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SPATIAL AND TEMPORAL DISTRIBUTION OF *PSEUDOMONAS AERUGINOSA* SPECIE IN SURFACE LAGOON WATER

***Ebohon J. O., Agwu O. A., Oluwagunke T. O., Bello I. A., Lampejo A. M., Mohammed H. A.**

Department of Biological Oceanography, Nigerian institute for Oceanography and Marine Research, Victoria Island, Lagos State

*Corresponding authors' email: joyosiuare@gmail.com

ABSTRACT

Pseudomonas aeruginosa is a ubiquitous bacterium in diverse environment, and has been implicated in various ecological and clinical activities. The present study assessed the spatial and temporal prevalence of *P. aeruginosa* in Lagos Lagoon surface water by isolation and enumeration of the culturable bacteria on two selective media (Cetrimide and Acetamide agar) and species identification was done by applying different biochemical test. Enumeration of the *P. aeruginosa* isolate observed throughout this study in the twelve stations sampled ranged from 2.5 – 57000.0 CFU/100ml. The percentage population density was highest (54.75%) at the peak of rainy season (July) while the other sampling period had less than 20%. The two stations (5 and 7) with 25.33% and 24.82% population density are notable for high anthropogenic activities. The counts of *P. aeruginosa* were not correlated with any of the physico-chemical parameters tested. The temporal and spatial percentage population density indicate possible higher contamination during rainy season due to increased runoff and that higher human activity obviously contributes to contamination respectively. This highlights potential consequence to human health and seafood safety as well as the possible ecological roles particularly in the breakdown of pollutants and other biotechnological benefits.

Keywords: *Pseudomonas aeruginosa*, Lagoon, surface water, pollution

INTRODUCTION

Pseudomonas aeruginosa is a gram negative, motile, rod shaped and facultative anaerobic bacterium of the family Pseudomondaceae. It should be emphasized that this microbe is extremely versatile and can adapt to a wide range of habitats. This adaptableness accounts for its ubiquitous presence in the both natural and other environment including soil, water, animal hosts, hospital settings, soap, and even distilled water hence, they have an extensive impact on ecology, agriculture and commerce (Tripathy *et al*., 2006; Sivri *et al*., 2013; Januário *et al*., 2020; Vuki´c Luši´c *et al*., 2021). *P. aeruginosa* of both clinical and environmental isolates produce virulence-associated traits, hence are considered potential pathogens. As quintessential opportunistic pathogen, they cause diseases in plants and animals. They are usually implicated in infections in immunocompromised patients involving urinary and respiratory tracts, skin and bloodstream. They exhibit high antibiotic resistance which hinders treatment (Grosso-Becerra *et al*., 2014; Anversa *et al*., 2019). This bacterium is an important specie for studying in the marine environment with regards to the role it plays during the contamination of recreational waters (Pellett *et al*., 1983), sea water and drinking water as well as outbreaks of opportunistic pseudomonas infection (Khan *et al*., 2007).

Surface water play a crucial role in spread of pathogenic agents and also due to the increasing colonization and population of the coastal zone, it has introduced a proclivity for the discharge of untreated sewage into the aquatic environment. These microbes may find their way into water through surface runoff/domestic waste water, postures during rainfall (Collins *et al*., 2005) or by direct deposition of fecal matter with access to steam channels (Eyles *et al*., 2003). These can be transferred to humans by several roots like recreation; irrigation of crops and vegetables. This form of aquatic pollution is perhaps responsible for the greatest number of human morbidities and mortalities worldwide. Hence the need for regular monitoring and quality evaluation of coastal water.

We observed dearth of data on the occurrence of *P. aeruginosa* as a measure of water quality in our coastal surface waters. Previous studies had focused on the occurrence of Total and Feacal coliform bacteria; therefore, the objective of this study was to evaluate the occurrence, distribution of *Pseudomonas aeruginosa* in surface water of Lagos Lagoon. The effect of physicochemical parameters was also studied. Such a study is vital and important as it shall provide a framework for practical measures to guide local authorities for coastal water management, control of anthropogenic activities around the coastal areas and would enlighten the general populace to mitigate and control the impact of pollution on water ecosystem and population. The results of this study will also highlight the potential ecological and biotechnological benefits *P. aeruginosa* in the Lagos Lagoon surface water.

MATERIALS AND METHODS

Study Area

A total of twelve stations with anthropogenic activities were selected along Lagos Lagoon. The names and location coordinate of the stations is presented in Table 1.

Table 1: Names of Sampling Stations and location co-ordinate

STATION	CO-ORDINATES
NIOMR JETTY (1)	N06°25.148' E003°24.455'
ATLAS COVE (2)	N06°24.789' E003°23.849'
SABON-KWOJI (3)	N06°25.847' E003°22.975'

FOLAWIYO (4)	N06°25.781' E003°22.272'
CROWN FLOUR MILL (5)	N06°25.971' E003°21.826'
IJORA (6)	N06°27.824' E003°22.689'
EBUTE-ERO (7)	N06°27.758'E003°23.018'
OKO BABA (8)	N06°28.795' E003°23.527'
UNILAG (9)	N06°30.977' E003°24.321'
CIVIL SERVICE CLUB (10)	N06°26.542' E003°24.412'
LEKKI SHOPRITE AREA (11)	N06°26.292' E003°27.041'
LEKKI SEWAGE DISPOSAL (12)	N06°27.007' E003°27.979'

Surface water Sampling

Surface water samples were taken from twelve stations of the Lagos Lagoon for four sampling periods as follows; May 2019, July 2019, October 2019 and January 2020 to cover the two (wet and dry) main seasons. The surface water samples were aseptically collected using 500ml sterile bottles and were kept in an ice chest box, they were then transported to the laboratory and analyses were carried out within 5 hours.

Measurement of physicochemical parameters

Sampling and analyses of some physicochemical parameters such as water and air temperature, pH, salinity, and dissolved oxygen were done by using standard methods given by APHA (2005)

Bacterial isolation, enumeration and identification

To isolate and enumerate *P. aeruginosa* species from the water samples two selective media were employed (cetrimide and acetamide agar). These were performed using the traditional spread plate method, after appropriate serial dilution of water samples buffered peptone water, then 0.1ml of selected dilutions were inoculated into the petri dishes containing the selective agar for evenly spreading. The plates were then incubated for 37^0C for 24 to 48 hours and the bacterial colonies enumerated. Colonies were then sub cultured on nutrient agar to obtain pure colonies. Preliminary identification of the pure cultures were carried out by applying different biochemical tests alongside observation of colonial characteristics including pigment production which were observed more on acetamide media. All strains were identified according to Bergey's manual of systemic bacteriology (Pallerony, 1984), gram staining, oxidase, citrate and catalase production were also carried out.

Statistical Analysis

For statistical analysis, bacteria counts were subjected to analysis of variance (ANOVA), correlation coefficient and DMRT post-hoc was analyzed using SPSS v.20 computer software program. Tests were carried out at 5% significance level.

RESULTS AND DISCUSSION

Physico-chemical parameters during the sampling Period

Water temperature, air temperature,pH,salinity and dissolved oxygen were measured *in situ* using a probe connected to a multipara meter Horiba-u20(Table 2).Water temperature did not show any seasonal variation, the temperature was maintained throughout the sampling period (26-27^0C) while the air temperature varied between 24^0C to26^0C.The pH values were relatively stable .The salinity values shows spatial variations, low salinity values were observed in stations 8,9,11 and 12 ranging from 6.73-9.44%.Mean values of dissolved oxygen varied greatly from 2.26mg/l at station 5 to 8.63mg/l at station 12.

Table 2: Mean ±SD of some physico-chemical parameters of Lagos Lagoon surface water during the study

STATIONS	AIR TEMP.	WATER TEMP	PH	SALINITY	D.O
1	25.13 ±0.85	26.13 ±0.63	7.99 ±1.53	15.82±12.69	7.06±6.27
2	25 ±0.82	26.25 ±0.5	7.91 ±1.22	16.87±15.70	6.01±4.53
3	25.13 ±0.85	26.5 ±1.29	7.93 ±1.18	12.41±10.82	7.41±5.47
4	26.13 ±0.63	26.5 ±0.58	7.77 ±1.39	11.99±10.32	4.97±5.00
5	25.5 ±0.58	26.63 ±0.48	7.95 ±1.45	12.13±10.22	2.26±2.19
6	24.75 ±0.96	26.25 ±0.5	7.69 ±1.15	11.80±12.52	6.25±5.77
7	25.88 ±1.65	26.88 ±0.63	7.61 ±1.12	11.14±11.38	6.72±5.43
8	26.25 ±0.96	27 ±0.82	7.68 ±1.33	9.44±10.57	7.13±3.31
9	25.88 ±1.44	26.75 ±0.96	7.28 ±1.44	6.73±7.57	6±4.38
10	25.25 ±1.89	26.63 ±1.49	7.75 ±1.15	11.21±11.18	6.69±4.97
11	26 ±1.41	27.38 ±0.75	7.81 ±0.99	8.80±9.55	8.11±8.08
12	26.38 ±1.89	27.75 ±0.96	7.73 ±0.99	8.04±8.87	8.63±5.88

Total viable count of *Pseudomonas aeruginosa* in all stations during the entire sampling period

The presence of *Pseudomonas aeruginosa* was noted in most of the stations during the four sampling periods except at station 10 and 11 during the October and January sampling respectively (Table 3). The total viable count observed for the entire study ranged between 10 and 7.60 x10^4 cfuml^{-1}. Evaluating the temporal occurrence of the organism, July sampling period presented the maximum viable count (1.67 x 10^4 cfuml^{-1}) while the other three sampling periods had 1.89 x 10^3, 4.88 x 10^3 and 5.50 x 10^3 cfuml^{-1}. On the other hand, assessing the total viable count at the various sampling stations, the spatial mean viable count ranged from 8.96 x10^2±7.64 to 2.21 x10^4±1.4 cfuml^{-1}. Although no viable

Pseudomonas aeruginosa was present at Station 10 during a sampling period, a mean of 3.47 $x10^3$±4.5 $cfuml^{-1}$ was recorded for the entire study at that station. Nevertheless, Station 11 which also had no viable count of the organism during the January sampling period recorded the least spatial mean (8.96 $x10^2$±7.64 $cfuml^{-1}$) during the study.

Table 3: Total viable count ($cfuml^{-1}$) of *Pseudomonas aeruginosa* in all stations during the entire sampling period

STATIONS	MAY	JULY	OCTOBER	JANUARY	SPATIAL MEAN
1	10 ±0	1.00 $x10^4$±0	5.67 $x10^2$±3.2	9.25 x 10^2±7.8	**2.86 $x10^3$±4.8**
2	60 ±5.7	1.50 $x10^3$±5.0	4.00 $x10^3$±1.4	9.00 x 10^3±0	**3.64 $x10^3$±3.9**
3	1.48 $x10^3$ ±1.3	3.15$x10^4$±2.2	3.00 $x10^2$±2.8	1.27 x 10^3±1.5	**8.64 $x10^3$±1.5**
4	7.90 $x10^2$ ±1.4	1.00 $x10^4$±6.0	2.15 $x10^3$±4.9	2.29 x 10^4±3.9	**8.95 $x10^3$±1.0**
5	5.06 $x10^3$ ±4.4	3.58 $x10^4$±6.1	3.20 $x10^4$±0	1.56 x 10^4±1.4	**2.21 $x10^4$±1.4**
6	2.55 $x10^2$ ±6.4	1.00 $x10^4$±0	1.28 $x10^3$±5.3	4.50 x 10^2±2.1	**2.99 $x10^3$±4.7**
7	6.87$x10^3$ ±6.5	7.60 $x10^4$±1.1	2.10 $x10^3$±8.2	1.63 x 10^3±8.2	**2.17 $x10^4$±3.6**
8	1.29 $x10^3$ ±8.5	4.50 $x10^3$±4.0	1.01$x10^4$±4.1	1.20 x 10^4±8.4	**6.98 $x10^3$±4.9**
9	1.41 $x10^3$±1.0	7.00 $x10^3$±4.4	1.35 x 10^3±7.1	7.75 x 10^2±3.9	**2.63 $x10^3$±2.9**
10	2.73 $x10^3$±1.9	1.00 $x10^4$±0	0	1.15 x 10^3±4.9	**3.47 $x10^3$±4.5**
11	1.85 $x10^3$±7.2	1.00 $x10^3$±0	7.33 x 10^2±3.1	0	**8.96 $x10^2$±7.64**
12	9.58 $x10^2$±6.9	1.55 x 10^3±2.1	3.98 x 10^3±2.5	3.50 x 10^2±3.5	**1.71 $x10^3$±1.6**
TEMPORAL MEAN	**1.89 x 10^3**	**1.67 x 10^4**	**4.88 x 10^3**	**5.50 x 10^3**	

Temporal Percentage Population density

The percentage population density of the *Pseudomonas aeruginosa* species at the various sampling period in this study is presented in Figure 1. The first sampling in May had the lowest population density of 6.27%. A remarkable increase to 54.75% was observed in July, with a subsequent decrease to 19.17% and 19.82% in October and January respectively.

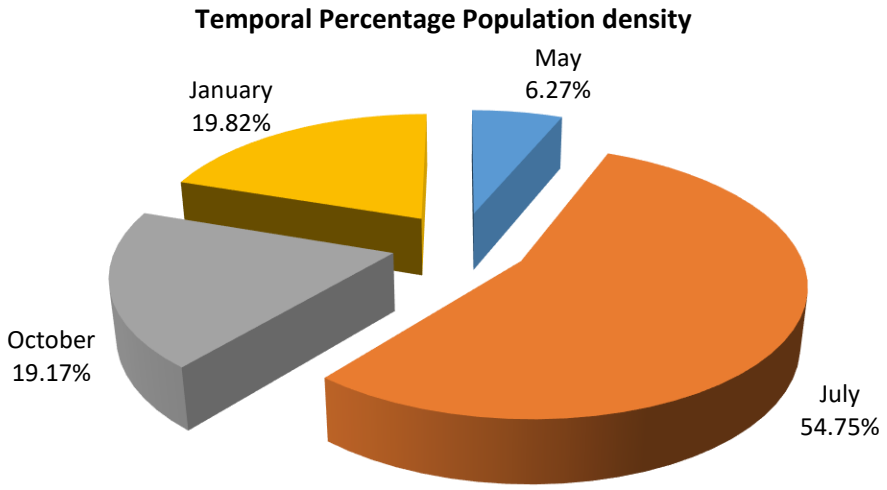

Figure 1: Temporal percentage population density

Spatial Percentage density

The percentage population density of *P. aeruginosa* in all the stations varied during this study as depicted in Figure 2. The spatial percentage population density varied with Stations 5, 7 and 4 having 25.33%, 24.82% and 10.27% respectively while the other Stations had below 10%. The least population density (1.37%) was noted at Station 11.

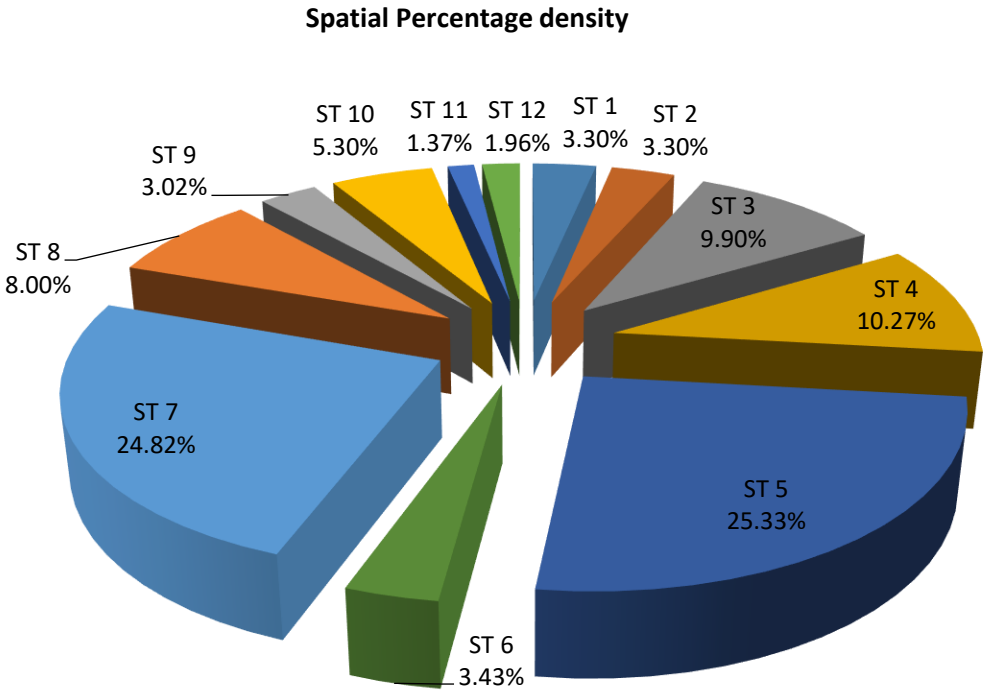

Figure 2: Spatial percentage density

Correlation of *Pseudomonas aeruginosa* viable count with some physico-chemical parameters

The Correlation coefficient in Table 4 shows that there was no significant correlation ($p>0.05$) between the occurrence of *P. aeruginosa* and all the tested physico-chemical parameters of the water although there was significantly weak correlation between pH and Salinity (r = 0.296*, $p<0.05$).

Table 4: Correlation coefficient of the physico-chemical parameters with *P. aeruginosa* from the Lagos Lagoon

	Air Temp	Water Temp	pH	Salinity	DO	*P. aeruginosa*
Air Temp	1					
Water Temp	.746**	1				
pH	.394**	0.188	1			
Salinity	0.219	0.09	.296*	1		
DO	0.06	0	-0.155	-.636**	1	
P. aeruginosa	-0.187	-0.156	0.064	-0.092	-0.22	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Environmental status of Lagos Lagoon is swayed by various factors which includes; industrialization, urbanization, intense agricultural activities, sewage discharge etc. The presence of *P. aeruginosa* is known to increase with such activities; therefore, this present study was carried out to find out the distribution (both temporal and spatial) of *Pseudomonas aeruginosa* in surface water of this remarkable Lagoon.

The prevalence of *P. aeruginosa* in the surface water of Lagos Lagoon was evident in the total viable counts recorded in the various stations during this study (Table 3). Januário *et al.* (2020) had earlier stated that, enumeration of microbes is a major means of assessing water quality as it indicates microbiological threats. The result of the present study is also in accordance to various research works around the world that has observed *P. aeruginosa* in diverse water bodies. Adingra *et al* (2012) noted the presence of *Pseudomonas aeruginosa* alongside other pathogenic bacteria from surface water collected from Grand-Lahou Lagoon Cote d' ivoire. Suzuki *et al* (2013) reported 2-46 CFU/100 ml of *P. aeruginosa* in Kyotake and Yae Rivers of Miyazaki city, Japan. Neiwolak and Opieka (2000) isolated *P.aeruginosa* from 0-1060 Cfu/ml from Czarna River, Poland. The high count of *Pseudomonas* in coastal Lagoons was attributed to different activities which includes recreational activities because their ubiquity in these water bodies is incomparable to the minimal 7.6% *P. aeruginosa* positive samples from 251 water samples from public water supply in municipalities in São Paulo State, Brazil as reported by Anversa *et al.* (2019).

Analysis the temporal distribution of *P. aeruginosa* in this study, the percent population density in the month of July (Fig 1) was remarkable. It is noteworthy that this is usually the peak of rainy season. Adingra *et al*., (2012) had earlier reported high level of incidence of *P. aeruginosa* from the Grand-Lahou Lagoon Cote d' ivoire during the rainy season. During rainy season, there is regular rainfall, as a result of these; there is maximum dilution and high influx of sewage which brings about turbidity and less penetration of sunlight. This is contrary to reduction in water flow, increased nutrient concentration, organic matter and reduction in water volume which brings about minimum dilution observed in dry season as noted by Castillo *et al*. (2004). Nonetheless, differing to these present results, Vuki´c Luši´c *et al*. (2021), reported a high prevalence of this bacterium in warmer season. Also,

Shivin *et al* (2015) alongside Marsalek *et al* (1994) noticed higher level of *Pseudomonas* in summer in Kshipra River and water of River St. Clair in Sarnia respectively.

The spatial population distribution of *P. aeruginosa* in this study displayed two stations (5 and 7) with greater than 20% population density compared to the other stations that had population density of 10% and below. It is notable that these two stations have high human and industrial activities; this agrees with earlier findings that prevalence *P. aeruginosa* is usually in accordance with hydrocarbon, pesticides and fecal pollution alongside other anthropogenic activities as compared to relatively low prevalence in uncontaminated sites (Crone *et al*., 2019). According to a report of Januário *et al*. (2020), the mean counts of *P. aeruginosa* from water collected near a sewage treatment plant ranged between 135.8 CFU/100 mL and1800 CFU/100 mL while water samples from a downstream site had 2–33 CFU/100 mL. In addition, Shivi *et al.* (2015) observed *P.aeruginosa* in Kshipra River with relation to anthropogenic activities.

The environmental parameters in the natural environment, affects the survival, adaptability and bacterial numbers/counts (Aboulkacem *et al.,* 2007), in this study, the count of *P. aeruginosa* indicated that there was no significant correlation ($p > 0.05$) with all the physic-chemical parameters of the water (Table 4). Aside these water parameters, the growth and development of *P. aeruginosa* was also noted to be influenced by nutrient accessibility, variation in water flow, water hardness and presence of disinfectants (Vuki´c Luši´c et al., 2021).

P. aeruginosa represents a significant public health menace causing a number of plant and animal diseases with elevated antibiotics resistance cases nevertheless, there is increasing studies of their biotechnological potentials (Sivri *et al*., 2013; Grosso-Becerra *et al.*, 2014). Wei *et al.* (2020) reported a wide distribution of virulence genes (*lasB, phzM, toxA, ExoU,* and *ExoS* genes) among *P. aeruginosa* isolates and confirmed that they are indeed potential pathogens. Meanwhile, Chatterjee *et al*. (2017) stated that Pseudomonads of environmental origin have high genetic diversity resulting in their ability to produce variety of secondary metabolites of which has been implicated in bioremediation or bio control, these include non-ribosomal peptides, bacteriocins, and quinolones. Agwu *et al*. (2012) also isolated *Pseudomonas aeruginosa* from clinical and environmental samples and discovered that these bacteria possess capacity to synthesize surfactants that could be of high industrial value. Bhawsar and Singh *et al*. (2014) isolated *P. aeruginosa* from Kosi Dam, India, and identified its role in breaking down hydrocarbon, and they also discovered that *P. aeruginosa* produces two pigments- pyocyanin and pyoverdin. Kaszab *et al*. (2021) also buttressed that, due to the absence of some virulence genes and the non-hemolytic characteristics of environmental *P. aeruginosa* isolates, they can be categorized as 'non-pathogenic' and used for bioremediation and plant disease protection. All these considerations suggests that the occurrence of *P. aeruginosa* in Lagos Lagoon surface water presented in this study could indicate public health issues since there is possible contamination of fish and sea food obtain therein, including risk to swimmers. Although there could also be some ecological benefits.

CONCLUSION

Pseudomonas aeruginosa is ubiquitous in surface water of Lagos Lagoon with particular prevalence in stations with high anthropogenic and industrial activity. This highlights potential implication to public health as well as the imperative ecological roles particularly in the breakdown of pollutants, hence there need for regular monitoring to ascertain human health and seafood safety, it also presents opportunities for isolation of *P. aeruginosa* isolates with excellent ecological and biotechnological potentials.

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