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Research Article

Seasonal Changes of Microbial Load in Some Sea Foods from Buguma and Ekerekana Creeks, Niger Delta, Nigeria

Abstract

Background and Aim: Niger delta environment has been exposed to organic and inorganic contaminants from industries and domestic wastes, thereby enhancing the capacity of the ecosystem into harboring a sizeable population of microorganisms. Most of these microorganisms are found in the water column, bottom sediment and water film, resulting in their elevated levels in seafood. Proper understanding of the transfer of microorganisms through the food web is essential to predict the exposure of seafood consumers to possible health consequences associated with its consumption in the coastal areas. Hence, the present study examines the microbial content of some sea foods such as: periwinkle (*Tympanotonus fuscatus*); mudskipper (*Periophthalmus papilio*); mullets (*Liza falcipinis*) Oyster (*Crassotrea gasar*) and water from Ekerekana and Buguma creeks, in the Niger delta, Nigeria.

Methods: Seasonal variations in microbial content in some seafood namely: Periwinkle (*T. fuscatus*); mudskipper (*P. papilio*); mullet (*L. falcipinnis*) and Oyster (*C. gasar*) from Ekerekana and Buguma creeks, Niger delta, Nigeria were assessed. The sea foods were sampled monthly from both creeks for a period of eight months. Standard methods were employed in the evaluation of microbial load in each species.

Results: The result indicated that the microorganisms isolated: total heterotrophic bacteria count, total vibro count; and total coliform count were more predominant in the wet season when compared to dry season months. Total coliform count bacteria exhibited some measure of elevation in the dry season months, an indication that they can be prevalent in both seasons depending on the type of human activities that is prevalent in the area.

Conclusion: This study has shown that industrial and domestic wastes discharged into Ekerekana and Buguma creek resulted in high concentrations of pollutants in the water body, which promotes the growth of microorganisms in the water column and sea foods, this may pose a health hazard to humans as a final consumer of these organisms There is therefore the need to formulate appropriate policies and regulations for safeguarding the ecosystem from adding undesirable microbial population.

Introduction

Seafood constitutes an important food component for a large proportion of world population especially those living in coastal areas [1,2]. In recent years, the Niger delta, environment have been exposed to organic and inorganic contaminants from industries and domestic wastes, especially oil related activities which are predominant in the region, thereby enhancing the capacity of the ecosystem into harboring a sizeable population of microorganisms [3]. The continuous release of these pollutants has resulted in an enriched microbial community. These microbes often find surfaces or organs of aquatic organisms for colonization [4]. Hayat et al. [5], noted that the number and type of microorganisms found on freshly caught seafood are influenced by location, season and rate of environmental pollution.

Bacterial species of enteric origin namely; *Bacillus spp. E. coli*, *Shigella spp. Staphylococcus aureus and Listeria monocytogenes* can be isolated from coastal water, as a result of human activities, such as improper waste disposal which include human, domestic and industrial wastes [6,7]. Most of these bacterial organisms are usually

isolated from waters which contain fecal materials [8-10]. Pathogenic bacteria in marine waters are most abundant in the sediments, they can also be found on the surface film, as well as in the water column [11]. As a result, fin and shell fishes found in marine environment often show elevated levels of these bacteria, which can cause disease in fish as well as human host that consume them. In most Niger delta communities, periwinkle, oyster, mullets and mudskipper are delicacies, they are in high demand among the populace living in these areas [12,13].

However, proper understanding of the transfer of microorganisms through the food web is essential to predict the exposure of consumers of this seafood to possible health consequences associated with their consumption. A large proportion of people living in rural and urban communities of Niger delta consume on regular basis sea foods harvested from the rivers, creeks and estuaries, laden with history of pollution consequent of domestic and industrial activities. Oysters and periwinkles are filter – feeding organisms capable of accumulating microorganisms in high concentrations. These organisms filter the surrounding water and consequently assimilate

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all the contaminants present in these environments. Consumers of these sea foods are therefore exposed to microbial and chemical pollutants in the ecosystem. Conversely, mudskipper and mullets are prominent aquatic animals found in brackish water zone of the Niger delta. They are particularly adapted to coastal environments, where they are widely consumed as food fish, because of their position in the food chain, they can equally accumulate microorganisms from their immediate environment. Hence the present study examines the seasonal variation in microbial content of some sea foods such as: periwinkle (*T.fuscatus*), mudskipper (*P.papilio*), mullets (*L.falcipinis*), Oyster (*C.gasar*) and water from Ekerekakana and Buguma creeks, in Niger delta, Nigeria.

Materials and Methods Study Area

The study was carried out in Ekerekana and Buguma creeks, Niger delta, Nigeria. Ekerekana creek is located in Okrika local Government Area or Rivers State, Nigeria and lies between longitude 70° and 60° E and latitude 40° and 50° N. While Buguma creek is located in Asari Toru local Government area of the state. It is situated between longitude 6° 47 E and latitude 40° 59 N (Figure 1).

Sampling period

The sampling was carried out bimonthly between July 2012 and February 2013; consisting of four wet season months (July-October) and four dry season months (November February).

Collection of samples

The different species used in the study were sampled from the creeks based on their life cycle, feeding and behavioural pattern. These species were chosen based on their availability all year round. Ten samples of each specimen were collected in each of the sampling months. The periwinkles (T. fuscatus) were handpicked from the sediment in the creeks at low tide. The oysters (C. gasar) were collected with the aid of knife from the roots of the mangroves during low tide. Specially designed traps were used in the collection of mudskipper (P.papilio), while mullets Liza falcipinnis were collected from the creeks using a seine net. They were kept in a sterile isothermal container and transported to the laboratory for microbiological analysis. At the laboratory, these samples were extensively washed and rinsed with normal saline solution to remove dirt, debris and surface contaminants. The edible parts of oysters were removed from the shell with a sterilized sharp knife, while that of periwinkle was removed with the aid of a specially fabricated sterile needle. The fleshy part of mullets and mudskippers were cut with sterilized knife. These samples were transferred separately one at a time to a sterile blender for homogenization and serial dilution.

Water samples were collected monthly with sterile plastic containers from both creeks, in all sampling months. The containers were rinsed three times with the water samples to be collected at the site before the collection was made. The water and the sea foods were later transferred to the laboratory for microbiological analysis.

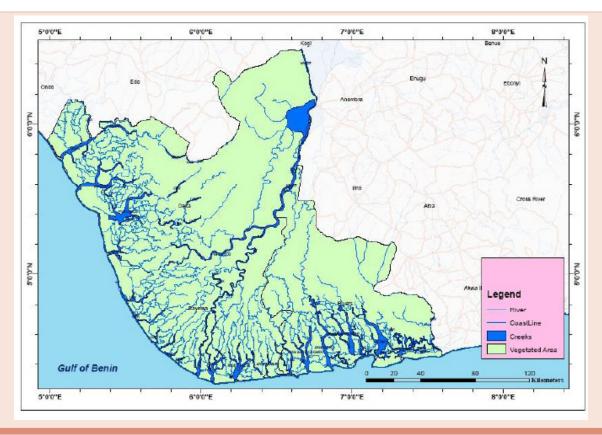


Figure 1: Map of Niger Delta, Nigeria.



Microbiological analysis

Total hetorotrophic bacteria count; Total vibrio count; Total Salmonella/ Shegella count and Total Coliform count were analysed in water and seafood samples namely periwinkle, oyster, mudskipper and mullets

Samples of the seafood (the fleshy edible part) were hygienically transferred to a sterile stomacher (Model BA 6021, Seward Medical, UK) according to the method described by Aluyi et al. [14]. This was later homogenized, using 225ml sterile 0.1% peptone water for a period of two minutes. Serially, tenfold dilution up to 10⁵ of the homogenates were made by transferring 1ml of fresh sterile dilutors to plates of surface-dried Nutrient Agar for total bacteria count and Salmonella-Shigellae Agar for salmonella count. All plates were incubated at 37°C for 48 hours. In analysis of total coliform, the 5-tube MPN (Most Probable Numbers) methods described by Gerhardt et al. [15], was employed. The tubes for total coliforms were incubated at 37°C for 24 hours. Evaluation of bacterial number was done by plant count following the methods described by APHA [16].

Enumeration of vibrio spp. was done by using the medium Thiosulphate citrate bile salt agar (TCBS). Aliquot (0.1ml) each of water sample and each of the seafood was pipette aseptically into TCBS plates in triplicates and spread with a sterile glass rod. The vibrio spp. was later enumerated using standard microbiological techniques [17].

Data analysis

The data generated from the study was collated and analyzed in each of the seafood and water samples from both creeks and were presented in tabular form and student *t*-test was used to show differences between season and sample sites.

Results

The results of population densities of colony forming unit's permillimeter of Total Heterotrophic bacteria (THB) count that are found in water and seafood samples in the wet and the dry season months of study are presented in Table 1. The result indicated that the values of THB in Ekerekana creek were significantly (p<0.001) higher than that of the Buguma creek in both wet and dry season months. The overall average values of THB (5.5x10⁵ cfu/ml) in wet season were significantly (p<0.005) higher than the value of 3.5x10⁵ cfu/ml obtained in the dry season months. The average values of total vibrio count (TVC) in the sampled water and seafood from Ekerekana creek was significantly (p<0.005) higher than that of Buguma creek in wet and dry season respectively (Table 2). The overall average values (3.46x10⁵ cfu/ml) in wet season were significantly higher (p<0.0001) than the average values (1.95x105 cfu/ml) obtained during the dry season. The seasonal variation in the population of Total Shigella counts (TSC) in water and sea foods from Ekerekana and Buguma creeks is presented in Table 3. During the wet season, the average value (4.58x105 cfu/ml) of TSC in Ekerekena creek was significantly (p<0.05) higher than the average value (2.65x10⁵⁾ obtained in Buguma creek. Conversely, the same trend was equally observed during the dry season, with the average value of TSC (3.51x10⁵ cfu/ml) in Ekerekana creek which was significantly (p<0.005) higher than the value of 2.78x10⁵ cfu/ml recorded in Buguma creek. The overall average value of TSC in the wet season was significantly (p<0.05) higher than the dry season months. The values of Total coliform in Ekerekana creek were significantly (p<0.005) higher than that of the Buguma creek

Months	Sample	Wet Season			Dry Season	
		Ekerekana	Buguma	Months	Ekerekana	Bugum
July	Water	6.3 x 105	3.0 x 105		4.1 x 105	2.1 x 10
	Mudskipper	8.1 x 105	5.2 x 105		5.1 x 105	3.2 x 10
	Periwinkle	7.6 x 105	4.8 x 105	December	7.2 x 105	3.6 x 10
	Mullet	6.7 x 105	6.2 x 105		2.4 x 105	2.1 x 10
	Oyster	6.2 x 105	6.2 x 105		3.2 x 105	2.2 x 10
	Water	6.0 x 105	4.0 x 105		5.6 x 105	3.6 x 10
	Mudskipper	7.4 x 105	5.1 x 105		4.2 x 105	2.8 x 10
August	Periwinkle	7.8 x 105	4.2 x 105	January	5.3 x 105	2.3 x 10
	Mullet	5.8 x 105	5.2 x 105		2.6 x 105	2.4 x 10
	Oyster	5.2 x 105	4.7 x 105		3.4 x 105	2.9 x 10
	Water	5.8 x 105	4.1 x 105		4.3 x 105	3.2 x 10
	Mudskipper	7.0 x 105	3.2 x 105		5.2 x 105	3.1 x 10
September	Periwinkle	6.2 x 106	3.0 x 105	February	5.7 x 105	3.1 x 10
	Mullet	5.9 x 105	3.2 x 105		2.9 x 105	2.2 x 10
	Oyster	6.4 x 105	3.1 x 105		3.8 x 105	2.7 x 10
	Water	7.1 x 105	5.1 x 105		3.9 x 105	1.9 x 10
	Mudskipper	7.6 x 105	4.6 x 105		5.8 x 105	3.1 x 10
October	Periwinkle	6.8 x 105	3.6 x 105	November	6.9 x 105	2.7 x 10
	Mullet	7.2 x 105	3.4 x 105		2.1 x 105	2.6 x 10
	Oyster	6.9 x 105	3.8 x 105		3.9 x 105	2.9 x 10
Avei	age	6.7x105***	4.3x105**		4.3x105***	2.7x105*
Overall	Overall average		5.5x105***		3.5x105***	

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Table 2: Total vibrio count (cfu/ml) in water and sea food samples from Ekerekana and Buguma Creeks at different season months.

Months	Sample	Wet Season		Months	Dry Season	
		Ekerekana	Buguma	WOITINS	Ekerekana	Buguma
July	Water	3.0 x 105	1.8 x 105		1.9 x 105	1.0 x 105
	Mudskipper	4.0 x 105	2.4 x 105		1.1 x 105	1.2 x 105
	Periwinkle	5.4 x 105	3.1 x 105	December	2.5 x 105	1.4 x 105
	Mullet	4.2 x 105	3.9 x 105		2.4 x 105	1. 2x 105
	Oyster	3.7 x 105 2. 9 x 105 2.8 x 10 3.2 x 105 1.6 x 105 2.4 x 10 4.8 x 105 2.1 x 105 1.7 x 10 3.6 x 105 1.6 x 105 January 2.5 x 10 5.2 x 105 4.7 x 105 3.9 x 10 3.9 x 105 3.3 x 105 4.2 x 10 3.0 x 105 1.4 x 105 2.0 x 10	2.8 x 105	1. 2 x 105		
	Water	3.2 x 105	1.6 x 105		2.4 x 105	1.1 x 105
	Mudskipper	4.8 x 105	2.1 x 105		1.7 x 105	1.2 x 105
August	Periwinkle	3.6 x 105	1.6 x 105	January	2.5 x 105	1.1 x 105
-	Mullet	5.2 x 105	4.7 x 105		3.9 x 105	2.1 x 105
	Oyster	3.9 x 105	3.3 x 105		4.2 x 105	2.8 x 105
	Water	3.0 x 105	1.4 x 105		2.0 x 105	1.1 x 105
	Mudskipper	4.2 x 105	1.6 x 105		1.8 x 105	1.4 x 105
September	Periwinkle	3.9 x 105	1.9 x 105	February	3.2 x 105	1.1 x 105
	Mullet	3.6 x 105	3.4 x 105		2.6 x 105	2.2 x 105
	Oyster	3.4 x 105	3.4 x 105		2.5 x 105 2.4 x 105 2.8 x 105 2.4 x 105 1.7 x 105 2.5 x 105 3.9 x 105 4.2 x 105 2.0 x 105 1.8 x 105 3.2 x 105	2.1 x 105
October	Water	3.8 x 105	7.8 x 105		1.8 x 105	1.4 x 105
	Mudskipper	4.9 x 105	2.1 x 105		1.9 x 105	1.4 x 105
	Periwinkle	4.1 x 105	2.7 x 105	November	2.3 x 105	1.2 x 105
	Mullet	4.1 x 105	3.4 x 105		2.0 x 105	1.9 x 105
	Oyster	4.2 x 105	3.6 x 105		2.2 x 105	2.0 x 105
Av	erage	4.01x105***	2.91x 105**		2.39 x105**	1.51 x105
Overall average		3.46 x 105***			1.95x105***	

*significant at p<0.05; **significant at p<0.005; ***significant at p<0.001.

Table 3: Total Salmonella/Shigella count (cfu/ml) in water and sea food samples from Ekerekana and Buguma Creeks at different season months.

Months	Sample	Wet Season		Months	Dry Season	
		Ekerekana	Buguma	Wionths	Ekerekana	Buguma
July	Water	9.0 x 105	4.0 x 105		6.7 x 105	3.6 x 105
	Mudskipper	2.0 x 105	1.1 x 105		1.2 x 105	1.9 x 105
	Periwinkle	2.4 x 105	1.4 x 105	December	4.7 x 105	3.2 x 105
	Mullet	7.2 x 105	4.0 x 105		3.6 x 105	3.0 x 105
	Oyster	5. 6 x 105	2. 9 x 105		4. 2 x 105	3. 1 x 10
	Water	8.2 x 105	3.2 x 105		1.8 x 105	1.9 x 105
	Mudskipper	2.0 x 105	1.0 x 105	January	1.6 x 105	2.8 x 105
August	Periwinkle	2.2 x 105	1.1 x 105		2.1 x 105	3.4 x 105
	Mullet	6.4 x 105	3.2 x 105		3.6 x 105	2.9 x 105
	Oyster	3.5 x 105	4.0 x 105		3.2 x 105	2.7 x 105
	Water	8.5 x 105	4.5 x 105		4.7 x 105	1.9 x 105
	Mudskipper	2.0 x 105	1.0 x 105		2.3 x 105	3.4 x 105
September	Periwinkle	2.4 x 105	1.2 x 105	February	2.4 x 105	3.4 x 105
	Mullet	2.4 x 105	2.1 x 105		3.9 x 105	2.4 x 105
	Oyster	3.9 x 105	3.2 x 105		2.4 x 105 3.9 x 105 3.4 x 105	2.7 x 105
	Water	9.1 x 105	6.2 x 105		6.8 x 105	4.8 x 105
	Mudskipper	3.4 x 105	1.4 x 105		1.2 x 105	1.1 x 105
October	Periwinkle	3.1 x 105	1.6 x 105	November	4.8 x 105	2.4 x 105
	Mullet	4.6 x 105	3.4 x 105		4.1 x 105	2.4 x 105
	Oyster	3.7 x 105	2.6 x 105		3.8 x 105	2.6 x 105
Averag	ge	4.58x 105**	2.65 x 105*		3.51x105**	2.78x105
Overall average		3.62x105*			3.15x105*	

in both wet and dry season months. The overall average of the total coliform count between dry and wet seasons were almost within the same range with no significant difference (p>0.05) between the wet and dry season months (Table 4).

Discussion

In the two creeks studied in this work, the seafood analyzed contains more bacteria species in the wet season months (July – October) than the dry season months (November – February). This

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Table 4: Total Coliform count (cfu/ml) in water and sea food samples from Ekerekana and Buguma Creeks at different season months.

Months	Sample	Wet Season		Months	Dry Season	
		Ekerekana	Buguma		Ekerekana	Buguma
July	Water	1.4 x 105	1.0 x 105	December	2.47x 105	1.7 x 105
	Mudskipper	2.4 x 105	1.4 x 105		1.1 x 105	1.0 x 105
	Periwinkle	3.6 x 105	1.6 x 105		2.8 x 105	1.6 x 105
	Mullet	2.4 x 105	2.1 x 105		2.8 x 105	2.1 x 105
	Oyster	2. 4 x 105	2. 6 x 105		2. 6 x 105	2. 4 x 105
	Water	1.4 x 105	1.0 x 105	January	2.4 x 105	1.4 x 105
	Mudskipper	2.6 x 105	1.6 x 105		1.7 x 105	1.5 x 105
August	Periwinkle	3.8 x 105	1.8 x 105		1.8 x 105	3.8 x 105
	Mullet	2.4 x 105	2.0 x 105		2.7 x 105	2.2 x 105
	Oyster	2.6 x 105	2.5 x 105		2.8 x 105	2.4 x 105
September	Water Mudskipper Periwinkle Mullet	1.4 x 105 2.3 x 105 3.6 x 105 2.1 x 105	1.4 x 105 1.2 x 105 1.6 x 105 1.9 x 105 1.6 x 105	February	1.40x 105 3.6 x 105 1.7 x 105 2.9 x 105	1.4 x 105 1.2 x 105 1.2 x 105 2.7 x 105 2.9 x 105
	Oyster	1.9 x 105	1.0 X 100		2.4 x 105 1.7 x 105 1.8 x 105 2.7 x 105 2.8 x 105 1.40x 105 3.6 x 105 1.7 x 105	2.0 X 100
	Water	2.6 x 105	1.1 x 105			1.2 x 105
	Mudskipper	3.8 x 105	1.6 x 105	November		1.2 x 105
October	Periwinkle	4.2 x 105	1.8 x 105			1.2 x 105
	Mullet	1.9 x 105	1.5 x 105			2.4 x 105
	Oyster	1.6 x 105	1.3 x 105		2.8 x 105	2.4 x 105
Ave	erage	2.52x105**	1.63x105**		2.45x105**	1.89x105**
Overall	average	2.0	8x105ns		2.17	7x105ns

*significant at p<0.05; **significant at p<0.005; **significant at p<0.001; ns not significant.

may be due to increase in concentration of bacterial flora in the surrounding water, as fish and shell fishes have a tremendous ability to concentrate bacteria from their immediate surrounding waters [18]. In the present study, Vibrio spp isolated in the sea foods is more predominant in Ekerekana creek compared to Buguma creek during the study months. This is similar to the results of Yang et al. [19], when comparing two coastal areas of Eastern China. This result is also comparable with that of Zimmerman et al. [20], in the Gulf of Mexico waters. Vibiro species were also isolated from Buguma, but at lower concentrations, this supports the findings of Omenwa et al. [21], in the same creek. This may be due to human activities, such as bathing, defecation by the local populace, coupled with waste from cottage industries, which are common phenomenon in the area. Conversely, Winfield and Groisman [22], reported that increased level of human activities could bring about the elevation of organic matter resulting in high microbial load in the water, therefore high microbial population in an aquatic environment, is an indication of the input of microorganisms from domestic and industrial sources consequent of human activities.

Salmonella spp is one of the most important food-borne pathogens and are indicators of sewage contamination and it is found to be associated with a number of non-human hosts such as reptiles [23]. This bacteria species has been reported to survive and persist in the aquatic environment and has been detected in seafood in different creeks of Niger Delta [24] and causes new born meningitis and infantile diarrhoea [24]. In addition, Staphylococcus aureus was isolated in seafood sampled from Ekerekana creek, this is in agreement with that reported by Okonko et al. [25], while comparing the microbial quality of sea foods from two different areas.

The seasonal changes observed in the various microbial groups in both creeks could be as a result of the influence of the physicochemical properties and human activities. Earlier findings have shown slightly elevated levels of faecal Coliforms in creeks during the wet season than dry season [26], these observations were attributed to the effects of low salinity, high turbidity and low temperatures which are typical of wet season months [27].

The total heterotrophic bacteria (THB), Total Vibrio counts, (TVB) and Total Salmonella and Shigella count (TSC) of the creeks revealed higher counts in the wet months (July to October) than the dry season months (November to December). This observation may be due to more favourable physical and chemical conditions such as No.7, S042, pH and temperature occasioned by allochthonus materials from the sampling area of the creek during the season [28]. The increase in the bacterial population is attributed to the stimulatory effect of additional carbon and energy sources in the form of effluents, and crude oil, which leads to an enrichments of the oil degrading microbial population, this is made possible by hydrocarbon utilizing bacteria [29]. Also, Eze [30], observed a similar trend in inland water in Port Harcourt. More hydrocarbon utilizing bacteria (HUB) counts were recorded in the wet season than the dry season. This result is in tandem with the higher concentrations of THC recorded in these creeks, during the wet season, confirming that these organisms require hydrocarbons as their sole source of energy [31]. It should be noted that microorganisms, especially bacteria play a major role in hydrocarbon degradation in aquatic environments. The presence of these hydrocarbon degraders in the river might result in commensality or complimentary degradations in the aquatic ecosystem [32]. Also, Njoku et al. [34], noted that apart



from the natural input of carbon into aquatic habitats, hydrocarbon compounds can enter the ecosystem through human activities, such as transportation and oil explorative activities.

However, the total coliform count did not change as such between both seasons and in both creeks. This could be attributed to the toxic effects of hydrocarbons on organisms which at certain concentrations could also be either inhibitory on stimulatory in different conditions [33,34]. Although some hydrocarbon organism are known to contain plasmids with the relevant genes for the degradation of different hydrocarbons [34].

Moreover, there are occasional rainfalls in the coast of Niger delta in the dry season months, whose run-off carries some load of debris, faecal and organic matter into the creeks resulting in higher values of total coliform count bacteria as recorded in this study. Earlier studies by different authors suggests that rainfall pattern in a particular zone may influence the bacteria invertebrate interactions in the aquatic environment, possibly by changing the concentration of bacteria organisms in surface waters, feeding habits of the aquatic organisms and depuration dynamics [35-37].

Conclusion

This study has shown that the total heterotrophic bacteria count total Vibro count; and total coliform count were more predominant in the wet season compared to dry season months, total coliform count bacteria exhibited some measure of elevation in the dry season months an indication that they can be prevalent in both seasons depending on the type of human activities that is prevalent in the area. Also, these isolated organisms were more in population in Ekerekana creek compared to Buguma creek. This may have resulted from industrial and domestic wastes discharged into Ekerekana and Buguma creeks which resulted in the presence of high concentrations of pollutants in the water body. The complex combination of toxic substances that is simultaneously present in the environment may act synergistically and therefore impose a higher toxicity burden on the ecosystem by stimulating and promoting growth of microorganisms in the water column and sea foods such as periwinkle, oyster, mullet and mudskippers which may pose a health hazard to humans as a final consumer of these organisms. Also, continued discharge of improperly treated effluent may further compound the worsening environmental problem of the creek and cause ecological imbalance of the Niger Delta basin. There is therefore the need to formulate appropriate policies and regulations for safeguarding the ecosystem from adding undesirable microbial population.

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