele and Owoseni, 2012; Ayandiran *et al*., 2014 and Njoku *et al*., 2015.

There is a death of information on microbial communities of Awba dam reservoir, a manmade lake in the University of Ibadan, Nigeria used for water supply and fisheries. The lake is recently proposed to be used for ecotourism despite receiving waste water from living quarters of the university and science laboratories and run-offs from adjoining farmlands. Ajani *et al*., 2016 using culture dependent method determined total aerobic plate count, isolation and characterization of E. coli O157: H7 str. EDL933 strains. To identify the bacteria composition and diversity of the Awba dam reservoir with more accuracy and predict their ecosystem function, we

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investigated the microbial diversity using 16S rRNA gene clone library. This will enhance effective management of the reservoir and contribute to data on tropical bacterioplankton.

## MATERIALS AND METHODS

### Description of Study Area

Awba dam reservoir (Figure 1) is located at the University of Ibadan (latitude 7º27'3''N, 3º53'30''E; longitude 3º53'N and 3º54'E) at an altitude of 185m above sea level (Ajani *et al*., 2016). The Awba dam was constructed in 1974 at a point where Awba stream flowed through a natural valley. The dam is about 8.5m high, 110m long with a crest of about 12.2m. The reservoir has a maximum length of about 700m and a maximum depth of 5.5m with a surface area of about 6ha (Ajani *et al*., 2016). The water level remains mostly constant throughout the year and excess water made to spill away during raining season. The water of the reservoir is still with occasional multidirectional movement due to wind effect. Station 1 (S1) is located at the lotic part, the Awba stream, while Station 2(S2) is in Awba reservoir. All samples were collected in replicates.

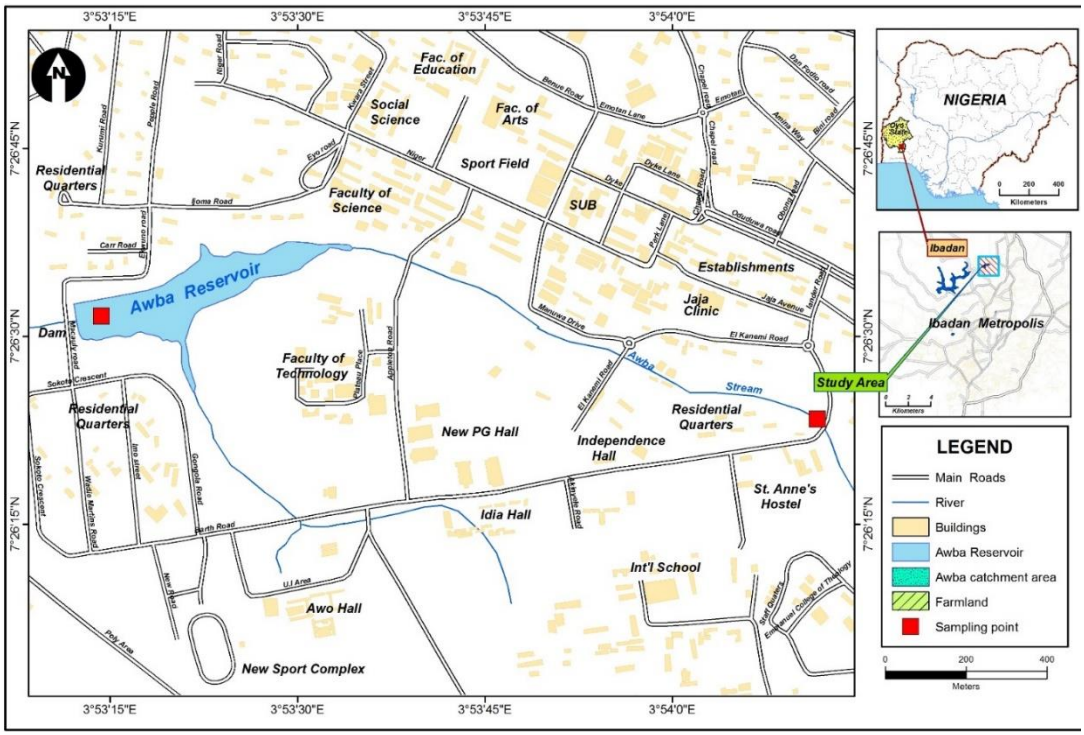


**Fig. 1.** Map of study area at University of Ibadan, Oyo State, Nigeria.

### Water Sample Collection and Analysis

Surface water samples (0 – 0.5 m) were collected in triplicate from each station in dry season using sterilized 1L polycarbonate bottles. The samples of water were kept on ice and transported to the laboratory. All water samples were processed within 4hours of collection. Water samples (200 ml) were passed through 5 µm pore size filters to remove larger plankton and particles. The filtrates were then passed through 0.22 µm pore size polycarbonate membrane filters (Millipore) to concentrate the bacterioplankton biomass (Zhengui *et al*., 2012). Filters were stored at -80°C until further analysed. Temperature of the surface water was determined using a mercury-in-glass thermometer. Unfiltered water samples were used to measure conductivity, total dissolved solids (TDS), phosphate, nitrate, $P^H$, and dissolved oxygen (DO) according to APHA (1998).

### DNA Extraction and Purification, PCR Amplification, Cloning and Sequencing

The total genomic DNA was purified using a soil DNA extraction kit (Q Biogene). It involved a bead beating step and filter that contained the concentrated bacteria cut into small pieces to represent the soil (Percent *et al*., 2008). Total extracted genomic DNA was quantified using spectrophotometry and the quality of the DNA was revealed through Agarose gel electrophoresis on 1% gels followed by ethidium bromide staining and visualization by UV transillumination (Percent *et al*., 2008). PCR amplification of the genes 16S rRNA of the obtained total genomic DNA was carried out using universal primer pair FD1(5'-AGAGTTTGATCCTGGCTCAG-3'), and RP2(5'ACGGCTACCTTGTTACGACTT-3') (Weisburg *et al*., 1991). Gel electrophoresis was used to determine the amplification products and molecular cloning of environmental 16S rRNA genes via TOPO cloning (Invitrogen) was done. The environmental 16S rRNA gene clone libraries were analyzed through the following: growing liquid cultures of the clone, PCR screening, RFLP analysis and presumed DNA preparation. All the above methods were adopted as previously described by Percent *et al*., 2008. Automated sequencing was accomplished at the sequencing facility MCLAB Molecular Cloning

Laboratories located in San Francisco, CA. Identification of each clone was by identifying its nearest neighbour using the sequence match and classifier tools at the Ribosomal Database Project (RDP) (http: //rdp.cme.msu.edu/) and identity up to genus level confirmed based on 97% sequence similarity score. Relative abundance of each taxonomic unit was also calculated using the Naive Bayesian assignment of RDP classifier. Average sequence length for the 158 representatives was 1052bp. Sequences were aligned with MUSCLE algorithm and phylogenetic analyses carried out with MEGA 5.05. Based on alignment analysis, phylogeny was inferred using the Maximum Likelihood method under a GTR substitution model (G+I) and validated by bootstrapping with 100 replicates. Midpoint rooting was used in forming trees. The evolutionary distances in the units of the number of base substitutions per site. Tree branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

### Statistical Analysis

Chao1 estimator (Chao, 1984), Shannon-Wiener diversity index (Shannon and Wiener, 1963), Pielou Evenness (Pielou, 1975) and Simpson index of diversity (Simpson, 1949) were used to analyze the community structure of the microbes. Community similarities were determined by Sorenson's coefficient (Sorenson, 1948). Student's t test was used to determine statistically significant difference in physico-chemical parameters of the reservoir.

## RESULTS

### Physico-chemical parameters

The temperature ranged between 22 – 28ºC with higher mean temperature (26.7 ± 1.15ºC) recorded in S2 (lentic site) Table 1. The pH was between 7.45 to 8.8. The conductivity was between 202 and 294 µs/cm, with higher mean value in S2 (275 ± 18.1 µs/cm). Higher mean TDS was recorded in S2 (125.3 ± 35.97 mg/L). The dissolved oxygen concentration during period of study was between 2.03 to 18.29 mg/L and mean DO of S1 (lotic site; 16.3 ± 2.88 mg/L) differed significantly (t=0.05; P≥ 0.05) from S2 (4.07± 2.88 mg/L). Higher mean nitrate concentration in S2 (25.5± 2.68 mg/L) which was threefold the concentration detected at S1(7.26 ± 1.91 mg/L) differed significantly (t=0.02; P= ≥ 0.05). The mean phosphate concentration was 2.56±0.18 mg/L in S2 and 1.44±0.59 mg/L in S1. The mean nitrate concentration of S2 exceeded the National Environmental Standards and Regulations Enforcement Agency (NESREA) acceptable limit.

**Table 1**

Physico-chemical Parameters of Awba reservoir and stream, southwestern Nigeria

| Physico-chemical Parameters | Awba stream | Awba reservoir | NESREA |
|---|---|---|---|
| Temperature (ºC) | 24.8 ± 2.47(22-26.5) | 26.7 ± 1.15(26-28) | 35.00 |
| $P^H$ | 7.85 ± 0.20(7.7 -8.1) | 7.9 ± 0.62(7.5 -8.8) | 6.50 – 7.50 |
| Dissolved Oxygen(mg/L) | 16.3 ± 2.88(14.2-18.3) | 4.07 ± 2.88(2.0 – 6.1) | 7.50 |
| Conductivity(µs/cm) | 225 ± 30.8(202-260) | 275± 18.1(258-294) | N/A |
| Total Dissolved Solids(mg/L) | 106.1± 29.8(66.4-138) | 125.3± 35.97(74.8-156) | N/A |
| Nitrate(mg/L) | 7.26 ± 1.91(5.91-8.61) | 25.5 ± 2.68(23.6-27.4) | 10.00 |
| Phosphate(mg/L) | 1.44 ± 0.59(1.02-1.86) | 2.56 ± 0.18(2.43-2.69) | <05.00 |

### Bacterial Community Compositions in Awba Reservoir and Stream

A total of 191 16S rRNA gene clones were obtained from the libraries of bacteria from the study area (S1, 95 clones; S2, 96 clones). Of these clones, 158 (S1=80; S2=78) representatives of each set of distinct same-length band were chosen as Operational taxonomic Units (OTUs) and 156(S1=80, 100%; S2=76, 97%) were sequenced.

The identified sequenced clones belong to Proteobacteria, Actinobacteria, Bacteriodetes, Lentisphaerae, Spirochaetes, Acidobacteria, Firmicutes, Chloroflexi, Cyanobacteria, Verrucomicrobia and Planctomycetes divisions (Figures 2a and b). The contribution of these bacterial groups and subgroups to the sequenced OTUs is shown on Table 2. The bacterial community from the S1 contained members within 17 classes compared to 12 classes in S2. The bacterial groups that were most encountered in the clone libraries were Proteobacteria, Bacteriodetes, Actinobacteria and Firmicutes. The Proteobacteria dominated in both sampling stations (S1:57.5%; S2:63.12%). The Betaproteobacteria was the most dominant subdivision of the Proteobacteria in both stations (S1=63.04%; S2= 43.75%) Figure 3. In Station1, the Betaproteobacteria OTUs mainly belonged to Burkholderiaceae, Rhodocyclaceae, Comamonadaceae and Methylophilaceae (Table 3) and the OTUs were closely related to *Zoogloea* Itzigsohn 1868, *Polynucleobacter* Heckmann and Schmidt 1987, *Cupriavidus* Makkar and Casida 1987, *Limnohabitans* Hahn et al. 2010 and *Hydrogenophaga* Willems et al. 1989. In Station 2, members of Betaproteobacteria OTUs encountered belonged to Rhodocyclaceae (*Zoogloea*), Comamonadaceae (*Limnohabitans*), Neisseriaceae (*Vogesella* Grimes et al. 1997) and Burkholderiales_incerte_sedis (*Ideonella* Malmqvist et al. 1994) Table 4.

The Alphaproteobacteria were more abundant in the clone library of Station 2(23.7%) and the OTUs were closely related to *Caulobacter* Henrici and Johnson 1935, *Azospirillum* Tarrand et al. 1979 and *Methylobacterium* Green and Bousfield 1983. However, in S1, the Alphaproteobacteria OTUs were related closely to *Methylobacterium, Methylocystis* Bowman et al. 1993 and *Novosphingobium* Takeuchi et

al. 2001. The Gammaproteobacteria had similar contribution to the two clone libraries (S1= 7.5%; S2= 6.58%) with sequences closely related with *Legionella* Brenner et al. 1979. The Deltaproteobacteria group had OTUs closely related to *Desulfobulbus* Widdel 1981 and *Anaeromyxobacter* in S1 and *Bacteriovorax* Baer et al. 2000 in S2. Bacteriodetes was the second predominant phylum and constituted 16.25% and 23.68% of the bacterial communities in S1 and S2 respectively. In S2, members of class Flavobacteria were more dominant in phylum Bacteriodetes and were closely related to *Flavobacterium* Bergey et al. 1923. However, in S1, members of classes Sphingobacteria, Bacteroidia and Bacteriodetes_incertae_sedis were equally encountered. The OTUs were closely related to *Paludibacter* Ueki et al. 2006, *Terrimonas* Xie and Yokota 2006 and *Flavobacterium*. The Firmicutes were observed more in S1 with sequences closely related to *Bacillus* Cohn, 1872 and *Pediococcus* Claussen 1903. The phylum Actinobacteria had greater occurrence in S1 (6.25%) and was closely related to *Ilumatobacter* Matsumoto et al. 2009. The remaining bacterial groups were encountered occasionally. Spirochaetes, Acidobacteria, Chloroflexi, Cyanobacteria and Verrucomicrobia were only observed in station 1 while Planctomycetes occurred only in 2.

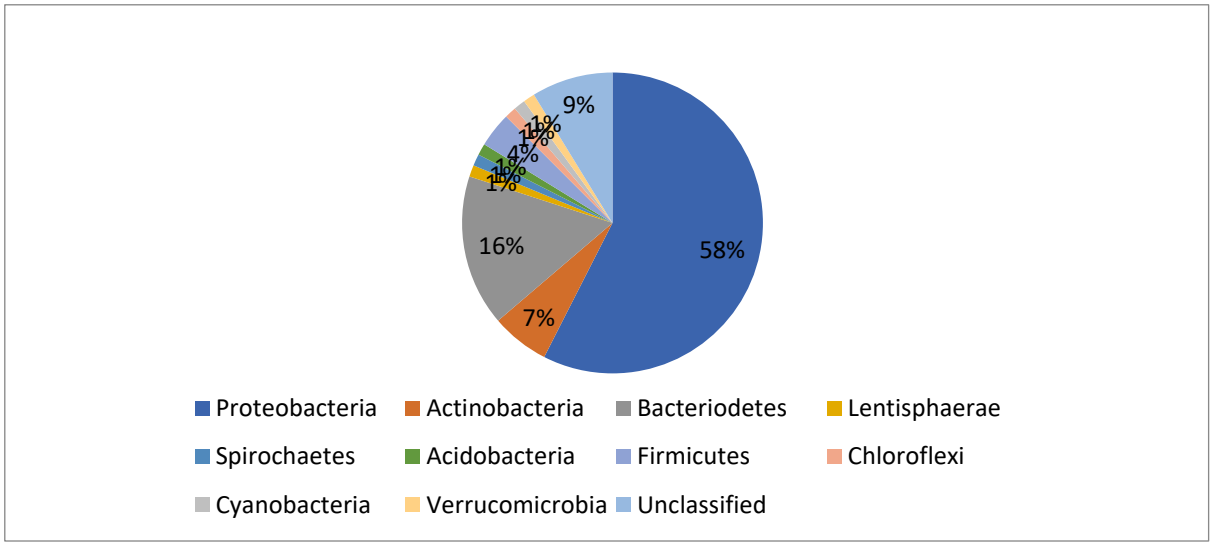


**Fig. 2. a** Percentage (%) contribution of different bacterial divisions to Awba stream (Control site, Station 1) Clone Library.

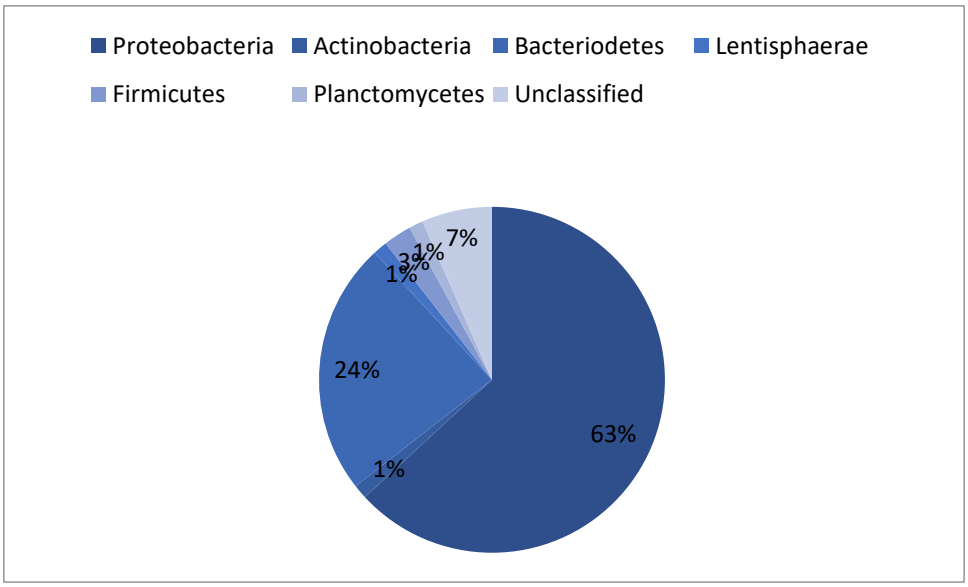


**Fig. 2. b** Percentage (%) contribution of different bacterial divisions to Awba Reservoir (Station 2) Clone Library.

**Table 2**

Relative abundance of clones (clone percentage in the community) in Awba Stream and Reservoir, southwestern Nigeria

| Phylum/Class | Number of Clone (%) | |
|---|---|---|
| **Phylum Proteobacteria** | **Awba Stream** | **Awba Lake** |
| Class Alphaproteobacteria | 6(7.5) | 18(23.68) |



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| | | |
|---|---|---|
| Class Betaproteobacteria | 29(36.25) | 21(27.6) |
| Class Gammaproteobacteria | 6(7.5) | 5(6.58) |
| Class Deltaproteobacteria | 5(6.25) | 4(5.26) |
| **Phylum Actinobacteria** | | |
| Class Actinobacteria | 5(6.25) | 1(1.32) |
| **Phylum Bacteriodetes** | | |
| Class Sphingobacteria | 3(3.75) | 4(5.26) |
| Class Flavobacteria | 3(3.75) | 12(15.79) |
| Class Bacteroidia | 5(6.25) | 2(2.63) |
| Class Bacteriodetes_incertae_sedis | 2(2.5) | ___ |
| **Phylum Lentisphaerae** | | |
| Class Lentisphaeria | 1(1.25) | 1(1.32) |
| **Phylum Spirochaetes** | | |
| Class Spirochaetes | 1(1.25) | ___ |
| **Phylum Acidobacteria** | | |
| Class Holophagae | 1(1.25) | ___ |
| **Phylum Firmicutes** | | |
| Class Bacilli | 2(2.5) | 1(1.32) |
| Class Clostridia | 1(1.25) | 1(1.32) |
| **Phylum Chloroflexi** | | |
| Class Anaerolinae | 1(1.25) | ___ |
| **Phylum Cyanobacteria** | | |
| Class Chloroplast | 1(1.25) | _ |
| **Phylum Verrucomicrobia** | | |
| Class Verrumicrobia | 1(1.25) | ___ |
| **Phylum Planctomycetes** | | |
| Class Planctomycetacia | ___ | 1(1.32) |
| **Unclassified** | 7(8.75) | 5(6.58) |

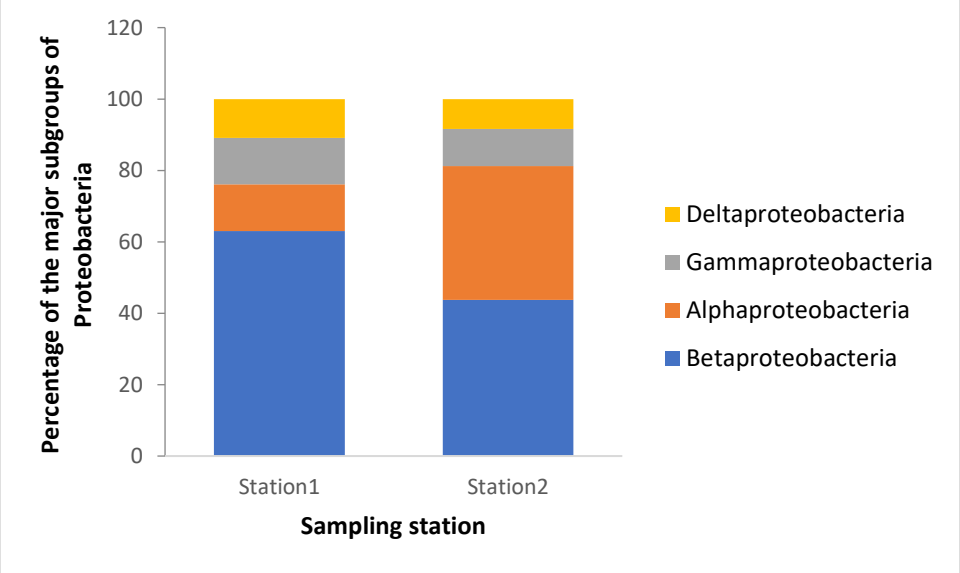


**Fig. 3.** Percentage contribution of different subgroups of phylum Proteobacteria in Awba stream (Station 1) and reservoir (Station 2).

**Table 3**

Major bacterioplankton taxa encountered in Awba Stream (27/73 clones).

| Phylum | Class | Family | Genus/Species | Acc. No | Sequence similarity |
|---|---|---|---|---|---|
| Acidobacteria | Holophagae | Holophagaceae | *Geothrix* sp. Coates *et al*. 1999 | U009352515 | 100% |
| Proteobacteria | Alphaproteobacteria | Methylobacteriaceae | *Methylobacterium* | U009352516 | 100% |
| Proteobacteria | Betaproteobacteria | Methylophilaceae | *Methylotenera* Kalyuzhnaya *et al*. 2006 | U009352517 | 100% |
| Firmicutes | Bacilli | Bacillaceae 1 | *Bacillus* | U009352518 | 100% |
| Bacteriodetes | Bacteroidia | Porphyromonadaceae | *Paludibacter* | U009352521 | 100% |

| | | | | | |
|---|---|---|---|---|---|
| Bacteriodetes | Sphingobacteria | Chitinophagaceae | *Terrimonas* | U009352526 | 98% |
| Proteobacteria | Gammaproteobacteria | Legionellaceae | *Legionella* | U009352530 | 97% |
| Proteobacteria | Betaproteobacteria | Burkholderiaceae | *Polynucleobacter* | U009352531 | 100% |
| Proteobacteria | Deltaproteobacteria | Desulfobulbaceae | *Desulfobulbus* Widdel 1981 | U009352532 | 100% |
| Proteobacteria | Betaproteobacteria | Burkholderiaceae | *Cupriavidus* | U009352537 | 100% |
| Proteobacteria | Alphaproteobacteria | Methylocystaceae | *Methylocystis* | U009352539 | 100% |
| Proteobacteria | Betaproteobacteria | Burkholderiaceae | *Polynucleobacter* | U009352541 | 100% |
| Actinobacteria | Actinobacteria | Acidimicrobiales | *Illumatobacter* | U009352542 | 99% |
| Bacteriodetes | Bacteroidia | Porphyromonadaceae | *Paludibacter* | U009352545 | 100% |
| Proteobacteria | Betaproteobacteria | Comamonadaceae | *Limnohabitans* | U009352548 | 100% |
| Proteobacteria | Deltaproteobacteria | Desulfobulbaceae | *Desulfobulbus* | U009352550 | 100% |
| Bacteriodetes | Flavobacteria | Flavobacteriaceae | *Flavobacterium* | U009352552 | 100% |
| Proteobacteria | Alphaproteobacteria | Methylobacteriaceae | *Methylobacterium* | U009352555 | 100% |
| Proteobacteria | Alphaproteobacteria | Sphingomonadaceae | *Novosphingobium* | U009352557 | 97% |
| Proteobacteria | Betaproteobacteria | Rhodocyclaceae | *Zoogloea* | U009352559 | 100% |
| Bacteriodetes | Bacteroidia | Porphyromonadaceae | *Paludibacter* | U009352565 | 100% |
| Firmicutes | Bacilli | Lactobacillaceae | *Pediococcus* | U009352566 | 100% |
| Protobacteria | Betaproteobacteria | Comamonadaceae | *Hydrogenophaga* | U009352571 | 100% |
| Proteobacteria | Betaproteobacteria | Rhodocyclaceae | *Zoogloea* | U009352573 | 99% |
| Bacteriodetes | Bacteroidia | Porphyromonadaceae | *Paludibacter* | U009352576 | 99% |
| Proteobacteria | Betaproteobacteria | Rhodocyclaceae | *Zoogloea* | U009352580 | 100% |
| Proteobacteria | Deltaproteobacteria | Cystobacteraceae | *Anaeromyxobacter* | U009352587 | 100% |

**Table 4**

Major bacterioplankton taxa encountered in Awba Lake (20/71 clones).

| Phylum | Class | Family | Genus | Acc. No | Sequence similarity |
|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Caulobacteraceae | *Caulobacter* | U009352456 | 100% |
| Proteobacteria | Betaproteobacteria | Burkholderiales_incerte_sedis | *Ideonella* | U009352457 | 93% |
| Bacteriodetes | Flavobacteria | Flavobacteriaceae | *Flavobacterium* | U009352458 | 100% |
| Proteobacteria | Betaproteobacteria | Rhodocyclaceae | *Zoogloea* | U009352460 | 100% |
| Proteobacteria | Betaproteobacteria | Comamonadaceae | *Limnohabitans* | U009352462 | 100% |
| Proteobacteria | Deltaproteobacteria | Bacteriovoracaceae | *Bacteriovorax* Baer et al. 2000 | U009352471 | 99% |
| Proteobacteria | Alphaproteobacteria | Sphingomonadaceae | *Novosphingobium* | U009352472 | 100% |
| Bacteriodetes | Flavobacteria | Flavobacteriaceae | *Flavobacterium* | U009352473 | 100% |
| Bacteriodetes | Bacteroidia | Porphyromonadaceae | *Paludibacter* | U009352475 | 99% |
| Firmicutes | Bacilli | Bacillaceae 1 | *Bacillus* | U009352477 | 100% |
| Proteobacteria | Alphaproteobacteria | Caulobacteraceae | *Caulobacter* | U009352478 | 100% |
| Proteobacteria | Alphaproteobacteria | Caulobacteraceae | *Caulobacter* | U009352480 | 100% |
| Proteobacteria | Alphaproteobacteria | Rhodospirillaceae | *Azospirillum* | U009352484 | 98% |
| Proteobacteria | Betaproteobacteria | Neisseriaceae | *Vogesella* | U009352485 | 100% |
| Proteobacteria | Betaproteobacteria | Comamonadaceae | *Limnohabitans* | U009352486 | 100% |
| Proteobacteria | Betaproteobacteria | Neisseriaceae | *Vogesella* | U009352488 | 100% |
| Proteobacteria | Betaproteobacteria | Rhodocyclaceae | *Zoogloea* | U009352489 | 100% |
| Proteobacteria | Alphaproteobacteria | Methylobacteriaceae | *Methylobacterium* | U009352493 | 100% |
| Bacteriodetes | Flavobacteria | Flavobacteriaceae | *Flavobacterium* | U009352495 | 100% |
| Bacteriodetes | Flavobacteria | Flavobacteriaceae | *Flavobacterium* | U009352499 | 100% |

The phylogenetic relationship between the sequences derived from Awba stream and Awba Lake is shown on Figure 4. The sequences between the two stations clustered and the phylogenetic tree was cut into major clusters (figure 4: dashed line), each with >90% bootstrap values at the ancestor nodes, showed some pattern of inter-relatedness of sequences recovered from the stations. Cluster Aw1, comprising just 16 sequences (3from S1 ; 13 from S2) was a mix-bag of 10 bacterial families from 9 different classes, with only *Limnohabitans, Fluviicola* O'Sullivan et al. 2005 and *Paludibacter* Ueki et al. 2006 confidently assigned at genus level. Cluster Aw2, comprising 12 sequences (8 from S1; 4 from S2) was a mainly phylum Proteobacteria–driven group, with *Vogesella* and *Novosphingobium* identified to genus. Each of these sequences predicted to be close-related within the tree belonged to different families of Proteobacteria. Cluster Aw3, with its 22 sequences clustered (7from S1; 15 from S2) has some similarity to Aw2, but with increased Proteobacteria dominance and very visible Bacteroides content.

The largest grouping of sequences based on the cut tree level used, Aw4, comprising 69 sequences( 39 from S1; 30 from S2), is a Proteobacteria and Bacteroidetes–dominated cluster interspersed with very few Actinobacteria (mainly Actinomycetales family) and Firmicutes (mainly Bacillaceae). The fusion of

these sequences, from 27 families and 8 classes, reflected the dominance of Alpha and Betaproteobacteria classes which makes up half of the sequences within the cluster, and the relative composition in the entire water body itself.

Furthermore, in these major clusters, specific genera were consistently and confidently (>97% similarity) identified often. These included *Flavobacterium* and *Paludibacter* genera from Bacteroidetes; *Methylobacterium*, *Caulobacter*, *Azospirillum* and *Novosphingobium* were the oft-identified genera of Alphaproteobacteria; *Limnohabitans, Hydrogenophaga*, *Vogesella* and *Curvibacter* for Betaproteobacteria and *Bacillus* for Firmicutes phylum. Comamonadaceae, Cryomorphaceae, Burkholderiales_incertae_sedis, Methylobacteriaceae and Flavobacteriaceae were the most common families. Interspersed between these major clusters are sequences showing the longest branch lengths from ancestors and most variations in the entire phylogenetic tree (starred in tree). The best assigned among these were from genera *Ideonella* and *Zoogloea*. Also, interspersed here are very small pairs of sequences which generally mirrored the major clusters.

The two stations had large proportions of clones (S1=63%; S2=72%) with low similarity to known sequences on the database. It is generally accepted that at least 97% similarity at genus level and at least 95% at family level is needed. The Shannon Weiner and Evenness indices were higher in S2 (H=2.44, E=0.95) while the Chao 1 estimator and Simpson index obtained in S1 ($S$ = 51.7, D = 13.95) were higher (Table 5). Sorenson's coefficient used for determining community similarities was 0.44 indicating quite a bit of species overlap/similarity between the stations.

**Table 5**

Diversity indices of bacterioplankton in the Awba dam reservoir

| Diversity Index | Awba stream | Awba dam reservoir |
|---|---|---|
| Shannon H | 1.87 | 2.44 |
| Evenness | 0.64 | 0.95 |
| Simpson D | 13.95 | 10 |
| CHAO 1 | 51.7 | 21 |

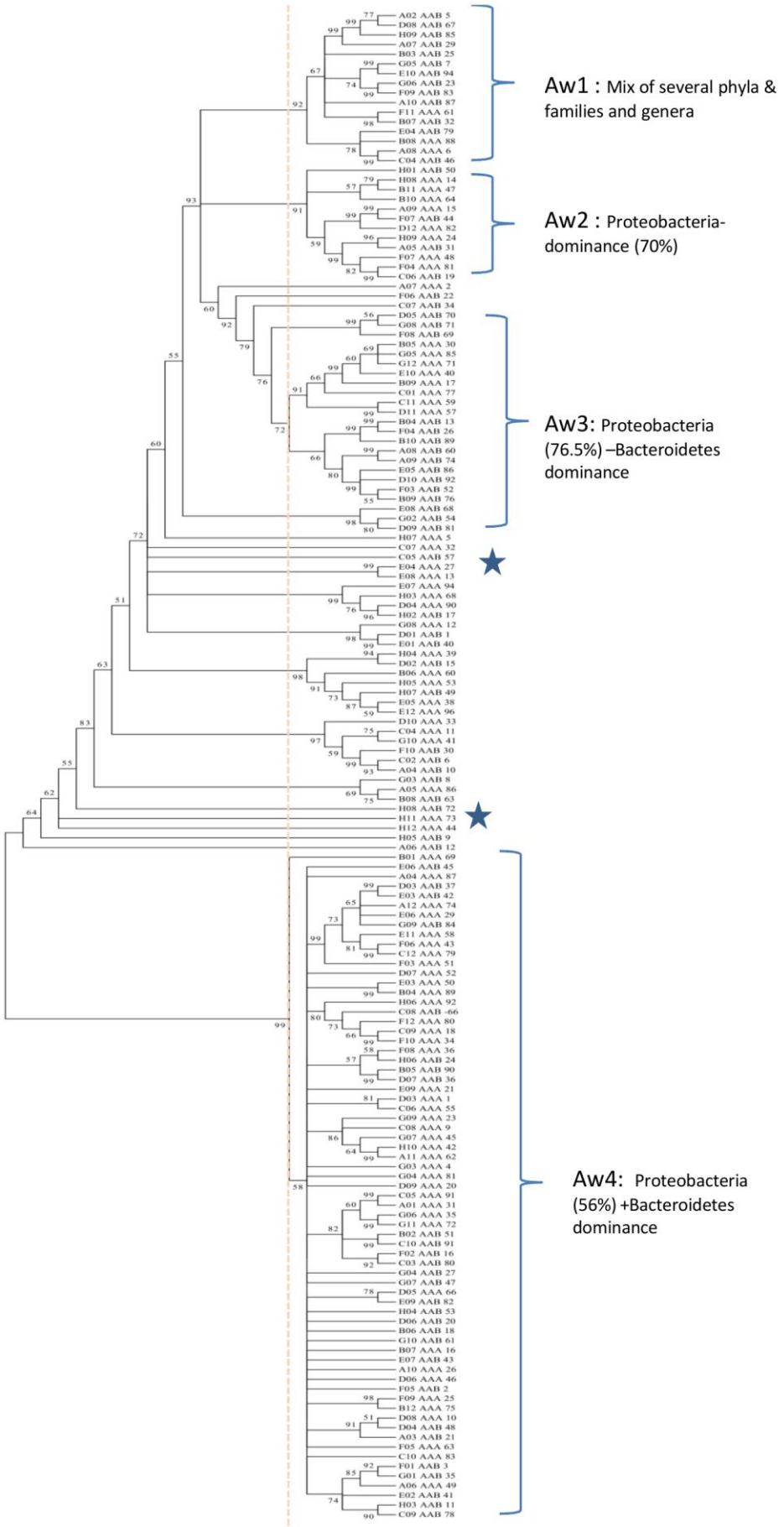


**Fig. 4 ESM_1.tiff** Phylogenetic tree (bootstrap consensus) of bacterioplankton sequences recovered from Awba dam reservoir, Ibadan, southwest Nigeria. Branches corresponding to partitions reproduced in less than 50% bootstrap

replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. Scale shows number of base substitutions per site. For example, A02 AAB5 identifies A02 as second plate and AAB5 as clone 5 in Awba stream (control site); A08 AAA6 identifies A08 as eight plate and AAA6 as clone 6 in the Awba reservoir

## DISCUSSION

Most of the physico-chemical parameters of the study area met the requirements of NESREA except the nitrate concentration of Awba reservoir that exceeded the acceptable limit and instances of low DO (2.03 mg/L), suggests organic pollution by wastewater received from residences and laboratories and run-off from farm lands. According to Ogunfowokan *et al*., 2006, runoff and organic matter decomposition in surface water produced inorganic nutrients such as ammonia, nitrate and phosphates with resultant effects of eutrophication and other serious ecological impairments of such water body. High nitrate levels in water have serious health implications and can result in several diseases. At concentration levels greater than 10 mg/L, there is an increased risk of babies developing infant methaemoglobinaemia, a disease commonly known as 'blue baby' syndrome (Canter, 1996).

In the lotic and lentic sites, six and four phyla, respectively formed less than 10% of the bacterial community while, two phyla constituted over 70%(stream) and 85%(reservoir). The phylogenetic diversity of the minority bacterial group been higher might be of ecological significance in the evolution of Awba stream and reservoir. Fang *et al.*, 2015 made similar observation on BCC of Lake Sayram. Other previous investigations that showed low abundance bacteria played important roles in the biogeochemical cycles include Bodelier *et al*., 2013and Hausmann *et al*., 2016. The 8.75% (stream) and 6.58% (reservoir) of the 16SrRNA clones that were not classified into any phylum under the Bacteria domain and the >60%(stream) and >70%(reservoir) of the clones that showed low similarity (< 97% similarity) to any known genus points to large number of unknown pelagic bacteria in the parent stream and reservoir. This further confirmed the need for more research to be carried out on the BCC of the tropical freshwater and southern hemisphere to know whether regional differences occur in freshwater bacteria. Similar observation of about 50% of the OTUs detected in Lake Tanganyika not been closely related to genotypes previously detected in freshwater environment occurred (De Wever *et al*., 2008).

The dominance of Proteobacteria and Bacteroidetes in the BCC of Awba stream and reservoir agreed with previous freshwater studies including Zwart *et al*., 2002 and Van der Gucht *et al.*, 2005. However, the proportion of dominant populations were different between the two libraries e.g. class Alphaproteobacteria formed 25.4% and 7.8% of Awba stream and reservoir, respectively; class Actinobacteria formed 6.3% and 1.4% of stream and reservoir; class Flavobacteria formed 3.8% and 16.9% of stream and reservoir, respectively. This variation in the bacterioplankton community composition of the Awba stream and reservoir reflect the differences in their ecosystems. This corroborate the findings of Yan *et al*., 2015, whose results indicated that bacterioplankton communities were both taxonomically and functionally different between backwater and riverine sites of the three gorge reservoirs.

According to Debroas *et al*. (2009), the taxa occupying bigger proportion in bacterial communities are correlated to the metabolic properties, thus Proteobacteria (*Zoogloea*, *Caulobacter*, *Polynucleobacter*, *Limnohabitans*, *Methylobacterium*) and Bacteroidetes (*Paludibacter*, *Flavobacterium*) that were frequently encountered could play important roles in metabolism of the Awba stream and reservoir. The genus *Zoogloea* (encountered frequently in both sites) occur free living in organically polluted fresh water and waste water at all stages of treatment and has been suggested as an indicator organism for these environments. *Zoogloea* sp. has ability to lower biochemical oxygen demand in activated sludge system by as much as 72 per cent within a 3-hr period (Butterfield, 1935). *Zoogloea ramigera* Itzigsohn 1868 is used in the biosorption of metals in water sources. The low DO and the high nitrate concentration of the Awba reservoir also suggests organic pollution which may have favored the growth of *Zoogloea*. The genus *Polynucleobacter* maintains both free-living and symbiotic ecotypes with an apparently ubiquitous distribution in freshwater ecosystems. These bacteria comprise <1% to 70% (on average about 20%) of total bacterioplankton cells in various freshwater habitats. Fang *et al*., 2014 stated that *Polynucleobacter* possesses genes related to the degradation of pesticides used in agriculture. Though the reason for the apparent absence/ non-detection of *Polynucleobacter* in the reservoir during study period is not known, according to Sangwan *et al*., 2016 metabolic differences in energy metabolism, substrate utilization, and the concentration of available nutrients (e.g., ammoniacal nitrogen) seem to have a stronger influence on the *Polynucleobacter* population structure. *Limnohabitans* is a recently established betaproteobacterial genus and is a group of environmentally important ''not-easily cultivable'' freshwater bacteria from the BetI lineage (Hahn *et al*., 2010). This nitrogen cycling microbe represents one of the most important bacteria groups in many freshwater habitats (Zeng *et al*., 2012). *Caulobacter* sp. encountered only in the Awba reservoir during study period plays an important part in biogeochemical cycling of organic nutrients. *Methylobacterium* spp. are normal inhabitants of drinking water distribution systems and buildings, including hospitals. Further, a substantial proportion of *Methylobacterium* spp. isolates are chlorine-resistant, form biofilm, resistance to high temperature and belong to the group of amoeba-resisting bacteria of drinking water (FalkinhamIII *et al*., 2016). *Methylobacterium* species are a cause of health care-

associated infections, including infections in immunocompromised hosts (Kovaleva *et. al*., 2014).

The genus *Flavobacterium* were encountered more in Awba reservoir than lotic site and according to Starliper (2011), the genus *Flavobacterium* (Bacteriodetes) has both non-pathogenic and pathogenic species including *Flavobacterium columnare* (Bernardet and Grimont 1989) (typically occurs in warm waters, 20°C –25°C) which cause fish diseases known as columnaris disease. Groundwater that was contaminated with arsenic also contained the genus *Flavobacterium* (Paul *et al*., 2015). Although there is little or no study on fish disease in Awba dam reservoir, Adeogun *et al*., 2016 reported that the sediment was significantly contaminated with arsenic compared to the reference site. *Paludibacter* is Gram-negative, strictly anaerobic, chemoorganotrophic and non-motile genus (Ueki *et al*., 2006). Other taxa encountered in the bacterial community composition of the study area include *Cupriavidus* spp., which are ubiquitous environmental organisms that are mostly found in soil, in water, and on plant. *Cupriavidus* species are environmental gram-negative, non-fermentative bacilli of low virulence but can cause infection related to contaminated infusates or in immunocompromised hosts, including transplant recipients and patients with HIV infection or leukemia (Steinberg and Burg, 2015). Resistance to metals by some species has been described, however the low number encountered in the lotic part may not implicate heavy metal pollution. *Legionella* occur commonly in soil and bodies of fresh water and is the bacteria known to cause legionellosis or Legionnaires' Disease. In those environments they generally pose little threat because natural conditions (bacteria eaters, UV light, etc) help to keep their colonies at safe levels. *Legionella* becomes an issue when they infiltrate a manmade water system that presents the conditions necessary for amplification and aerosolization (Field *et al*., 2002). *Bacillus* species can be obligate aerobes, or facultative anaerobes. *Bacillus* includes both free-living and parasitic pathogenic species. Bacilli cause an array of infections from ear infections to meningitis, and urinary tract infections to septicemia. Mostly they occur as secondary infections in immunodeficient hosts or otherwise compromised hosts. *Bacillus* spp. produce a variety of bioactive metabolites that exert antagonistic actions against pathogens. *Pediococcus* are facultative anaerobes, Gram -positive lactic acid bacteria and homofermentative (Todar, 2015). They are native to plant materials and fruits and ferments sugar.

The phyla that are encountered only in the Awba stream include Chloroflexi and the OTUs belonged to class Anaerolinae, an anaerobe consisting a group of physiologically diverse filamentous chemo-organotrophic bacteria (Yamada *et al*. 2006). *Anaerolineae* had been identified as one of the core populations, and for most of the cases, the dominating proportion of anaerobic digestive systems (Narihiro *et al*., 2012). Verrucomicrobia is found in a variety of environments, some species are acidophilic, aerobic methanotroph; some are capable of degrading diverse polysaccharides and fixing nitrogen. Recently isolated members are thermoacidophilic methylotrophs (Hedlund, 2010). The cyanobacterial phylum encompasses oxygenic photosynthetic prokaryotes of a great breadth of morphologies and ecologies; they play key roles in global carbon and nitrogen cycles. The phylum Spirochaetes consists of a large group of motile bacteria which are widespread in the environment and are highly prevalent disease-causing agents . They can also be free-living or host-associated, pathogenic or non-pathogenic, and aerobic or anaerobic (Paster, 2011).

Sequence clustering further established specific importance of a few alpha - and betaproteobacteria genera and families as a driving force at the sampling sites. The sequences in Aw1despite being from varied lineages, showed enough relatedness to be grouped together with relatively-high confidence. This may be an indication of possible genetic exchange, DNA transfer and speciation events in such an environment. This unexpected deep diversity in Aw1 clustered sequences may make them worthy of further research, along with sequences having the longest branch lengths, to determine the novelty of their classification lineage or speciation-level.

The diversity indices indicated that the Awba reservoir showed higher diversity than lotic part and this agreed with the previous studies, Dumestre *et al*., 2001and Yan *et al*., 2015. They suggested that the lake-like conditions characterized by low flow velocity, low turbidity and greater nitrogenous nutrients may support a greater variety of bacterioplankton lineages. The degree of similarities of the bacterial community of lotic and lentic parts suggests that apart from receiving microbes from parent waterbody, other sources (e.g. physico-chemical parameters) contributed to structuring the BCC of the Awba reservoir.

## CONCLUSIONS

This preliminary analysis of the bacterial composition and diversity of Awba stream and reservoir using 16S rRNA clone library revealed biogeochemically significant bacteria and disease-causing agents to aquatic and terrestrial organisms including human. We recommend that the University authorities should provide effluent management systems at the residential quarters and laboratories to forestall further degradation of the environment and to prevent public health epidemics. A remarkable portion of the operational taxonomic unit could not be classified into any known taxonomic unit, thus further research may be required to determine the novelty of their classification lineage.

## AUTHORS' CONTRIBUTION

Conceptualization, A.A.A. and S.A.N.; methodology, S.A.N.; data collection A.A.A.; data validation, A.A.A. and S.A.N.; data processing A.A.A.; writing—original draft preparation, A.A.A.; writing—review and editing, A.A.A.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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