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Research Article

Comparative Analysis of Microbial Load of Water in Emelda, Boys, Girls, and Post Graduate Hostel of Nnamdi Azikiwe University

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Abstract

Water is a clear, colorless, tasteless liquid that is composed of hydrogen and oxygen. Water in its natural form contains a number of microbes and pollutants due to industrialization and other anthropogenic activities; the purity of water is critically threatened because of the increasing relevance of these factors. Water quality is crucial to human health and therefore the necessity of water analysis is felt desirable. The main aim of this research is to make a comparative analysis of water from Post graduate, Emelda, Girls and Boys hostel of Nnamdi Azikiwe University. Water samples were collected from four different hostels and analyzed using standard culture methods. The samples were cultured on Nutrient Agar, Eosin Methylene Blue Agar, *Salmonella Shigella* Agar, Sabouraud Dextrose Agar using pour plate method. Seven different isolates were cultured and they include *Escherichia spp*, *Pseudomonas spp, Klebsiella spp, Enterobacter spp, Salmonella spp,* and *Aspergillus spp*. Total bacteria count, total coliform count and total fungi count were also analyzed on the water samples. The isolated bacteria were identified using biochemical tests which includes; Catalase test, citrate test, indole test, motility test, and gram Staining was also carried out. This research showed that the microbial load was highest in girls' hostel water, followed by the Emelda hostel and post graduate hostel. Boys' hostel had the lowest microbial load. This clearly shows that the water samples from these hostels are contaminated and they are unsafe for consumption. There is a need for improved sanitation and also the public should be educated on the importance of water quality monitoring in order to prevent contamination.

Keywords: Microbial Load; Water; Hostel; Bacteria

Introduction

Following the theory of creation, it is clear that water is as old as man. From time immemorial, man has resorted to the use of this unique commodity for domestic and other purposes. Water covers approximately 71% of Earth's surface, with continents and Islands accounting for the remaining 29% [1]. Water is a very essential commodity. It influences the survival of all living things. The importance of water has made it to be regarded as one basic necessity of life [2]. Water as defined by the English dictionary (2016) is a substance of molecular formula H_20 found at room temperature and pressure as a clear liquid. Water is a basic necessity of life; in the absence higher animals survive only but a few hours or days. It has several uses such as washing, cooking, and swimming and among others. Out of these uses, drinking water seems to be the most sensitive as it could have a direct deleterious impact on the health of humans according to the World Health Organization (2018).

Water that is consumed should be from clean, safe, and healthy sources. This implies that people should have access to safe and potable water for consumption from improved sources and within considerable walking distance, obtaining water that is of good quality and in sufficient quantity from sources of water supply [3]. Perhaps the greatest danger associated with drinking water is contamination by human excrement [4]. Water is potable when it is colourless, odorless, or tasteless and also free from poisonous, corroding, staining substances as well as disease-causing organisms [5]. It is present naturally as rain and found in rivers, lakes, and seas; its solid form is ice and its gaseous form is steam. Following the theory of creation, it is clear that water is as old as man. From time immemorial, man has resorted to the use of this unique commodity for domestic and other purposes. Water is absolutely essential to life, not only human life but all life, including animals and vegetables. Most of the biochemical reactions that occur in metabolism and growth of living cells involve water, all taking place in water [6].

Man uses water not only for drinking purposes but also for bathing, washing, laundering, fire protection, swimming, and wildlife propagation. Water is an essential resource to living things. It is both the most common and the most precious substance on Earth. Without water, there can be no life. From the foregoing, it is clear that water demand is ever-increasing among humans. There are indicators of water consumption. For instance, population size, activities embarked upon, pattern of water consumption and general behavior of people define largely the pattern of water consumption in particular areas [2]. It is a basic necessity of life for both plants and animals [7]. Mankind cannot survive without water as even the human body is made of about 70% water [8]. Although a human can do without food for 28 days, man cannot do without water for three days [9]. Quality drinking water is essential for life. Alternative sources of water such as rainwater and groundwater have become major sources of drinking water for people living in new settlements and some residents who do not have access to treated water in Ghana. The need to assess the quality of water from some of these alternative sources has become imperative because they have a direct effect on the health of individuals [10].

Contaminants such as bacteria, viruses, heavy metals, nitrates, and salt have polluted water supplies as a result of inadequate treatment and disposal of waste from humans and livestock, industrial discharges, and over-use of limited water resources [11]. Even if no sources of anthropogenic contamination exist, there is potential for natural levels of metals and other chemicals to be harmful to human health. This was highlighted in Bangladesh where natural levels of arsenic in groundwater were found to be causing harmful effects on the population [12]. Unfortunately, this problem arose because the groundwater was extracted for drinking without a detailed chemical investigation. The natural water analyses for physical and chemical properties including trace element contents are very important for public health studies [13,14]. The presence of *Escherichia coli* in drinking water denotes that the water has been contaminated and therefore presents a potential health risk to households that use them untreated [15]. Studies have proven that over one billion people in the world lack access to safe drinking water and about 2.5 billion people do not have access to adequate sanitation services at all [16].

Natural water contains microorganisms from soil and possibly from animals or sewage [17]. Natural groundwater is usually of good quality, but this can deteriorate due to inadequate source protection and poor resource management [18]. Borehole water is not entirely pure and its purity depends on geological conditions of the soil and in particular anthropogenic activities in the area in which improper waste disposal, leachate from landfills and dumpsites often polluting groundwater supply thereby resulting in the transmission of bacteria and diseases [19,20].

The atmospheric water includes rainfalls. All water bodies consist of a variety of bacteria and other microorganisms like algae and fungi which inhabit these natural water bodies [21,22]. Some of these microorganisms are indigenous to this natural water and some are transient, entering the water from the external environment [23]. Tap water, as one of the water sources, is mostly used domestically. The increase in drinking water from different hostels in Nnamdi Azikiwe University has made it necessary to investigate the microbial content of water [24]. Water is a potential carrier of pathogenic organisms that can endanger human life. Most drinking water sources are contaminated with different pollutants like feces, animal, and plant wastes, making such water unfit for drinking if not treated [25]. The pollution of water with pathogenic organisms can only be detected by carrying out microbiological assessment of such water. Microorganisms play an important role in water quality. Microbes that are health hazards do occur in water and can cause illness or even death [7]. Defective plumbing was the cause of outbreak during the world fair in Chicago 1933 Microorganisms [26] that are concerned with water-borne diseases are *Salmonella* species, *Shigella* species, *Escherichia coli*, and *Vibrio cholera*. The presence of fecal coliforms of *Escherichia coli* and those listed earlier are indicators of contaminated water [27].

Water-borne diseases are those diseases that are transmitted through the direct drinking of contaminated water with human or animal excreta [28]. Some of the organisms remarkable for their role in the outbreak of water-borne diseases include *Escherichia coli*, *Salmonellosis*, Typhoid, Cholera, Giardiasis, Amoebic dysentery, and *Campylobacter enteritis* [29]. Several studies have revealed the significance of water quality, sanitation, and hygiene in explaining the occurrence of water-borne diseases. For instance, in Ile Ife, Nigeria, an attempt to assess and map the incidence of water-borne diseases found that most of the reported cases were due to environmental factors such as poor environmental sanitation, indiscriminate waste disposal, low topography, and swamps that led to high microbial growth, water hardness, and high pH of wells and borehole water in the area [30].

Microbial contamination is a global issue that affects millions of people every year. In Nigeria, the quality of drinking water is a major concern, especially in university hostels where large numbers of students live in close quarters [24]. Water-borne diarrhoeal cases are preventable through changes to the environment which includes interventions to increase the availability of clean water for drinking and to improve sanitation hygiene practices [31]. Numerous studies have underscored the significance of microbial water quality assessment, emphasizing its role in preventing waterborne illnesses [32]. Water quality is a paramount concern for public health, and in communal living environments such as school hostels, understanding the microbial load in water sources is of critical importance. Microbial contaminants in water can give rise to a spectrum of waterborne diseases, posing a potential threat to the well-being of residents.

Furthermore, water is required for consumption in homes, offices, schools, and administrative institutions. However, the level to which water demand differs across institutions has not been given attention in available studies especially as it relates to educational institutions specifically in residential hostels that are provided for students in tertiary institutions. Water being an indispensable commodity is largely demanded for consumption in residential hostels for various purposes [33]. Available studies have shown that water is a serious necessity for students in hostels in tertiary institutions across the world [34]. When students have access to basic amenities and services, they are likely to be in better health to face their academics [35]. When residents in hostels in tertiary institutions are not provided with sufficient water and required quantity, their attitude towards sanitation becomes a problem [36]. Further used water accessibility and availability as a variable in determining the level of housing satisfaction of students in tertiary institutions.

Access to facilities and amenities in schools influences the academic performance of a student to an extent [37]. Students in residential hostels demand water for laundry, sanitation, cleaning of surfaces, and drinking [38]. Water consumption in student hostels is increasing astronomically and its pattern of consumption is tied to activities, water availability, and so on [39]. Apart from water accessibility, another important feature that is concerned with water consumption is water quality and quantity; this is because the quality and quantity of water influences water demand [2]. When available water for consumption is not sufficient in terms of quantity and quality, consumers are likely to turn to other sources in order to satisfy water needs. Water consumption is mostly predicated upon activities, household size, income, and seasons [40]. Hostel residents in Port Harcourt depend on boreholes for

water needs. Water quality largely influences water consumption. In Calabar, Nigeria, there are several tertiary institutions that provide residential accommodation to their students.

These hostel residents demand water for laundry, personal hygiene, and other purposes [41]. This study employs a comparative approach to assess and analyze the microbial load in different water sources within hostels in Nnamdi Azikiwe University and also identify potential sources of contamination [24]. A detailed knowledge of water quality in these hostels is essential so that drinking water can be adequately treated and the contamination of its source can be prevented. Previous research has highlighted the influence of factors such as source water quality and maintenance practices on the microbial content of water in various settings [42]. Understanding microbial load in water sources within hostels is essential for implementing effective water quality management strategies.

Aim of the Study

The aim of this study was to compare the microbial load and quality of water sources in the postgraduate, female, and male hostels. Specifically, the study sought to determine the levels of microbial contamination in water samples from each hostel, identify the predominant microorganisms, and statistically compare the microbial loads across the three hostels. The findings are intended to evaluate the potential health risks associated with the consumption of untreated water from these sources and to provide evidence-based recommendations on safer water alternatives or appropriate treatment methods for the benefit of the hostel residents.

Materials and Methods

Study area

The experiment was carried out in the Microbiology Laboratory in Nnamdi Azikiwe University, Along Enugu-Onitsha Expressway, Ifite Road, Awka.

Collection of samples

Water samples were collected from four different hostels of Nnamdi Azikiwe University. Sterile universal sampling bottles were used to collect the samples from girls, boys, post graduates and Emelda hostel. The samples were later brought to the laboratory for analysis. The samples were differentiated using alphabets to label the bottles. The water in the bottles was then sterilized. The samples were examined within three hours of collection.

Preparation of media

Nutrient Agar, EMB Agar, SDA Agar, and SSA Agar were prepared according to the manufacturer's guideline.

Sample analysis

0.1 ml of the water samples labeled A, B, C, D were poured into sterile Petri dishes marked with sample type and media names. The molten media was then poured into the Petri dishes containing the sample and mixed by rotating clockwise and anticlockwise gently to avoid spillage. It is done near a lit Bunsen burner to avoid contamination. The culture plates were incubated at 37°C aerobically for 24 - 48 hours for bacteria and 48 - 72 hours for fungi. Developing colonies on Nutrient agar and SDA plates were counted to obtain total viable colonies.

Plate count

The culture plates were examined after 24 hours of incubation and counted. The number of bacteria colonies on the plates which had between 30 - 300 colonies were counted and identified based on their morphological characteristics in all media at the end of the incubation periods [43].

Sub culturing of pure isolates

EMB agar and SSA were used in the sub-culturing of the bacteria isolates to identify specific bacterial species. The preparation of the media was according to the manufacturer's guideline.

The molten media we're poured into sterile Petri dishes in the presence of a Bunsen burner to prevent contamination, after which it was left to cool and solidify. Using a sterile wire loop, six discrete colonies that were identified based on their morphological characteristics [44], were inoculated into the solidified agar, in the presence of a Bunsen burner. They were inoculated by picking the colonies from the mixed culture with the wire loop and gently streaking on the surface of the agar. After that it was incubated for 24 hours at 37°C.

Characterization and identification of bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests [45].

Gram staining

A drop of distilled water was placed on clean, grease free slides and the pure isolates were transferred to the drop and smeared using a sterile wire loop in the presence of a Bunsen burner. The film was allowed to air dry. The film was fixed by passing it through the Bunsen flame three times without exposing the dry film directly to the flame. The slides were flooded with Crystal Violet solution for 60 seconds and washed off briefly with distilled water. Then they were flooded with iodine solution which acts as a mordant for about 60 seconds and washed with distilled water. Excess water is removed and about 95% alcohol applied for 15 seconds, the slides were washed with distilled water and drained. The slides were counter stained with safranin for 60 seconds and rinsed with water and allowed to air dry. The slides were then viewed under an oil immersion lens microscope (x100) and results read.

Biochemical test

Catalase

This test is used to differentiate those bacteria that produce the enzyme catalase. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a sterile wire loop. A drop of 3% hydrogen peroxide was added on the slide and mix. A rapid evolution of oxygen within 5 - 10 seconds as evidence of bubbling and a negative result shows no Bubbles [46].

Citrate utilization test

This test is based on the ability of organisms to utilize citrate as the sole carbon source. 2.4g of the Simmon's citrate agar was weighed using a weighing balance and poured into 100 ml of distilled water in a conical flask. The solution was evenly distributed into labeled test tubes and clogged with cotton Wool and sealed with aluminum foil and masking tape and sterilized at 121°C for 15 minutes. The media was allowed to cool and gel, after which the organisms were stabbed into the media using a sterile needle in the presence of Bunsen burner and incubated for 24 hours at 37°C. A positive test was indicated by a change from green to blue color on the surface of the Simmon's citrate agar slant. No color change indicated a negative reaction.

Indole test

It is a test performed on bacterial species to determine the ability of organisms to convert tryptophan to indole. 1.5g of peptone was weighed using a weighing balance and poured into 100 ml of distilled water in a conical flask and stirred until a homogenous solution was formed.

The peptone water was distributed into labeled test tubes and clogged with cotton Wool and sealed with aluminum foil and a masking tape and sterilized at 121°C for 15 minutes. The broth was left to cool, after which the organisms were inoculated into broth using a sterile wire loop in the presence of a Bunsen burner and incubated for 24 hours at 37°C. Few drops of the indole solution was added to the test tubes containing the broth and left to stand for 15 minutes and the results read.

Motility test

This test is used to differentiate motile bacteria from non-motile. With a sterile straight needle, a colony of a young (18 - 24) hours culture growing on agar medium was collected. Singly stab down the center of the tube to about half the depth of the medium. Incubated at 37°C and examined daily for seven days. Result shown is as a result of moving the bacteria away from line of inoculation.

Sugar fermentation test

It is used to identify a bacterium by its ability to ferment a specific carbohydrate (sugar). Glucose and lactose were used to carry out this test. 3g of peptone was weighed using a weighing balance and poured into 200 ml of distilled water in a conical flask and stirred until a homogenous solution was formed. 1g each of the glucose and lactose sugars was measured and added into labeled conical flasks containing 100 ml each of peptone water, 9 ml each of the sugar solutions were put into labeled test tubes containing Durham's tubes and sterilized for 10 minutes to avoid denaturing the sugars. The organisms were inoculated into the solution after cooling and incubated for 24 hours at 37°C. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in color of medium from green to yellow while gas production was observed by presence of gas in Durham's tubes.

Results

The result showed that sample B had the highest microbial growth and sample A had the lowest microbial growth compared to other water samples. The result of the calculation of the colony forming unit on the nutrient agar plate range from 1.5 x 10^3 cfu/ml to 5.6 x 10^2 cfu/ml.

Samples	Source	Odour	Taste	Colour
A	Boys Hostel	-	-	-
B	Girls Hostel	-	+	-
C	Emelda	-	+	-
D	Post Graduate	-	-	-

Table 1: Shows result of physical analysis carried out on the water samples.

Key: Negative = -; Positive = +.

Sample	Nutrient agar	Eosin methylene blue agar	*Salmonella Shigella* agar	Sauboraud dextrose agar
A	56	32	31	55
B	150	120	90	130
C	96	77	38	123
D	110	52	45	31

Table 2: Shows the number of bacterial colonies that grew on the culture plate after 24 hours of incubation.

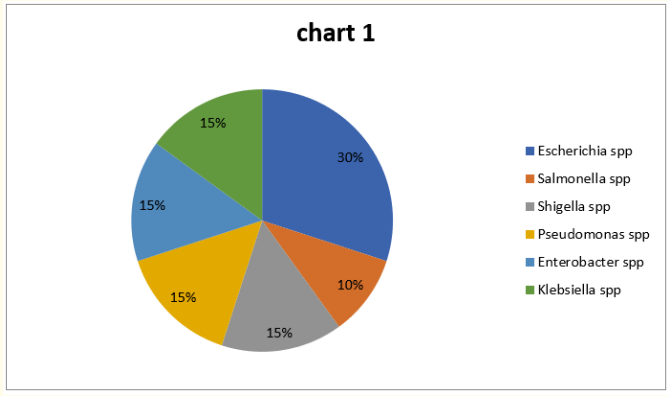

Figure 1: Percentage occurrence of bacteria the water sample.

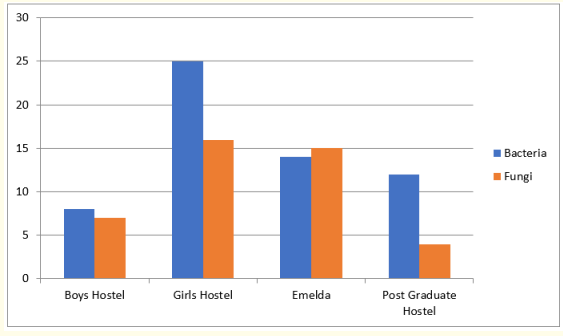

Figure 2: Bar chart showing the bacteria and fungi count on the water sample.

Organism A	Organism B	Organism C	Organism D	Organism E	Organism F	Organism G
Sample A	Sample B	Sample B	Sample A	Sample B	Sample B	Sample B
Sample B	Sample C	Sample C	Sample C	Sample C	Sample C	Sample C
Sample C	Sample D	Sample D	Sample D	Sample D	Sample D	
Sample D						

Table 3: Shows the sample types that contained these organisms.

From the table above it showed that organism A was found in all the water samples.

Organism	Catalase Test	Citrate Test	Indole Test	Glucose Test	Lactose Test	Motility Test	Gram stain	Rod/ Cocci	Probable
A	+	_	+	+	+	+	_	Rod	*Escherichia spp*
B	+	+	+	+	_	+	_	Rod	*Salmonella spp*
C	+	_	+	+	_	_	_	Rod	*Shigella spp*
D	+	+	_	_	+	+	_	Rod	*Pseudomonas spp*
E	+	+	+	+	+	+	_	Rod	*Enterobacter spp*
F	+	+	+	+	+	_	_	Rod	*Klebsiella spp*

Table 4: Microscopic and biochemical test for identification of the bacterial isolates.

Key: Negative = -; Positive = +.

Colony Morphology	Microscopy	Probable
On Sabouraud Dextrose Agar plate, it appeared powdery with a velvety texture. The colour was whitish after 2 days of incubation.	It showed characteristic features such as the conidiophores which are unbranched	*Aspergillus spp*

Table 5: Microscopy and colony morphology.

Discussion

The water samples obtained from four different hostels of Nnamdi Azikiwe University were contaminated. All isolates were characterized using standard morphological and biochemical tests [47]. Bacterial isolates were identified using Bergey's manual [48]. The total bacteria count recorded highest count of 1.2 x 10^3 Cfu/ml in sample B and lowest in sample A with total bacteria count of 5.6 x 10^2 Cfu/ml. The presence of total coliform in water samples are therefore an indication that opportunistic pathogenic bacteria such as *Salmonella spp*, *Shigella spp*, and *Escherichia coli* may be present [49].

These pathogens and opportunistic microorganisms could cause diseases such as Cholera, typhoid fever, dysentery and gastroenteritis.

Some physical tests were carried out for testing of its physical appearance such as colour, odour and taste. (Table 1). Biochemical tests such as catalase test, indole test, motility test were carried out to confirm the bacteria present .Gram staining narrowed down the organisms gotten down into Gram negative rods which includes; *Escherichia spp, Salmonella spp, Shigella spp, Klebsiella spp, Pseudomonas spp* and *Enterobacter spp*. One genera of fungi was also isolated from the water sample which is *Aspergillus spp*. In last decade, several more studies have resulted in increased knowledge on the occurrence of fungi in drinking water [50]. The probable bacterial isolates that were isolated includes; *Escherichia spp, Salmonella spp, Enterobacter spp, Shigella spp, Klebsiella spp, and Pseudomonas spp*. (Table 5). Many of the fungi isolated from drinking water includes; *Aspergillus spp, Candida albicans* and *Cladosporium spp*, [51] and *Aspergillus spp* was isolated in this research.

This research showed that *Escherichia spp* had the highest percentage occurrence on the water sample. The total bacteria count ranges from the highest in sample B and lowest in sample A. The Organism A-F that was viewed under the microscope was all gram negative rods. All the water samples analyzed were contaminated with bacteria isolates and they have been implicated in water related diseases [52]. The importance of public health of clean drinking water requires objective test methods to establish high standards of water safety and evaluate the effectiveness of the treatment procedure and routinely to monitor water for the detection of actual indicator Enteropathogens such as *Escherichia coli* and *Salmonella spp*. The *Escherichia coli* presence is the most frequently used indicator organism of fecal Pollution in water. [53]. From the result obtained *Escherichia spp* had the highest occurrence. The presence of *Escherichia coli* in the water samples indicates presence of other pathogenic bacteria such as *Salmonella spp* [54]. The result obtained from this research showed that the water samples were contaminated.

Conclusion

This research was carried out to analyze the Microbial load of water samples from four different hostels. This study observed that the water samples were contaminated with bacteria pathogens that could impact on public health. The high Microbial load particularly in girls hostel and Emelda hostel water samples make them unsuitable for consumption. The microbial populations especially for coliform bacteria encountered from some samples analyzed were above the limits for drinking water set by the World Health Organization. There is a need to increase awareness among residents of the hostels towards preventive and control approaches to minimize the danger associated with contaminated water.

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