TEMPORAL AND SPATIAL EFFECTS ON MICROBIAL LOAD IN SEDIMENTS AND WATER OF POLLUTED ECOSYSTEM

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ABSTRACT
Seasonal effects on the microbial load of sediment and water at different locations along Bonny Estuary of Niger Delta were investigated for a year spanning both the wet season and dry season. The total fungi count in the sediment and water at different locations showed no significant difference ($P \geq 0.05$) during both seasons while the hydrocarbon utilizing fungi in the sediment and water showed a significant difference in both seasons ($p \leq 0.05$). There was a significant difference in the bacterial concentration in the sediment and water. This shows that the growth of certain microbial types may be favoured by different seasons. The fundamental role of micro-organisms in marine habitat have not been given better attention. Also, their distribution and diversity remain poorly understood in the marine environment. It is, therefore, necessary to investigate the effect of seasons and locations on microbial load in the marine ecosystem of the Niger Delta area to ensure the nutrient dynamics of the aquatic ecosystem
Keywords: Microbes, ecosystem, marine, environments.

INTRODUCTION

The Bonny Estuary of Niger Delta is one of the water bodies in Rivers state, Nigeria. It receives an indiscriminate effluent discharge and oil spills from the heavily industrialized and highly populated Port-Harcourt metropolis. The geological weathering, the industrial processing of ore and metals; petroleum exploitation, vessel repair facilities, industrial wastes, oil spills, and human faces (Chaerun *et al*., 2004; Munian-Mujika *et al.*, 2002; Loisy *et al*., 2005; Lees, 2000) are some of the reasons pollution occurs in this area. This can impact negatively the aquatic organisms, water, and sediment. Bacteria, fungi, and other soil microorganisms play important roles in the ecological process and nutrient dynamics of the aquatic ecosystem (Levy-Broth and Winder, 2010). However, most contaminants are discharged into aquatic ecosystems which are likely embedded as particles and accumulate in sediments and water (WHO, 2006). A large reservoir of bacteria and fungi in sediments exists and acts as an overlying water column (WHO, 2006) and leads to adverse ecological effects and danger to human health. The release of these contaminants from sediments and water may result in the re-suspension of particulates.

The extent of the risks and concentration of microorganisms in relation to seasons and locations deserve more investigations on account of the complexity of biochemical activities that alter their availability in water, tissues, and sediments.

Microorganisms are present everywhere and can survive in the environment for an extended period (Dowd and Maier, 1999). Microorganisms are the agents that are mostly responsible for the formation of carbon in most aquatic ecosystems (Baldrian and Stursova, 2011), however, little is known of their responses to the seasonal variation and different locations in aquatic ecosystems.

In microbial communities, fungal and bacteria/bacterial composition has been far less studied (Tedesoo *et al.*, 2014; Okereke *et al.*, 2017). The spatial and temporal distributions of microorganisms in water and soils are related to the nutritional and physicochemical features of their habitat, such as organic matter content (Burke *et al.*, 2009), pH (Fierer and Jackson 2006), aggregate size (Lauenroth and Bradford 2012), water content, seasons and temperature, which also influence their survival and dispersal (Whitford, 1996). However, studies have not shown the effect of different seasons and locations on bacterial concentration in a marine environment. There have been several reports concerning their specific functional groups (Roseline *et al*.,2003, Koidi *et al*.,2007, Courty *et al*., 2008; Okereke *et al.*, 2017) or studies limited to particular soil or litter horizons (Coince *et al*.,2013). Thus, the understanding of seasonal variations and locations of microbial concentrations becomes necessary for the prediction of its response to climate changes.

MATERIALS AND METHODS

Study site

The sampling site is located at latitude 4º 49` 52.32º to 4º 46` 12.72 north and longitude 7º 1 57.36 to 7 4 57 which transverse the Azuabie creek in Abuloma south-west of Port Harcourt and adjacent to – Okirika LGA, Oginiba and OKUJAGU axis, Rivers State. Azuabie creek is one of the major transportation links to Bonny Island and open seas with some built-up communities and forest (wetland)

Collection of Water Samples

Water samples were carried out monthly for a period of one year between May 2015 to May 2016 at mapped locations of Okujiagu, Slaughter, Abuloma, and Oginigba. The 50ml of surface water from each of the locations was collected with a

sterile conical flask. The depth of water from each location ranged from 3-5m at low tide while that at Oginigba was collected at 5m because it is an upper stream. This was done at three different points at each location per sampling trip. Samples were taken to the laboratory within 30min-1h of collection. The water samples were analysed for the microbial load.

Collection of Sediment Samples

The sediment samples were collected at three different points per month per location (Slaughter, Okujagu, Abuloma, and Oginigba) at a depth of 3-5m with van Veen grab. Samples were released into foil and taken to the laboratory for microbial analysis. Samplings were carried out during different seasons (May 2015 to May 2016). Collection points were geo-referenced with GPS (Global Positioning System). In each month, three samples of 20g were collected from three different points at each location. The sediment samples were taken to the laboratory for microbial analysis.

Sample Preparation

Total Heterotrophic Bacterial Count

This test was done to screen for the total viable and culturable aerobic, mesophilic, and heterotrophic bacteria present in each sample (water) in terms of colony forming unit per ml (CFU/ml), and colony forming unit per g (CFU/g) It was done by spreading 0.1 ml of the dilutions (10^{-1}, 10^{-2}, 10^{-3}', and 10^{-4}) of each water sample in duplicates, aseptically with a sterile glass rod on freshly prepared media and labeled nutrient agar plates. The plates were incubated at 37°C for 24h following Tsuneo, 2010 techniques.

Hydrocarbon Utilizing Bacterial Count

This test was done to enumerate the proportion of heterotrophic bacteria found in the samples that can use hydrocarbon compounds as carbon sources. The 0.1ml of each of the prepared dilutions was spread onto the mineral salt agar with a sterile hockey stick. A sterile filter paper was dabbed into crude oil and placed aseptically on the opposite side of the plate where the agar was poured and incubated for 24h at 35°C following Tsuneo, 2010 techniques.

Total Heterotrophic Fungal Count

This test was carried out to enumerate the total heterotrophic fungal species contained in the effluent samples. In this, 0.1ml of each of the dilutions of each sample was spread on different freshly prepared acidified potato dextrose agar and incubated for 3-5 days. After incubation, the total heterotrophic fungal content for each sample was recorded (Tsuneo, 2010).

Hydrocarbon Utilizing Fungal Count

This test was performed to estimate the proportion of the heterotrophic fungal species in the samples that can utilize hydrocarbon as a carbon source. This was done by spreading 0.1ml of each dilution of the samples onto different agar plates containing acidified mineral salt medium. A filter paper dabbed with crude oil was inserted under the cover of the Petri plates and incubated also at 28°C for 3-5 days.

Vibrio Count

This was done using the thiosulphate citrate bile salt agar. Each of the dilutions from each sample was plated on freshly prepared thiosulphate citrate bile salt plates in duplicates using the spread plating technique and incubated at 35°C for 24h. After incubation, greenish and yellowish colonies, which are tentative for *Vibrio* species were isolated and purified.

Faecal Coliform Count

This was done using the most probable Number method (Tsunea, 2010). For a given sample, 0.1ml, 1ml, and 10ml of that sample were introduced each in Lactose Broth media of single strength and double strength respectively. These tubes were incubated for 24 h. After incubation, tubes with a gas bubble showing positive were counted and their number determined using the MPN (most probable number).

STATISTICAL ANALYSIS

The Experimental design used for this analysis was factorial. Data obtained were subjected to two-way analysis of variance (ANOVA) and means separated at 95 percent with Tukey HSD significant difference using Minitab 23 statistical package.

RESULTS

A total load of different microbial contaminants on sediment and water body from different locations

Table 1 showed the spatial mean values of microbial load in sediment. The microbial load concentration ranges from Oginiba 2.50±1.22, 2.25±1.31, 24±2.94 (THB) (HUB) (TFC) to Okwujagu 734.75±36.04, 726.25±36.93, 283.75±8.50 cfu/g (THB) (HUB) (TFC) respectively. HUF and FCC ranges between 1.0±0.00, 18.00±1.15 (Oginiba) and 72.00±3.39, 119.25±16.75 cfu/g (Slaughter). Abuloma had the least (VC) concentration of 1.55±0.10 and the highest value of 2.15±0.09 (Okwujagu). In the water, Oginiba location had the least microbial loads and higher loads in the slaughter location except for (TFC) recorded with the least value of 25.0±5.0 (slaughter) location and a higher value 95±7.07 (Abuloma) location with significant differences (Table 2).

Table 1. The spatial mean value of microbial load in sediments of the study area (Mean ±SE)

Location	Microbial Contaminants					
	THB	**HUB**	**TFC**	**HUF**	**VC**	**FCC**
Abuloma	312.5±14.93^{b}	345±18.48^{b}	47.5±1.44^{c}	19±0.40^{b}	1.55±0.10^{a}	97.75±1.31^{ab}
Oginiba	2.50±1.22^{c}	2.25±1.31^{c}	24±2.94^{d}	1.0±0.00^{b}	1.62±0.22^{a}	18.00±1.15^{c}
Okwujagu	734.75±36.04^{a}	726.25±36.93^{a}	283.75±8.50^{a}	17.75±1.03^{b}	2.15±0.09^{a}	46.5±2.06^{bc}
Slaughter	575.5±32.40^{a}	575±56.78^{ab}	75.5±6.34^{b}	72.00±3.39^{a}	1.55±0.22^{a}	119.25±16.75^{a}

Means within the column with different superscripts are significant at p<0.05

Key: THBC - Total heterotrophic bacterial count, HUBC - Hydrocarbon utilizing bacteria count, TFC - Total fungal counts, HUFC - Hydrocarbon utilizing fungal counts, FCC - Faecal coliform count, VC - Vibrio Count (all in x10^{7}cfu/g)

Table 2. The spatial mean value of microbial load in the water of the study area (Mean ±SE)

Locations	Microbial Load					
	THB	**HUB**	**TFC**	**HUF**	**VC**	**FCC**
Abuloma	95.0±5.00^{a}	145±5.00^{b}	95±7.07^{a}	57.5±3.53^{ab}	9.9±0.14^{a}	625±475^{a}
Oginigba	50±0.00^{b}	50.00±0.00^{c}	45±7.07^{b}	37.5±3.53^{b}	4.75±0.35^{c}	260±10.00^{a}
Okujagu	95.0±5.00^{a}	110±14.14^{b}	95±7.07^{a}	55±7.07^{b}	7.8±0.28^{b}	600±500^{a}
Slaughter	110±10.00^{a}	195±5.0^{a}	25.0±5.0^{b}	80.0±5.0^{a}	10.5±0.50^{a}	625±527^{a}

Means within the column with different superscripts are significant at p<0.05

Key: THBC - Total heterotrophic bacterial count, HUBC - Hydrocarbon utilizing bacteria count, TFC - Total fungal counts, HUFC - Hydrocarbon utilizing fungal counts, FCC - Faecal coliform count, VC - Vibrio Count (all in x10^{7}cfu/g)

Total Heterotrophic bacteria

The result of the concentration of Total hetero trophic bacterial count in water and Sediment at different seasons is represented in figure 1. The result shows sample (water and sediment) has a significant effect (p<0.005) in both seasons. The THB (total hetero trophic bacterial) counts in sediment at Slaughter(575.5±32.40^{a})was higher than that of water at Abuloma, Okujagu, and Slaughter while at Oginigba location it was lower(50±0.00^{b}) in water sample compared to other locations (Figure 2).

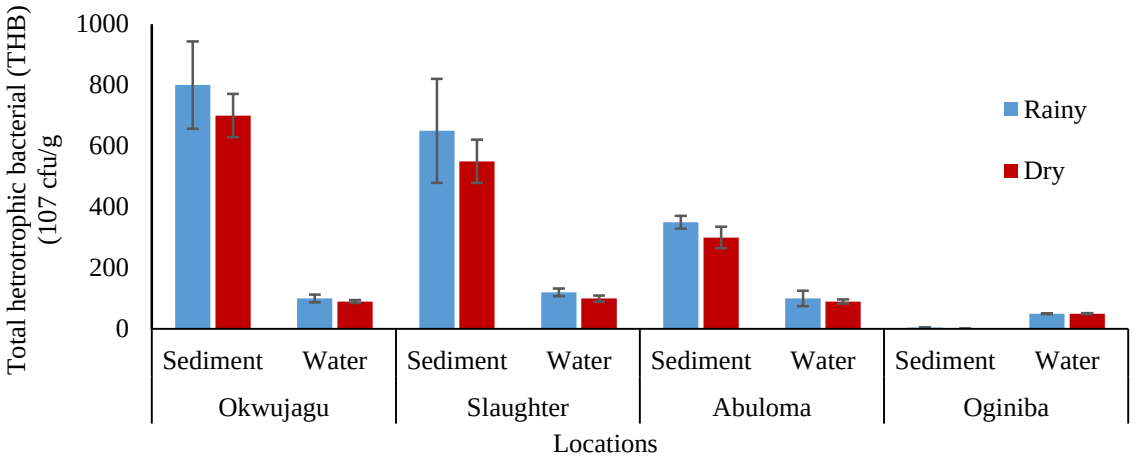

Fig. 1: Total hetrotrophic bacterial count (THBC) in sediment and water body from different locations at different seasons

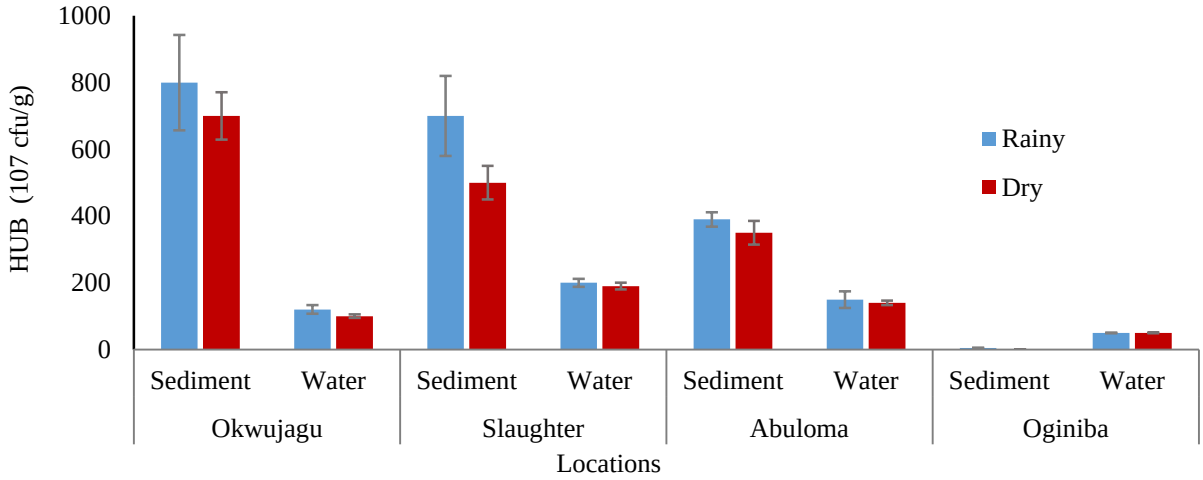

Fig. 2: Hydrocarbon Utilizing Bacteria (HUB) in sediment and water body from different locations at different seasons

Hydrocarbon utilizing bacteria counts was high in sediment at Okujagu and Slaughter than in water (Figure.2). There were significant differences in the hydrocarbon utilizing bacteria in sediment than in water. There was a significant ($p<0.05$) increase in the hydrocarbon utilizing bacteria in the sediment than water during raining season. The hydrocarbon utilizing bacteria was higher in sediment at Okujagu, Slaughter, and Aboluma than in Oginigba during raining season.

Total Fungal Count

The fungi count was higher in sediment and water in Okujagu in both seasons than in other locations. There was a significant increase in the total fungi count in Abuloma, Slaughter, and Oginigba ($P \geq 0.05$) while in Oginiba, it was higher in water than in sediment for both seasons (Figure3). Oginigba recorded a higher concentration of total fungi counts in water than in sediment during the rainy season. These showed significant differences ($P \geq 0.05$) at different seasons and locations. At the Slaughter location, the total fungi count was higher in sediment than in water compared to Oginigba which recorded high fungi in water than in sediment at both seasons.

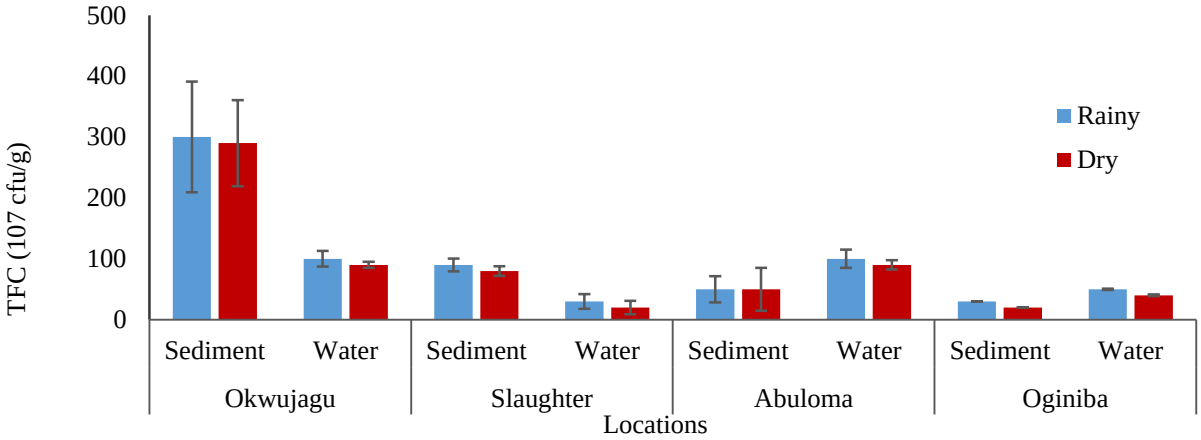

Fig. 3: Total Fungal Counts (TFC) in sediment and water from different locations at different seasons

Hydrocarbon Utilizing Fungi Count

The hydrocarbon utilizing fungal count was higher in wet seasons than in dry seasons. This was presented in (Figure 4). There was no significant difference in the hydrocarbon utilizing fungal counts ($p>0.05$) in all locations at both seasons. There was an increase in the hydrocarbon utilizing fungi concentration in water at the four stations than in the sediment, and during the wet season than during the dry season. The hydrocarbon utilizing fungi count showed a significant decrease in both water and sediments in Okwujiagu, Slaughter and Abuloma compared to Oginigba while at Oginiba the concentration of hydrocarbon utilizing fungi reduced further

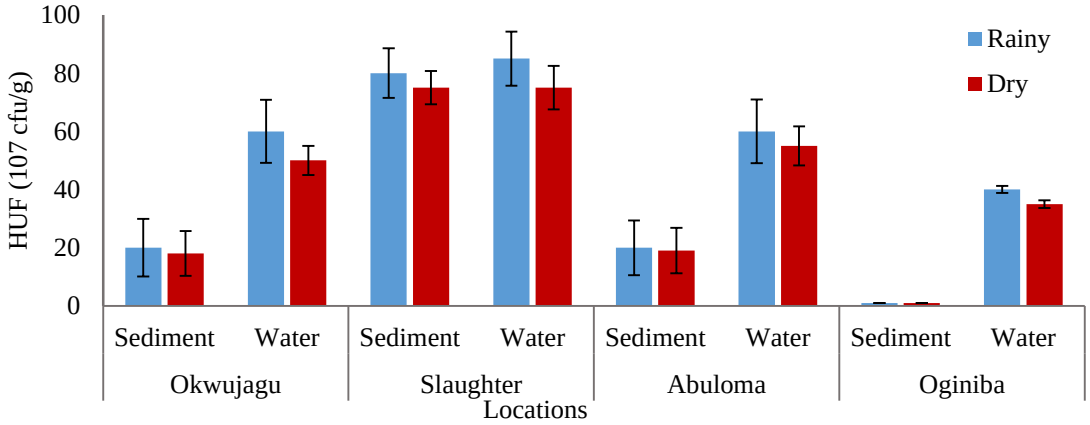

Fig. 4: hydrocarbon utilizing fungi count (HUF) Counts in sediment and water from different location at different seasons

Vibrio Count

The vibrio count has presented in Figure.5. The *Vibrio* count was higher in water than in sediment at all the locations. There were significant differences ($p\leq0.5$) in the vibro count in the water during the dry season in all locations. The vibro count in the water during rainy seasons was higher than in the sediment at both locations.

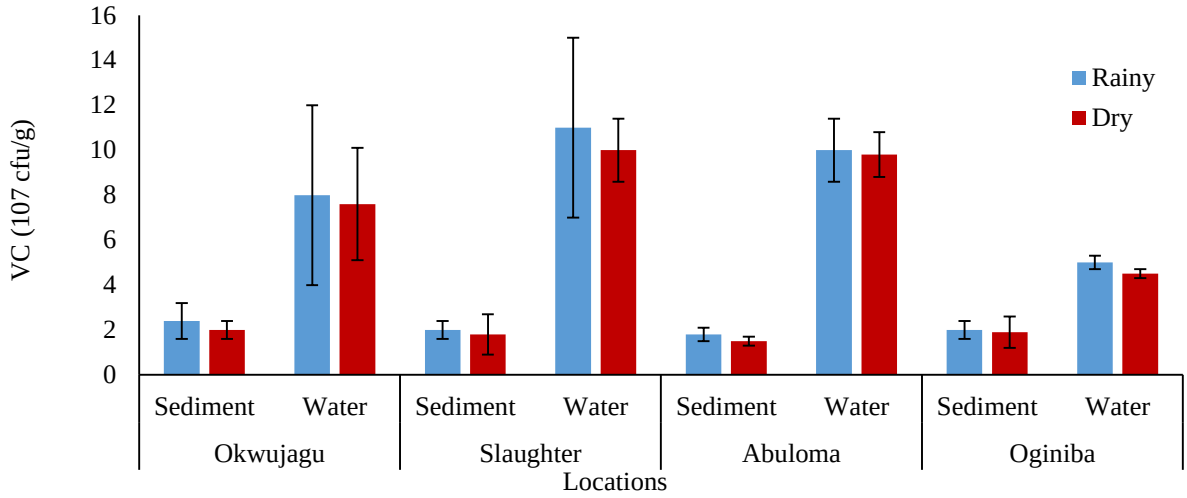

Fig. 5: Total vibro counts in sediment and water from different locations at different seasons

Feacal Coliform

The feacal coliforms were higher in water than the sediments at the four stations of Okujagu, Slaughter, Abuloma, and Oginigba, and during the wet season than during the dry season. There was a significant difference in the fecal coliform count between water and sediment. The feacal coliform count at both locations (Slaughter and Abuloma) in the sediment during the dry season showed no significant difference.

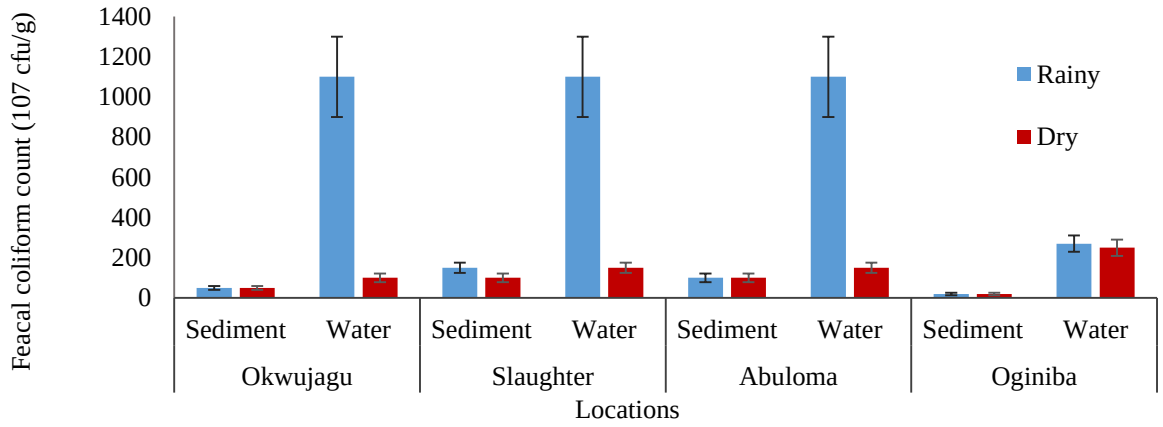

Fig. 6: Total Faecal Coliform Counts in sediment and water from different locations at different seasons

DISCUSSION

Most shellfish responded to changes in concentrations of contaminants in water as a result of the integration of contaminants from the water column over time (Dekock and Kramer, 1994; Gunther and Daurs 1997, Gunther *et al.,* 1999). When enteric bacterium (*E.coli*) is excreted into the water they die at a slower rate than pathogenic bacteria such as *Salmonella* and *Shigella* and their presence might indicate the presence of other pathogens in water (Madiaga *et al.,* 2000). The seasonal changes observed in the various microbial groups in both seasons could be because of the influence of the physicochemical properties and human activities.

When enteric bacterium (*E.coli*) is excreted into the water they die at a slower rate than pathogenic bacteria such as *Salmonella* and *Shigella* and their presence might indicate presence of other pathogens in water (Madiaga *et al.,* 2000). The significant differences in the total heterotrophic bacteria for different locations in water and sediment in both seasons could also be because of human activities done in those locations. These activities include bathing, washing clothes, boats, or other materials, disposal of faecal matters, sewage discharge, and discharge of oil spills. The seasonal changes observed in the various microbial groups in both seasons could be because of the influence of the physicochemical properties and human activities.

The high concentration of the THB (total heterotrophic bacteria) in the sediment at Okujagu and Slaughter could be large as a result such as the discharge of chemical substances- metals, pesticides, and organochlorine components from industrial and municipal treatment processes (Gunther *et al.,* 1999). The increase in the hydrocarbon utilizing bacterial population in the sediment during the rainy season could also be a result of the stimulatory effect of additional carbon and energy sources in the form of effluents, and crude oil in this creek which leads to an enrichment of the oil-degrading microbial population, which is made possible by hydrocarbon utilizing bacteria.

The high population of total fungal count recorded during the rainy season could be due to their heterotrophic nature which enables them to utilize nearly all natural organic matters. This creek is known to receive nutrient-laden waste materials from sewage and inland waters which build up in the water and enrich the sediment. These, provide a sufficient source of nutrients for the proliferation of organisms. The high fungal count could also be because of fungal activities such as biochemical cycling of nutrients in the water column, degradation of compounds, and contribution to the food web in the creek. This could also be a result of an increase in water volume which led to low salinity and increased microbial population. Rojaniaka and Ramlingappa, (2008) had earlier observed the occurrence of a higher fungal population during the rainy season. He noted a high population of fungi when the salinity was as low as 1.8 – 16.8 %). Microorganisms are natural habitats of soil and water, indeed the aquatic environment is a potentially good habitat for many fungal species (Zhangi, 2015). The high content of the total fungi in all locations of the creek is in tandem with the work of Rojaniaka and Ramlingappa (2008) and Okereke, *et al.* (2017) who stated that fewer fungi would be expected in the lesser aerobic sediment environment than in the more aerobic surface water. Recently, Zhangi *et al.* (2015) reported a tremendous fungal increase in sediment and stated that the marine environment is a potentially good habitat for many fungal species. This contradicts the work done by Rojaniaka and Ramlingappa (2008) in which surface water yielded 8 genera and 13 species of aquatic fungi while water samples collected from near the bottom recorded fewer (3 – 4 genera or species). This increase in fungal concentration also contradicts this study because Okwujiagu, Abuloma, and Slaughter recorded the highest total fungi counts in sediment while water samples collected from near the bottom recorded were poor. The fungi counts were more abundant in wet seasons than in dry seasons, suggesting they are more susceptible to environmental changes. Fungi like other micro-organisms are ubiquitous in all types of natural waters (El-Hissy *et al.,* 2000). According to Rojaniaka and Ramlingappa (2008), there are approximately 1.5 million fungal species on earth, about 3000 species are known to be associated with aquatic habitats and only 465 species occur in marine waters. He noted a high population of fungi when the salinity was as low as 1.8 – 16.8%). Although there are no studies that specify the effect of different seasons and locations on microbial diversity, however, in marine ecosystems, seasons can be considered a contributing factor to fungal occurrence and abundance. The total fungal count was also high in the sediment at Slaughter than in water at a different location. This could also be because of the continuous discharge of the blood of animals from the local abattoir into the creek.

The high population of hydrocarbon fungal count recorded during the rainy season could be due to the heterotrophic nature of the fungal which enables them to utilize nearly all natural organic matters. Although there are no studies that specify the effect of different seasons and locations on microbial diversity, however, in marine ecosystems, seasons can be considered as a contributing factor to fungal occurrence and abundance because of the high population at a particular season. This creek is also known to receive nutrient-laden waste materials from sewage and inland waters build up from the water column, degradation of compounds, and contributes to the food web in the creek. The difference in hydrocarbon count in the sediment was found between seasons and locations which may have an impact on the global climate changes.

This is in line with the work of Voriskova and Baldrian (2013), who stated that seasonal changes in the fungal community, with significant differences in their relative abundance, depending on the sampling time, not on a geographic point/s.

The high hydrocarbon utilizing fungal count(s) in Water at all the studied stations during wet seasons could be due to their heterotrophic nature which enables (s) them to utilize nearly all-natural organic matter. These, provide a sufficient source of nutrients for the proliferation of these organisms. It could also be a result of its activities such as biogeochemical cycling of nutrients in the water column, degradation of compounds, and contribution to the food web in the creek. Okereke, et *al*. (2017) had earlier observed the occurrence of a higher fungal population in sediment than in water during the rainy season He noted a high population of fungi when the salinity was as low as 1.8 – 16.8 %.). However, in marine ecosystems, seasons and locations can be considered contributing factors for fungal occurrence and abundance (Okereke *et al*., 2017). Seasonal changes and different locations of this fungal count are important to understand as well as their responses to global climate changes. This is in line with the work of Okereke *et al.,* (2017). WHO stated seasonal changes in the fungal community, in which the fungal community structure was dynamic,

with significant differences in their relative abundance depending on the sampling time but not on a geographic point.

The presence of *Vibrio* in both sediment and water was in line with some researchers who stated that *Vibrio* spp are adapted to survive in the marine environment (Yang *et al*., 2008 Zimmerman *et al.*, 2007). The persistence of *Vibrio in the water* in this study may be generally attributed to their presence in the environment (Kreger *et al.*, 2001). The high Vibrio count recorded in water at Okujagu, Slaughter, and Abuloma was higher than in sediment in both seasons. This could also be because of human activities such as bathing, washing of clothes, boats, or other materials, disposal of faecal matters, and sewage discharge done in those locations. This is in agreement with the work of Kreger *et al.* (2001), Winfield and Groisman (2003), and Okereke *et al*., (2017) who also reported that increased level of human activities could bring about the high microbial load in the water, which indicate the input of microorganisms from domestic and industrial sources.

It is pertinent to note that faecal coliform is derived not only from human sources of faecal pollution but also from wild and domestic animals including birds (Kator and Rhodes, 2001). The significant differences in the feacal coliform for different locations in water and sediment in both seasons could be because of human excreta deposited in these locations. Conversely, Winfield and Groisman (2003) reported that an increased level of human activities could bring about the elevation of organic matter resulting in a high microbial load in the water, which will lead to a high microbial population in an aquatic environment. This is an indication of the input of microorganisms from domestic and industrial sources which is a consequence of human activities.

CONCLUSION

This study shows that the microbial load was affected by the seasons and that the wet seasons have a higher load compared to the dry seasons Consequently, an understanding of the seasonal variations vis-a-vis locations and levels of microbial load would be necessary for the prediction of its response to climate change which may affect the microbial counts.

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