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Physico-chemical properties and microbial ecology of the Lagos Lagoon, Nigeria.

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ABSTRACT: The Lagos city is an urbanized commercial nerve center of Nigeria with complex ecological environment. The Lagos Lagoon is a typical Nigeria coastal aquatic environment of salt and brackish water with complex microbial diversity. The physical and chemical properties of surface water affected the distribution pattern of microbes. The temperature and pH are relatively stable. The averages of pH values were pH 7.28 and pH 6.55 for February and June period of sampling respectively, while temperature average 31°C during this period. The salinity and conductivity of the water were high during the month of February. The chlorides content determined by Mohr titration method was within permissible level of 250mg/l specified by WHO. The calcium content and suspended solid value were relatively high during the period of sampling.

During the study, Agboyi creek shows the highest microbial count of 8.5×10^3 CFU per 100ml to the lowest of 1.5×10^3 CFU per 100ml recorded for station 2 special. The highest coliform count was observed in stations 2 and Agboyi creek respectively with 7.1 x 10^3 CFU per 100ml while other stations varied with low coliform counts. In the sediment, high values up of 3.3×10^3 CFU per gram sediment samples was obtained. Various microbial species encountered during the study and their respective percentage distributive occurrence in the Lagoon were *bacillus spp.* (31.21%); *B. megaterium (16.7%)*, Micrococcus spp. (10,40), Klebsiella spp. (9.25), Enterobacter spp. (9.25), Escherichia coli (8.09), *B. polymyxa* (4.62), *Veillonella spp.* (2.89), Streptococcus spp. (1.73), Pseudomonas spp. (1.73), Proteus vulgaris (1.73), Aeromonas hydrohila subsp. Anaerogenes (1.69), Bifidobacterium adolescentis (0.57), and Moraxella bovis (0.57). The study shows that, the Lagos lagoon is largely inhibited by antibiotic resistant organisms and enteric bacteria which may have some health implications.

Keywords: Bacteria; Coliforms; Lagos lagoon; Microorganisms; Nigeria; Ecological Pattern.

Introduction

The Lagos Lagoon is situated in the Western part of Nigeria. It is a great expanse of shallow water covering an area of about 208 square kilometers. In most places, the Lagoon is usually less than 1.5 meters deep (Akpata and Ekundayo, 1978). The central body of the Lagoon is located between longitude $3^{\circ}23'$ and $3^{\circ}40'E$ and Latitude $6^{\circ}22'$ and $6^{\circ}28'N$. It is the largest of the four Lagoon system of the Gulf of Guinea Coast (Webb., 1958).

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The importance of the Lagoon and estuaries as sources of fisheries products and as desirable recreation areas is now being recognized with increasing awareness. This is coupled with global increase in population and resultant waste generated (Halasi-kun, 1981; Webb, 1958). Lagos city was formerly the federal capital of Nigeria with notable air and sea ports of entry into the country. The high level of urbanization and industrialization of the city and its environs with the inevitable generation of domestic and industrial waste have led to biological consequences in the coastal aquatic environment. The Lagoon is a marine environment which is dominated by certain species of microorganisms. The marine microbial populations of marine environment are composed primarily of bacteria, but viruses, yeast and moulds are also found (Meyers, 1967; Jones, 1976; Sinidu, 1974; Fell, 1976; Seiburth *et al.*, 1978; Prescott *et al.*, 2002).

There is paucity of information and data on the microbial population of the Lagos lagoon. Most of the information relating to microbial isolate are based on studies of Akpata and Ekundayo in which they reported the prevalence of *Escherichia coli* in the Lagos Lagoon specifically at sewage disposal sites but less so in areas close to sea water with high salinity (Akpata and Ekundayo, 1978). The relative abundance of microorganisms in the Lagoon is related to the instability of the physical and chemical factors of the Lagoon. High microbial population has been recorded for the dry season (Olaniyan, 1969). A wide range of sediments types from silty-sand to shelly mud were found in the Lagos Lagoon (Ajao, 1990). However, the most abundant marine microbial populations are those which inhabit coastal Lagoon or estuarine sediments with constant large inputs of allochtlous organic matter. In those sediments and detritus, the total number of bacteria ranges from 3 to 10×10^9 cells per gram and their biomass from 2 to 5mg per gram of sediment (Longhurst, 1981).

The study on ecological distribution pattern of microorganisms in the Lagos lagoon thus reflect the social, health and economic trend of this notable Nigerian aquatic environment of the highly commercialized city of Lagos and possible solutions to some environmental problems.

Materials and Methods

Method of Sample Collection From Study Site.

A sizeable area of the Lagos lagoon was mapped out as the study site. Twenty-four stations were selected for study and sampled in February and June, with the assistance of the Nigerian Institute for oceanography and Marine research (NIOMR). A large map designated by NIOMR staff was employed to locate the sample points. Experienced NIOMR Field Assistants assisted in doing this with aid of a compass. Surface water and sediment samples were collected from this sampling points (Fig. 1).

Surface Water Collection

Surface water was collected within a depth of one foot into sterile plastic container employing a Ruttner standard water sampler, 8cm diameter and 50cm long, capacity 500ml. Approximately about a litre of water was collected from each sampling point. The samples were labeled and sealed, and immediately kept in a cooler containing ice on board the boat for preservation. During time of collection, the temperature of the water was measured with the aid of a thermometer calibrated in degree centigade (°C) and the depth at which the water was collected was measured in meters.

Sediment Collection

Sediment samples were collected at same site where the water samples were taken, with aid of a "Metal grab". Portion of the collected sediment was apportioned unto sterile plastic nylon seals and also kept in the cooler.

Laboratory Procedures

The surface water samples were analysed in the laboratory for the physical and chemical properties using standard techniques. This parameters include the determination of pH, chloride, salinity, suspended

solids (SS), total organic carbon (TOC), calcium and conductivity. Filter paper method was used for suspended solid (SS), while TOC)) was estimated by UV absorbance at 254nmin filtered samples. The microbial isolates recovered from both the surface water and sediments samples were equally characterized for identification purposes and occurrence at different sites in the lagoon. This samples were analysed within 36 hours of sample collection. The total microbe and colliform counts were determined employing milipore membrane filters (sartorius, GmbH), for surface water while a pour plate technique was employed to determine the total microbial count for sediment.

Procedure for identification of bacteria isolates

Different colonies farmed on the plate count media were picked with the aid of sterilized inoculating loop and streaked on nutrient agar plates. These were incubated at 37°C for 18-24 hours. After incubation a gram stained film of each discrete microbial colony was prepared. Each bacterial isolate was identified based on their morphological characteristic, their colour, arrangement of vegetative cell and possession of spores (Robert *et al.*, 1984). Each isolate was preserved on nutrient agar slopes for further characterization and full identification purposes.

The major microbiological techniques and biochemical tests intensified for the identification ppurpose include the gram stain, spore stain, biochemical utilization of various carbohydrate sugars, such as fermentation of mannitol, glucose, fructose, lactose, sucrose, raffinose arabinose and their ability to reduce nitrate. The cultural characteristics of each isolate on eosine methylene blue (EMB), citrate utilization, mannitol fermentation, haemolysis on blood sgar, and ability to produce catalase were also determined. Nutrient broth and brain heart Infusion (BHI) were routinely used for the cultivation of micro-organisms during the study.

Antibiotic sensitivity test

The *in-vitro* antibiotic susceptibility testing of bacteria isolates was performed using the standardized agar diffusion method described by Bauer *et al.*, (1996). Paper disc medium (PDM), antibiotics sensitivity agar (AB BIODISK, Solna Sweden) was the plating medium used.

The antibiotic discs (AB BIODISK, Solna, Sweden) that were used for this test included gentamicin, $30\mu g/disc$: nalidixic acid $30\mu g/disc$;Ttetracycline, $30\mu g/disc$ Trimethoprim + Sulfamethoxazole $1 - 2 + 23.8\mu g/disc$; Trimethoprim, $1.2\mu g$; Spectinomycin; $30\mu g$ Ampicillin, $10\mu g$; Sulfamethoxazole, $23.8\mu g$; Chloraphenicol, $30\mu g$; Streptomycin $30\mu g$.

The diameter of the zone of growth inhibition was measured by means of a ruler in millimeters from the underside of the plates. The portion between the point and the area showing no visible growth that could be seen with the miaded eye was taken as the zone of growth inhibition. The AB Biodisk manual for interpretive zone diameter standard were used to interpret the diameter of zone inhibition. Isolates were then scored as either sensitive or resistant.

Results

The result of the physical and chemical properties of surface water during the month of February and June is as shown in Table 1. The pH and temperature are relatively stable. The average values for pH are pH 7.28 and pH 6.55 for February and June respectively, while temperature averages 31°C for these periods (Table 1). The salinity and conductivity of the water were high during the month of February. The chloride content determined by Mohr titration method was within permissible level of 250mg/l specified by WHO. The calcium content was high, the average recorded ranged from 138.68mg/l to 151.28mg/l for the periods of sampling collection. The suspended solid value (SS) ranged from 15.31mg/l to 17.16mg/l for the months of February and June respectively.

Table 2 reflects the bacterial population including the coliform load of the surface water of the lagoon during the month of February and June. The total microbial load varied from station to station with Agboyi creek recording the highest count of 8.5 x 10^3 CFU per 100ml of Lagoon water during the month of February and the lowest microbial load of 2.25 x 10^3 was recorded in station 5.

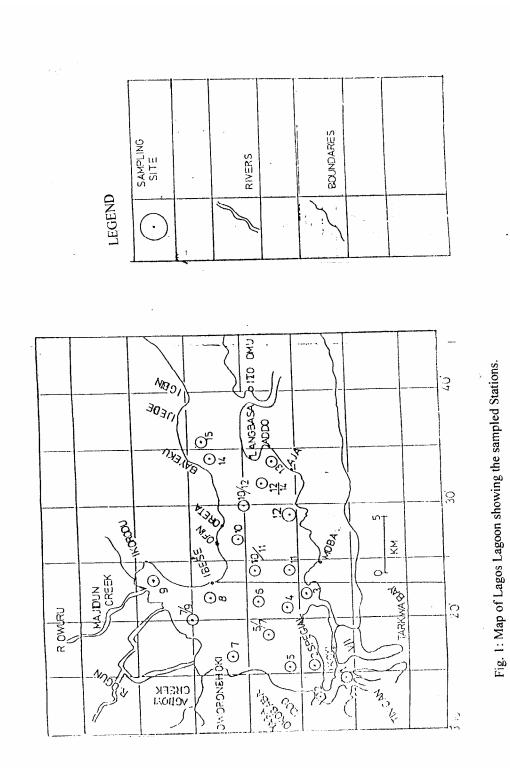


Table 1:

Table 1: Physical and chemical properties of the surface water of the Lagos Lagoon.
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Stations		Surface	Water		Sedi	ment
	February		June		February	June
	Total Microbial Count cfu/100 ml x 10 ³ dil. factor	Coliform Count cfu/100 ml x 10 ³ dil. factor	Total Microbial Count cfu/100 ml x 10 ³ dil. factor	Coliform Count cfu/100 ml x 10 ³ dil. factor	Total number/gram cfu/100 ml x 10 ³	Total number/gram cfu/100 ml x 10 ³
1	6.5	2.5	2.7	0.21	0.92	0.70
2	7.2	7.1	2.0	0.35	2.3	1.2
2 Special	6.0	2.8	1.5	0.67	3.6	2.5
3	3.5	0.5	3.4	2.4	5.2	3.3
4	4.0	0.10	4.5	1.8	5.9	3.5
5	2.25	0.30	1.55	0.37	2.4	2.4
6	6.0	0.1	2.6	0.70	1.2	1.3
7	2.5	0.4	2.0	0.19	9.1	5.1
5/7	4.3	1.7	2.0	1.00	3.7	2.2
7/9	3.40	0.48	3.6	0.37	5.4	6.4
8	4.5	0.50	2.8	1.10	8.4	6.3
9	3.5	1.5	3.5	0.80	23.0	8.0
10	3.7	0	3.6	0.28	11.0	8.1
11	3.0	0.20	3.0	1.7	6.2	6.2
10/11	_	_	_	_	33.0	15.0
12	8.3	0	3.4	1.4	4.1	2.4
12/10	5.4	0.70	4.4	1.7	5.1	3.0
12/14	6.4	0.40	5.3	3.2	0.96	0.91
9 MJR	2.8	2.0	3.2	0.50	8.2	5.4
AGR	8.5	7.0	3.2	0.20	4.6	2.0
OGR	5.2	0.30	3.2	0.20	4.6	2.0
13	2.3	0	3.3	0.80	3.5	2.3
14	2.7	0.20	2.7	0.60	31.0	12.0
15	3.5	0.3	2.9	0.15	3.5	3.3

Table 2: Microbial load of the Lagos Lagoon

The above figures reflect the total bacteria count compared with the coliform count which ranged from a high of 7.1 x 10^3 recorded in station 2 and Agboyi creek respectively to low coliform counts of zero (0) in each of the stations 10, 12 and 13. Similar values were recorded during the other sample periods. The bacterial population of the sediments collected from various stations was also shown in Table 2. Station 10/11 recorded the highest microbial load of 3.3×10^8 CFU per gram of sediment sample during the month of February. Other values varied from station to station.

Identification of microbial isolates

Microorganisms of diversed nature were recovered from the Lagos lagoon. Bacterial isolates recovered from the Lagoon can be categorized into 12 different genera. The predominant species were *Bacillus spp.*, which constituted (31.21%) of the total isolates. This was followed by *bacillus megaterium* (16.76%). Other species encountered were *Micrococcusmegaterium*, *Enterobacter spp.*,(9.25%). *Klebsiella spp.* (9.25%); *Escherichia coli* (8.09%); *Bacillus polymyxa*(4.62%) and *veillonela spp.* (2.69%). The following species were also recovered in less frequency. These are: *Proteus vulgaris* (1.73%), *Steptococcus spp.* (1.73%). Other microbes encountered were *Pseudomonas spp.*(1.73%); *Bifidobacterium adolescentis* (0.57%) and *Moraxella bovis* (0.57%) (Table 4).

The *in-vitro* antibiotic sensitivity test was performed for all the bacteria isolates. Table 4 shows the pattern of antibiotics resistance. A total of 77 strains were tested with 46(60%) of the strains showing multiple antibiotic resistance.

Discussion

The specific aims of this study are to isolate, identify and determine the occurrence of microorganisms in the surface water and sediment samples from the Lagos Lagoon (Fig. 1). Furthermore, some parameters of ecological and health importance to human populations were determined. Lagos city being an highly urbanized and commercial nerve center of the country, Nigeria, the study of this nature therefore becomes necessary. The stations that fall in an estuarine area in this study include 9MJR (Majidun Creek), AGR (Agboyi Creek) and OGR (Ogun River). Each of this stations has low salinity during the month of February and June compared with relatively high salinity in other stations. The reason for this may be due to the influx of fresh water from the rivers into the Lagos Lagoon marine environment and the low level of dissolved ionisable particles in the estuarine areas.

Table 1 shows that the Lagoon salinity was low during the month of June compared with relatively high salinity recorded for February in most of the designated stations. These periodic changes in salinity could have influenced seasonal distribution of microorganisms (Olaniyan, 1957; Sandison and Hill, 1966; Fagade and Olaniyan, 1974). This study confirmed localized contamination of Lagos Lagoon with faecal coliform bacteria which corroborated the findings of Akpata and Ekundayo (1978). The level of contamination with coliform bacteria was high in areas where there was low salinity and low in areas with high salinity.

The pH temperature were relatively stable. The chloride value which is within the 250mg/l maximum permissible concentration was considered to be within the permissible average of WHO, 1971. So also the mean value of suspend solid (SS) for February was within the limit of European communities (EC) of 25mg/l (Chapman, 1989). However, the calcium content for both periods was high. The conductivity value of the Lagoon was high compared with the desirable level of 25ms/cm (25°C) that is internationally acceptable (WHO, 1971).

Organisms belonging to the genus *bacillus* constitute about 52.59% of total bacterial isolates of both surface water and sediments. The reason for the predominance of bacillus in the Lagoon may be due to inherent nature of this organisms which is associated with its ability to survive in hostile environment, its nonfastidious nutrient requirement and the sporogenous nature of the organism. Enteric gram negative short rods, lactose fermenters such as *Klebsiella sp., Enterobacter spp., Escheriachia coli* constituted 26.59% of entire organism cultured from both the surface water and sediment samples suggesting recent contamination of this body of water with human sewage. The prevalence of enteric and multiple antibiotic resistant organisms in the water may have significant health implication as previously discussed.

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Table 3: Morphological and cultural characteristics of bacterial isolates.

Table 3:

	Surface	Water		Sediment	Overall% Total of bacterial spp.	Pattern of Antibiotic	Resistance
	February	June	February	June		No. of strains tested	No. % showed multiple resistance
Isolates	No. of isolates (% Total)	No. of isolates (% Total)	No. of isolates (% Total)	No. of isolates (% Total)			
Bacillus spp.	10 (41.66)	7 (14.28)	25 (24.49)	12 (24.49)	31.21	35	24 (68.57)
Bacillus megaterium	5 (20.89)	6 (12.24)	9 (16.98)	9 (18.97)	16.76	14	10 (71.43)
Micrococcus spp.	3 (12.5)	8 (16.32)	4 (7.54)	5 (10.20)	10.40	7	2 (28.57)
Klebsiella spp.	2 (8.33)	7 (14.28)	2 (3.77)	5 (10.20)	9.25	4	3 (75)
Enterobacter spp.	1 (4.16)	5 (10.20)	4 (7.54)	6 (12.24)	9.25	5	3 (60)
Escherichia coli	2 (8.33)	6 (12.24)	2 (3.77)	4 (8.17)	8.09	4	2 (50)
Bacillus polymyxa	-	5 (10.20)	1 (1.88)	2 (4.08)	4.62	1	-
Veillonella spp.	-	3 (6.12)	-	2 (4.08)	2.89	-	-
Streptococcus spp.	-	1 (2.04)	1 (1.88)	1 (2.04)	1.73	1	-
Pseudomonas spp.	1 (4.16)	1 (2.04)	-	1 (2.04)	1.73	1	-
Proteus vulgaris	-	-	2 (3.77)	1 (2.04)	1.73	2	2 (100)
Aeromonas hydrophila subspecies anaerogenes	-	-	1 (1.88)	1 (2.04)	1.16	1	-
Bifidobacterium adolescentis	-	-	1 (1.88)	-	0.57	1	-
Moraxella bovis	-	-	1 (1.88)	-	0.57	1	-

Table 4: Nature of bacterial species isolated from the Lagos Lagoon

In conclusion, this study shows that the Lagos lagoon is populated with diversed species of microorganisms mainly pathogenic bacteria and that the pathogenic organisms are more likely to be found in areas of low salinity than in areas of high salinity. The antibiotic sensitivity of bacteria encountered in the Lagoon could be linked to the clinical and epidemiological control of disease causing organisms in these area. The prevalence of certain species of organisms in certain areas of the Lagos lagoon may also be of some epidemiologic significance. Lagos city is densely populated, highly industrialized and also possessed major parts of entry into the country and this warrants the knowledge of the ecological study of the area. The results obtained in this study should be helpful to ecologist and health-care administrators in the proper monitoring of our natural waters for sustainable economic development. Moreover, the data obtained during the study could be used as a guide for future research work in this area.

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