

INVESTIGATION OF COLIFORMS IN LAGOS LAGOON ECOSYSTEM WITH A

FOCUS ON SALINITY AND POLLUTION LEVELS

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Abstract: *Pollution of the Lagos Lagoon is one of the major ecological concerns in Lagos metropolis. The ecological survey of coliform was performed to determine the level of pollution in Lagos Lagoon. Water samples were collected from Iddo, Makoko, Unilag, Oyingbo, and Marina sample stations. The physicochemical and bacteriological analyses were performed under standard procedures. The values of the water samples ranged from; 4.2±0.019 to 7.7±0.016 for pH; 26.7 ±0.012 to 28.9±0.125 for temperature (°C); 0.38±0.016 to 0.56±0.044 for turbidity (nephelometric turbidity unit (NTU)) and 541.8±13.207 to 40672.02±1252.930 for salinity(mg/L). The selected physicochemical parameter results were analyzed using the one-way ANOVA test. The test revealed that the mean values of the parameters were not significantly different for the five sample stations. The pairwise test revealed no significant difference between the sample stations in the Lagos Lagoon. The predominant bacteria isolated belonged to the following genera: Klebsiella, Escherichia, Providencia, Salmonellae, Enterobacter, Aeromonas, Pseudomonas, and Citrobacter. The total viable counts ranged from 1.0 x* 10^7 cfu/mL to 2.5 x 10^9 cfu/mL, and the total coliform counts ranged from 1.0 x 10^5 cfu/mL to 2.5 x 10^6 *cfu/mL. The study revealed high coliform count in Lagos Lagoon. Therefore, the study stresses the need for authorities to implement laws and regulations to prohibit the discharge of untreated wastes into waterbodies to control faecal pollution.*

Keywords: *Lagos Lagoon; Coliforms; Salinity; Pollution; Physiochemical Parameters; Escherichia coli*

1. Introduction

A major constituent of all living matter, water makes up approximately two-thirds of the human body [1]. It is crucial to prioritize water quality to protect the environment and all living organisms. The guidelines for water quality offer crucial information on the parameters that affect water quality and the toxicological threshold values that demand monitoring. Strict adherence to these guidelines is nonnegotiable if we are to achieve a cleaner, healthier future for our planet. Several physical and chemical parameters influence the aquatic environment, including temperature, rainfall, pH, salinity, dissolved oxygen, and carbon dioxide. Others are total suspended and dissolved solids, total alkalinity and acidity, and heavy metal contaminants [1]. These parameters are the limiting factors

for the survival of aquatic organisms (flora and fauna). The Lagos lagoon is a very important natural resource of Lagos state, Nigeria. Lagoon water is brackish (slightly salty). There is great socioeconomic significance to this water, which is regarded as one of the most productive aquatic ecosystems in the world [2]. A total of nine Lagoons are integrated into the Lagos Lagoon system: Yewa, Ologe, Badagry, Iyagbe, Lagos, Kuramo, Epe, Lekki, and Marhin. A broad range of communities make up the Lagos Lagoon waterfront, including Makoko, University of Lagos, Ilaje, Oworonshoki, Ogudu, Bayekun, Agboyin, Moba, Ofin, Ikorodu, Ibeche, Aja, Lekki peninsula, Banana Island, and Ikoyi [3]. As a result of the massive molluscan and fishing exploitation and commerce in the Lagoon, the Lagoon plays a significant role in the human community. However, humans can also damage the ecological balance of the environment by exerting stress upon it [4]. 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 microtidal environment. As

inputs, the system receives domestic sewage, industrial wastewater, sawdust, particulate wood wastes, gasoline, cooling water from a power plant, and automobile exhaust emissions [5]. Lagos Lagoon contains many enteric bacteria, including *Klebsiella* sp., *Enterobacter* sp., and *Escherichia coli* [6, 7, 8]. The lagoon may contain these bacteria due to contamination from sewage, abattoir waste, or animal manure. The goal of this study is to assess the pollution level of the Lagos Lagoon using an ecological coliform survey.

2. Materials and methods

2.1 Collection of Samples for Analysis

Water samples were collected from five sample stations, namely Marina, Iddo, Oyingbo, Unilag, and Makoko. Samples were collected using sterile wide-mouthed bottles (75mL and 500mL), the bottles were kept in a sterile container packed with ice and transported to the laboratory for physiochemical and microbiological analysis within 24 hours. Figure 1 is satellite image of Lagos State showing the Lagos Lagoon and sampling stations.

Fig. 1. Satellite image of Lagos State showing Lagos Lagoon and the sampling stations

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2.2 Physiochemical Analysis

Water samples from the five (5) sample stations were subjected to physicochemical analysis. The selected physicochemical parameters analyzed included pH, temperature turbidity, and salinity. All of these were carried out using standard methodologies described previously by American Public Health Association (APHA) [9, 10] and World Health Organization (WHO) [11].

2.3 Microbiological Analysis

The samples from Lagoon were subjected to 10-fold serial dilutions [12]. Aliquots (0.1ml) of appropriate dilutions for each sample were plated by the pour plate and spread methods in triplicates onto nutrient agar and MacConkey (MCA) [13]. These were incubated at 37 ºC for 24 hours. The colonies were counted with the aid of a colony counter and the colonial morphologies were noted. Pure bacteria colonies were maintained on nutrient agar slant and stored at 4 °C until needed. The identification of the bacteria isolates was by morphological examination under the microscope and biochemical tests following the methods of [14, 15]. The isolates were identified using Bergey´s Manual of Systematic Bacteriology [16].

2.4 Statistical Analysis

The results obtained for physicochemical analysis were expressed as mean ± standard deviation using SPSS. The selected physicochemical parameters determined (pH, temperature, turbidity, and salinity) for five lagoon locations were compared using one-way analysis of variance (ANOVA) [17], which assesses whether the means of groups are statistically different from each other.

3. Results

3.1 Physiochemical Analysis

The results of the physicochemical analysis are presented in Tables 1-4. The pH changes in the samples from the five sample stations are presented in Table 1. The pH changes of the water samples from sample stations ranged from 4.2 ± 0.019 to 7.7±0.0164. The stations had water sample's pH values ranging from 5.5 ± 0.01 to 7.4±0.05 for Iddo, 6.6±0.044 to 7.1±0.035 for Makoko, 6.6±0.024 to 7.2±0.0012 for Unilag, 4.2±0.019 to 6.9 \pm 0.009 for Oyingbo, and 5.7 \pm 0.009 to 7.7±0.0164 for Marina. The highest reported acidity level was 4.2±0.019 from Oyingbo station in the first week of May, followed by 5.5 ± 0.01 from Iddo station in the same week, 5.7±0.009 from Marina station in the first week of June, and the lowest at 6.6 ± 0.024 and 6.6 ± 0.044 from Unilag and Makoko stations in the same second week of June.

The results of the temperature $(°C)$ recorded by the samples obtained from stations are shown in Table 2. The temperature of the water samples ranged from 26.7±0.012 to 28.9±0.125. Water samples from Unilag station had the highest temperature of 28.9 ± 0.125 (1st week, June) followed by water samples from Oyingbo and Makoko stations with temperatures of 28.8 ± 0.068 (2nd week, June) and 28.8 ± 0.057 (1st week, June), respectively. The lowest temperature was obtained from water samples from Iddo station with temperature of 26.7 \pm 0.012 (1st) week, May). In general, there was not much of a temperature variation between the water samples from the five stations.

The results of turbidity (NTU) samples from the stations are presented in Table 3. The turbidity of the water samples from stations ranged from 0.38±0.0164 to 0.56±0.044. Water samples from Marina station had the highest turbidity of 0.56 ± 0.044 (1st Week, June) followed by water samples from Unilag station with 0.54 ± 0.012 (2nd Week, June), and water samples from Makoko station had the least turbidity of 0.42 ± 0.007 . (1st Week, June). Salinity results (mg/L) of samples from the

stations are shown in Table 4. The salinity of the water samples from stations ranged from 541.8 ±13.207 to 40,672.0±1252.930. Water samples from Marina station had the highest salinity of 40,672.0±0.1252.930 $(1st Week, May) followed by water$ samples from Oyingbo station with salinity of $31,569.6\pm1811.469$ (2nd Week, May) and Makoko station had the least salinity of $10,113.8\pm 634.482$ (2nd Week, May).

The error bars for salinity, turbidity, temperature, and pH are shown in Figures 2 through 5. Except for the pH values for the samples taken from the Oyingbo station, which are out of control and need to be investigated.

The matrix scatter plot in Figure 6 indicates that the four variables being studied are not significantly correlated. In other words, there is no relationship between the values of temperature, turbidity, pH, and salinity across different stations. Therefore, a high temperature in one station does not necessarily mean that the levels of turbidity, pH, or salinity will also be high in that same station. This observation is also supported by the data presented in Table 5.

The one-way ANOVA test indicates that there is no significant difference between the five stations' means for pH, temperature, turbidity, and salinity ($p >$ 0.05) (Table 6). Any detected discrepancies may therefore be the result of random variation. At a 5% level of significance, there are no differences in the stations' mean temperatures or mean turbidities. At the 5% level of significance, there is a significant difference in the mean salinity and pH in the stations.

According to the Least Significant Difference (LSD) test, there is a noticeable difference in the pH level of water samples from all stations (Iddo and Oyingbo, Makoko and Oyingbo, Unilag, and Oyingbo, Marina and Oyingbo). It is important to investigate the pH level of water samples from the Oyingbo Lagoon as it significantly differed from the other stations.

There were variations seen in the salinity of the water samples across different sites. However, a pairwise test was conducted, and it was found that the mean value concentrations at each station did not significantly differ from one other. The pvalues that were found were higher than 0.05.

Table 1.

 $Unit = C$

Unit = Nephelometric Turbidity Unit (NTU)

Table 4.

*Unit=mg/*L

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Fig. 2. Error Bars for Temperature at different stations; 3-Error Bars for Turbidity at different stations; 4-Error Bars for pH at different stations; 5-Error Bars for Salinity at different stations & 6- Matrix plot, CI-Confidence interval

Bivariate correlation among Temperature, Turbidity, pH, and Salinity Correlations

3.2. Total Counts from Lagoon samples

The individual coliform count varied from station to station and the level of pollution was measured by coliform content. Table 7 revealed the total coliform counts of water samples from the stations. This present study obtained the total number of coliforms ranging from 1.0×10^5 to $2.5 \times$ 10⁶ cfu/mL. The highest total coliform count of 2.5 x 10^6 cfu/mL was obtained from water samples collected from Unilag station followed by water samples from Makoko station with 2.25 x 10^6 cfu/mL and water samples from Iddo station had the least counts of 7.5 x 10^5 cfu/mL. The total viable counts were presented in Table 8 and counts ranged from 1.0×10^7 to $2.5 \times$ 10⁹ cfu/mL for the water samples from the stations. Water samples from Unilag station recorded the highest viable counts of 2.5 x 10^9 cfu/mL followed by water samples from Marina station with total viable counts of 2.25 x 10^9 cfu/mL and the least total viable counts of 5.0×10^8 cfu/ml obtained from water samples from Iddo station.

3.3 Identification of the Test Organisms

Table 5.

Pure cultures of bacterial isolates were identified based on their biochemical characteristics. The biochemical characteristics of the isolates were described in Table 9. The predominant bacteria isolated belonged to the following genera: *Klebsiella, Escherichia, Providencia, Salmonellae, Enterobacter, Aeromonas, Pseudomonas* and *Citrobacter*.

3.4 Distribution pattern of identified coliforms

Table 10 displays the distribution pattern of identified coliforms that were isolated from various sampling stations in Lagos Lagoon. The highest distribution was observed for *Escherichia coli* and *Enterobacter aerogenes*, which occurred in four locations.

This was followed by *Escherichia vulneris*, *Klebsiella pneumoniae*, *Klebsiella liquefaciens*, *Citrobacter diversus*, and *Enterobacter intermedius*, which were present in three locations.

Klebsiella terrigena, *Escherichia fergusonii*, *Plesiomonas shigelloides*, *Salmonella arizonae*, *Klebsiella planticola*, *Enterobacter asburiae*, *Aeromonas hydrophila,* and *Pseudomonas aeruginosa*

occurred in two locations. On the other hand, *Klebsiella aerogenes*, *Providencia alcalifaciens*, and *Salmonella enteritidis* had the lowest distribution, occurring in just one location.

Table 6.

One-way ANOVA Descriptives

4. Discussion

Water resources can be protected based on findings of values derived from different

physiochemical parameters and ecologically suitable toxicological thresholds [18]. In this present study, four physiochemical parameters were selected

and measured. The negative logarithm of a solution's hydrogen ion concentration, known as pH, thus serves as a measure of determining whether a liquid is acidic or alkaline [19]. According to Karuppasamy and Perumal [20] and Rajasegar [21], fluctuations in pH values of the water samples can generally be attributed to elements like $CO₂$ removal by photosynthesis through bicarbonate degradation, dilution of seawater by

freshwater influx, low primary productivity, reduction of salinity and temperature, and decomposition of organic materials. The chemical form, solubility, and toxicity of a contaminant to exposed biota may be affected by the pH of the environment in which it is deposited [22]. The structure and operation of the ecosystem can be significantly impacted by changes in pH, both directly and indirectly.

Table 7.

Table 8.

Total coliform counts from stations

Unit = Colony-forming unit (cfu/mL)

Total viable counts from stations

Unit = Colony-forming unit (cfu/mL)

The pH range of water samples is likely dependent on the lagoon's salinity regime. This is in line with Ajao's assertions [22]. A pollutant in pH-controlled environment may influence the chemical form, the solubility and its toxicity to exposed biota [22]. pH variations have a significant impact on the ecosystem's structure and function, both directly and indirectly. The salinity regime of the lagoon appears to be a major determinant of the very narrow pH range seen in the study locations.

This is consistent with the findings of Ajao [22]. The author reported that the salinity regime in the brackish environment appears to be the primary determinant of the study area's very narrow pH range. The present study indicated that the pH changes

of the water samples from sample stations ranged from 4.2±0.019 to 7.7±0.0164.

Water from Tendo Lagoon, in the Western Region of Ghana, had a slightly acidic pH [23]. The pH levels observed in this study were more acidic than the range (7.0-9.28) reported by Seu-Anoï et al. [24] in the lagoon but comparable to the mean value (6.7 ± 0.5) recorded by Adiyah et al. [25] at an upstream station in the Tano River. It is important to note that extreme pH values can have negative effects on fish mortality, fish taste, and the solubility of toxic metals.

Temperature is important because it goes a very long way affecting the amount of oxygen that dissolves in water. As the temperature drops, more oxygen will dissolve in the water. Moreso, it affects the migratory patterns, growth, nutrition, reproduction, and distribution of aquatic organisms [26]. The water will dissolve more oxygen as the temperature drops [27]. It also affects aquatic organisms'

migratory behaviors, growth, feeding, reproduction, and dispersal [28]. The rainy season, in which the sampling was done may have contributed to the low temperatures usually experienced across all of the stations, particularly during the first week of May. This finding corroborated the results obtained by Nandita et al. [8].

Due to the cold weather, less sunlight, and cloudy atmosphere during the wet months, the water temperature drops [29]. Solarin [31] measured air and water temperatures in Lagos lagoons over three years ranging from 25.0 to 33.2°C and 25.0 to 32.4°C, respectively. Onyema et al. [32] measured temperatures between 27.0 and 31.0°C in the same area. The findings of this study did not corroborate those of Vanden-Bossche and Bernacsek [33], who noted that surface water temperatures ranged from 27.5 to 34.0°C in the Malonda lagoon in Congo, from 25.0 to 32.0°C in the Ebrie lagoon in Cote d'ivoire, and from 18.0 to 34.3°C in Ghana's brackish water lagoons.

Table 9.

Biochemical characteristics of the isolates from Lagoon samples

1-Gram stain; 2-Cellular Morphology; 3-Catalase; 4-Oxidase; 5-Indole; 6-Motility ;7-Methyl red; 8-VP; 9-Citrate; 10-Urease; 11- Casein; 12-Starch; 13-Gelatin; 14-H₂S; 15-NO₃; 16-Growth on MAC; 17-Glucose; 19-Sucrose; 19-Galactose; 20-Salicin; 21-Lactose; 22-Maltose; 23-Mannitol; 24-Inositol; 25-Sorbitol; 26-Arabinose; 27- Xylose; 28-Trehalose

A-*Klebsiella terrigena*; B-*Escherichia coli*; C-*Escherichia fergusonii*; D-*Escherichia vulneris*; E- *Plesiomonas shigelloides*; F-*Salmonella arizonae*; G-*Klebsiella planticola*; H-*Klebsiella aerogenes*; I-*Klebsiella pneumonia*; J-*Providencia stuartii*; K-*Providencia alcalifaciens*; L-*Klebsiella liquefaciens*; M-*Enterobacter asburiae*; N-*Enterobacter aerogenes*; O-*Aeromonas hydrophila*; P-*Pseudomonas aeruginosa*; R-*Salmonella enteritidis*; S-*Citrobacter diversus*; T-*Enterobacter intermedius +:* Positive; -: Negative, G-Gram, ±- variable

Isolates	Iddo	Makoko	Unilag	Oyingbo	Marina
Klebsiella terrigena	$+$			\pm	
Escherichia coli	$\overline{+}$	┿		\pm	
Escherichia fergusonii					
Escherichia vulneris	\pm				
Plesiomonas	\pm				
shigelloides					
Salmonella arizonae					
Klebsiella planticola	\pm				
Klebsiella aerogenes					
Klebsiella pneumoniae	\pm				
Providencia stuartii					
Providencia					
alcalifaciens					
Klebsiella liquefaciens	$+$				
Enterobacter asburiae					
Enterobacter aerogenes	$\ddot{}$				
Aeromonas hydrophila					
Pseudomonas	$^{+}$			+	
aeruginosa					
Salmonella enteritidis					
Citrobacter diversus					
Enterobacter	ᆠ				
intermedius					

Table 10. Distribution pattern of identified coliforms isolated from the sampling stations in Lagos Lagoon

+ =present, - =absent

The ability of the light to pass through water is measured by turbidity, it can be used to determine how turbid the water is. An estimation of the suspended particles in the water is provided by measuring turbidity [34]. Turbidity of water samples collected was high in this present study. Clear water does not always indicate healthy water, even if high turbidity is frequently an indication of poor water quality and land management. Extremely transparent water may indicate extremely acidic conditions or excessive salinity levels [35]. High levels of turbidity may be caused by rainwater infiltration, cloudiness, less light penetration, washes, salt, sand, and high levels of organic matter, as well as low transparency from suspended inert particulate matter [36]. Low levels of turbidity may be caused by a clear atmosphere, water evaporation, and high light penetration. Cote d'Ivoire's lagoon system and coastal rivers' waters

were physicochemically characterized, according to Kouame et al. [37]. Kouame et al. [37] found that regardless of the sample period, the temperature and pH of every river or lagoon remained constant. 29.71°C for temperature and 7.51 for pH are the average values. Based on the high standard deviation results, the same authors reported that the variable range for conductivity $(5584.28\mu S \text{ cm}^{-1})$ and salinity (4.91) is unusually broad. In this study, the water samples from five selected stations ranged in pH from 4.2 ± 0.019 to 7.7±0.0164, temperature from 26.7±0.012- 28.9±0.128, turbidity from 0.38±0.0164- 0.56 ± 0.044 , and salinity from 541.8±13.207-40,672.0±1252.930. The pH, temperature, and salinity values found in this investigation were in close agreement with those published by Kaoume et al. [37]. The results of this study supported their findings.

Salinity reflects the amount of fresh water

mixed with seawater. Usually, it is measured in terms of total dissolved solids (TDS) or electrical conductivity (EC) [38]. As a result of heavy rains that diluted the salt concentration, the salinity values decreased across the stations in June. Reports of Edokpayi et al*.* [39] corroborated with results in this study. It was stated by Paramasivam and Kannan [40] that salt level at any point within an estuary depends on factors such as topography, tide state (high or low, spring or neap), time of the year controlling rainfall etc., and the extent of freshwaters flow. Intertidal fauna is most likely affected by salinity variations caused by dilution and evaporation, which act as a limiting factor in living organism distribution [41,42]. Water molecules often become sequestered in hydrated ionic structures at high salinities, forming icelike lattices that limit water movement. Due to the reduced availability of water molecules for interactions with enzymes, high salt conditions decrease their activity [43]. At high salinities, water molecules frequently become caught in hydrated ionic frameworks, generating ice-like lattices that restrict water transport. High salt conditions inhibit the activity of enzymes because there are fewer water molecules available for interactions [Karan et al. 43]. Emmanuel et al. [44] observed a variation in the salinity of Lekki Lagoon (0.007 - 4.70%), indicating that the lagoon was not completely a freshwater system. Kusemiju [45] also confirmed that Lekki Lagoon's salinity range of 0.05 - 0.30‰ was not completely fresh. The enteric Gramnegative rod organisms identified in this study include *Klebsiella* spp., *Enterobacter* spp., *Providencia* spp., *Escherichia* spp., and *Escherichia coli. Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli* enteric, all of which are Gram-negative short rods and lactose fermenters, were isolated by Ajayi and Akonai [6]. They found that a significant number of the total organisms cultivated from samples taken

from the Lagos Lagoon indicated that this body of water had been contaminated by a human sewage source. This may be an indicator that the Lagoon has served as the ultimate sink for disposal of untreated domestic sewage [6]. Similar research conducted by Akinyemi and Buoro [46] on Lekki lagoon, a branch of Lagos lagoon, found that *Proteus vulgaris* was the most predominant bacteria there, along with *Staphylococcus epidermidis*, *Vibrio parahaemolyticus*, *Clostridium perfringens*, *Bacillus* sp., *Escherichia coli*, *Salmonella* sp., and *Streptococcus* sp. *Salmonella* serotypes include *Salmonella mississippi*, *Salmonella paratyphi*, and *Salmonella* Concord as well as *Escherichia coli*, *Enterococcus faecalis*, sulfitereducing anaerobes, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and others have also been isolated from the lagoon [47, 48]. The metabolic activity of microorganisms is significantly influenced by the waste discharged into the Lagoon [49, 50]. According to the World Health Organization (WHO), drinking water should contain less than 20 CFU/mL heterotrophic bacterial counts with no coliform bacteria, fecal coliforms, E. *coli*, nterococci, and *P*. *aeruginosa* [51]. The majority of the water samples, especially those from the Makoko sampling site, had high total coliform count values which suggested that untreated sewage and residential effluents had been mixed with the water. *E*. *coli* in water samples suggests pathogens may be present [48]. As the largest lagoon ecosystem in Nigeria, the Lagos lagoon provides important ecosystem services that support resident organisms and the welfare of coastal populations [52, 53]. Continued untreated trash disposal would be harmful to the ecosystem as well as to human health [54].

5. Conclusion

The findings in this study underscore the urgent need for decisive action. The high

pollution levels in the sampling stations pose a significant threat to the inhabitants of this ecosystem such as fish, lobsters, and other living components. Furthermore, the consumers of seafoods sourced from the Lagos lagoon are at risk of foodborne diseases, especially if the seafoods are not properly cooked. While the government's policy banning untreated wastewater and other wastes disposal into the lagoon is commendable, stringent enforcement measures are imperative for its effectiveness and the safeguarding of public health and environmental integrity.

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7. References

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