



Microbial Analysis of Borehole Drinking Water in St. Thomas Aquinas Parish Government Reserved Area, (G.R.A) Awka.

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ABSTRACT

The prevalence of microorganisms in full scale water supply systems raises concerns about their pathogenicity and effects to public health. Clean tap water is essential for public health safety. The source water quality, the drinking water distribution system, and building water supply systems in buildings, greatly influence the microbial community in tap water. Given the importance of drinking water biosafety, the study of microbial diversity from source water to tap water is essential. This study is aimed at evaluating and determining the microbial quality of the borehole drinking water at St. Thomas Aquinas Parish G.R.A Awka. The water samples were collected from three different tap outlets, using three clean sterile containers, the taps were swabbed externally using a sterile cotton wool dipped in 75% alcohol, after which it was opened and allowed to run for five minutes respectively before the water samples were collected and sent immediately to the Microbiology laboratory in Nnamdi Azikiwe University Awka for analyses. The samples were cultured for both bacteria and fungi, with different agar medium using the pour plate, membrane filtration and fungi microscopy methods respectively. The results showed that the water sample had no bacteria growth after 24 hours of culture a total of seven fungal growth was counted, and three different isolates were identified (*Aspergillus sp.*, *Fusarium sp.*, *Penicillium sp.*), in the fungal agar medium after three days of culture The fungal colonies were too few to be counted using the standard method of fungi colony count (TFC=N/VD × 10).

INTRODUCTION

Background of study

Water is the essential factor for life after oxygen, if this essential factor is not available some organisms die early, some resistant stage, while some other dies late. People obtain their drinking water from surface and underground sources. (Oyango *et al.*, 2018). However, both surface and ground water could become contaminated by biological and chemical pollutants arising from point and non-point sources. Surface water sources like lakes, rivers and stream. The qualities of a surface water rapidly alter as a response to alteration in the environment. For example, high level of nutrients (Nitrogen and Phosphorus) cause eutrophication in surface water sources and massive growth of algae, leading to the growth of other microorganisms and cause turbidity in surface water. The sixth Sustainable Development Goal is to ensure universal access to safe and cheap drinking water for over 800 million people who do not have access to basic services, as well as to improve the accessibility and safety of services for over two million people. (Ahmad *et al.*, 2017), (Odoh *et al.*, 2014). Groundwater is the world's largest and most important source of fresh drinkable water on a global scale. Groundwater provides drinking water to an estimated 1.5 billion people worldwide and has shown to be the most reliable supply for satisfying rural water needs in Sub-Saharan Africa. Water quality issues, such as pollution, arise when groundwater is tapped from boreholes, especially when these wells are drilled near beneficiary communities and within homes (Akoth *et al.*, 2015). South Asia, East Asia, and Sub-Saharan Africa are home to the majority of these people (Kamara *et al.*, 2018), (Likambo, 2014). According to WHO data, unpotable water, inadequate sanitation, and hygiene are responsible for 9.1% of global infections and 6.3 percent of all deaths. In many developing countries, microbial pollution affects all types of water sources, including piped water supply. Waterborne diseases affect 37.7 million people each year, with 1.5 million children dying from diarrhea and 73 million working days lost owing to infection (Pal *et al.*, 2018), (Clasen *et al.*, 2014), (Fan *et al.*, 2019). Challenges of safe drinking water accessibility are many include urbanization, economic problems, and climate changes (Grady *et al.*, 2014), (Omarova *et al.*, 2019), (Edokpayi *et al.*, 2017). Two-fifth of Africans don't have a developed water supply, 60.2% have access to a better drinking water source than 39.8% of Africans, and 36% have access to developed sanitary facilities. Pathogenic microorganisms from animal and human wastes in the water that has been obtained from various researches include bacteria, viruses, and parasites. The most prominent waterborne infection is diarrhea, which is caused by the consumption of unpotable water (Sila *et al.*, 2019), (Uzoigwe *et al.*, 2012), (Akinde *et al.*, 2016). Since from the recorded history water play vital role in the spreading of various bacterial, viral and protozoan diseases. Drinking water contaminated by a wide variety of microorganism. Some of these microbes may be pathogenic and cause different diseases in human. It is also an established fact that drinking water if polluted can spread dangerous disease like hepatitis, cholera, dysentery, typhoid and diarrhea. According to United Nations report in developed countries 95% of the population has access to clean and safe drinking water and 90% have adequate, proper manage and safe disposal. In developing countries, the situation is different. The United Nations estimated that at least 2.5 billion populations of the developing countries

have no proper manage sanitation system and above half of these people have no access to safe drinking water (Foka *et al.*, 2019). Unwanted changes in microbial quality of drinking water can have adverse effects on distribution system and consumers. For example, during distribution, excessive growth of bacteria can lead to deterioration of drinking water quality in terms of safety, consumer's perception, and operational aspects (Sun *et al.*, 2014). Changes in microbial water quality are a result of complex interactions between various organisms regulated by access to available growth limiting nutrients, response to environmental conditions, such as water temperature, presence of potential residual disinfectant and other inhibitory substances, attachment of bacteria to pipe walls, particle deposition, sedimentary suspension and biofilm formation. The aim behind the concept of biological stability is that minimum change in water quality is occurring during drinking water distribution, or at least not to a degree that affects consumer's safety or aesthetic perception or cause technical failure. To achieve this and limit bacterial growth during transport, drinking water is distributed in numerous countries with disinfectant residuals, using different substances (free chlorine, chlorine dioxide, monochloramine) at varying concentrations (Gillespie *et al.*, 2014). Adverse health effects of disinfection by products and altered water taste have, however, led several countries to use for water distribution without the addition of disinfectant to the produced drinking water. (Vital *et al.*, 2012, Lautenschlager *et al.*, 2013, Prest *et al.*, 2014). In the latter case, minimum change in water quality is achieved in the first place by controlling the water quality with extensive water treatment strategy, and secondly by distributing water in well-maintained piping systems. Therefore, this study is to determine the wholesomeness of the borehole drinking water at St. Thomas Aquinas Parish, Government Reserved Area, (G.R.A), Awka.

Aim of Study:

This research is aimed at evaluating and determining the microbial quality of borehole drinking water from St. Thomas Aquinas Parish, Government Reserved Area, (G.R.A), Awka.

MATERIALS AND METHOD

Study Area

This study was carried out in the Microbiology laboratory of Nnamdi Azikiwe University Awka.

Sample Collection

Samples were collected from Three different tap outlets with a sterile container from St. Thomas Aquinas Parish G.R.A Awka. The samples were collected using a clean sterile container, the tap was swabbed externally using a cotton wool dipped in 70% alcohol, and were opened and allowed to run for five minutes, before the samples were collected and sent immediately to the laboratory for analyses. Three micro pipettes were used to collect samples from different water samples. The sample containers were labeled sample A, B and C in the Microbiology laboratory and were analyzed.

Materials

Equipment and reagents

The equipment and reagents used are; Nutrient agar, salmonella shigella agar, Sabouroud Destrose agar, Eosin Methylene Blue agar petri dishes, alcohol (70%), cotton wool, aluminum foil, spatula, micropipette, electronic weighing balance, beakers, conical flask, measuring cylinder, cotton wool, foil, gas cylinder, inoculating loop, glass slide, crystal violet, membrane filter, iodine, hydrogen peroxide, lactophenol cotton blue, distilled water, autoclave, microscope, test tube racks, masking tapes, Electronic microscope.

Preparation of Culture Media

The culture media employed in this sample are Nutrient agar, Sabouraud dextrose agar (SDA), Eosin methylene blue agar (EMB) and Salmonella Shigella agar (SSA). All culture media was prepared according to the manufacturer's instructions.

Morphological Characterization

Morphological characteristics of bacterial and fungal colonies were done based on the criteria of Berger's Manual of Determinative Bacteriology. These include the shape of colonies, elevation, optical characteristics, margin, opacity and pigmentation. Fungi were described based on their shape, spore, color or pigmentation, size and hyphae development.

Preparation of Agar Medium

From the manufacturer's standard, 5.6g of agar powder was measured using an electronic balance and suspended into 200ml of distilled of water in a conical flask. The mixture is mixed until all components are dissolved completely. The mixture was then covered and then sterilized by autoclaving at 121 degrees for 15 minutes. It was then allowed to cool to 45-50 degree Celsius (but not gel).

Sample Culture

Pour Plating

0.1 ml of the sample was collected from the sterile container using a micropipette and put inside the sterile petri dish, The prepared Sabouroud Destroyed agar is poured over the sample and is rotated gently to ensure even distribution over the plate. The agar plate is covered and allowed to solidify, after

which it is allowed to incubate at 25 °C for three days. For the Nutrient agar, Eosin methylene blue agar and Salmonella Shigella agar, it was allowed to incubate at 37 °C for 24 hours.

Membrane Filtrations

The membrane filter was sterilized using a cotton wool dipped in ethanol and assembled, Then the filter paper was placed inside it. The membrane filter was placed on a beaker, after which the water samples were poured into the sterile membrane filter and allowed to sieve out into the beaker. Then the filter paper was removed from the membrane filter and placed in an already prepared Nutrient Agar plate. It covered and allowed to incubate at 37 °C for 24 hours.

Fungi Microscopy

A small portion of the fungi was picked from the culture plate containing the fungi colony using a sterile wire loop, and was transferred aseptically on a sterile and clean glass slide, The slide was stained with lactophenol cotton blue to make the fungi structures more visible. A coverslip was placed on the slide and then viewed under the microscope using x40 magnification.

RESULTS

The study showed that there was no bacterial growth on any of the agar plates after 24 hours of culture using the Nutrient agar and Salmonella Shigella agar but there was growth of fungi on the three water samples using the Sabouroud destrose agar, after three days of culture. The amount of growth on the Sabouroud destrose agar (SDA) plate was very few to be counted.

Table 1: Number of Bacterial Colonies On Different Agar Medium

Agar medium	Probable Organisms	Number Of Growth
Nutrient Agar Medium	<i>Escherichia coli.</i>	Nil
Salmonella Shigella Agar Meduim	<i>Salmonella sp., Shigella sp.,</i>	Nil
Eosin Methylene Blue Agar Meduim	<i>Escherichia coli, Enterobacter sp.,</i>	Nil

Table 2: Morphological Characterization of Fungal Colonies

Isolates	Type of organism	Macroscopic characteristics	Microscopic characteristics	Suspected organisms
C.1-1	Filamentous mold	Colourless and smooth conidiospores, white fluffy appearance.	Small spherical spores.	<i>Aspergillus sp.</i>
C.1-2	Filamentous mold	Initially white and fluffy in appearance	Presence of branched hyphae with spherical chains of conidiospores.	<i>Penicillium sp.</i>
C.1-3	Filamentous mold	White and fluffy	Presence of septate hyphae	<i>Fusarium sp.</i>

Discussion

The study aimed to investigate the presence of microorganisms in borehole drinking water, considering the potential implications for water quality and human health. Water samples were collected from three different tap outlets at St. Thomas Aquinas Parish G.R.A Awka. The Sample processing involved filtration, culturing, staining, and microscopy for fungal identification. A total of seven fungal isolates were gotten from the samples, with three different colonies. While the presence of fungi in borehole water is common, the study highlighted the need for monitoring and understanding their impact on

water quality. Certain fungi can act as indicators of environmental changes or potential contamination, mycotoxins produced by fungi pose potential health risks, emphasizing the importance of assessing their levels in drinking water. The study raises awareness about the potential health implications of fungal presence in borehole drinking water, immunocompromised individuals may be particularly vulnerable to fungal contaminants, guidelines for fungal limits in drinking water should be considered to safeguard public health.

Conclusion

In conclusion, the research on microbial analysis in borehole drinking water revealed the fungi colonies present in the borehole water source. The microbial analysis showed that the samples are biologically satisfactory based on World Health Organization standards, which shows that the water is safe for drinking.

Recommendation

Recommendations for improved water management strategies and continued research in this area are essential for developing effective measures to address fungal contamination in borehole drinking water. As water quality remains a critical aspect of public health, ongoing efforts are crucial to enhance our understanding of fungal dynamics and implement strategies for the safe provision of drinking water from borehole sources.

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