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Bacterial Indicators of Contamination in Highly Impacted Segment of Tropical Lagoon, Southwest Nigeria

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ABSTRACT: Increasing deterioration of coastal waters is a major public health concern in many regions of the developing world. This study investigates the impact of water quality on the proliferation of bacterial pathogens in surface water, sediment and tissues of fish, *Sarotherodon melanotheron* from the Makoko axis of Lagos Lagoon. Analysis of physicochemical characteristics and microbial quality of samples followed standard methods and procedures. Measured water quality parameters (water temperature, turbidity, pH, salinity and dissolved oxygen) which showed no significant variation ($p>0.05$) across study stations were within the Nigerian Federal Environmental Protection Agency set limit The highest total bacterial count (TBC) ($214.09\pm43.95 \times 10^5$ CFU g^{-1}), total coliform counts (TCC) ($91.15\pm15.05 \times 10^4$ CFU g^{-1}) and total faecal coliform (TFC) ($36.22\pm12.98\times10^3$ CFU g^{-1}) were recorded in fish muscle tissue while the lowest TBC ($52.39\pm39.72 \times 10^5$ CFU ml^{-1}), TCC ($33.45\pm33.94 \times 10^4$ CFU ml^{-1}) and TFC ($0.19\pm0.18\times10^3$ CFU ml^{-1}) were recorded in water sample. The bacterial species with the highest percentages of occurrence in water, sediment, fish gill and fish muscle tissue were *Klebsiella pneumoniae* (22.11 %), *Enterobacter aerogenes* (32.37 %), *Escherichia coli* (32.97 %) and *E. coli* (29.00 %) respectively. A very strong positive correlation (r = 1.00) was obtained between TBC in water and salinity as well as with dissolved oxygen levels. Likewise, the TBCs in fish parts (muscle tissue and gill) were positively correlated with the water temperature and turbidity. On the other hand, a negative correlation was obtained between pH and TBC in fish muscle tissue (r = -0.81) as well as with fish gut (r = -0.77). The relatively high counts of pathogenic bacteria species recorded during the study have serious public health implications.

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The contribution of the seafood sector to the global food supply and its role as an important source of animal protein to the general populace cannot be over emphasized. However, fish species inhabiting coastal waters that receives untreated waste and sewage from domestic sources and city centers might be contaminated with pathogens. According to Gunnarsdóttir *et al.* (2013), sewage effluent entering coastal waters contains among others, diverse pollutants including viral and bacterial pathogens, noxious substances, as well as organic and inorganic wastes. Previous studies cited by Srinivas *et al.* (2018) noted that the indiscriminate dumping of untreated wastes in water bodies beyond their self-purifying capacity and the resultant deterioration in the physical, chemical and biological parameters of this ecosystem is a major water quality challenge. Environmental factors, including salinity, temperature, nutrients and light however play an important role in the survival and sometimes the proliferation of pathogens in aquatic ecosystem (Saxena *et al.*, 2015). The impact of environmental quality on fish health might therefore serve as an indicator of the general contamination in the aquatic environment. Over the years, numerous reports on Lagos coastal waters emphasized a steady

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deterioration with far reaching consequences on ecosystem health (Usese *et al*., 2017; 2019; Lawal-Are *et al*., 2021; Moruf *et al*., 2022). The Lagos Lagoon, a brackish water body located along the coastline of Southwestern Nigeria serves as a habitat for a wide array of fishes and source of income to fisher folks from the surrounding coastal communities. Notably, poorly treated or untreated waste water containing compounds far above the national set limit enters the Lagos Lagoon system frequently (Olarinmoye *et al*., 2016; Aniyikaiye *et al*., 2019). In particular, the Makoko segment of the Lagos lagoon, which started off as a fishing settlement and now a home to many has been under intense pressure and siege from prevailing social issues. It is characterized by numerous stilts buildings and shanties on the lagoon, swamps and floating school with little or no public service and sanitation. According to Oyinloye *et al.* (2017), available communal latrines are usually shared by approximately 15 households. Hence, over population, direct discharge of untreated effluents and sewage including human excreta from households as well as unregulated solid waste dumps are among the multiple human induced stressors negatively impacting the area.

Currently, studies examining bacterial pathogens and the relationships with deteriorating water quality are lacking. The paucity of information on the microbial indicators of contamination in important fish species used as food by man in relation to the declining environmental quality calls for concern. This study therefore investigates the abundance of pathogenic bacteria in water, sediment and tissues of *Sarotherodon melanotheron* from Makoko axis of the Lagos Lagoon in relation to habitat quality.

MATERIALS AND METHODS

Sample collection: Samples of surface water and sediments were obtained from three (3) locations which are active disposal sites for untreated sewage, domestic and solid waste discharge around Makoko segment of the Lagos Lagoon (Fig. 1). Adult samples of fish, *S. melanotheron* (n = 22) were obtained with the help of a professional fisherman from the sampling stations. The fish samples were wrapped in aluminum foil and placed in ice so as to keep the temperature at approximately 4°C and transported to the Aquatic Toxicology and Ecophysiology Laboratory of the Department of Marine Sciences, University of Lagos.

Laboratory procedure: Fish samples were dissected and filleted using sterile dissecting scalpel and forceps to remove the desired tissues. Tissue samples were homogenized using sterile mortar and pestle; after which they were transferred into different aluminum foil and labeled appropriately. The samples were kept in a refrigerator at a temperature of 4 – 8 ^{o}C prior to further analysis.

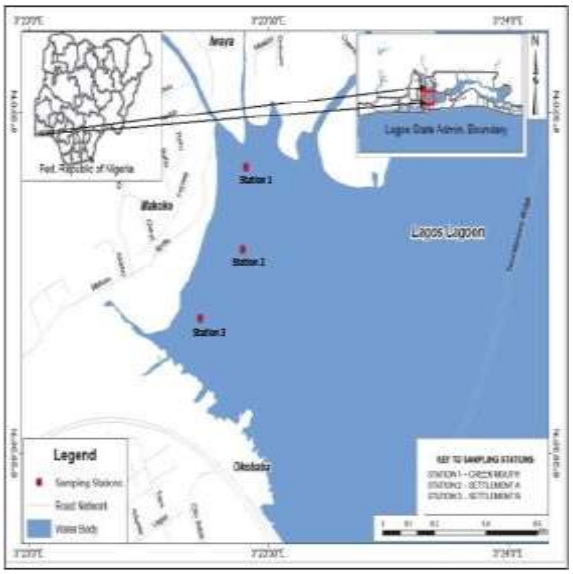

Fig 1: Map of Lagos Lagoon showing Makoko sampling Stations

To account for the environmental conditions of the Lagoon at the time of sampling, *in situ* determination of water temperature, pH, salinity, turbidity and the dissolved oxygen (DO) was carried out as described in APHA (2005).

Culture and enumeration of bacterial indicator in samples: Prior to analysis, the samples were brought out of the refrigerator to attain ambient temperature. One millilitre of water from the three stations and 1g of sediment and fish tissues each, weighed with Ohaus top balance were diluted serially in 9ml of sterile dilution water into seven (7) folds (10^{-1} to 10^{-7}). Three different aliquots of dilutions of samples were inoculated unto sterile plates of molten nutrient agar (Merck, Darmstadt, Germany), MacConkey agar (Biomark Laboratories, India) and Eosin methylene blue agar (Himedia, India) in duplicates with the aid of micropipette fitted with sterile tips. To ensure even distribution of the inoculum, the plates were swirled and thereafter, allowed to set. The inoculated plates were then incubated at 37^{o}C for 24 hours. The developed colonies were counted in duplicate using colony counter. The culture plates with discrete colonies that fell within acceptable limits were taken and recorded. Standard pour plate technique as described by Dubey and Maheshwari (2014) was employed for bacteriological analysis of surface

water, sediment, gills and muscle tissues of fish collected from the three sites in the lagoon for total heterotrophic bacteria, total coliform and faecal coliform population in colony forming unit per gram/ml of samples. The mean colonies counted were then multiplied by the dilution factor to give the total number of bacteria population in CFU g^{-1} or CFU ml^{-1} of the samples analysed.

Isolation of pure cultures and biochemical identification of isolates: The inoculated plates were then incubated at 37ºC for 18-24 hours. Standard streaking technique was employed for isolation of pure cultures; where the discrete colonies were streaked on sterile, dried, nutrient agar plates with the aid of sterile inoculating loop. The pure cultures were observed for cultural characteristics and cellular morphology (under x100 oil immersion objective) as well as prepared for preliminary identification and biochemical tests (Dubey and Maheshwari, 2014). Cellular and biochemical identification of the pure cultures were carried out using standard techniques described by De Vos *et al.* (2009). These includes: gram staining, catalase, oxidase, motility, urease, citrate, coagulase, indole, hydrogen sulphide production, gas production and sugar fermentation tests.

Statistical analysis: Analysis of variance (ANOVA) and Duncan multiple post hoc tests were used to compare the differences between means at $p < 0.05$ level of significance. All statistical analyses were conducted using SPSS version 17.

RESULTS AND DISCUSSION

The summary of the results obtained for water quality parameters from the sampling sites during the study is presented in Figure 1. Statistically, there was no significant difference ($p>0.05$) for the measured parameters (water temperature, turbidity, pH, salinity and D.O.) across study stations. Nkwoji (2016) recorded relatively uniform values for surface water temperatures in the Lagos Lagoon and attributed it to the conservative nature of this parameter in tropical waters. In the present study, turbidity values ranged between 8.37 mgL and 20.93 mg/L. Surface water pH levels ranged between 5.80 and 6.19 with the highest value in Station 3. Similar observations in other parts of Makoko Creek have been reported by Adejumobi *et al.* (2019). The slightly acidic pH levels recorded were within the Nigerian Federal Environmental Protection Agency (FEPA) set limit of 6.0 – 8.5. The salinity levels in the surface water ranged between 11.84 ‰ and 13.58 ‰. The relatively higher mean values of dissolved oxygen (1.46 mg/L) recorded in station 3 could be attributed to the dilution by floodwater and a reduction in the resident time of the polluted water in this segment of Makoko Creek. Areas of pronounced inputs of organic wastes such as station1 and station 2 recorded lower values of dissolved oxygen. This could be attributable to the consumption of the dissolved oxygen by aerobic microorganisms which biodegrade the organic wastes. This observation agrees with Nwabueze *et al.* (2020) on the negative effects of waste products on aquatic fauna.

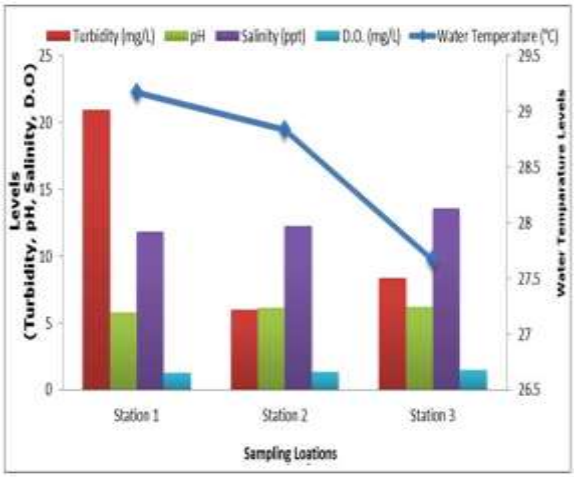

Fig. 2: Physicochemical characteristics of surface water from Makoko sampling stations

The bacterial loads in water, sediment and fish from Makoko segment of the Lagos Lagoon are presented in Table 1. The highest total bacterial count (TBC) ($214.09\pm43.95 \times 10^5$ CFU g^{-1}), total coliform counts (TCC) ($91.15\pm15.05 \times 10^4$ CFU g^{-1}) and total fecal coliform (TFC) ($36.22\pm12.98\times10^3$ CFU g^{-1}) were recorded in fish muscle tissue while the lowest TBC ($52.39\pm39.72 \times 10^5$ CFU ml^{-1}), TCC ($33.45\pm33.94 \times10^4$ CFU ml^{-1}) and TFC ($0.19\pm0.18\times10^3$ CFU ml^{-1}) were recorded in water sample. There was no significant difference ($P > 0.05$) between the total bacterial and total coliform counts amongst examined samples. This observation is in line with the report of Zhang *et al.* (2016) who recorded high population of bacteria in the muscle tissue of *Eriocheir sinensis* when compared to the population of bacteria in Lake Tai water of China. Lawal-Are *et al.* (2022) and Moruf (2022) also reported high bacterial loads in muscle tissue and gut of different mangrove crabs inhabiting the study area. As noted in a recent study, the intestinal microbiota of an aquatic biota is a reflection of the surrounding environment (Sun *et al.*, 2020). Furthermore, the difference in the intestinal microbiota might be attributed to the bacterial composition of a particular food source and among various diet available to the organism (Sun *et al.*, 2020).

Table 1. Mean± standard error values of bacterial loads in water, sediment and fish tissues from Makoko segment of the Lagos Lagoon

Samples	Total Bacterial Counts $\times 10^5$	Total Coliform Counts $\times 10^4$	Total Fecal Coliform $\times 10^3$
Water (CFU ml^{-1})	52.39 ± 39.72^a	33.45 ± 33.94^a	0.19 ± 0.18^a
Sediment (CFU g^{-1})	139.92 ± 12.42^{ab}	30.69 ± 22.69^a	2.21 ± 2.01^a
Fish gills (CFU g^{-1})	112.67 ± 33.07^a	77.87 ± 21.87^a	17.52 ± 13.84^a
Fish muscle tissue (CFU g^{-1})	214.09 ± 43.95^b	91.15 ± 15.05^a	36.22 ± 12.98^a

***Keys**: Mean ± Standard error; Values with different superscripts across column are significantly different at (P < 0.05)*

In this study, thirteen (13) bacteria species consisting both gram-positive and gram-negative bacteria were isolated from the various samples (Table 2). The isolates were identified as *Enterobacter aerogenes*, *Bacillus cereus*, *B. subtilis*, *Citrobacter sp*., *Escherichia coli*, *Klebsiella pneumonia*, *Lactobacillus sp*., *B. licheniformis*, *Micrococcus sp*., *B. megaterium*, *B. pumilus*, *Salmonella typhi* and *Staphylococcus aureus*. The bacterial species with the highest percentages of occurrence in water, sediment, fish gill and fish muscle tissue were *Klebsiella pneumoniae* (22.11 %), *Enterobacter aerogenes* (32.37 %), *Escherichia coli* (32.97 %) and *E. coli* (29.00 %) respectively. Consistently, these bacterial phyla were also detected in the intestine of *E. sinensis* in Lake Tai, and the surroundings (Liu *et al*., 2013). A number of other bacterial species belonging to the family Enterobacteriaceae, such as *Salmonella typhi*, were also prevalent during the study. The coliforms which are gram-negative, oxidase – negative, non – sporing rods are the most frequently used indicators of faecal pollution (Ibemenuga *et al.*, 2014). The *E. coli* isolated in the fish samples appeared in large numbers and may be attributed to faecal contamination of water in which fish live and mishandling which occurs after fish harvest.

Table 2. Mean± standard error values of bacterial loads in water, sediment and fish tissues from Makoko segment of the Lagos Lagoon

Bacteria	Water		Sediment		Fish gill		Fish muscle tissue	
	Number	%	Number	%	Number	%	Number	%
Bacillus cereus	12	5.77	1	0.48	1	0.37	12	5.19
B. licheniformis	0	0.00	1	0.48	0	0.00	0	0.00
B. megaterium	1	0.48	0	0.00	0	0.00	1	0.43
B. pumilus	1	0.48	0	0.00	0	0.00	0	0.00
B. subtilis	44	21.15	23	11.11	23	8.42	34	14.72
Citrobacter sp.	0	0.00	0	0.00	0	0.00	1	0.43
Enterobacter aerogenes	34	16.35	67	32.37	12	4.40	23	9.96
Escherichia coli	12	5.77	34	16.43	90	32.97	67	29.00
Klebsiella pneumoniae	46	22.11	34	16.43	68	24.91	34	14.72
Lactobacillus sp.	12	5.77	12	5.80	0	0.00	1	0.43
Micrococcus sp.	0	0.00	0	0.00	0	0.00	1	0.43
Salmonella typhi	34	16.35	23	11.11	67	24.54	56	24.24
Staphylococcus aureus	12	5.77	12	5.80	12	4.40	1	0.43

The correlation analysis between the physicochemical parameters and bacterial indicators of contamination in water, sediment and fish is shown in Table 3. A very strong positive correlation (r = 1.00) was obtained between TBC in water and salinity and as well as with dissolved oxygen levels. Likewise, the TBCs in fish parts (muscle tissue and gill) were positively correlated with the water temperature and turbidity. Negative correlation was however obtained between pH and TBC in fish muscle tissue (r = -0.81) as well as fish gut (r = -0.77).

This finding is in consonant with the report of Haque *et al*. (2019) who noted that higher total coliform and faecal coliform were strongly associated with some physicochemical parameters such as temperature, turbidity and dissolved oxygen among others. In an earlier study, it was shown that the immediate depletion of dissolved oxygen and subsequent impairment of the quality of the water body tend to occur during the decomposition of the incoming effluents by total heterotrophic bacteria (Garnier *et al*., 1991). According to Al-Kareem *et al*. (2015), water temperature can be recognized as an important factor that influences bacterial growth.

Conclusion: It can be concluded that the water of the Makoko sampling station is contaminated with domestic waste and faecal materials. The cumulative effects of these wastes have resulted in significant changes in the physicochemical and microbial characteristics of the water, with adverse impacts on biological communities and its capacity to provide ecological services. The study suggests the need for a regular monitoring and effective management strategy that will reduce further pollution.

Table 3. Correlation coefficient between the physicochemical parameters and bacterial indicators of contamination in water, sediment and fish from Makoko parts of the Lagos Lagoon

	Temp.	*Turbidity*	*pH*	*Salinity*	*D.O.*	*Water TBC*	*Sediment TBC*	*Tissue TBC*	*Gills TBC*	*Water TCC*	*Sediment TCC*	*Tissue TCC*	*Gills TCC*	*Water TFC*	*Sediment TFC*	*Tissue TFC*	*Gills TFC*
Temp.	1																
Turbidity	0.56	1															
pH	-0.76	-0.96	1														
Salinity	-1.00	-0.57	0.77	1													
D.O.	-1.00	-0.58	0.77	1.00	1												
Water TBC	-1.00	-0.50	0.71	1.00	1.00	1											
Sediment TBC	0.31	-0.62	0.39	-0.29	-0.28	-0.38	1										
Tissue TBC	1.00	0.63	-0.81	-1.00	-1.00	-0.99	0.23	1									
Gills TBC	1.00	0.58	-0.77	-1.00	-1.00	-1.00	0.28	1.00	1								
Water TCC	-0.98	-0.37	0.60	0.97	0.97	0.99	-0.51	-0.95	-0.97	1							
Sediment TCC	0.34	-0.59	0.36	-0.32	-0.32	-0.41	1.00	0.26	0.32	-0.54	1						
Tissue TCC	0.09	-0.78	0.58	-0.07	-0.06	-0.16	0.97	0.00	0.06	-0.30	0.97	1					
Gills TCC	0.58	-0.35	0.09	-0.57	-0.56	-0.64	0.95	0.51	0.56	-0.74	0.96	0.86	1				
Water TFC	-0.99	-0.41	0.64	0.98	0.98	1.00	-0.46	-0.97	-0.98	1.00	-0.49	-0.25	-0.71	1			
Sediment TFC	0.27	-0.65	0.42	-0.26	-0.25	-0.34	1.00	0.19	0.25	-0.47	1.00	0.98	0.94	-0.43	1		
Tissue TFC	0.31	-0.61	0.38	-0.30	-0.29	-0.38	1.00	0.23	0.29	-0.51	1.00	0.97	0.96	-0.47	1.00	1	
Gills TFC	0.58	-0.36	0.10	-0.56	-0.56	-0.63	0.95	0.50	0.56	-0.74	0.96	0.86	1.00	-0.70	0.94	0.96	1

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