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Bacteriological Investigation of Cafeteria Wastewater and Soil of Dumpsite in Madonna University, Elele Rivers State, Nigeria

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Abstract

Wastewater is any water that has been adversely affected in its qualities by human anthropogenic activities, which have led to human and environmental threats. The study, however, was designed to Bacteriologically investigate the cafeteria wastewater and dumpsite in Madonna University Elele, Rivers state. A total of six (6) cafeteria wastewater and six (6) soil samples were aseptically collected and brought to the Microbiology laboratory at Madonna University. The population of culturable bacterial isolates was carried out by performing ten-fold serial dilutions using 1ml from 10^4 samples and inoculating unto Nutrient agar, MacConkay agar, *Salmonella Shigella* agar, using pour plate method and incubated for 24hrs at 37^{o}C for bacterial growth. Bacterial isolates were subjected to biochemical identification and characterization. Total culturable heterotrophic bacterial counts from the girl's hostel cafeteria wastewater ranged from (2.5 x 10^4 cfu/ml - 4.6 x 10^4 cfu/ml). At the same time, the boy's hostel cafeteria wastewater ranged from (1.2 x 10^4cfu/ml - 3.3 x 10^4 cfu/ml). Total culturable heterotrophic bacterial counts from the girl's hostel cafeteria wastewater soil dumpsite ranged from (1.4 x 10^4 cfu/ml -2.9 x 10^4 cfu/ml). While the boys' hostel cafeteria wastewater soil dumpsite ranged from (1.9 x 10^4cfu/ml - 4.7 x 10^4 cfu/ml). The bacterial identified and frequency of occurrence is *Staphylococcus* spp 6(33.3%), *Escherichia coli* 5(27.8%), *Salmonella* sp 2(11.1%), *Shigella* sp 1(5.6%), *Bacillus* sp. 2(11.1%) and *Acinetobacter* sp 2(11.1%) respectively. This study has revealed that cafeteria wastewater and dumpsite harbours bacteria organisms that could be pathogenic to humans and their environment if not properly handled. The study equally revealed a higher bacterial load on the dumpsite from the boy's hostel due to their anthropogenic activities. Therefore, there should be an adequate holistic pre-treatment of the wastewater before disposal into the receiving environment to avoid breeding disease-causing bacteria organisms.

Keywords: Wastewater, Cafeteria, Bacteria, Dumpsite, Environment.

1: Introduction

The amount of wastewater or effluents with huge microbial loads are directly released into open spaces, open water sources or as underground infusion without treatment is a significant threat to the health of the inhabitants in communities where such practices are carried out or performed (Ogunfowokan et al., 2005; Agwa et al., 2013). Cafeteria wastewater in undeveloped Countries

disposes of their produced waste without any treatment. This waste accumulates, causing various types of harm and spreading microorganisms directly or indirectly from different sources. Primarily, they emanated from contaminated equipment's used in food processing, contaminated hands from food handlers and other possible sources (Ofor et al., 2009).

The consequence of untreated waste on ecological and Public Health requires regular monitoring and adequate legislation (Idris-Nda et al., 2013).

Ecological stresses on microorganisms rising from food processing, washing with cleaner, preserving additives, and cooking food and pigment may prompt transformation in the ecosystem and raise different drug resistance. Consequently, the new microbial quality and amount of wastewater released from the Cafeteria might be considered a source of contamination. Therefore, wastewater is stated as any waste that has been adversely affected in its quality by anthropogenic influence (Idris- (Nda et al., 2013; Adeniran et al., 2017).

The composition of wastewater varies widely depending on its source, which can also be characterized by its physical, biological, and chemical constituents (Akindele et al., 2015).

It consists of liquid wastes discharged by domestic residences, commercial properties, industry, and agriculture and can encompass a wide range of potential contaminants and concentrations (Idris-Nda et al., 2013).

This wastewater often harbours microorganisms capable of producing disease conditions to its host in this context bacteria family (Akindele et al., 2015). If wastewater is not managed correctly, it could be a hazard to the health of human populations and the environment.

Wastewater also contains vast quantities of bacteria, most of which are harmless to man. However, a pathogenic form that causes diseases such as typhoid, dysentery, and other intestinal disorder may be present in the wastewater (Absar, 2006).

Water conflicts arise primarily due to the need to manage the resource, which is becoming scarcer with time. The benefits of water reclamation and reuse are recognized as preventing the pollution of surface and groundwater (Hespanhol,1992). This wastewater is discharged unlawfully without any form of pre-treatment before discharging into the recipient's ecological system, adversely affecting the composition of the soil, such as organic matter, minerals, gases, fertility, and organisms that support life (Pong, 2015). Soil is a product of several factors: the influence of climate, relief (elevation, orientation, and slope of terrain), organisms, and the soil's parent materials (original minerals) interacting over time (Pong, 2015; Yu et al., 2015).

It continually undergoes development through numerous physical, chemical, and biological processes, including weathering with associated erosion (Yu et al., 2015). Land used for dumping waste is called a dumpsite. In developing countries, waste is deposited on land or discharged into water. When waste is dumped on land, microorganisms such as bacteria and fungi proliferate using the components of the waste degrading the waste's organic materials (Kumar et al., 2016).

The microbial ecology of dumpsite soil can help us predict the consequences of such waste dump on soil living organisms and activities (Kumar et al., 2016; Alexander, 2016).

According to Karen et al. (2003) and Karen & Wei (2017), health is at risk for those who live within five kilometres of a (dumpsite) as they are being exposed to air pollutants emitted by the waste, causing lung cancer, respiratory diseases as well as deaths. These are prominent, particularly in younger adults. Therefore, it is imperative to underscore the microbial population in Cafeteria wastewater and its adverse effect on the receiving environment, particularly the soil environment.

2. Materials and Methods

2.1: Study Area

This study was carried out in the Cafeteria located at Madonna University, Elele, Rivers State, between June-August 2022.

2.2: Sample Collection

A total of six (6) Cafeteria wastewater and six (6) soil samples from the dumpsite were collected from two (2) different cafeteria points (female hostel, male hostel) under aseptic conditions. The soil samples were aseptically collected by 12-15cm in diameter using a spatula and a sterile polythene bag. Clean tap water and soil from different dumpsites were collected. During collection, the sterile plastic container was rinsed three (3) times with clean water before filling it with the wastewater samples. After that, the sample was brought to the laboratory for further microbiological investigation.

2.3: Microbiological Analysis of Wastewater and Soil Samples

2.3.1: **Serial dilution**

One millilitre of each of the wastewater samples was added to 9ml of 0.1% peptone water diluents, giving rise to a 10^{-1} dilution factor. After good shaking, further serial dilution of ten (10) –fold (v/v) was performed by transferring 1ml of the previous solution to newly or freshly prepared peptone water diluents to a level of 10^{-8} dilutions factor.

An aliquot (0.1ml) of appropriate ten-fold serial dilutions (10^{-2}-10^{-8}) of each sample was transferred to plates of surface-dried MacConkey agar nutrients for bacteria, respectively.

2.3.2: Sub-Culture

After 24hrs, growth on the various plates was identified, morphologically counted, and recorded. Colonies were expected to be between 30 and 300 (Cheesbrough, 2006). Isolates were then subcultured on nutrient agar to obtain a pure culture. Plates were inverted and put in the incubator for18hrs. Agar slants were prepared to preserve the first and pure cultures. These slants were all preserved in the refrigerator (Cheesbrough, 2003).

2.3.3: Gram Stain Reaction

Gram staining was done as described by Cheesbrough (2006); Anele et al. (2019). "A loopful of water was placed in a grease-free sterile slide, and then a portion of the organism was spread to

make a smear. The smear was air-dried and heat fixed. The smear was covered with crystal violet and allowed to stand for 60secs, the stain was washed off, and excess water was drained. The smear was covered with Gram's iodine and allowed to stand for 60secs. The excess iodine was drained off and rinsed gently. 75% alcohol was also used as a decolourizer and spread on the smear until the drops from the slide were a pale violet colour for 20secs. The slide was washed gently with water. The smear was counterstained with safranin for 2mins. It was washed with water, and the smear was allowed to blot dry. A drop of the immersion oil was placed on the smear, and the slide was viewed under the microscope at the 100x objective lens. Gram-positive cells appeared purple under the microscope, and Gram-negative cells appeared pink under the microscope.

2.3.4: Biochemical Test

The biochemical test that was carried out is as follows: Indole test, Sugar fermentation test, Oxidase test, Citrate test, Catalase test, Methyl Red Voges Proskauer test (MRVP), Motility test and Triple Sugar Iron test as described by Cheebrough (2006); Nworie et al. (2012) and Anele et al. (2019).

2.4: Statistical analysis

The results obtained from this study were edited, coded and subjected to different statistical investigations. The mean occurrence was determined for various samples. Analysis of variance (ANOVA) was used to determine the significance at 95% internal ANOVA.

3. Results

3.1 Total Culturable Heterotrophic Bacterial Counts from Wastewater Samples

The total culturable heterotrophic bacterial counts of Cafeteria wastewater from the girls' and boys' hostel of Madonna University, Rivers State, are shown in Table 1. Total culturable heterotrophic bacterial counts from the girl's hostel cafeteria wastewater ranged from 2.5 x 10^4 cfu/ml to 4.6 x 10^4 cfu/ml. While the boy's hostel cafeteria wastewater ranged from 1.2 x 10^4cfu/ml to 3.3 x 10^4 cfu/ml.

Table 1: Total Heterotrophic Bacterial counts in wastewater samples from Madonna University Cafeteria, Rivers State.

Samples code	Location Girls Hostel (cfu/ml)	Location Boys Hostel (cfu/ml)
DKH 1	4.6 X 10^4	3. 3 X 10^4
DKH2	4.3 X 10^4	2.1 X 10^4

3.2. Heterotrophic Bacterial counts in soil from wastewater Dumpsite

The total culturable heterotrophic bacterial counts in soil from the Cafeteria wastewater Dumpsite from the girls' and boys' hostel of Madonna University, Rivers State, is shown in Table 2. Total culturable heterotrophic bacterial counts from the girl's hostel cafeteria wastewater soil dumpsite ranged from 1.4 x 10^4 cfu/ml to 2.9 x 10^4 cfu/ml. In contrast, the boy's hostel cafeteria wastewater soil dumpsite ranged from 1.9 x 10^4cfu/ml to 4.7 x 10^4 cfu/ml.

Table 2: Total Heterotrophic Bacterial counts in soil from wastewater Dumpsite in Madonna University, Nigeria Rivers State

Samples Code	Location Cafeteria Girls Hostel (cfu/ml)	Location Boys Hostel(cfu/ml)
DKH 1	2.9×10^{-4}	4.7×10^{-4}
DKH 2	1.4×10^{-4}	2.9×10^{-4}
DKH 3	1.6×10^{-4}	1.9×10^{-4}

KEYS: Cfu= Colony Forming Unit

3.3. Frequency of Occurrence (percentage) of Bacterial Isolates in Cafeteria wastewater and Dumpsites

The percentage frequency of occurrence of bacteria isolated from Cafeteria wastewater and dumpsites is shown in (Table 3) from the results obtained, 55.6% of the isolates were from cafeteria wastewater. In comparison, 44.4% were identified from the dumpsites. The bacterial organisms isolated from the study and their frequency of occurrence include *Staphylococcus* sp (33.3%, n=6) as the most predominant. This was followed by *Escherichia coli* (27.8%, n=5), Salmonella sp (11.1%, n=2), Bacillus sp (11.1%, n=2) and *Acinetobacter* sp (11.1%, n=2), Shigella sp (5.6%, n=1) was the least predominant.

The bacterial frequency of isolation from Cafeteria wastewater is shown in (Table 4) among the isolates found in Cafeteria wastewater, *Escherichia coli* (30.0%) is the most predominant. This was followed by *Staphylococcus* sp (20.0%), *Bacillus* sp (20.0%), *Salmonella* sp (10.0%), *Shigella* sp (10.0%) and *Acinetobacter* sp (10.0%).

Table 3: Percentage occurrence of Bacterial isolates obtained from Cafeteria wastewater and Dumpsites in Madonna University, Rivers State

Bacterial Isolates	Frequency (%)	Wastewater (%)	Dumpsite (%)
Escherichia coli	5(27.8)	3	2
Staphylococcus sp	6(33.3)	2	4
Salmonella sp	2(11.1)	1	1
Shigella sp	1(5.6)	1	0
Bacillus sp	2(11.1)	2	0
Acinetobacter sp	2(11.1)	1	1
Total	**18(100.0)**	**10(55.6)**	**8(44.4)**

Table 4: Frequency of occurrence of Bacterial isolates obtained from Cafeteria wastewater in Madonna University, Elele Rivers State

Bacterial Isolates	Frequency (%)
Escherichia coli	3(30.0)

Staphylococcus sp	2(20.0)
Salmonella sp	1(10.0)
Shigella sp	1(10.0)
Bacillus sp	2(20.0)
Acinetobacter sp	1(10.0)
Total	**10(100.0)**

Table 5: Frequency of occurrence of Bacterial isolates obtained from Cafeteria Dumpsite in Madonna University, Elele Rivers State

Bacterial Isolates	Frequency (%)
Escherichia coli	2(25.0)
Staphylococcus sp	4(50.0)
Salmonella sp	1(12.5)
Acinetobacter sp	1(12.5)
Total	**8(100.0)**

4. Discussion

Indiscriminate discharge of untreated cafeteria wastewater into the recipient's ecological system could lead to oxygen depletion, excessive nutrients, undesirable metals and other environmental pollution that could endanger the safe living of the organisms. These can also enhance the increased level of pathogenic microbes, which could render the environment un-conductive if proper standard measures are not followed. The study termed to bacteriologically investigate cafeteria wastewater and dumpsite soil in Madonna University Elele, Rivers State. A total of 18 bacterial organisms comprising six (6) different species were isolated. The results obtained in (Table 3) showed that bacteria such as *Escherichia coli*, *Staphylococcus* sp, *Salmonella* sp, *Shigella* sp, *Bacillus* sp, and, *Acinetobacter* sp were isolated from cafeteria wastewater and dumpsite soil located at boys and girls hostel in Madonna University, Elele, Rivers State. This result agrees with previously reported results (Rabah et al., 2008; Agwa et al., 2013).

Some bacteria isolated from the water samples have been reported as causative agents of various diseases (Dennis et al., 2005). Such diseases include acute enteritis in infants caused by Escherichia coli (Muoghahu & Omocho, 2000) and typhoid fever caused by Salmonella sp (Wyant et al., 1999). Also, some of these isolated bacteria belong to Enterobacteriaceae, which can cause hand-to-mouth infections in men if hands are not properly sanitized. They also tend to cause other forms of enteric diseases in people when appropriate measures are not in place (Rusin et al., 2002).

The isolation of *Staphylococcus* sp, with the second highest percentage occurrence 6(27.27%), agrees with Sarmah et al. (2018). *Staphylococcus* sp is a significant component of the usual floral of the skin and nostrils, which probably explain its high prevalence as a contaminant, as it can be quickly discharged by several human activities, including sneezing, talking, and contact with moist skin (Itah et al., 2004).

The total culturable heterotrophic bacterial counts obtained from the Cafeteria wastewater in the boys' hostel ranged from 1.2 x 10^4 cfu/ml to 3.3 x 104cfu/ml. In contrast, the girls' hostel ranged

from 2.5 x 10^4 to 4.6 x 10^4. This result agrees with Adeniran et al. (2017), who reported similar bacterial counts on wastewater obtained from domestic activities. In this study, the wastewater from the girls' cafeteria hostel had more bacterial load than that of the boys' hostel cafeteria due to the level of hygienic conditions and anthropogenic activities of the cafeteria providers. In this light, the cafeteria providers in the boys' hostel pay more attention to hygienic practices. Meanwhile, those girls neglect hygienic practices, which could lead to an increase in bacterial load by extension which can cause disease in the environment (Akindele et al., 2017).

Furthermore, the total culturable heterotrophic bacterial counts obtained from the cafeteria wastewater dumpsite soil in the boys' hostel ranged from 1.9 x 104 to 4.7 x 104cfu/g. While the girls' hostel wastewater dumpsite soil from 1.4 x 104 to 2.9 x 104cfu/g. This result agrees with Akindele et al. (2017), who reported similar bacterial counts on the soil where such activities are being carried out. In this study, the boys' hostel wastewater dumpsite soil is termed to have a more bacterial population. This observation may be that soil had been negatively affected due to untreated and unlawful discharge of this wastewater into the soil environment. They were coupled with the fact that cafeteria wastewater in the boys' hostels is discriminately discharged into the soil. The boys also contribute to the increased bacterial load in the soil environment due to their anthropogenic operations, such as urinating at the same site, creating more suitability for the bacterial increase. These activities, in turn, contaminate the groundwater source, thereby exposing or subjecting the occupant to several ill-health conditions.

Therefore, leading to oxygen depletion, an increase in soil pH, temperature, and, most times, makes it impossible for indigenous bacterial organisms to survive, causing a decline. Similarly, the cafeteria wastewater dumpsite soil in girls' hostels was not much affected because of how their dumpsite is being handled. In order words, it connotes that the girls take appropriate sanitary measures more than the boys, incredibly inappropriate urination. The boys usually discard other wastewater from their laundry, bathing, or waste from the kitchen, which can increase every element of the soil, thereby affecting the inhabitation of such soil's ecological biosphere.

Agwa et al. (2013) isolated similar bacteria genera from streams receiving raw abattoir waste. The bacteria identified include Staphylococcus sp, Escherichia coli, Pseudomonas sp, Salmonella sp, Enterobacter sp, Bacillus sp, Acinetobacter sp, and Proteus sp. The bacteria identified by biochemical characterization and their frequency of occurrence showed *Escherichia coli* 5(27.8%), *Staphylococcus* sp 6(33.3%), *Salmonella* sp 2(11/1%), *Shigella* sp 1(5.6%), *Bacillus* sp 2(11.1%), and *Acinetobacter* sp 2(11.1%). In s similar study, *Salmonella* type, *Escherichia coli*, *Shigella* sp and *Citrobacter* sp were isolated from domestic wastewater and soil in Ijebu Igbo Southeast, Nigeria (Adeniran et al., 2017; Akindele et al., 2015).

Some of these bacterial organisms isolated from this study are regarded as potential pathogenic bacteria organisms to humans and their environment if not correctly handled. Most of them could cause food-borne diseases and other human health abnormalities. Thus, it results in severe consequences that could lead to life loss. More so, some of them play a particular role in the environment where they are found, especially in the case of *Acinetobacter* sp. They help fertilize the soil environment, thus improving soil fertility and productivity. *Acinetobacter* sp is an oxidase-negative member of the group assigned to the genus *Acinetobacter* (Baumann et al.,1965;

Baumann, 1968). Before the isolation of a large number of strains from soil and water in our laboratory, most of the available strains of *Acinetobacter* had been isolated from clinical specimens (eyes, ears, nasopharynx, abscesses, blood, wounds, genital samples, stools, urine, etc.), and had assigned a variety of specific names (Baumann et al., 1965; Baumann et al., 1968). Recently, and with increasing frequency, these bacteria have been implicated in cases of postoperative meningitis, respiratory tract infections, endocarditis, and septicaemia, particularly after extensive antibiotic treatment (Chassignol, 1961; Henderson, 1965; Baumann, 1968).

5. Conclusion

Recycling wastewater is a widely available option to overcome the water supply shortage. The breeding of pathogenic bacteria, which could lead to environmental pollution and insufficient provision of sanitation and wastewater disposal facilities, is likely to lead to environmental and public health challenges. There should be adequate wastewater pre-treatment before discharging into the environment. Because of this, wastewater treatment and recycling methods will be vital to provide safety to the receiving ecological inhabitant. Also, human activity has been revealed to be a contributing factor that triggers an increase in bacterial load in wastewater and dumpsite. This study has revealed that cafeteria wastewater and dumpsite harbours bacteria organisms that could be pathogenic to humans and their environment if not correctly handled. The study revealed a higher bacterial load on the dumpsite sample collected from the boys' hostel due to their anthropogenic activities. This study gives baseline data for bacteriological analysis of cafeteria wastewater and dumpsites in Madonna University Elele Rivers State. Relevant Environmental agencies should endeavour to monitor wastewater and dumpsite in their localities.

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