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Analysis of Microbial load and Litter Characterisation of Araromi Beach Sand, Ondo State, Nigeria

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ABSTRACT: Increasing development of coastal areas and recreational use of beaches, has been linked with greater threats associated with public health hazards with respect to sediment and sand present in these coastal areas. This study assessed the microbial and litter characteristics of Araromi Beach Sand, Ondo State, Nigeria using appropriate standard methods. Data obtained show that Twenty-nine microbes comprising 17 bacterial species, seven fungal species and five yeast species were isolated. The microbial load ranged from 1.45 × 10^{-4} CFU/g to 12.4 × 10^{-4} CFU/g which were found to be within the permissible limit (8 CFU/g to 250 CFU/g) as stated by the WHO. However, Microbial load showed significant difference (t=0.011) between the areas of high tourist activities and low tourist activities. Also, the beach sand litters were categorized into seven groups. However, the most common type of litter were Organic wastes in the form of dried leaves and tree branches (59%), followed by Plastic/Polystyrene (32%). The average litter density ranged from 10.00 litter/m^2 in July to 21.57 litter/m^2 in December. There was no provision for waste disposal and toilet facilities. Araromi beach falls under the Moderately Dirty category with Clean Coast Index values of 8.52 and 6.81 in the dry and wet seasons respectively. Overall, the results of this study shows that Araromi beach is still in an underdeveloped state with the host community the key contributors to the amount of waste found on the beach. It is therefore recommended that to attract more visitors and improve the overall beach experience, the local community and government should invest in regular beach clean-ups and waste management programs to keep the beach in a pristine condition.

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Many tourist sites are usually found inside of cities in tranquil settings to satisfy recreational demands of city dwellers and residents of neighbouring towns. Many others are established outside the cities to offer a respite from the bustling and boisterous city life (Odunlami and Ijeomah, 2016). One of the most common types of tourism to offer such respites is Coastal tourism which is based on a unique resource combination at the interface of land and sea offering amenities such as water, beaches, scenic beauty for swimming, boating, sunbathing and surfing (Obinwanne and Okpoko, 2015). However, the sustainability of beaches is under threat due to a range of factors, including climate change, coastal development, waste and litter as well as pollution (Lee and Park, 2019; Kim, *et al.*, 2021). With increasing development of coastal areas and recreational use of beaches, there has also been greater threats associated with public health hazards with respect to swimming water, sediment and sand present in these coastal areas (Oyelade, *et al.*, 2018). Sands and sediments of the coastal zone provide a

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habitat where microorganisms can persist, and even flourish under the right conditions serving as one of the indicators available to users, regarding the beach's environmental and sanitary quality (Halliday and Gast, 2011). These microorganisms are usually introduced into the soil by sources such as litter from tourists and local resident activities, animal and human faecal matter as well as adjoining industries. In terrestrial beach environments, infectious diseases can spread through direct contact with sand-borne microbes or through the movement of microbes from water to sand in the swash or intertidal zone. All kinds of pathogenic microbes, including bacteria, protozoa, fungi, and viruses, become trapped, perish or survive, occasionally multiply in sand, then infect people or escape into the water (Heaney, *et al.*, 2012). Allochthonous microorganisms (Faecal Indicator Bacteria; E. coli, faecal coliforms, and enterococci) have the potential to be pathogenic to humans, leading to mild to serious health issues if contacted (Efstratiou, 2018) particularly for those with advanced age, diabetes, immunodepression (transient or permanent), and respiratory problems (Valério, *et al.*, 2022).. Direct faecal deposits by humans as well as warm blooded animals is common (Valério, *et al*., 2022) on a beach where there are no proper management structures in place. A significant amount of enteric bacteria, frequently pathogenic, are directly contributed to the sand by domestic pets and wild animals with the most common ones being dogs, cattle, some wild animals, and birds (such as seagulls) (Nguyen, *et al*., 2018; Ryu, *et al*., 2012; Shanks, *et al*., 2010). Other potential contributions include bathers themselves, who might be a significant source of infections. When walking on sand, a person discharges a sizable amount of bacteria, particularly *S. aureus* and enterococci. According to Efstratiou, (2018), users of sandy beaches may contract infections ranging from minor, self-limiting illnesses to severe diseases (Gastrointestinal symptoms, diarrhoea, respiratory issues, rash, eye conditions, earaches, infection of recent cuts, and fungal infections are among the illnesses that have been reported) as a result of contact with polluted sand (Weiskerger and Brando (2020). However, the likelihood of contracting an infection from such contact depends on the level of pollution, the pathogen's virulence, the length of exposure, and the person's immune system.

Marine litters has a significant global impact by posing risks to both people and the environment. According to Jambeck, *et al*. (2015) 80% of these litters have their origins on land. However, despite its importance, it has only gained real recognition during the past few years (Williams and Rangel-Buitrago, 2019). Because of so much negligence, most coastal areas have become places used by people as public toilets, sewage and refuse disposal and abandonment of broken-down ships and boats (Okoye, 2014). All these have an overall effect on the quality of the beach and people's willingness to visit such tourist destinations.

Nigeria has a coastline measuring approximately 853 km^2, with about 25% of the national population and cover a total of nine states including Ondo State (Oladele, *et al*., 2018). Araromi sea-side is found in the south western part of Nigeria and has the potential as a form of coastal tourism to bring about an increase in the income of the community thereby also contributing to the GDP of the nation. Yet, like many coastal regions around the world, Araromi beach serve as a sink for domestic and industrial waste resulting from various activities such as inappropriate litter disposal and management practices which can affect the quality and state of the beach affecting aesthetic values, spread of water and soil related diseases as well as intervening in the normal systems of the environment (Agwu and Oluwagunke, 2014). Hence, the objective of this paper is to assess the microbial and litter characteristics of Araromi Beach Sand, Ondo State, Nigeria ith a view to ascertain it overall quality and cleanliness.

MATERIALS AND METHODS

Description of Study Area: The study was carried out at Araromi beach in Ilaje local government area, in the coastal areas of Ondo State, southwest Nigeria. It has a landmass of $1357km^2$ and an average projected population of 445,200 (National Bureau of Statistics, 2019; Samuel, *et al.*, 2022). The vegetation of the area is predominantly mangrove swamp, with low grasses covering expanse of undulating lowland of mud and silt deposit in south and rainforest vegetation with abundant raffia palms in the north of the local government. Araromi is a densely populated town whose major occupations include fishing, canoe making, farming, lumbering, net making, mat making, and trading. One of the main human activities is also sand mining along the waterways of Araromi beach, but for the Ilaje, farming is still their primary source of income (Akingbe, 2013).

Data Collection: Data for the study was collected through both Field survey and Laboratory analysis.

Beach Litter Assessment and Quantification: Litter assessment was conducted following standardized protocols of Marine Litter Monitoring Guidance (Hanke, *et al.*, 2013). The beach sand area was divided into three from the coast line (DI was 0m – 20m from the coast line, DII was 20m – 40m from the coast line and DIII was 40m – 60m from the coast line) all parallel to the shoreline within the sand sector. In each of the division, fifteen 2m x 2m quadrant was established (totalling 45) to carry out total enumeration of beach sand litters (Thanh-Khiet, *et al.,* 2022). Within each quadrant, all items larger than 0.5 cm on the surface were observed and classified into any of the following seven categories: (i) plastic/polystyrene, (ii) glass/ceramics, (iii) cloth/textile, (iv) organic matter, (v) processed wood, (vi) metal and (vii) rubber (Ertaş, 2020; Asensio-Montesino., *et al.*, 2021). For each quadrat, litters were quantified and the density expressed as items per square meter of beach surface (items/ m^2). Percentage litter cover and bare ground was estimated visually. The survey was conducted for six (6) months covering three months in the dry season (December - February) and three months in the wet season (May - July).

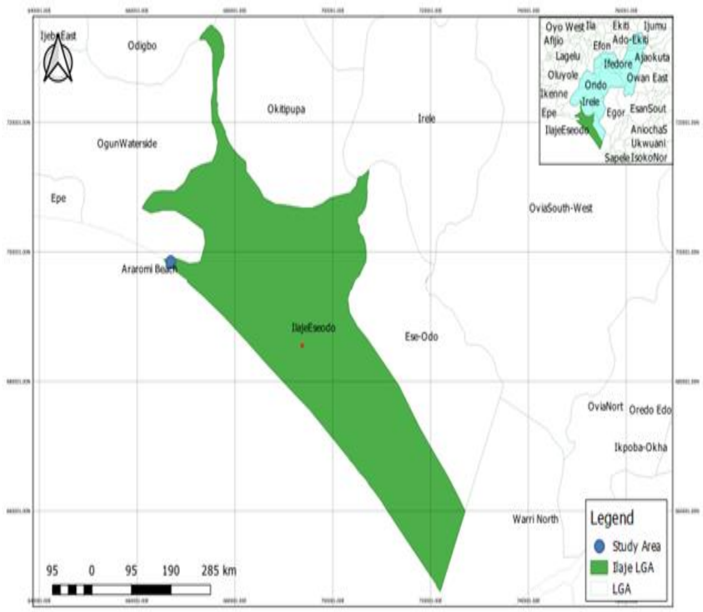

Fig 1: Location Map of Araromi beach, Ondo State, Nigeria

Clean Coast Index Calculation: The Clean Coast Index (CCI) is a good tool for measuring the quality of a beach. In order to determine the level of Araromi beach's cleanliness, the total litter counts were tallied to calculate for the Clean Coast Index (CCI) (Alkalay, *et al*., 2007). The CCI was calculated as thus; CCI = Total litters on sampling unit/ Total area of sampling unit, multiply by K. Where K is the correction coefficient of 20 to make sure that the value of the resulting index would not fall between 0 and 1 (Asensio-Montesinos, *et al.,* 2021; Munari, *et al.,* 2016). The CCI value from 0-2 signify "very clean", 2-5 signify "clean", 5-10 signify "Moderately dirty", 10-20 signify "Dirty" and >20 signify "Extremely Dirty" (Vlachogianni, *et al.,* 2018).

Laboratory Analysis: Prior to laboratory analysis, the sampling sites were divided into two; areas where there are high tourists' activity and areas where there is little or no tourist activities (Brandão, 2019). Samples were collected once in the months of December, January and February (dry season) and May, June and July (wet season) from top soil profile (10cm) where there is a high microbial population and activity following the World Health Organization Manual for Recreational water and Beach Quality Monitoring and Assessment (WHO, 2021). Sand samples were collected in clean, dry and sterile plastic containers using sterilized spatula in order to reduce the chances of external contamination which may affect the results of the laboratory analysis. The samples were then stored and transported to the laboratory for analysis.

Characterization of Bacterial and Fungal Isolates: To obtain pure colonies, the representative bacterial and fungal colonies on each plate were isolated and sub-cultured. The pure bacterial and fungal isolates were grown on nutrient agar and sabouraud dextrose agar slants respectively, and then kept in the refrigerator at 4oC. The bacterial isolates were presumptively identified after their biochemical tests were determined using Bergey's Manual of

Determinative Bacteriology (Ajadi and Thonda, 2017).

Cultural Characteristics: The cultural characteristics and the total number of colonies on agar plate were recorded. The total colony counts were multiplied with the dilution factor. Microbial counts were expressed as colony forming units per ml (cfu/ml) of water samples. The colonial appearance of each bacterial isolate on the plate was observed and classified based on the following characteristics; colour, shape, edge, elevation, surface and opacity (Ajadi and Thonda, 2017).

Biochemical Identification of the Isolates: Biochemical tests were carried out for the identification of bacterial isolates. These includes catalase test, coagulase test, methyl red, Voges-Proskauer test, oxidation/fermentation test, nitrate reduction test, sugar fermentation (glucose, sucrose, maltose, mannitol, lactose) test, reaction on sulphide in dole motility medium. These biochemical tests were carried out, 18 to 24 h both culture of each isolate were used. An un-inoculated sterile medium was used as control in all the tests carried out. (Dubey and Maheshwari, 2002)

Identification of Fungal Isolates: Identification of fungi was done by examining the fungi under the microscope. Seven days' pure culture of fungi was used for the microscopic identification. A wet mount of the culture was prepared on a clean slide by adding a drop of cotton blue lactophenol reagent. The slide was then covered with a cover slip and viewed under the microscope at the X40 objective lens.

Statistical Analysis: Data obtained were tabulated on Microsoft Excel sheet and subjected to both descriptive (frequency table, bar chart etc) and inferential analysis. Micobial count data for the two stratified activity areas as well as the two seasons were analysed using a paired t-test to check for significant difference. Also, One-Way ANOVA was used to test for significant difference in the beach sand litter density across the transects as well as between the months of study.

RESULTS AND DISCUSSION

Microbial Species Isolates of Araromi Beach Sand: A total of 29 microbial species were isolated and identified in the beach sand samples collected. These comprises of 17 bacterial species, 5 Yeast species and 7 Fungi species (Table 1). Some of the common bacterial species isolated includes *Esherichia coli, Clostridium histolyticum, Salmonella pullorum;* Yeast species includes *Camdidia spp, Schizosaccharomyces spp, Asteromyces spp;* Fungi species includes *Mucur mucedo, Aspergillus flavus, Botryoderma spp* and *Memnoniella spp.* In areas of high tourist activities, 17 microbes were identified comprising of 9 bacterial species, 5 fungi species and 2 yeast species. In areas of low tourist activities, 21 microbes comprising of 10 bacterial species, 6 fungi species and 5 yeast species were isolated and identified. However, two bacterial species (*Bacillus subtilis* and *Enterobacter spp)* two yeast species (*Candidia spp* and *Kluveromyces spp)* and four Fungi species (*Aspergillus flavus, Aspergillus niger, Mucur mucedo, and Rhizopus stolonifera)* were common to both areas.

Additionally, of the 29 identified microbes, 18 are pathogenic while the remaining 11 are non-pathogenic. Twelve of the bacterial species are pathogenic while 5 are non-pathogenic. Also, three each of the fungi and yeast species are pathogenic while 4 fungi and 2 yeast species are non-pathogenic. This put the beach at a high risk destination for visitors.

Some key pathogenic microbes found during the course of the survey such as *Clostridium histolyticum, Salmonella pullorum, Bacillus subtilis, Esherichia coli, Geotrichum albidun, Candidia spp, Aspergillus flavus* and *Mucur mucedo* had been reported by many authors such as Ajadi and Thonda, (2017); Brandão, (2019); Halliday and Gast, (2011); Sabino, *et al*., (2011); Pinto, *et al.*, (2012); Viji, *et al*., (2019); Oyelade, *et al*., (2018); Weiskerger and Brandão, (2020) to cause illnesses such as urinary tract infections, Gas gangrene, gastroenteritis, Candidosis, Mucormycosis to name a few.

Furthermore, some of the microbes are allochthonous (originating from a place other than where it is found such as Faecal Indicator Bacteria: E. coli, faecal coliforms, and enterococci) while some are autochthonous (native species). Sources of these microbes may include tourist wastes, domestic wastes, fishing waste, feral and domestic animals waste and decaying wood. This was evidenced by the herds of cattle observed to be grazing near the beach site with their faecal droppings littering the area as well as tourists and local communities' faeces seen on beach sands. This is in support of the statement by Valério, *et al*. (2022); Nguyen, *et al*., (2018); Ryu, et al., (2012); Whitman, *et al.* (2014) that gut, skin, and mucosae of animals, faecal droppings of feral animals as well as sewage produce pathogens which are introduced to the beach by point and nonpoint sources of marine pollution.

Table 1: The isolated microbes in Araromi beach sand samples and their health implications

Types of Isolate	A	B	Pathogenic	Non-pathogenic
Bacteria Species				
Clostridium histolyticum	+	-	P	-
Salmonella pullorum	+	-	P	-
Klebsiella aerogenes	-	+	P	-
Bacillus subtilis	+	+	-	NP
Esherichia coli	+	-	P	-
Flavobacterium spp	+	-	-	NP
Staphylococcus saprophyticus	+	-	P	-
Thiobacillus spp	+	-	-	NP
Staphylococus epidemidis	+	-	P	-
Chromobacterium lividum	-	+	P	-
Staphylococcus hominis	-	+	-	NP
Leuconostoc spp	-	+	-	NP
Alcaligenes odorans	-	+	P	-
Enterobacter spp	+	+	P	-
Corynebacterium spp	-	+	P	-
Proteus vulgaris	-	+	P	-
Pseudomonas aeuriginosa	-	+	P	-
Yeast Species				
Geotrichum albidun	-	+	P	-
Schizosaccharomyces spp	-	+	-	NP
Asteromyces spp	-	+	P	-
Candidia spp	+	+	P	-
Kluyveromyces spp	+	+	-	NP
Fungi Species				
Aspergillus flavus	+	+	P	-
Mucur mucedo	+	+	P	-
Aspergillus niger	+	+	-	NP
Aspergillus fumigatus	+	-	-	NP
Rhizopus stolonifer	+	+	P	-
Memnoniella spp	-	+	-	NP
Botryoderma spp	-	+	-	NP

NOTE: + present, - absent, P=Pathogenic, NP= Non-pathogenic

Microbial Load of Beach Sand Samples: Sample A (high tourist activity area) has a higher concentration of microorganism per gram of beach sand than sample B (low tourist activity area) Table 2. The bacterial load of Sample A ranged from 12.00×10^{-4} CFU/g to 12.80×10^{-4} CFU/g with a mean concentration of 12.4×10^{-4} CFU/g; the Yeast load ranged from 9.20×10^{-4} CFU/g to 9.60×10^{-4} CFU/g with a mean concentration of 9.4×10^{-4} CFU/g and the Fungi load ranged from 1.00×10^{-4} CFU/g to 6.00×10^{-4} CFU/g with a mean concentration of 3.5×10^{-4} CFU/g. In Sample B, the Bacterial load ranged from 9.60×10^{-4} CFU/g to 10.80×10^{-4} CFU/g with a mean concentration of 10.2×10^{-4} CFU/g, the Yeast load ranged from 8.00×10^{-4} CFU/g to 8.20×10^{-4} CFU/g with a mean concentration of 8.10×10^{-4} CFU/g and the Fungi load ranged from 1.40×10^{-4} CFU/g to 1.50×10^{-4} CFU/g with a mean concentration of 1.45×10^{-4} CFU/g. The t-test result indicated a significant difference ($p<0.05$) in the level of microbial loads in samples A and B. This implies that tourist activities and local festivals contribute significantly to the microbial loads in the area. This means that tourist can easily ingest these microbes thereby causing sickness. However, the highest microbial loads found in both samples retrieved (18.4×10^{-4} cfu/g) were shown to still be within the permissible limit for the presence of microbes within beach sand according to the World Health Organization's Guidelines on Recreational Water Quality (2021) which has a permissible limit of 60 cfu/g. It is also supported by findings of (Sabino, *et al.*, 2011).

Beach Litter Composition and Characteristics: A total of 2749 items of litters were visually observed, counted and identified along all three transects over the course of the survey and classified into seven groups (Table 3). Organic waste (composed mainly of dried coconut leaves, coconut husks, dried tree branches, cutler back bones, crab shells, conifer cones, cow dungs etc.) had the highest litter amount of 1542 (56.09%), followed by plastic/polystyrene with 696 items (25.32%) while Glass/Ceramics had the least items of 50 (1.82%).

The mean seasonal litter items was higher in the dry season (479±173.23 items) than the rainy season (383±82.71 items). December had the highest litter items (672) while June had the least litter items (293)..

Table 2: Microbial Load of beach sand both in the dry and wet seasons

Season	Microbial Species/group	Mean of sample A	Mean of sample B	T-Value	P-Value
Dry	Bacterial Count CFU/g	18.4×10^{-4}	16.2×10^{-4}	15.607	0.011
Wet	Bacterial Count CFU/g	12.4×10^{-4}	10.2×10^{-4}	14.562	0.001
Dry	Yeast Count CFU/g	12.4×10^{-4}.	10.10×10^{-4}	16.435	0.000
Wet	Yeast Count CFU/g	9.4×10^{-4}.	8.10×10^{-4}	15.765	0.000
Dry	Fungi Count CFU/g	6.5×10^{-4}	4.45×10^{-4}	15.342	0.025
Wet	Fungi Count CFU/g	3.5×10^{-4}	1.45×10^{-4}	16.421	0.002

Note: Sample A=High tourist activity, Sample B=Low tourist activity, CFU/g= Colony Forming Unit per gram of Beach sand; 10^{-4} = Dilution factor

This high composition is due to the high composition of coconut tree which due to wind action causes branches to break and scatter along the beach as well as the collection and peeling of coconut by people in the area.

The cow dung seen was as a result of herders that move in numbers in the area to graze their cattle and to drink water. This is in agreement to a study conducted on Malotong Beach in Indonesia by Sulistiawati, *et al.* (2020) which indicated that the activity of the local population indirectly, has the potential to produce a wood litter or the fragments of plant branches from the surrounding area. The low composition of plastic litter can be tied to the fact that Araromi beach is not a main destination for recreation with few parties thrown on the beach once in a while and recycling system put in place in the community which consists of a bin for collecting PET bottles. Some exotic wastes such as cosmetic tubes, polystyrene plates were also observed. This proves that although Araromi beach is not a major tourist destination, it still sees to a certain degree of patronage and use. This is in tandem with the findings of Öztekin *et al*. (2019) who reported that plastic litters accounted for 32% of the total beach litters representing the second most abundant litter category. However, it contradict the reports of Ertaş, (2020); Asensio-Montesinos, et al. (2021) in which plastic litters were the most abundant litter category accounting for about 60-80%. The beach sand litter density was highest in December with a value of 21.57 litter/m^2 followed by July with a value of 10.00 litter/m^2 and lowest in February with a value of 8.52 litter/m^2 (Figure 2). Also, the seasonal litter density was higher in the dry season (10.64±3.85 litter/m^2) than the wet season (8.53±1.84 litter/m^2) (Figure 3). Test of homogeneity showed significant difference in the monthly and seasonal litter density (p = 0.000).

Table 3: Table showing the quantity of beach litter observed during the study period

Litter Groups	Number of litters observed							
	Dry season			Wet season				
	Dec	Jan	Feb	May	June	July	Total	Percentage
Plastics/ Polystyrene	141	118	130	151	21	135	696	25.32
Textile	36	0	0	21	15	12	55	2.00
Rubber	3	6	9	4	5	10	37	1.35
Organic waste	381	295	183	205	222	256	1542	56.09
Paper/Wood	57	3	6	13	15	26	205	7.46
Metal	42	6	6	6	13	11	164	5.97
Glass/Ceramics	12	0	3	3	2	5	50	1.82
Total	**672**	**428**	**337**	**403**	**293**	**455**	**2749**	**100**
Seasonal mean	479±173.23			383±82.71				
Monthly Density	14.93	9.51	7.48	8.96	6.51	10.11		
Seasonal Density	10.64			8.53				

Beach Cleanliness/Clean Coast Index of Araromi Beach: The result of the clean coast index calculated revealed that during the dry and wet seasons the CCI values were 8.52 and 6.81 which means that the beach is “Moderately dirty” in both seasons. In the dry season December had the highest CCI value of 11.94 indicating that the beach was Dirty while February had the lowest value of 5.99 indicating “Moderately dirty”. However, in the wet season the CCI values recorded were within the same “Moderately dirty” category in all the three months covered (5.21 – 8.09). In accordance with CCI study index by Alkalay *et al*., (2007), this current study observed that Araromi beach fell in the “Moderate dirty” category during the survey conducted both during the dry and wet seasons. It can be deduced that the low CCI index value of Araromi beach signifies low turnout of tourists. This further proves that Araromi beach is not a major tourist destination with low exposure. However, in December that the CCI fell in the "Dirty" category. This is due to a higher number of beach users as a result of it being a festive month with both locals and tourists accessing the beach more during this period. Overall, Araromi beach is still relatively clean compared to similar studies conducted in Homa beach (Ertas, 2020) and Black sea trenches (Öztekin *et al*., 2019) where there were high tourist activities.

Table 4: Araromi Beach sand Clean Coast Index for Dry and Wet seasons

Season	Months	CCI Value	Decision
Dry	December	11.94	Dirty
	January	7.61	Moderately dirty
	February	5.99	Moderately dirty
Dry season index		**8.52**	**Moderately dirty**
Wet	May	7.16	Moderately dirty
	June	5.21	Moderately dirty
	July	8.09	Moderately dirty
Wet season index		**6.81**	**Moderately dirty**

Conclusion: The study showed that Organic waste in the form of dried coconut leaves and tree branches was the most abundant litter category with Plastic litter being the second most abundant. The microbial load is still within the permissible limit as stated by the World Health Organization's Guidelines on Recreational Water. However, it is still imperative that surveillance measures be put in place to keep the microbial load at the barest minimum. The result of this study, indicates that Araromi beach lacks waste management and toilet facilities. It is therefore recommended that to attract more visitors and improve the overall beach experience, the local community and government should invest in regular beach clean-ups and waste management programs to keep the beach in a pristine condition.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the first author/ corresponding author.

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