International Journal of Environmental Protection and Policy
2017; 5(6-1): 23-32
http://www.sciencepublishinggroup.com/j/ijepp
doi: 10.11648/j.ijepp.s.2017050601.14
ISSN: 2330-7528 (Print); ISSN: 2330-7536 (Online)

Microbial Estimation and Characterization of Wastewater and Sludge in Awka Metropolis, Nigeria

Bassey Effiong Edet[1], Gwana Adamu Mohammed[2, *], Buhari Bagudu Yawuri[3], Alhaji Bashir Mohammed[4], Abubakar Musa[4], Abba Mala Galtimari[5], Hauwa Lawan Badawi[6], Mustapha Ahmadu[7], Fanna Ali Garra[2]

[1]Department of Applied Microbiology and Brewery, Nnamdi Azikwe University, Awka, Nigeria

[2]Laboratory Unit, Department of Animal Health and Production Technology, Mohamet Lawan College of Agriculture, Maiduguri, North – Eastern Nigeria

[3]Department of Science Laboratory Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Nigeria

[4]Department of Basic Sciences and Technology, Mohamet Lawan College of Agriculture, Maiduguri, North, Eastern Nigeria

[5]Department of General Studies, Mohamet Lawan College of Agriculture, Maiduguri, North – Eastern Nigeria

[6]Heritage Unit, Department of Heritage and Monument Sites, National Commission for Museums and Monuments, Maiduguri, Nigeria

[7]Department of Remedial Sciences, Mohamet Lawan College of Agriculture, Maiduguri, North – Eastern Nigeria

Email address:

edetbassey69@gmail.com (Bassey E. E.), admuwana@yahoo.com (Gwana A. M.), hutawas@yahoo.com.uk (Buhari B. Y.), malhajibashir@gmail.com (Alhaji B. M.), abbakmusa@gmail.com (Abubakar M.), abbamgaltimari@gmail.com (Abba M. G.), elbadawi@yahoo.com (Hauwa L. B.), ahmadumustapha960@gmail.com (Mustapha A.), micfanna@gmail.com (Fanna A. G.).

[*]Corresponding author

To cite this article:

Bassey Effiong Edet, Gwana Adamu Mohammed, Buhari Bagudu Yawuri, Alhaji Bashir Mohammed, Abubakar Musa, Abba Mala Galtimari, Hauwa Lawan Badawi, Mustapha Ahmadu, Fanna Ali Garra. Microbial Estimation and Characterization of Wastewater and Sludge in Awka Metropolis, Nigeria. *International Journal of Environmental Protection and Policy*. Special Issue: The Role of SLT: Environmental Impact Assessment And Statement Concept. Vol. 5, No. 6-1, 2017, pp. 23-32. doi: 10.11648/j.ijepp.s.2017050601.14

Received: March 23, 2017; **Accepted**: March 24, 2017; **Published**: August 21, 2017

Abstract: This study focuses on the estimation and characterization of microorganisms isolated from wastewater and sludge in Awka metropolis, Nigeria. The samples were collected randomly from four (A, B, C and D) respective areas, analyzed microbiologically by homogenizing the samples under aseptic conditions to obtain a homogenous mixture, the isolation techniques used was pour plate methods as described by Cheesbrough, (2010); Joanne *et al*, (2011); APHA, (1989). The samples were diluted serially, inoculated on to MacConkey agar and Sabouraud Dextrose agar media each and microorganisms were isolated; wastewater had a range from 44 to 100 colonies and total of 284 (71 ± 24.30), CFU / ml (10^{-4}) of 1.0×10^7 to 8.0×10^6 with total count of 19.4 (4.85 ± 2.96) $\times 10^6$, while sludge was ranges from 60 to 120 colonies with a total of 358 (89.5 ± 29), CFU / ml (10^{-4}) ranged from 1.0×10^7 to 7.0×10^6 with total count of 15.2 (3.8 ± 3.1) $\times 10^6$. The colony and the morphology of the isolates were recorded, the bacterial isolates were characterized by motility tests, Gram staining and identified by biochemical tests. The fungal isolates were microscopically examined using a wet mount procedure. From the results obtained 8 bacterial and 7 fungal isolates were identified from both the wastewater and the sludge; *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Micrococcus leteus, Enterococcus faecalis, Serratia mercescens* and *Yersinia enterocolitica*. *Acrophialophora fusispora, Epicoccum Purpurascens, Rhizopus microsporus, Exophiala spinifera, Cladosporium cladosporiodes, Aspergillus niger* and *Phaeoacremonium parasiticum.* Results also showed that *staphylococcus aureus* have the highest percentage of occurrence in wastewater, while in sludge *Micrococcus species* have the highest percentage of occurrence.

Keywords: Biochemical Test, Culture Media, Domestic, Industrial, Microorganism Morphological, Sludge, Wastewater

1. Introduction

Wastewater is defined as any water that has been used, such as for domestic or industrial use and contains waste products [1, 3, 5]. These waste products are most often liquid or solids and they can be biological, chemical or radioactive [2, 4]. In addition to having adverse health implications, wastewater contamination can also have natural and ecological affects. Wastewater is also any water that has been adversely affected in quality by anthropogenic influence [5, 6]. Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water and from sewer inflow or infiltration [7]. These may include the degradation of ecosystems such as a decrease in important aquatic plants that help preserve the condition of waterways or biodiversity loss such as loss of aquatic life like fish and crustaceans that are an important part of animal and human diet [8, 9, 21].

In a large waterway, such as a river or stream that has a continuous flow and a renewable source of fresh water, a small amount of contaminant may not make a considerable impact as there is a natural process of bacteria breakdown if water temperature, dilution and solar radiation are optimal [10, 12, 13]. Streams and rivers, which wind through rocks, pebbles, gravel and sand, also have a natural filtration system that can help to break down contaminants [11]. In addition, a certain amount of nutrients is actually helpful in the growth process of aquatic plants, but excessive nutrients can also hasten algae growth which then leads to a decrease in dissolved oxygen [4, 14, 15]. This overgrowth of algae clouds the water and prevents sunlight from permeating, leading to the destruction of important organisms, plant and animal life.

Municipal wastewater (also called sewage) is usually conveyed in a combined sewer or sanitary sewer, and treated at a wastewater treatment plant [16]. Treated wastewater is discharged into receiving water via an effluent pipe. Wastewaters generated in areas without access to centralized sewer systems rely on on-site wastewater systems. These typically comprise a septic tank, drain field, and optionally an on-site treatment unit. The management of wastewater belongs to the overarching term sanitation just like the management of human excreta, solid waste and storm water (drainage) [16, 17, 18].

Sewage is a type of wastewater that comprises domestic wastewater and is therefore contaminated with feces or urine from people's toilets, but the term sewage is also used to mean any type of wastewater [19, 20]. Sewerage is the physical infrastructure, including pipes, pumps, and screens, channels etc. used to convey sewage from its origin to the point of eventual treatment or disposal [21]. Sludge is a mixture of water and solids separated from various types of water as a result of natural or artificial processes. The generic sludge term generally used for untreated sludge, includes sewage sludge (sludge from urban waste water), septic tank sludge and industrial sludge [19, 23].

Over one hundred (100) different enteric pathogens may be found in waste water. These include viruses, parasites, fungi and bacteria, all of which may be associated with waterborne disease. The detection and identification of the many different types of microbial pathogens known to contaminate sludge and wastewater would be a difficult, time consuming and an immensely expensive undertaking if attempted on a regular basis [19, 24, 25]. To avoid the necessity of such vast undertakings, indicator microorganisms, commonly faecal coliforms, are used to determine the relative risk of the pre - sence of pathogenic microorganisms in a sample. Indicator organisms have the disadvantage that they often have survival characteristics which are different to a variety of pathogens, in particular enteric viruses and protozoa [5, 6, 28]. There are a number of established methods for the detection of most microbial pathogens in wastewater. Most established detection methods either rely on the culturing of pathogens using an artificial medium or cell culture, or, when they cannot be cultured, through direct detection usually involving the use of microscopy with major limitations, associated with the time taken to isolate and/or identify the pathogen, the determination of pathogen numbers in a sample and the accuracy of detection [1, 24, 28, 29]. Methods are currently under development which may allow the rapid and accurate detection of a range of microbial pathogens from a single water sample [7, 28]. *Escherichia coli* have been used as indicators of faecal contamination of water sources [5]. *E. coli* is used as its growth characteristics and behaviour in the environment are relatively well known, excreted by warm blooded grown on selective media at 44°C, ability to be cultured at elevated temperatures has leaded them to be known as the thermo - tolerant Coliforms and they have become the mainstay indicator for the water industry [13, 15, 29]. The examination of the presence, estimation, behaviour and / or incidence of a number of potential indicator organisms in water environment and compared their concentrations with the occurrence of pathogen (Coliforms, Enteric viruses, *Aeromonas spp*, *Salmonella spp*, *Gardia spp*, *Cryptosporidium spp*, etc) became necessary. This study is to estimate and characterize the microorganisms isolated from wastewater and sludge in Awka metropolis, Nigeria.

2. Materials and Methods

2.1. Study Site

The study was conducted at the microbiology general laboratory of Nnamdi Azikwe University, Awka, Nigeria. The study lasted from July to August 2016.

2.2. Sample Collection

Four different samples of wastewater were collected from Juhel parental drugs, Crunchies, St. Joseph the worker and Science village. Four different samples of sludge were also

collected from Ifite, Thinkers lodge, Nonny's lodge and Divine lodge, all in Awka South LGA. These samples were immediately transported to the laboratory of the applied microbiology and brewing department, Nnamdi Azikwe University, Awka, Nigeria, were the research study was conducted.

2.3. Materials Used

2.3.1. Culture Media used for the Study

The media used for this experiment were MacConkey Agar and Sabouraud dextrose agar.

2.3.2. Media Preparation

The media prepared were the MacConkey Agar and Sabouraud dextrose agar and were prepared aseptically and standard operation procedures been observed.

(i) MacConkey Agar (MCA)

Nine point four (9.4 g) of MacConkey Agar was measured using an electrical weighing balance. They were subsequently poured into conical flask and were dissolved in 250 ml of distilled water. The media was autoclaved at 121°C for 15 minutes and allowed to cool at 45°C respectively.

(ii) Sabouraud Dextrose Agar (SDA)

Sixteen point three (16.3 g) of Sabouraud dextrose agar was weighed into conical flask and was dissolved with 250 ml of distilled water. Zero point five (0.5 g) of Chloramphenical was added to the SDA to inhibit the growth of bacteria. The media were boiled using a bursen burner and stirred continuously using a sterile glass rod for a homogenous mixture to be obtained. The conical flask were tightly closed with a sterile cotton wool and wrapped with a foil paper to avoid contamination. They are autoclaved at 121°C for 15 minutes; the media are allowed to cool at 45°C respectively

2.3.3. Isolation technique (Pour Plate Method)

One ml of each of the four samples each of wastewater and sludge were transferred into 9 ml of sterile water of different test tubes using a serial dilution method ($10^{-1,}$ $10^{-2,}$ 10^{-3}, 10^{-4} and 10^{-5}). The procedure was repeated in all the samples collected. Each sample was allowed to stand for two minutes, and was shaken so that there will be homogenous distribution of the microorganism with the aid of a sterile pipette, 0.1 ml of each solution was transferred into different sterile Petri dishes, before the media are poured into the Petri dishes using a pour plate technique. The inoculated plates were Incubation at 37°C for 24 hours for bacteria and at 37°C for 72 hours for fungi. The plates were observed for colonies and other morphological appearances.

2.3.4. Sub - Culturing

The mixed cultures of the microorganisms were sub cultured into fresh medium of MCA and SDA plates. A pure culture of the bacteria and the fungi were obtained after the subculture and were stored in the bijou bottles at 4°C for bacteria and room temperature for the fungi.

2.3.5. Characterization and Identification of Bacterial and Fungal Isolates

(i) Bacterial Identification

The bacterial isolates were characterized and identified based on their morphology and biochemical reactions as described by the methods of Cheesbrough (2010), APHA, (1989) and that of Joanne *et al*, (2011).

a. Colony morphology

The isolates were characterized based on their morphologically based on shape, colour, elevation, margin, and texture.

b. Biochemical reactions

Various biochemical tests were being carried out for the identification of bacterial isolates that were isolated in the wastewater and the sludge sampled.

i. Gram staining

This was carried out to differentiate gram positive organism from gram negative ones. A wire lop was sterilized in Bunsen burner flame and allowed to cool, then a loopful of the growth was collected from the stored pure culture sample and smeared on a clean grease free glass slide. Then a drop of normal saline was added, emulsified, smeared and allowed to dry, and heat fixed by passing over a Bunsen burner flame three times. The smear was flooded with crystal violet stain for 30 - 60 seconds and then washed off. Then grams iodine was added for 1 minute pour off and then washed off. It was decolorized with acetone until no color runs off the slide. It was then washed off with water immediately. The slide is then flooded with safranin for 60 seconds and then washed off with clean water. The slide was kept to air dry and viewed under a microscope with x 100 objective lens (oil immersion lens) with a drop of Immersion oil and microscopically examined for presence of the Gram positive (G + ve), Gram negative (G - ve) or both organisms. Colour appearance; Purple or blue colour indicates a G + ve organism and red or pink colour is an indication of G – ve) organism respectively.

ii. Motility test

Motility test was aimed at identifying motile bacteria.

Nutrient agar are prepared and poured inside different test tubes, they are closed tightly with cotton and foil, and they are then autoclaved at 121 ^{o}C for 15 minutes. After the autoclaving, they are slanted and are allowed to solidified, and then the organisms are stabbed in the test tubes using a straightened sterile wire loop. The organism that grows from the stabbed position to the surface of the nutrient agar is motile, while those that do not move are non-motile.

iii. Catalase test

This was used to differentiate those bacteria that produce the enzyme Catalase.

A drop of hydrogen peroxide is placed on a glass slide, and the test organism is placed on the hydrogen peroxide. Catalase positive organisms produce bubbles, while Catalase negative organisms produce no bubble.

iv. Coagulase Test

This was used to identify pathogenic *staphylococcus aureus* which produces the Coagulase enzyme which causes

plasma to clot by converting fibrinogen to fibrin.

A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two thick suspensions. A loopful of plasma was added to one of the suspension, and mixed gently. The slide was examined for clumping or clotting of the organism within 10 seconds.

v. Urease test

This test was aimed to identify *Enterobacteriacaea* that produce Urease enzyme, which hydrolyze urea to give ammonia and carbon dioxide.

The test organism was heavily inoculated onto Christensen's urea broth in a bijou bottle using a sterile wire loop and incubated at 35°C – 37°C for 18 - 24 hours and examined, thereafter, a pink coloration in the medium showed a positive result.

vi. Indole Test

This test was carried out to know that bacterium that expresses the enzyme tryptophanase which can hydrolyze tryptophan into Indole pyruvic acid and ammonia. A sterile wire loop was used to inoculate a colony of test organism in to 2 test tubes containing 2 ml of peptone water which contained tryptophan. The tube was incubated at 37°C for 24 hours. Kovac's reagent was added to the medium. Observation of red coloration on the surface layer within 10 minutes showed a positive result.

vii. Voges Proskauer test

This test was used to show if the bacterium has butanediol fermentation and can split glucose to acetoin. Peptone bacteriological, Dipotassium Dihydrogen phosphate and glucose were mixed together, and after autoclaving, the test organisms were inoculated and incubated at 37°C for 3 days. 0.6 ml of Alpha naphtol was then added followed by 0.2 ml of potassium hydroxide. The formation of pink coloration shows a positive result.

viii. Methyl red test

This was carried out to identify bacterium based on their ability to produce and maintain stable acid end product from glucose fermentation. Peptone bacteriological, Dipotassium Dihydrogen phosphate and glucose were mixed together, and after autoclaving, the test organisms were inoculated and incubated at 37°C for 3 days, after which a few drop of methyl red solution was added to the solution. A formation of red coloration immediately showed a positive result.

ix. Sugar fermentation test

This test is used to determine the ability of bacteria to utilize different sugars, like glucose, fructose, lactose and sucrose. The sugar solutions were prepared and poured into test tubes with Durham's tube for gas collection. The sugar was autoclaved, after which a loopful of test organism was introduced into the sugar solution. A yellow coloration shows acid production, while the collection of gas bubbles in the Durham's tube shows gas production.

x. Citrate test

This test shows those bacteria that can utilize citrate as their only carbon source.

The test organisms were inoculated into test tubes containing prepared Simmons's citrate agar, then they are incubated at 37°C during 24 - 48 hours. A colour change from green to blue shows a positive result.

(ii) Identification of Fungi Isolates

The identification of the fungal isolates was done based on their cultural characteristics and by microscopic examination using a wet mount method.

Wet Mount Preparation

Procedure:

Place some drops of lactophenol cotton blue on a clean grease free glass slide. By using a sterile wire loop, the colony of the fungal culture was collected and placed on some drops of the lactophenol cotton blue and mixed thoroughly. A cover slip was placed on the top of the sample mixed with the lactophenol cotton blue, the slide is now ready for examination. This procedure was carried out to facilitate detailed study of the morphology and the natural arrangement of the conidia, sporangium and arthrospores of the culture.

2.4. Data Analysis

The data obtained from this research study were subjected to statistical tools of analysis using Pie chart for measurement of dispersion and or discrepancy within the variables being obtained and its' significance, as described by Stroud and Booth, (2001). The analysis of variance (ANOVA) was used to determine the differences between treatments at significance rate of $P < 0.05$ as described by Steel and Torrie (1980). The standard errors of treatment means estimated out using Statistical Analysis System (SAS, 2000).

3. Results

Environmental pollutions and contaminations study was carried out to estimate and characterize microbes found in wastewater and sludge in Awka metropolis, Anambra state Nigeria. Four samples of wastewater and four samples of sludge were analysed. The samples of the wastewater and sludge were found to contain various microorganisms. Out of the four samples of wastewater and sludge samples studied, results showed that the wastewater and sludge samples contain bacteria and fungi. The bacterial cultures yielded species such as *Staphylococcus aureus, Micrococcus leteus, Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Serratia marcesecens, Yersinia enterocolitica, Klebsiella pneumonia,*. While the fungal isolates were mainly molds *Acrophialophora Fusispora, Epicoccum Purpurascens, Rhizopus microsporus, Exophiala Spinifera, Cladosporium Cladosporiodes, Aspergillus niger, Phaeoacremonium parasiticum.* These results are presented in the tables below;

3.1. The Bacterial Count for Wastewater and Sludge Samples

The bacterial count of the wastewater and the sludge

samples were carried out as shown in table 1 and 2.

3.2. The Colonial and Morphological Characteristics of the Fungal Isolates from Wastewater and Sludge Samples

The colonial and the morphological characteristics of the fungal isolates were examined macroscopically as shown in table 3 and table 4.

3.3. Identification of the Fungal Isolates from Wastewater and Sludge Samples

The fungal isolates from both samples were identified using a wet mount method procedure, as shown in table 5.

3.4. The Morphological Characteristics of the Bacterial Isolates from Wastewater and Sludge Samples

The organisms were further characterized based on morphological characteristics such as colour, shape, elevation and margin, as shown in table 6 and table 7.

3.5. Biochemical Reactions of the Bacterial Isolates from Wastewater and Sludge Samples

The biochemical reactions were carried out to identify the bacterial isolates into the genus level, as shown in table 8 and table 9.

3.6. Sugar Fermentation by the Bacterial Isolates from Wastewater and Sludge Samples

This test was carried out to know those bacteria that can utilize various sugars, as shown in table 10 and table 11.

3.7. Percentage of Occurrence of the Bacterial Isolates from Wastewater and Sludge Samples

This result shows the organism that has the percentage of occurrence in the wastewater and the sludge samples, as shown in fig 1 and fig 2.

Table 1. *Bacterial count for wastewater samples.*

Samples	Number of colonies	CFU / ml (10^{-4})
A	60	6.0×10^6
B	44	4.4×10^6
C	100	1.0×10^7
D	80	8.0×10^6
Total (mean ± STDev)	284 (71 ± 24.30)	19.4 (4.85 ± 2.96) × 10^6

Keys: CFU = Coliform Unit, ml = Millitres, STDev. = Standard Deviation.

Table 2. *Bacterial count for sludge samples.*

Samples	Number of colonies	CFU / ml (10^{-4})
E	60	6.0×10^6
F	70	7.0×10^6
G	120	1.2×10^7
H	108	1.0×10^7
Total (mean ± STD)	358 (89.5 ± 29)	15.2 (3.8 ± 3.1) × 10^6

Keys: CFU = Coliform Unit, ml = Millitres, STD = Standard Deviation.

Table 3. *Colonial and morphological Characteristics of Fungal Isolates from wastewater samples.*

Samples	morphology / colour	suspected organism
A	Grayish-brown and slightly floccose	*Acrophialaphora spp*
B	Dark-brown to black velvety and globose	*Aspergillus spp*
C	Orange-brown, globose and verrucose	*Epicoccum spp*
D	Blackish brown and suede-like to floccose	*Cladosporium spp*

Table 4. *The Colonial and Morphological Characteristics of Fungal Isolates for Sludge.*

Samples	morphology / colour	suspected organism
E	Sub-globose to globose and brownish	*Rhizopus spp*
F	Mucoid and black in color	*Exophiala spp*
G	Whitish-grey and velvety	*Phaeoacremonium spp*

Table 5. *Identification of the Fungal Isolates from Wastewater and Sludge Samples.*

Texture	Size	Colour	Organism
Velvety, mucoid and yeast-like and raised.	The conidiophores are 1 – 3 µm long.	Black.	*Exophiala spinifera*
Velvety to coarse, rough woolly and cottony.	The conidiophores ranging from 900 – 1600 µm in length.	Dark brown to black.	*Aspergillus niger.*
Globose, suede-like and verrucose.	The conidia are globose mostly 15-25µm in diameter.	Orange brown.	*Epicoccum purpurascens*
Velvety to floccose and cottony. brown.	The conidiophores erect branched-sympodial elongation.	Olivaceous-brown to Blackish	*C. cladosporioides*
Sub-hyaline, woolly and smooth.	The sporangiophores are 400 µm high and 10 µm wide.	Brown – grey.	*Rhizopus microsporus*
Suede-like and rough and velvety.	The Conidia are hyaline (3.6× 1- 2µm), phialides (15 - 50µm).	Whitish–grey.	*Phaeoacremonium parasiticum.*
Rough walled and velvety.	The Conidiophores (15µm long, 2-5 µm wide).	Grayish brown with a black reserve.	*Acrophalophora fusispora*

Table 6. *Morphological Characteristics of the Bacterial Isolate in Wastewater Samples.*

Isolates	Shape	Margin	Colour	Elevation
A	Circular	Entire	Creamy	Raised
B	Circular	Entire	Yellow	Raised
C	Circular	Entire	Pink	Flat
D	Circular	Entire	Yellow	Flat

Table 7. *Morphological Characteristics for Bacterial Isolates in Sludge Samples.*

Isolates	Shape	Margin	Colour	Elevation
E	Irregular	Erose	Creamy	Flat
F	Circular	Entire	Creamy	Raised
G	Irregular	Entire	Creamy	Flat
H	Circular	Entire	Creamy	Raised

Table 8. *Biochemical Reactions Tests of the Bacterial Isolates for Wastewater Samples.*

Isolate	Motility	Grams' Reaction	Coagulase	Catalase	Methyl Red	Indole	VP	Citrate	Organism
A	-	- Rod	-	+	-	-	+	+	*Klebsiella sp.*
B	+	+ cocci	+	+	+	-	+	+	*Staphylococcus aureus.*
C	+	- Rod	-	+	+	+	-	-	*Escherichia coli.*
D	+	- Rod	-	+	-	-	+	-	*Pseudomonas sp.*

Table 9. *Biochemical Reactions of Bacterial Isolates for Sludge Samples.*

Isolate	Motility	Grams' Reaction	Coagulase	Catalase	Methyl Red	Indole	VP	Citrate	Organism
E	-	+ Cocci	+	+	-	-	-	-	Micrococcus spp.
F	-	- Rod	-	+	-	-	-	-	Yersinia spp.
G	+	- Rod	+	+	-	+	+	+	Serratia spp.
H	+	+ Cocci	-	-	-	-	-	-	Enterococcus spp.

Table 10. *Sugar Fermentation of the Bacterial Isolates from Wastewater Samples.*

Isolates	Glucose	Lactose	Sucrose	Organisms
A	AG	AG	AG	*Klebsiella spp.*
B	AG	AG	AG *aureus.*	*Staphylococcus*
C	AG	AG	-	*Escherichia coli*
D	-	-	-	*Pseudomonas sp.*

Keys: AG = Acid and Gas, - = Negative.

Table 11. *Sugar Fermentation of the Bacterial Isolates from Sludge Samples.*

Isolates	Glucose	Lactose	Sucrose	Organisms
E	A	-	A	*Micrococcus spp.*
F	AG	-	-	*Yersinia spp.*
G	AG	-	AG	*Serratia sp.*
H	AG	AG	AG	*Enterococcus sp*

Keys: AG = Acid and Gas, - = Negative.

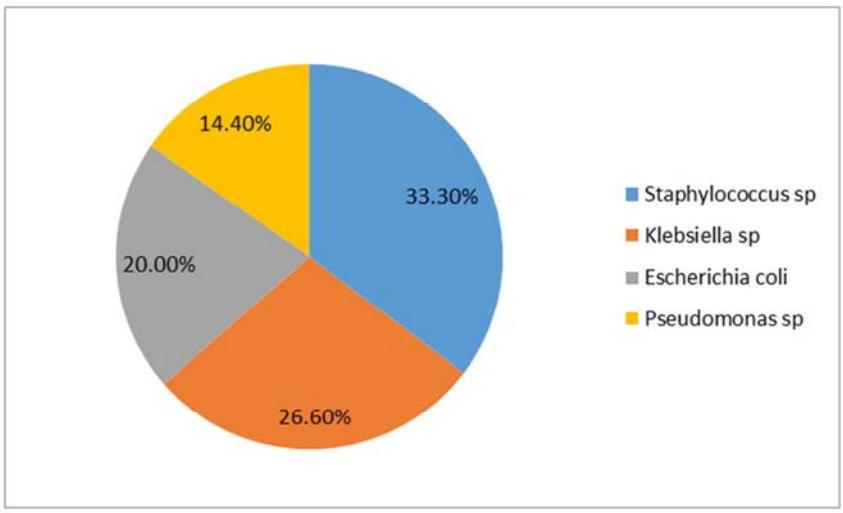

Figure 1. *The Occurrence of Bacteria in Wastewater.*

Figure 1 showed the Pie chart (in percentage values) of bacteria in wastewater; that Staphlococcus specie had 33.3%, Klebsiella specie had 26.6%, Escherichia coli had 20% and lastly Pseudomonas specie had 14.4% respectively. Thus, Staphlococcus specie was the highest in occurrence, followed by Klebsiella specie, then E. coli, and then lastly the least was Pseudomonas specie occurrence, with a p-value of 0.041.

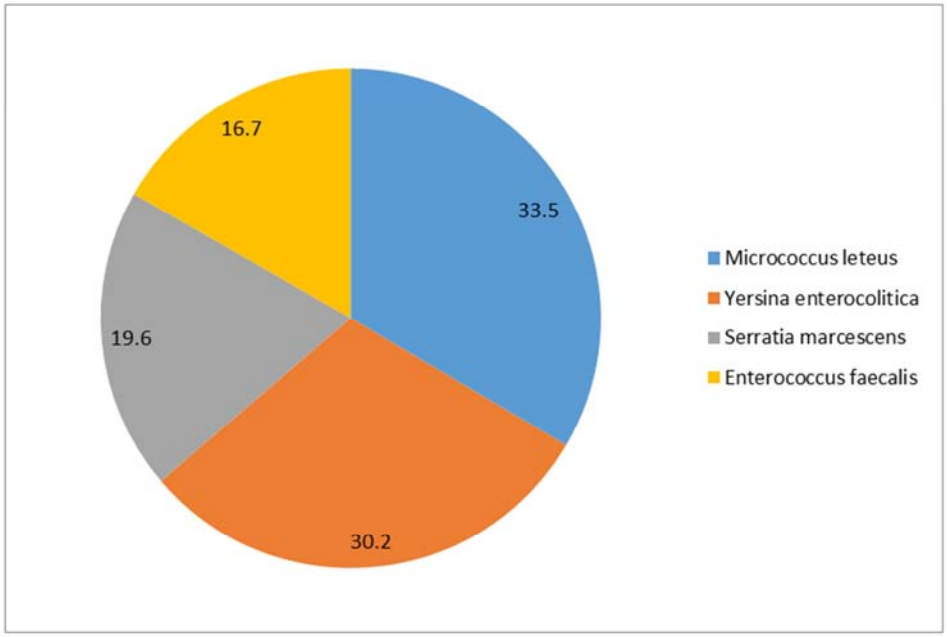

Figure 2. *The Occurrence of Bacterial Isolates from Sludge.*

Figure 2 shows the Pie chart (in percentage values) of bacteria in sludge; that *Micrococcus leteus* has the highest percentage of occurrence in sludge (33.5%), which is followed by *Yersinia enterocolitica* with a percentage of 30.2%, while *Serratia marcescens* has a percentage of 19.6% and 16.7% for *Enterococcus faecalis* with a p-value of 0.051, which shows significant differences in their occurrences.

4. Discussion

The climate change periodically, due to human activities and industrial wastes contaminating the environment, thereby forming wastewaters and sludge with an impact to the biodiversity and the extreme ecological of the study area especially the climatic variation could have the resultant effect in modifying Awka town ecosystem, this is in line with the statement made by Gwana *et al*, (2017) and Umaru *et al*, (2016). More than one hundred (100) different enteric pathogens may be found in wastewater and sludge. According to Gwana *et al*, (2017) who stated that water is essential to life, used for domestic and industrial purposes and a medium in which most of the organisms' lives and therefore, the growth of any organism is directly to type of the water, quality and quantity. These include viruses, parasites, fungi and bacteria, all of which may be associated with waterborne disease, many different types of microbial pathogens known to contaminate sludge and wastewater, thereby contaminating the environment making it unfriendly and health risks. Symptoms such as profuse diarrhoea and usually containing blood and mucus do occurred, abdominal cramps and dehydration appear within some hours after ingestion, and may last for up to future night.

Wastewater is defined as any water that has been used, such as for domestic or industrial use and contains waste products. Food borne outbreaks of the disease are usually linked to the use of raw, contaminated products in salads or foods that have not been properly washed with clean portable water and cooked before consumption. According to Savichtcheva and Okabe (2006) and Wadh et al, (2002) who stated that where water is contaminated with faeces of animal origin, this pathogen may be present, considers the group *Shigella species* as a threat to human health in cases where fresh produce is irrigated with contaminated water and then consumed raw. The detection and identification of the many different types of microbial pathogens known to contaminate sludge and wastewater, thereby contaminating the environment making it unfriendly, health risks, etc, this in order to assess and state the environmental impacts caused by this contamination process would be a difficult, time consuming and an immensely expensive undertaking if attempted on a regular basis to be conducted.

As a result, this study was carried out to estimate and characterize microbes found in wastewater and sludge in Awka metropolis, Anambra state Nigeria. Four samples (A, B, C and D) of wastewater and four samples (E, F, G and H) of sludge were analysed. The samples of the wastewater and sludge were found to contain various microorganisms. The bacterial isolates were identified as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Serratia marcescens, Yersinia enterocolitica, Micrococcus leteus, Enterococcus faecalis, and Klebsiella pneumoniae,* while the molds were, *Acrophialaphora fusispora, Aspergillus niger, Epicoccum purpurascens, Cladosporium cladosporiodes, Rhizous microsporus, Exophiala spinifera, and Phaeoacremonium parasiticum.* As indicated in fig 1, *Staphylococcus aureus* was found to be the organism with the highest occurrence in wastewater with a percentage of (33.3%), it is followed by *Klebsiella pneumoniae* with a percentage of (26.6%), *Escherichia coli* 20% and *Pseudomonas specie* with 14.7% occurrence, with a p-value

of 0.041. Fig 2 shows that *Micrococcus leteus* has the highest percentage of occurrence in sludge (33.5%), which is followed by *Yersinia enterocolitica* with a percentage of 30.2%, while *Serratia marcescens* has a percentage of 19.6% and 16.7% for *Enterococcus faecalis* with a p-value of 0.051, which shows significant differences in their occurrences.

Among the isolates identified from the wastewater samples, *Staphylococcus aureus,* is of major public health concern, the organism is responsible for many food borne outbreaks, because these organism are able to produce enterotoxins as indicated by Wadhwa (2002). *E. coli* has been found to be one of the contaminants of wastewater, this organism has high rate prevalence in water samples, and it has been found to cause hemorrhagic colitis, gastroenteritis and kidney failure as pointed by APHA, (2009) and Bryan, (2007). In some countries, wastewaters are used as irrigation water for crops, this poses a more disease treat to human when this produce are consumed especially vegetables. Some of these microbes are also airborne spread and some are spread indirectly by termites. The presence of fungi in wastewater and sludge is also alarming, because these organisms are able to produce mycotoxins which are hazardous to human health.

In addition to having adverse health implications, wastewater contamination can also have natural and ecological affects. Wastewater is also any water that has been adversely affected in quality by anthropogenic influence. Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water and from sewer inflow or infiltration. These may include the degradation of ecosystems such as a decrease in important aquatic plants that help preserve the condition of waterways or biodiversity loss such as loss of aquatic life like fish and crustaceans that are an important part of animal and human diet as well.

In a large waterway, such as a river or stream that has a continuous flow and a renewable source of fresh water, a small amount of contaminant may not make a considerable impact as there is a natural process of bacteria breakdown if water temperature, dilution and solar radiation are optimal. Streams and rivers, which wind through rocks, pebbles, gravel and sand, also have a natural filtration system that can help to break down contaminants. In addition, a certain amount of nutrients is actually helpful in the growth process of aquatic plants, but excessive nutrients can also hasten algae growth which then leads to a decrease in dissolved oxygen, as being also reported by other researchers. This overgrowth of algae clouds the water and prevents sunlight from permeating, leading to the destruction of important organisms, plant and animal life as results of contamination with sludge wastewater in not sanitize. It is important to dispose and handle wastewater with care, because of dangerous microbes associated with them. Any contact with food and other things that are consumed by man are detrimental to human health risk factors.

4. Conclusion

The climate change periodically, due to human and industrial activities producing wastes that contaminating the environment, thereby forming wastewaters and sludge with an impact to the biodiversity. Many different enteric pathogens may be found in wastewater. These include viruses, parasites, fungi and bacteria, all of which may be associated with waterborne disease, and many different types of microbial pathogens known to contaminate sludge and wastewater, thereby contaminating the environment making it unfriendly and health risks. Symptoms such as profuse diarrhoea and usually with blood and mucus do occurred, abdominal cramps and dehydration appear within some hours after ingestion, and may last for up to future night.

The results of this study revealed that the four different samples of wastewater were collected from Juhel parental drugs, Crunchies, St. Joseph the worker and Science village and the four different samples of sludge were also collected from Ifite, Thinkers lodge, Nonny's lodge and Divine lodge, all in Awka South LGA that there are presences of Coliforms and other pathogenic microbes, which indicates health risks involved in handling of wastewater and sludge in Awka municipal, Nigeria. It is important to dispose and handle wastewater with care, because of dangerous microbes associated with them. Any contact with food and other things that are consumed by man are detrimental to human health. All wastewater from domestic and industrial use should be disposed properly to minimize the risk posed by these pathogenic microbes.

Recommendations

Based on the findings of this research study we strongly recommend the followings:

That all the wastewaters and sludge from industries should be channeled under ground to a place where there will not be any contact with human activities. Household wastewater should be disposed properly, avoiding using them as irrigation water. That Government is urging to enforce a strict law on sanitation concerning improper disposal of wastewaters by industries and also stop the use of wastewater as irrigation water in the country for farming purposes and introduce some means of irrigation water.

Acknowledgements

We acknowledge with duly respected, most honoured and most grateful to the management of: Department of Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University, Awka, Nigeria and Laboratory Unit, AHP Department, Mohamet Lawan College of Agriculture, Maiduguri for providing some of the materials and venues, and any other persons, few to mentioned, that helped and aided us in the course of carrying out this research study successfully.

Appendix I

Table A1. *Chi-square Goodness-of-Fit Test for Observed counts in Variable: Wastewater.*

Category	Observed	Test Proportion to chi-Square	Expected	Contribution
1	14.70	0.25	23.66	3.39160
2	20.00	0.25	23.66	0.56546
3	26.60	0.25	23.66	0.36599
4	33.33	0.25	23.66	3.95466
P-Value= 0.041				

Chart of observed and expected values for bacterial prevalence in wastewater.

Appendix II

Table A2. *Chi-square Goodness-of-Fit Test for Observed counts in Variable: Sludge.*

Category	Observed	Test Proportion to chi-Square Expected	Contribution	
1	16.7 0.25	24.95	2.72796	
2	19.6	0.25	24.95	1.14719
3	30.2	0.25	24.95	1.10471
4	33.3	0.25	24.95	2.79449
p-Value = 0.051				

Chart of observed and expected values for bacterial prevalence in sludge.

References

[1] Abbaszadegan, M., Hasan, M. N., Gerba, C. P., Roessler, B. R., Wilson, R., Kuennen, and E. Van Dellen. "The disinfection efficacy of a point-of-use water treatment system against bacterial, viral and protozoan waterborne pathogens", *Water Research,* Vol. 31, No. 4, 2000; pp: 574-582.

[2] Abu-Ashour, J., Joy, M. H. Lee, H. R., Whiteley, and S. Zelin. "Transport of microorganisms through soil", *Water, Air and Soil Pollution*, Vol. 75, No. 9, 2004; pp: 141-158.

[3] Al-A'ama, M. S., and Nakhla G. F. "Wastewater reuse in Jubail, Saudi Arabia", *Water Research*, Vol. 29, No. 6, 2005; pp: 1579 - 1584.

[4] Anderson, J. M. "Current water recycling initiatives in Australia: scenarios for the 21st century", *Water Science and Technology,* Vol. 33, Nol. 5, 2006; pp: 37-43.

[5] APHA. (1989) "Standard methods for the examination of water and wastewater", Port City 2nd edition, Press, Baltimore, 1989; pp: 27-44.

[6] Armon, R., and Kott, Y. "Distribution comparison between coliphages and phages of anaerobic bacteria (Bacteroides fragilis) in water sources, and their reliability as fecal pollution indicators in drinking water", *Water Science and Technology*, Vol. 31, No. 17, 2003; pp: 215-222.

[7] Asano, T., and Levine, A. C. "Wastewater reclamation, recycling and reuse: Past, present and future", *Water Science and Technology*, Vol. 33, No. 5, 2000; pp: 1-14.

[8] Ashbolt, N. J., Ball, A., Dorsch, M. C., Turner, P., Cox, A., Chapman, and Kirov., M. N. "The identification and human health significance of environmental aeromonads", *Water Science and Technology*, Vol. 31, No. 23, 2005; pp: 263-270.

[9] Aulicino, F. A., Mastrantonio, A., Orsini, P., Bellucci, C., Muscillo, M., and Larosa, G. "Enteric viruses in a wastewater treatment plant in Rome", *Water, Air and Soil Pollution*, Vol. 91, No. 4, 2001; pp: 327 - 334.

[10] Ayers, R. M., Stott, R., Mara, D. D., and Lee, D. L. "Wastewater reuse in agriculture and the risk of intestinal nematode infection", *Parasitology Today*, Vol. 8, No. 3, 2002; pp: 32-35.

[11] Bahlaoui, M. A., Baleux, B., and Troussellier, M. "Dynamics of pollution- indicator and pathogenic bacteria in high-rate oxidation wastewater treatment ponds", *Water Research*, Vol. 31, No. 42, 1997; pp: 630–638.

[12] Bahri, A., and Brissaud, F. "Wastewater reuse in Tunisia: Assessing a national policy", *Water Science and Technology*, Vol. 33, No. 9, 1996; pp: 87-94.

[13] Baker, K. H., and Bovard, D. S. "Detection and occurrence of indicator organisms and pathogens", *Water Environment Research*, Vol. 68, No. 6, 1996; pp: 406-416.

[14] Bales, R. C., Hinkle, S. R., Kroeger, T. W. and Stocking, K. "Bacteriophage adsorption during transport through porous media: Chemical perturbations and reversibility", *Environmental Science and Technology*, Vol. 25, No. 2, 1999; pp: 2088-2095.

[15] Basher, A., and Shahalam, M. "Wastewater effluent vs. safety in its reuse: State - of – the – art*", The Journal of Environmental Sciences*, Vol. 32, No. 7, 2007; pp: 35-42.

[16] Bitton, G., Koopman, B., and Jung, K. "An assay for the enumeration of total coliforms and Escherichia coli in water and wastewater", *Water Environment Research, Vol.* 67, No. 5, 1995; pp: 906-909.

[17] Bryan, F. L. "Diseases transmitted by foods contaminated by wastewater", *Journal of Food Protection*, Vol. 40, No. 43, 2007; pp: 45-59.

[18] Capuano, R. M., Siringan, M. A., Jan, R. Z., and Jurtshuk, P. "Enhanced activity of oligotrophic endogenous bacteria in clay - rich sediments by nutrient injection", *Geomicrobiology Journal*, Vol. 13, No. 32, 2000; pp: 165-179.

[19] Carducci, A., Arrighi, S., and Ruschi, A. 1999 "Detection of coliphages and enteroviruses in sewage and aerosol from an activated sludge wastewater treatment plant". *Letters in Applied Microbiology*. Vol. 19, No. 21, 1999, pp: 207-209.

[20] Cheesbrough, C. "Microbiological Techniques", In: District Laboratory Practice in Tropical Countries, Second Edition Update, Part 1, Cambridge University Press Publication, GB, 2010.

[21] Gwana, A. M., Bassey, E. E., Buhari, B. Y., Mala, A. M., Umaru, B. W., Mohammed, L. S., Shettima, U. K., Halima, M. B. "Role of Slt: Environmental Impact Assessments and Statements Concept. *International Journal of Environmental Protection and Policy*, Special Issue: The role of SLT: Environmental Impact Assessment and Statement Concept", Vol. 5, No. 6 - 1, 2017; pp. 1–7.

[22] Gwana, A. M, Umaru, B. W., Halima, M. B., Wanas, L. N. "Physico – Chemical Water Quality Analysis of Lake Alau, North – Eastern Nigeria", *Journal of Water Resources and Ocean Science*, Vol. 6, No. 1, 2017; pp: 14–22.

[23] Joanne, M. W., Linda, M. S. and Christopher, J. W. "Clinical Microbiology and Immunology", In: *Prescott's Microbiology*, 8th Edition, Mc Grow – Hill Companies, New York, USA, 2011; pp: 850–871.

[24] Savichtcheva, O. and Okabe, S. "Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives", *Water Research*, Vol. 40, No. 8, 2006; pp: 2463-2476.

[25] Steel, R. G. D and Torrie, J. K. "Analysis of Variance", In: *Principles and Procedures of Statistics*, Mc Grow Hill Book Company Incorporation, USA, 2nd Edition, 1980; pp: 125–130.

[26] Stroud, K. A and Booth, D. J. "Statistical Package", In: *Engineering Mathematics* WWW.Palgrave.Com/Stroud, Palgrave. GB, London, 5th Edition, 1980; Pp: 1130–1139.

[27] Umaru, B. W., Gwana, A. M., Wanas, L. N., Halima, M. B. "Assessments of Biological Water Quality and Condition Factor of Clarias gariepinus in Lake Alau, North – Eastern Nigeria", Vol. 1, No. 1, 2016; pp: 1–10.

[28] Wadhwa, S. G., Khaled, G. H., and Edberg, S. C. "Comparative Microbial Character of Consumed Food and Drinking Water", *Critical Reviews in Microbiology*, Vol. 28, No. 3, 2002; pp: 249-279.

[29] Welshimer, H. J. "Survival of Listeria monocytogenes in soil", *Journal of Bacteriology*, Vol. 80, 1960; pp: 316-320 (As cited by Beuchat, (1996);

[30] Smith, J. L. and Buchanan, R. L. (1992). "*Shigella*, In: Compendium of Methods for the Microbiological Examination of Foods" (Edited by Vanderzant, C. & Splittstoesser, D. F.). Pp. 423-431. Washington DC: American Public Health Association.