IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 2 Ver. IV (Mar -Apr. 2015), PP 78-84
www.iosrjournals.org

Physiochemical and microbiological assessment of Lagos lagoon water, Lagos, Nigeria

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Abstract: *An investigation was carried out here on physiochemical and microbiological assessment of lagoon water collected from different sampling stations where waste disposal is at large scale. A total of seven water samples were collected from three sample stations, Victoria Island, Okobaba saw mill and Makoko for analysis purpose. The pH, salinity (mg/L), dissolved oxygen (mg/L), temperature, biochemical oxygen demand (mg/L), acidity (mg/L$CaCO_3$) and alkalinity (mg/L$CaCO_3$) of the collected water samples were in the range of 7.21-7.72, 8.18-189.2, 2.20-5.12, 26.8-27.5, 0.89-3.12, 28-100 and 152-328 respectively. The Total bacterial count of water samples were in the range of $1.7x10^3$-$1.9x10^7$ in nutrient agar whereas the TBC values were the range of $8.7x10^2$ – $1.8x10^5$. The Total coliform count for samples from Victoria Island, Makoko and Okobaba saw mill sampling stations were in the range of 50- > 1800 (MPN/100ml).The bacteria isolated from samples include Escherichia coli, Bacillus sp., Aeromonas sp., Pseudomonas sp., Lactobacillus sp., Listeria sp. Klebsiella sp, Cornybacterium sp and Serratia sp. Aspergillus sp., Penicillium sp., Fusarium sp., Rhizopus sp. and Paecilomyces sp. were also isolated from the water samples. Susceptibility test of some of the isolates (E.coli, species of Staphylococcus, Klebsiella and Pesudomonas) revealed that most of the isolates are resistant to almost all the antibiotics tested. However, the isolates were sensitive to gentamycin and ofloxacin (92.3%).*

Key Words: *Lagos lagoon, TBC, TCC, E. coli, gentamycin*

I. Introduction

Pollution of the Lagos lagoon constitutes one of the environmental problems in Lagos metropolis. This lagoon is a shallow expanse of water with restricted circulation in a microtitdal environment. It receives inputs of domestic sewage, industrial waste waters, saw dust and particulate wood wastes, petroleum hydrocarbons, cooling water from a thermal power station and emissions from automobile exhaust (Okoye *et al*., 2010)[1]. Obasi *et al*. (2014)[2] conducted a microbiological and toxicological profiling of a pharmaceutical waste water entering the Lagos lagoon. The physicochemical characteristics of the pharmaceutical effluents showed that it was slightly acidic, with some considerable levels of electrical conductivity. There was also low level of dissolved oxygen and evidence of degradation action of microorganisms with BOD and COD levels of 36.0 and 72.0 mg/L, respectively. The effluent was low on heavy metals except for iron and manganese which were high in concentrations. Amaeze *et al*. (2012)[3] studied on the environmental pollution and fish diversity on Lagoon and showed that physicochemical properties of the water body were mostly within the National FEPA set limits except for Total dissolved solids and dissolved oxygen levels.

Kamaldeen and Wahaab (2011)[4], carried out studies on excreta disposal into Lagos lagoon, the outcome of the test conducted on water samples from Iddo Jetty showed that the pH value (7.9 to 8.9), recorded was still within acceptable standards for normal brackish water environment, but it is tending towards alkaline. Other parameters tested were higher, total Suspended Solids (TSS) recorded ranged from 2412ppm to 2815ppm. Total Dissolve Solid (TDS) was higher than 1990ppm. Conductivity was 310 uS to 510uS; these confirmed that excessive quantity of both suspended and dissolved matter were present in the lagoon.

The organisms associated with lagoon water are a result of the waste disposed. Most of the organisms are enteric bacteria. A study carried out by Ajayi and Akonai (2005)[5], reported that Lactose fermenting gram-negative organisms, such as *Klebsiella* sp., *Enterobacter* sp. and *Escherichia coli* were prevalent within Lagos Lagoon. Another study by Akinyemi and Buoro (2011)[6], on Lekki lagoon, an extension of Lagos lagoon, showed that *Proteus vulgaris* had the highest mean percentage of bacteria occurrence, with the presence of other organisms such as; *Staphylococcus epidermidis*, *Vibrio parahaemolyticus*, *Clostridium perfringens*, *Bacillus* sp., *Escherichia coli*, *Salmonella* sp. and *Streptococcus* sp. The presence of these organisms in Lagos lagoon could be as a result of sewage contamination, waste from abattoir or manure in the reservoir. *Escherichia coli*, *Proteus* sp., Enterobacter sp., *Aeromonas* sp., Klebsiella sp. and *Salmonella* sp., were the prevalent organisms found after the analysis of Iddo Jetty in Apapa Local Government (Ojolowo, 2011)[7].

Nwankwu, (1992,1993)[8.9], analyzed both water and fish samples from Lagos lagoon and all sites had a high count of heterotrophic bacteria, which is an indication that, Lagos lagoon is rich in organic matter and the highest microbial load was obtained at the sewage disposal point.

Results from Ajayi and Akonai, (2005)[5], proved that *Bacillus megaterium, Bacillus* sp., Micrococcus sp., *Klebsiella* sp., *Enterobacter* sp., *Escherichia* sp., *Bacillus polymyxa, Viellonella* sp., *Streptococcus* sp., *Proteus vulgaris, Pseudomonas* sp., *Aeromonas hydrophila, Bifidobacterium adolescentis* and *Moraxella bovis* were found in a sample site from Lagos lagoon and they all showed multiple resistance.

Bacteria indicator of fecal pollution such as *Escherichia coli, Enterococcus faecalis,* sulfite-reducing anaerobes, *Pseudomonas aeruginosa, Staphylococcus aureus* and Salmonella serotypes such as *Salmonella mississippi, Salmonella paratyphi* and Salmonella concord have also been isolated from the lagoon. (Adingra *et al.*, 2012).[10]

Several studies carried out on different lagoon sites have reported the presence of coliform, which is indicative of fecal contamination. Law and Othman (1985)[11], reported a high level of fecal coliform along Port Dickson, Malaysia. The most-probable number (MPN) of these fecal coliform on different sampling dates showed very high fecal coliform counts, ranging from 2,400 MPN/100ml to 92,000 MPN/100ml. This indicates the presence of high level sewage pollution at the recreational beach. According to a study carried out by Nwankwu (1992)[8], on Lagos lagoon, the sewage disposal point had the highest number of coliform but decreased drastically towards the sea where salinity were also high.

Ajayi and Akonai (2005)[5] also reported varying coliform count, ranging from 0 to 7.0×10^3 cfu, from 24 different sits within Lagos lagoon. Kamaldeen and Wahaab (2011)[4], reported high coliform count from iddo jetty in Apapa, the MPN/100ml of theses fecal coliform ranged for 53 - >1100.

A study was made here on physiochemical and microbiological assessment of Lagos lagoon water collected at three different sampling sites, Victoria Island, Okobaba saw mill and Makoko.

II. Materials And Methods

2.1 Study Area

Lagos lagoon is the largest of four lagoon systems of the Gulf of Guinea coast (Ajao et al., 1996)[12]. It stretches from Cotonu and extends to Niger Delta. The lagoon is about 50 km long and 3 to 13 km wide, it separates from the Atlantic Ocean by a long sand spit 2 to 5 km wide. Its surface area is approximately 6,354.7 km² (Amaeze *et al*., 2012)[3]. It is fairly shallow and piled with badges and boats. The lagoon receives discharge from Ogun and Osun River and empties into the Atlantic Ocean via the Lagos harbor. It is the ultimate sink of a number of industrial discharges/effluents and run-offs from the surrounding Metropolis (Ajao *et al*., 1996)[12]. Sampling points were chosen while taken in consideration the major polluted points mostly verified by questionnaires and also areas closer to effluents.

2.2 Sampling Technique:

A total of seven samples were collected from three sample stations, Victoria Island, Okobaba saw mill and Makoko. Sterile wide mouthed bottles were used for this purpose. About 500 ml of water was collected for each site and then after labeling, the bottles were kept in a container packed with ice and were transported to the laboratory within 4 hours.

Table 1: Description of collected water samples

Locations	Description of samples	Code
Victoria Island	Salty water with floating particles	A
Makoko Black Water (Surface Water)	Foul smell, black coloured water with particles	B
Makoko Fresh Water	Brown coloured water with particles	C
Makoko Black Water (Deep Water)	Foul smell, black coloured water with particles	D
Okobaba (1)	Collected at the edge of the site. Brown Coloured with floating particles	E
Okobaba (2)	Collected slightly at the middle. Brown Coloured water with floating particles	F
Okobaba (3)	Collected further from the middle. Brown Coloured water with floating particles	G

2.3 Chemical Assessment Of Collected Water Samples:

The water samples (A, B and E) were analyzed for temperature, pH, acidity, alkalinity, salinity, Dissolved oxygen and Biochemical oxygen demand using the standard procedures as described in APHA-AWWA-WEF, 2005[13].

2.4 Microbiological Assessment:

2.4.1 Analysis of Samples:

One (1) ml of each of water samples was transferred into 9 ml of distilled water and aseptically, serial dilution was performed to obtain water suspension up to 10^{-7} (Benson, 2005)[14]. One (1) ml of each dilution

was introduced into Nutrient agar, Potato Dextrose agar and MacConkey agar. The samples were inoculated using pour plate and spread plate methods, a sterilized glass spreader was used to aseptically spread the sample for spread plate technique. The nutrient and MacConkey agar plates were incubated at 37^0C for 24 hours while Potato Dextrose agar plates were incubated at room temperature for 3-5 days. The total bacterial count (TBC) was expressed in cfu/ml. Distinct colonies were isolated and sub-cultured into appropriate medium to obtain pure isolates. The isolates were stored at 4^0C.

2.4.2 Enumeration of Bacteria Using Nutrient Agar and MacConkey Agar

For this purpose, dilutions were made upto 10^{-7} for water samples. One ml of each dilution (10^1 -10^7) was inoculated on Nutrient agar medium and MacConkey agar medium using pour plate method. In either case, sterilized glass spreader was used to spread the suspension on the agar surface. The plates were incubated at 37^oC and the total bacterial count (TBC) was expressed in cfu/ml for water samples.

2.4.3 Identification of Isolates

The isolates were identified using gram staining and different biochemical tests such as catalase, coagulase, oxidase, citrate utilization test, urease, motility, indole, sugar (sucrose, glucose, galactose) fermentation test (Benson, 2005)[14]

2.4.4 Identification of Fungi

Fungi Isolates:

Cellular and Colonial morphology

Pure isolates were visualized by aseptically removing the cover of the plates to observe the growth morphology. Wet preparation was done using lacto phenol blue as mountant and it was viewed under a light microscope using x40 objective lens.

2.4.5 Enumeration of Total Coliform Count

Most Probable Number (MPN) method was employed to determine the presence of the microorganisms. 10ml of the double strength broth was added into 105 screw capped bottles of the same size. Each of the bottles contained an inverted Durham tube used to collect gas. Sterile pipettes were then used to discharge seven 10ml samples to 10ml double strength medium; seven 1ml samples to 5ml single strength medium; and 0.1ml sample to 5ml single strength medium. The bottles were then incubated at 37^oC and examined after 24-48 hours respectively. Acid growth and gas production were observed and the number of positive and negative tubes were obtained and compared with table standard (Kamaldeen and Wahaab, 2011)[4].

2.5 Antibiotic Sensitivity Test:

All bacterial isolates used for this test were inoculated using streak plate method in nutrient agar medium and allowed to incubate for 18 hours. Sensitivity disks containing conventional antibiotics such as Augmentin (20 µg), Amoxicillin (10 µg), Ciprofloxacin (5 µg), Cotrimoxazole (30 µg), Gentamicin (10 µg) and Nitrofurantoin (300 µg) manufactured by Abtek Biological Ltd., England were used to carry out the sensitivity test. A colony of the 18 hour culture was picked with an inoculating loop from each isolate on nutrient agar was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standard (a density of $1x10^8$ cells/ml) before inoculation (Benson,2005)[14].

Mueller-Hinton agar was inoculated with 0.5 ml suspension of each isolate adjusted to $1x10^8$ cells/ml using sterile spreader. Sensitivity discs containing antibiotics were placed on the surface using sterile forceps of each Mueller-Hinton agar plate evenly seeded with test organisms and it was incubated for 24 h at 37^oC.

III. Results

3.1 : Chemical Analysis of Water Samples:

The pH and temperature of the water samples were in the range of 7.21-7.72 and 26.8-27.5^{o}C respectively. The Salinity and Biological oxygen demand of the samples were in the range of 8.18-189.2 (mg/L) and 0.89-3.12 (mg/L) respectively whereas the acidity and alkalinity were in the range of 28-100 (mg/L$CaCO_3$) and 152-328 (mg/L$CaCO_3$). The results are expressed in Fig. 4.1 and Fig. 4.2

3.2 Enumeration of bacterial isolates

The TBC values of water samples in Nutrient agar were in the range of 1.7×10^3 –

7.2×10^4 whereas TBC values for water samples in MacConkey agar were in the range of $8.7x10^2$ – $1.8x10^{5.}$ The results are explained in Figure 4.3.

3.3 Identification of bacterial isolates

From the morphological and characteristics, it has been shown that the samples contain species of *Bacillus* , *Staphylococcus*, *Escherichia coli*, *Klebsiell*a sp., *Lactobacillus* sp., *Listeria* sp., *Pseudomonas* sp., *Aeromonas* sp., *Serratia* sp. and *Cornybacterium* sp.

3.4 Identification of fungal isolates

Cultural and morphological characteristics of fungi obtained from water samples were recorded. *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp. and *Paecilomyces* sp. were isolated from the water samples.

3.5 Determination of TCC of water samples

The TCC count for samples from Victoria Island, Makoko and Okobaba saw mill sampling stations were in the range of 50-> 1800 (MPN/100ml). The results are expressed in Fig. 4.4

3.6 Determination of antibiotic sensitivity

Susceptibility test of the isolates using the disc diffusion method are shown in Table 4.1. Most of the *E. coli*, *Staphylococcus* sp., *Pseudomonas* sp. and *Klebsiella* sp. were resistant to almost all the antibiotics tested. The pattern of susceptibility is as shown in Table 4.1. However, the isolates were sensitive to gentamycin and ofloxacin (92.3%) (CLSI, 2013).[15]

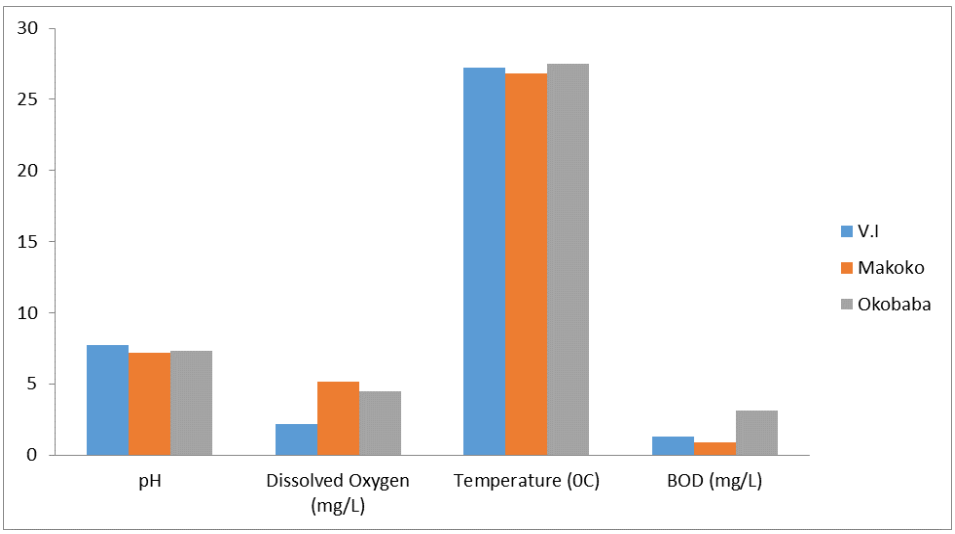

Figure 4.1: Determination of pH, Dissolved Oxygen, BOD and Temperature (Victoria Island, Makoko and Okobaba)

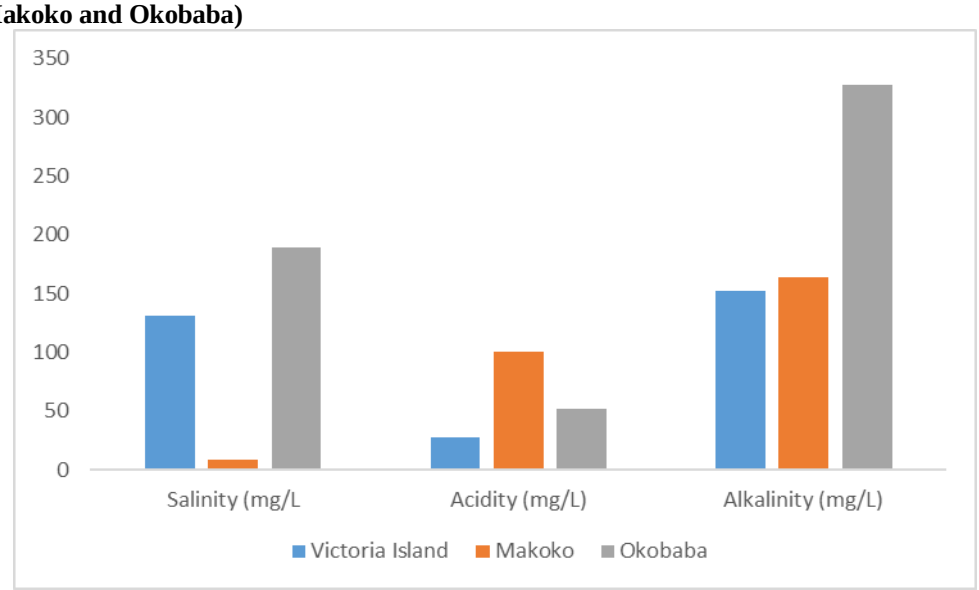

Figure 4.2: Determination of Salinity, Acidity and Alkalinity

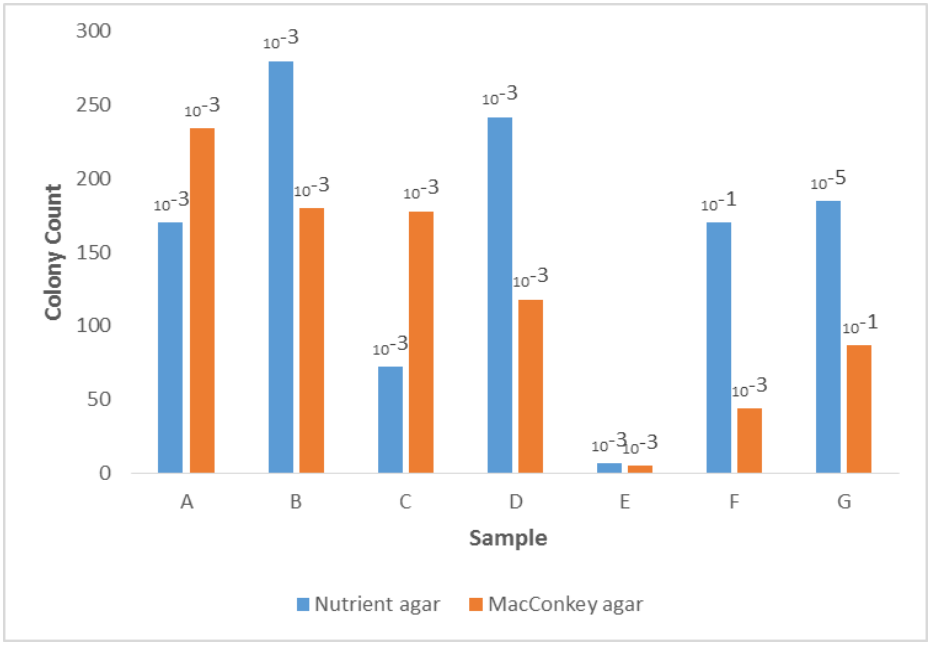

Figure 4.3: Total Bacterial Count (TBC) on Nutrient and MacConkey agar (colony count in 10^{-1}, 10^{-3} and 10^{-5} dilutions)

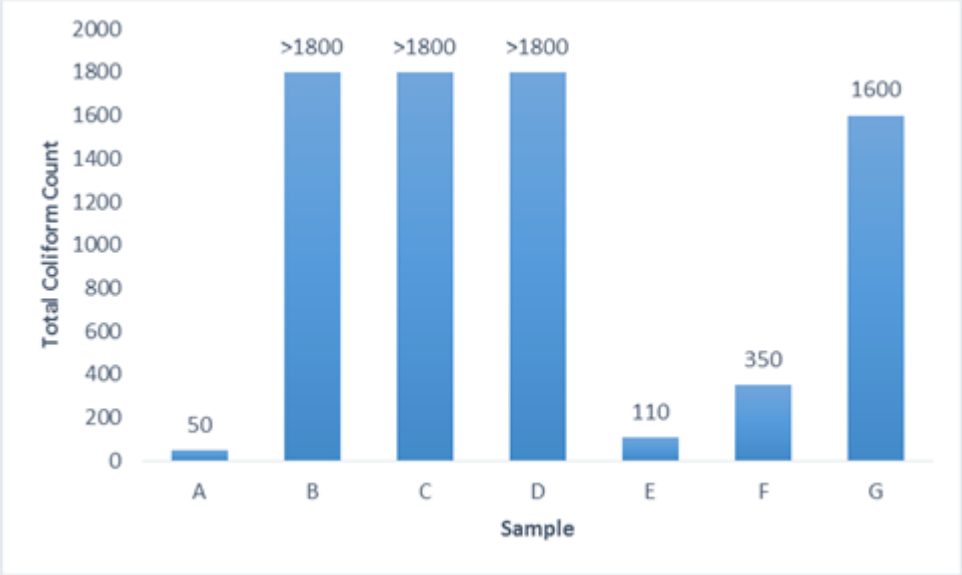

Figure 4.4: Total Coliform Count on MacConkey Broth

Table 4.1: Antibiotic sensitivity for *E. coli, Pseudomonas* sp., *Klebsiella* sp. and *Staphylococcus* sp. isolates obtained from water samples

Isolate code	GEN	COT	AUG	AMX	OFL	TET	NIT	ERY	CHL	NAL
A2	21(S)	-	R	R	30(S)	-	-	-	-	-
A3	15(S)	-	R	R	R	-	-	-	-	-
A5	13(I)	R	R	R	30(S)	R	R	-	-	R
E4	19(S)	-	27(S)	R	20(S)	17(I)	12(R)	20 (I)	R	-
B4	14(I)	R	R	R	21(S)	R	R	-	-	R
C3	11(R)	R	R	R	20(S)	R	R	-	-	R
D2	16(S)	R	R	R	10(R)	R	R	-	-	R
D3	17(S)	R	10(R)	14(I)	30(S)	R	R	-	-	R
D4	10(R)	R	R	R	20(S)	R	R	-	-	R
E6	13 (I)	R	R	R	22(S)	R	R	-	-	R
F5	22(S)	R	R	R	20(S)	R	R	-	-	R
G2	13(I)	R	R	R	20(S)	R	R	-	-	R
G3	14(I)	-	R	R	22(S)	-	-	-	-	-

GEN: Gentamycin, COT: Cotrimoxazole, AUG: Augmentin, AMX: Amoxicillin, OFL: Ofloxacin, TET: Tetracycline, NIT: Nitroflurantoin, ERY: Erythromycin, CHL: Chloramphenicol, NAL: Nalidixic acid, S-sensitive, I-Intermediate, R-resistant (diameter of zone ≤ 5 mm), "-" not carried out

A2,A3,G3- *Pseudomonas* sp., D2,D3,G2- *Klebsiella* sp., E4- *Staphylococcus* sp., A5,B4,C3,D4,E6,F5- *E. coli* isolates.

IV. Discussion

The pH and temperature of the collected water samples were in the range of 7.21-7.72 and 26.8-27.5^{o}C respectively. The Salinity and Biological oxygen demand of the samples were in the range of 8.18-189.2 (mg/L) and 0.89-3.12 (mg/L) respectively whereas the acidity and alkalinity were in the range of 28-100 (mg/L$CaCO_3$) and 152-328 (mg/L$CaCO_3$). Results of the chemical analysis of the collected water samples showed the high level of salinity in Okobaba sawmill and Victoria Island as a result of mixing of marine waters and also due to the high industrial activities. The low level of salinity at Makoko indicates the mixing of the bordering river waters. Dissolved oxygen was high in water sample from Makoko compare to other water samples and BOD value was low for Makoko water compare to other water samples. When BOD levels are high, dissolved oxygen levels decrease because the oxygen that is available in the water is being consumed by the bacteria. Since less dissolved oxygen is available in water (Victoria island and Okobaba) , fish and other aquatic organisms may not survive. The microbiological analysis revealed that the collected water samples contain organisms such as *E. coli*, *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp. and other organisms. Akoachere *et al.*, 2008[16] isolated 11 species of bacteria: *Bacteroides fragilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *E. coli*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Bacillus mycoides* and *Serratia marcescens* from Douala lagoon, Cameroon. The high TCC count for most of the water samples is an indication that the water body in the lagoon is polluted with untreated sewage (Adingra *et al*., 2012)[10]. Kamaldeen and Wahaab (2011)[4], reported high coliform count from Iddo jetty in Apapa , the MPN/100ml of theses fecal coliform ranged for 53 - >1100.

This is because of human activities surrounding the lagoon. WHO, 1992 [17]accepts the guide values of the investigated bacteria upto 500/100 ml for total coliforms, and 100/100 ml for both fecal coliforms and Enterococci. The high TCC values for most of the water samples especially from Makoko sampling station indicate the mixing of water with untreated sewage. Presence of *E. coli* in water samples indicates the possible presence of pathogens (Kamaldeen and Wahaab, 2011)[4]. Hennai *et al.*, 2012[18] studied on temporal and spatial distribution of faecal bacteria in a Moroccan lagoon. The results indicate that the deterioration of the microbiological quality of water derives from the cumulative impact of the reservoir of faecal contamination created in adjacent urban and suburban areas in the Oualidia sub – watershed, entering into the lagoon during storm water runoff. Santos *et al*., 2014 [19] studied the water quality of the Jansen lagoon through histological biomarkers and microbiological parameters. The high concentrations of thermotolerant coliforms in the analyzed water samples indicate domestic effluents in the lagoon's body of water compromising the site's environmental quality. The gill histological alterations are caused by environmental contamination by non-specific xenobiotics present at this site. Presence of pathogenic bacteria such as species of *Staphylococcus*, *Klebsiella*, *Pseudomonas* indicates the discharge of effluents from laundry and toilet. Lagos State has the highest population density of the four most industrialized states in Nigeria (Lagos, Rivers, Kano and Kaduna). It is also the state with the greatest concentration of industries, with well over seven thousand medium and large scale establishments (Oketola and Osibanjo, 2011)[20]. Being as Lagos lagoon is one of the most productive ecosystems in the world and the major revenue for aquatic life in the city, the continual disposal of untreated waste would not only pose as a threat health wise but also a reduction in the ecosystem. It can also lead in the production of mutant species of fishes. The isolated fungi from fish and water samples include species of *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* and *Paecilomyces.* Their presence is significant due to the fact that some are toxin producing organisms.

The general outlook of the antibiotic sensitivity profile of the isolates encountered showed multiple antibiotic resistance showed in Table 4.1, this includes both gram positive and gram negative organisms such as *Escherichia coli*, *Pseudomonas* sp, *Klebsiella* sp. and *Staphylococcus* sp. The presence of these multiple resistant species is evidence of the disposal of raw sewage and solid waste into the lagoon environment. Antibiotic resistance acquisition due to selective pressure is of public health concerns as resistance genes can be disseminated in nature and transferred to pathogenic counterparts of bacterial species by genetic mobile elements (Wellington *et al.*, 2013)[21]. Public awareness campaign on the evil of insanitary collection and disposal of faecal matter should be heightened to curb incessant dumping of excreta waste into the lagoon tune of canning disease that can impair public health. There should also be proper guidelines for industries to dump waste water to the lagoon.

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