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Bacteriological Analysis of Selected Industrial Wastewater in Akure Metropolis

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Abstract: Discharging untreated or inadequately treated wastewater from industries can cause health problems in many parts of the world. The present study was designed to enumerate and identify microorganisms in wastewaters from selected industries in Akure Metropolis. Waste water samples were collected from two industries in Akure Metropolis. The total heterotrophic bacteria count, fecal coliform count were used to determine bacterial contamination and also waste water samples were subjected to microbiological analyses. The bacteria isolated from waste water samples were *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Klebsiella oxytoca*, *Enterococcus faecalis*, *Proteus vulgaris*, *Lactococcus lactis*, *Listeria spp*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Micrococcus luteus* and *Erwinia caratova*. Wastewaters contain a large amount of pathogenic bacteria that can cause a real impact on human health. The bacteriological analysis of the wastewater indicates the presence of bacteria which suggests the water is not fit for consumption without proper processing. Thus, it calls for appropriate intervention, including awareness to the populace on the discharging of wastewater in order to minimize the potential health problems.
Keywords: Wastewater, Fecal coliform Bacteria, Microorganisms, bacteriological analysis.

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Introduction

Wastewater is one of the most critical problems of both middle and low income countries is improper wastewater management. Water is vital to the existence of all living organisms; however, this valued resource is increasingly being threatened as a result of upsurge in human population. Consequently, the demand for high quality water for both domestic and economic purposes has been noticeably on constant increase [1]. According to UNICEF report, about 800 million people in Asia and Africa are living without access to safe drinking water. Consequently this has caused many people to suffer from various diseases [2, 3]. However, access to safe drinking water has improved over the last decades in almost every part of the world especially Nigeria, but approximately 1.1 billion people still lack access to safe water and over 2.6 billion worldwide lack access to adequate water and Sanitation which causes water illnesses [4]. Wastewater that comprises of domestic wastewater is contaminated with faeces or urine from toilets, but the term sewage is also used to mean any type of wastewater. Industrial effluent contamination of natural water bodies has emerged as a major challenge in low income and densely populated countries like Nigeria. Estuaries and inland water bodies, which are the major sources of drinking water in Nigeria, are often contaminated by the activities of the adjoining populations and industrial establishments [5]. River systems are the primary means for disposal of waste, especially the effluents from industries that are nearby [6]. These effluent from industries contribute to water pollution and these can alter the physical, chemical and biological composition of the receiving water body [7]. Wastewater can originate from many sources such as; homes, businesses and industries. Storm water, surface water and ground water [8]. The greatest risk to public health from microbes in water is associated with consumption of wastewater that is contaminated with human and animal excreta [9].Wastewater which consist of human faeces can contain a variety of intestinal pathogens which cause diseases ranging from mild gastroenteritis to serious dysentery, cholera and typhoid. The most predominant waterborne disease, diarrhea, has an estimated annual incidence of 4.6 billion episodes and causes 2.2 million deaths every year [10]. Faecal coliform bacteria are used as an indicator for the presence of any of these water borne pathogens [11]. The presence of these indicative organisms is evidence that the water has been polluted with faeces of humans or other warm blooded animals [12].

2.0 Materials and Methods

2.1 Collection of wastewater sample

Wastewater sample was collected at septic tank of different companies using sterile container and transported to the laboratory for experiment.

2.2 Characterization and Identification of Bacterial Isolate

The isolates were characterized using colonial and cellular morphology as well as biochemical reactions as described in Benson's microbiological application. They were subsequently identified using cowan and steels manual for identification of bacteria [5].

2.3 Bacteriological Analysis

Nutrient agar, Eosine methylene blue, Shigella Salmonella agar and MacConkey agar were prepared using manufacturers direction. Bacteria isolates were characterized on the basis of the colonial morphology and Gram stain reaction. Biochemical tests such as catalase, Coagulase, Motility, Indole, Hydrogen sulphide, urease Meyhyl red, Vogesproskauer tests were carried out [8].

2.3.1 Total heterotrophic bacteria count

The spread plate method was used. Ten-fold serial dilution of each water sample was prepared aseptically in physiological saline of 10-1 up to 10-4 and 0.1 ml aliquot of each dilution was plated on nutrient agar plates. All incubations were conducted at 37°C for 24 hrs under aerobic conditions and plates containing 30 to 300 colonies were selected and counted. The number of colony-forming units per ml (cfu/ml) was calculated by multiplying the number of colonies by the dilution factor. Also, sub-culture was carried on MacConkey.

2.3.2 Faecal coliform count

Faecal coliform count was determined using Eosin Methylene Blue medium employing the streaking culture technique. A loopful of broth from positive tubes were streaked onto EMB agar plate for pure cultures. The plates were incubated at 37°C for 24 hrs. Colonies on EMB agar plate were further identified as fecal coliforms. On Eosin Methylene Blue (EMB) agar, E. coli strains appeared as greenish metallic sheen colonies.

Chapter Three

3.0 Result

3.1 Colonial characteristics of isolates

Tables 1 and 2 shows the colonial morphology of different isolates from wastewater from different industries in Akure Metropolis, Nigeria. In the table 1 and 2 the optical characteristics of all the isolates were either opaque, transparent and translucent the size were small, the shape were round, the colour was either white or cream the edge were smooth, even and entire, The elevation was either raised or flat, surface of the isolates were all smooth.

Summary of the microscopic and biochemical characteristics of isolates

Table 3 and 4 shows the summary of the microscopic and biochemical characteristics of isolates from wastewater in different industries in Akure Metropolis, Nigeria.The microscopy and the biochemical test is based on gram staining reaction, motility, catalase, oxidase, methyl red, VogesProskauer, citrate, urease, sugar fermentation test (such as Glucose and Lactose) and spore staining.

Table 1. Colonial characteristics morphology of isolates in waste water from industry A

Isolate Code	Optics	Size	Shapes	Edge	Elevation	Surface	Colour
IA1	Opaque	Small	Round	Entire	Raised	Smooth	White
IA2	Translucent	Small	Round	Smooth	Raised	Smooth	Cream
IA3	Opaque	Small	Round	Entire	Raised	Rough	White
IA4	Opaque	Small	Round	Even	Flat	Smooth	White
IA5	Opaque	Small	Round	Smooth	Flat	Smooth	Cream
IA6	Translucent	Small	Round	Entire	Raised	Smooth	White
IA7	Translucent	Small	Round	Smooth	Raised	Smooth	Cream
IA8	Transparent	Small	Round	Smooth	Raised	Smooth	Cream
IA9	Opaque	Small	Round	Smooth	Raised	Smooth	Cream
IA10	Translucent	Small	Round	Smooth	Raised	Smooth	Yellow
IA11	Opaque	Small	Round	Smooth	Raised	Smooth	White
IA12	Opaque	Small	Round	Smooth	Raised	Smooth	Cream
IA13	Opaque	Small	Round	Smooth	Raised	Smooth	Cream

Legend; IA1-IA13=First Isolate –Thirteen Isolate A13

Table 2.Colonial characteristics of isolates in waste water from industry B

Isolate Code	Optics	Size	Shapes	Edge	Elevation	Surface	Colour
IB1	Opaque	Small	Round	Entire	Flat	Smooth	Cream
IB2	Transparent	Small	Round	Entire	Raised	Smooth	Cream
IB3	Opaque	Small	Round	Entire	Raised	Smooth	Cream
IB4	Opaque	Small	Round	Entire	Raised	Smooth	Cream
IB5	Opaque	Small	Round	Entire	Raised	Smooth	White
IB6	Opaque	Small	Round	Entire	Raised	Rough	White
IB7	Transparent	Small	Round	Entire	Raised	Rough	White
IB8	Opaque	Small	Round	Entire	Raised	Smooth	White
IB9	Translucent	Small	Round	Smooth	Raised	Smooth	Cream
IB10	Opaque	Small	Round	Smooth	Raised	Smooth	Cream
IB11	Opaque	Small	Round	Smooth	Raised	Smooth	Cream

Legend; IB1-IB11= First Isolate –Eleven Isolate

Table 3. Microscopy and biochemical test for the bacteria isolated from industry A waste water

Isolate Code	Shape	Catalase	Coagulase Test	Gram Staining	Hydrogen Sulphide	Citrate	Urease	Motility Test	Arrangement	Methyl Red	Voges Proskaeur	EMB	MSA	SSA	Lactose	Sucrose	Arabinose	Glucose	Manitol	Maltose	Probable Micro-organism
IA1	Bacilli	+	-	-	-	+	-	+	Chain	-	+	P	C	C	AG	AG	AG	AG	AG	AG	*Enterobacterae rogenes*
IA2	Bacilli	+	-	-	-	+	+	-	Chain	-	+	P	C	C	AG	AG	AG	AG	AG	AG	*Klebsiellaoxytoca*
IA3	Bacilli	+	+	+	-	+	+	-	Chain	-	+	C	C	C	-	AG	A	AG	AG	-	*Bacillus subtilis*
IA4	Bacilli	+	-	-	+	-	+	+	Chain	+	-	C	C	C	-	AG	-	-	-	AG	*Proteus vulgaris*
IA5	Cocci	+	+	+	+	+	+	-	Cluster	-	-	C	Y	C	AG	A	AG	AG	A	A	*Staphylococcus aureus*
IA6	Bacilli	-	+	+	-	-	+	-	Cluster	-	-	C	C	C	-	A	A	AG	AG	A	*Bacillus cereus*
IA7	Cocci	+	-	+	-	+	-	-	Cluster	-	-	C	Y	C	AG	AG	A	AG	-	A	*Staphylococcus saprophyticus*
IA8	Cocci	-	-	-	+	+	+	+	Chain	-	-	C	C	C	AG	AG	AG	AG	A	A	*Lactococcuslactis*
IA9	Rod	+	-	-	+	+	-	+	Chain	+	-	C	C	B	AG	AG	AG	AG	A	A	*Salmonella typhi*
IA10	Cocci	+	-	+	-	-	+	-	Chain	-	+	C	C	C	AG	AG	AG	AG	A	A	*Enterococcus faecalis*
IA11	Cocci	+	+	-	-	+	+	+	Cluster	-	-	C	C	C	-	-	-	-	-	-	*Micrococcus luteus*
IA12	Bacilli	+	-	-	-	+	-	-	Chain	-	-	C	C	C	-	-	-	-	-	-	*Pseudomonas aeruginosa*
IA13	Bacilli	+	-	-	-	-	-	-	Chain	+	-	G	C	P	AG	AG	AG	AG	AG	AG	*Escherichial coli*

Keys:
A= Acid Production
Ag= Acid and Gas Production
B= Black
+ = Positive Reaction
- = Negative Reaction; P= Pink; G= Green;
Y= Yellow

Table 4. Microscopy and biochemical test for the bacteria isolated from industry B waste water

Isolate Code	Shape	Catalase	Coagulase Test	Gram Staining	Hydrogen Sulphide	Citrate	Urease	Motility Test	Arrangement	Methyl Red	Voges Proskaeur	EMB	MSA	SSA	Lactose	Sucrose	Arabinose	Glucose	Manitol	Maltose	Probable Micro-organism
IBI	Cocci	+	+	-	-	+	+	+	Cluster	-	-	C	P	C	-	-	-	-	-	-	*Micrococcus luteus*
IB2	Cocci	-	-	-	+	+	+	-	Chain	-	-	C	P	C	AG	AG	AG	AG	A	A	*Lactococcuslactis*
IB3	Bacilli	+	+	+	-	+	+	-	Chain	-	+	C	P	C	-	AG	A	AG	AG	-	*Listeria spp*
IB4	Bacilli	+	-	-	-	-	-	-	Chain	+	-	G	P	P	AG	AG	AG	AG	AG	AG	*Escherichia coli*
IB5	Cocci	+	+	+	+	+	+	-	Cluster	-	-	C	C	C	AG	A	AG	AG	A	A	*Erwiniacaratova*
IB6	Bacilli	+	-	-	+	-	+	+	Chain	+	-	C	C	C	-	AG	-	-	-	AG	*Proteus vulgaris*
IB7	Cocci	+	-	+	-	+	-	-	Cluster	-	-	C	Y	C	AG	AG	A	AG	-	A	*Staphylococcus saprophyticus*
IB8	Cocci	+	-	-	-	+	-	+	Chain	-	+	P	C	C	AG	AG	AG	AG	AG	AG	*Enterobacterae rogenes*
IB9	Bacilli	+	-	+	-	+	+	-	Chain	-	+	C	C	C	-	AG	A	AG	AG	-	*Bacillus subtilis*
IB10	Bacilli	+	-	-	-	+	-	-	Chain	-	-	C	C	C	-	-	-	-	-	-	*Pseudomonas aeruginosa*
IB11	Bacilli	+	-	-	+	+	-	+	Chain	+	-	C	P	B	AG	AG	AG	AG	A	A	*Salmonella typhi*

Keys:
A= Acid Production — **+ = Positive Reaction**
Ag= Acid and Gas Production — **- = Negative Reaction; P= Pink; G= Green;**
B= Black — **Y= Yellow**

Table 3a.Microoganisms isolated from industry A waste water

Isolate Code	Probable Microorganism
IAI	*Enterobacteraerogenes*
IA2	*Klebsiellaoxytoca*
IA3	*Bacillus subtilis*
IA4	*Proteus vulgaris*
IA5	*Staphylococcus aureus*
IA6	*Bacillus cereus*
IA7	*Staphylococcus saprophyticus*
IA8	*Lactococcuslactis*
IA9	*Salmonella Typhi*
IA10	*Enterococcus faecalis*
IA11	*Micrococcus luteus*
IA12	*Pseudomonas aeruginosa*
IA13	*Escherichia coli*

Table 4a.Microoganisms isolated from industry A waste water

Isolate Code	Probable Microorganism
IAI	*Micrococcus luteus*
IA2	*Lactococcuslactis*
IA3	*Listeria spp*
IA4	*Escherichia coli*
IA5	*Erwiniacaratova*
IA6	*Proteus vulgaris*
IA7	*Staphylococcus saprophyticus*
IA8	*Enterobacteraerogenes*
IA9	*Bacillus subtilis*
IA10	*Pseudomonas aeruginosa*
IA11	*Salmonella typhi*

Table 5. Bacteriological Analysis of Water

Sampling sites	Total Heterotrophic count	Total coliform count
Industry A	2.50×106	<100
Industry B	2.00×106	<100
WHO Standard	1.0×102	Zero
EPA Standard	1.0×102	Zero

Plate 1. Bacteria isolated by streaking method

4.0 Discussion

The bacteriological assessment of two industries reveals the presence of bacterial contaminants and this is in agreement with the findings of [13, 14].Industry A had the highest heterotrophic count 2.50×106 than industry B 2.00×106. There is a greater risk to public health from microbes that is associated with wastewater consumption that is contaminated with human and animal excreta. Hence wastewater can be faecally contaminated and can contain a variety of intestinal pathogens which cause diseases ranging from mild gastro-enteritis to the serious dysentery, cholera and typhoid. The most predominant waterborne disease, diarrhea has an estimated annual incidence of 4.6 billion episodes and causes 2.2 million deaths every year [15,16].

The presence of these indicative organisms is evidence that the waste water has been polluted with faeces of humans or other warm blooded animals [17].

The total coliform counts of all the water samples were generally high. They exceeded the standard requirement of 10 total coliform count per 100 ml for NSDWQ and zero total coliform count per 100 ml for WHO [18], The result showed that Gram negative bacteria are dominant than gram positive bacteria. *Enterobacteraerogenes*, *Klebsiellaoxytoca*, *Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus, Bacillus cereus,Staphylococcus Saprophyticus*, *Lactococcuslactis*, *Salmonella typhi*, *Enterococcus faecalis*, *Micrococcus luteus, Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria spp* and *Erwiniacaratova*.

The occurrence of *P. aeruginosa* and *S. aureus*, which was isolated from all the communities, was not surprising since P. aeruginosa is a particularly adaptable organism found in various habitats [19]. The alarming high number of total coliforms and faecalcolifoms per 100 mL obtained from the water samples indicates high level of faecal contamination of the river water which potentially poses a high health risk for the inhabitants of the community. This agrees with [1] who stated that high coliform counts are an indication of high faecal contamination. *Faecal streptococci* counts indicate more contamination with human excrement than animal excrement [20,21].

The total bacterial counts for all the water samples were generally high exceeding the limit of 1.0×102 cfu/ml which is the standard limit of heterotrophic count for drinking water [22]. The high total heterotrophic count is indicative of the presence of high organic and dissolved salts in the water [10]. The primary sources of these bacteria in water are animal and human wastes [23]. These sources of bacterial contamination include surface runoff, pasture and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks and sewage treatment facilities [24].

Similarly, the health guidelines for the use of wastewater in agriculture and aquaculture states that water to be used for irrigation of crops that is likely eaten uncooked and water to be used for sports and public parks in unrestricted regions should not exceed 103 per 100 mL faecal coliforms [20].

5. Conclusion

The outcome of this study has been shown that wastewaters contain a large amount of pathogenic bacteria that pose a real impact on human health, proof that the water is unsafe for consumption as coliform counts and the heterotrophic count were more than the international permissible standards recommended by World Health Organization. Thus, this water is inapt for drinking, bathing, washing of farm produce or for any other agricultural purpose except if it is adequately treated. Assessment wastewater treatment stations have to be build to prevent any further contamination It is evident that water borne diseases are due to improper disposal of industrial wastewater through surface runoff, therefore programmes must be organized to educate the general populace an also reservoir should be build to store wastewater for recycling.

Competing Interests

Authors have declared that no competinginterests exist.

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