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PHYSICO-CHEMICAL PARAMETERS AND MICROBIAL LOAD IN WATER, SEDIMENT AND ORGANISMS (*Nematopalaemon hastatus* and *Farfantepenaeus notialis*) IN COASTAL WATERS OF ONDO STATE

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Abstract
The impairment of coastal waters as a result of microbial pollution have had a tremendous impact on living organisms and human health, and is a major challenge confronting the water industry and regulatory authorities.Thus, the current study was carried out between May-July 2014, to assess the physico-chemical parameters and microbial load in water, sediments and tissue of Nematopalaemon hastatus and Farfantepenaeus notialisfrom two coastal water bodies of Ondo State (Ayetoro and Bijimi). The microbial load parameters that were analyzed are Total Heterotrophic Bacteria Count (THBC), Total Coliform Count (TCC), Total Shigella and Salmonella Count (TSSC), Total Vibrio Count (TVC), Total Fungal Count (TFC), and Escherichia Coli Count (ECC). The results of the analyses of the water samples showed that temperature had the highest value of 31.00±0.00°C in Bijimi, with the lowest value of 27.67±0.58 °C in Ayetoro. Turbidity range from 0.01±0.01 to 0.04±0.01 NTU,while conductivity had the least mean value of 1.77±0.06 µS/cm in Ayetoro and the highest mean value of 9.30±0.30µS/cm in Bijimi. pH range between 4.77±0.06 to 6.04±0.01. The result revealed that all the physico-chemical parameters of water determined except turbidity and conductivity showed no significant difference across the stations at P<0.05. The total count of heterotrophic bacteria was found to be highest in water, sediment and organismsat 128–650, 66-210 and 110-216 respectively, whileV. cholerae was not detected in water and organisms, but detected in sediments. The level of natural, human and anthropogenic microbial contamination into the coastal waters of Ondo state amongst the two stations follows the order sediment>water>organisms. This present study therefore reveals the risk associated with the health status of the Coastal water of Ondo State which may further deteriorates and become detrimental to the coastal community.

Keywords: *Nematopalaemon hastatus,Farfantepenaeus notialis. Heterotrophic Bacteria, Shigella and Salmonella, Escherichia Coli.*

Introduction
The occurrence and distribution pattern of microorganisms in the marine and brackish ecosystem plays an important role in the decomposition of organic matter and mineralization(Swarnakumar, 2008). These biological contaminants constitute the major cause of food and water borne diseases with varying degrees of severity, ranging from mild indisposition to chronic or life-threatening illness (Phyllis, 2007).

Potential sources of invasion into the marine environment includes industrial effluents, agricultural wastes, faecal materials from human and animal,seagulls, geese and other warm-blooded animals, river runoff, kitchen wastes, land runoff, untreated sewage wastes, accidental spillage, leakages of oil pipelineand nearby boating activities that re-suspend bottom sediment with bound *E. coli* (An *et al.,* 2002). In addition, environmental factors such as rainfall and onshore winds can also cause a dramatic increase in bacterial concentration and can exacerbate the contamination problem (Crowther *et al.,* 2001).Sediments also have the ability to impact the quality of water and can contain 100 to 1,000 times as many fecal indicator bacteria than the overlying water (Van Donsel and Geldreich, 1971), thus serving as reservoirs for fecal pollution (Crabill*et al.,* 1999). Recreational activity and wave action also have the ability to release fecal bacteria bound to sediment and contribute to poor water quality (Ishii *et al.,* 2007).All these microbial pathogens greatly affect the biological productivities, sustainability of living resources,and public healthof the ecosystem, thus causing economic loss. They are also pathogenic to humans and some have fatal infections.

Petroleum hydrocarbons, crude oil and other related organic pollutants have their negative consequence on the plants through the alteration of the physicochemical properties of the water body. The

aquatic habitats are killed by the toxic chemicals with the resultant disruption of the aquatic ecosystem and its food chain. The decomposition of the organic materials by micro-organisms in the aquatic ecosystem utilize the oxygen dissolved in water and reduce the level of dissolved oxygen of water to less than >2ppm, which in turn adversely affects the respiratory conditions, inhibits growth, and cause the death of the aquatic habitats, thus creating a significant reduction in the food chain of the aquatic ecosystem (Onuegbu, 2008).

Hence, the spatial and temporal distributions of the total and faecal coliforms as well as the pathogenic bacteria in water, sediments, and organisms is essential to assess the sanitary. Pathogens in coastal water bodies includes: bacteria, viruses and protozoans which cause diseases that vary from mild gastroenteritis to severe anthrax, botulism, and polio. The bacteria pathogens are enteropathogenic strains of *Escherichia coli, Salmonella typhi, Shigellaspp* etc.

The Ilaje coastal waters of Ondo State is one of the unexploited areas for its biodiversity and microbial studies. The increasing human settlements, urbanization, oil and gas exploration and exploitation, human and industrial wastes, and anthropogenic activities has led to the pollution of these environments, thereby warranting the need for the coastal water quality monitoring of the area. The present study is on the physicochemical properties and microbial analysis of Bijimi and Ayetoro waters, sediments and organisms of the Ilaje Local Government in order to know the relationship of microbes on the sediment and organisms in the water body with a view to contribute to the protection and conservation of the ecosystem.

Materials and Methods

The study areas (Ayetoro and Bijimi) fall within Latitudes 6.00° and 6° 30^{1} north and Longitudes 4° 45^{1} and 5° 45^{1} East of the Greenwich Meridian. They share boundaries with Okitipupa Local Government Area in the North; the Atlantic Ocean in the South; Ijebu Waterside Local Government Area (Ogun State) in the West and Delta state in the East. It consists of over five hundred settlement spreading over 3,000km^{2} and has over 180km long shoreline

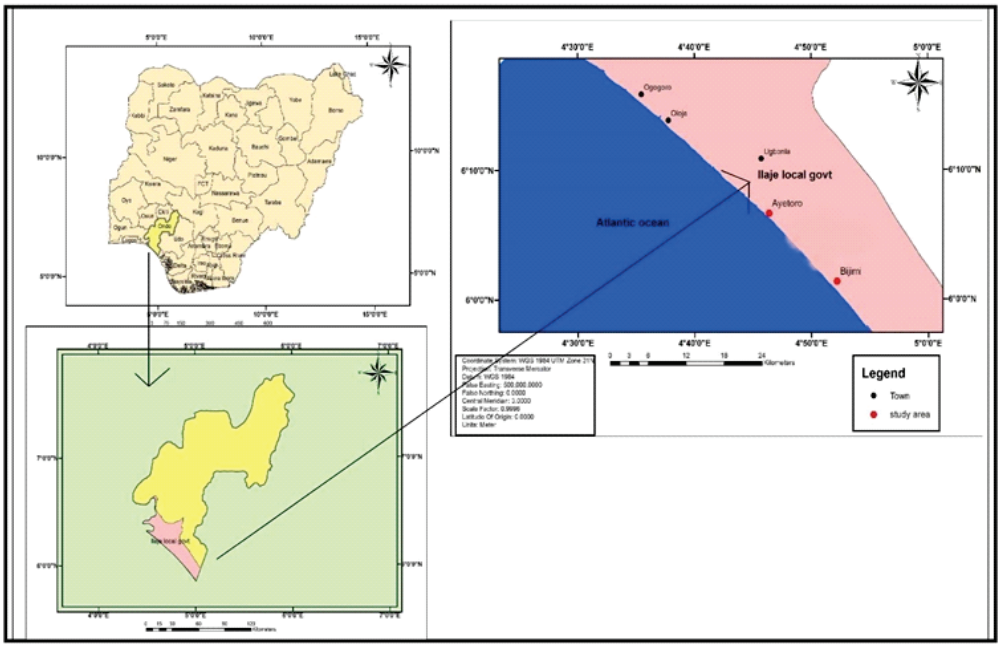

Figure 1: Map showing the study area.

Collection of Samples

The study was carried out in Ayetoro and Bijimi within the coastal area of Ondo State from May, 2014 to July 2014. Samples (water, sediments and organisms) were collected on monthly schedule between 10:00am to 11:00am. Water samples were collected in triplicate from each of the study area at sub-surface level 15-20cm, using 250 ml sampling bottles, while the sediments samples were collected manually in each of the study area in plastic bowls from three different points 50m apart with depth of 0.2m by using bottom grab. Shrimps were collected from the artisanal fishermen who employed Mallian traps for shrimp's capture, and all samples were transported in ice chest to the Laboratory for analysis. The Shrimps were sorted into different groups and identified to species level usingFAO identification guide (FAO 1981).

Determination of Physico-chemical Parameters of Water

Water temperature was measured in degree Celsius (^{o}C) in-situ using mercury in glass thermometer, while Turbidity, Conductivity, pH, DO, BOD, Salinity and COD were measured in the laboratory using Hanna Multi parameter Meter model HI 9828.

Determination of Total Microbial load

Preparation and sterilization of Culture media: Nutrient agar (NA) and potato dextrose agar (PDA) are the culture media of choice used for this investigation. The media were prepared by dissolving 28g of NA in a litre of dissolved water.39g of PDA was equally dispersed in a litre of distilled water separately. It was allowed to soak for few minutes, mixed thoroughly, cotton plugged, covered with aluminum foil and then sterilized in an autoclave at 121^{o}C for 15 minutes and allowed to cool about 45^{o}C before.

Sample Preparation/Isolation of Samples: About 1g of each sample was aseptically transferred separately into the sterile McCartney bottles containing 9ml of sterile distill water, as they were thoroughly mixed with sterile glass rod to form a homogenous suspension. 1ml aliquot of the sample suspension was pipetted into sterile test tubes, with each suspension serially diluted in another six sets of test tubes each to dilution ratio 10^{-6}. 0.1mlof the sample suspension from the 5th and 6th dilution was aseptically pipetted separately into different sterile petri dishes and thoroughly mixed with 20ml of cool (45^{o}C) molten agar media (NA and PDA media separately).The plates were gently swirled for even distribution with the media allowed set and incubated at optimum temperature (32 ^{o}C. At the end of the incubation period, the colonies forming from viable bacteria cells were counted and recorded appropriately per g/ml of each of the samples. Subculture was subsequently carried out to obtain pure culture of each isolates for further studies. Pure culture of individual samples was kept at 4^{o}C for biochemical test.

Identification of Bacteria Isolates by Cultural and Biochemical Test

Colonial characteristics of the isolated organisms were examined on solid agar surface after 18-24 hours of incubation. These characteristics includes; color, shape, opacity, translucency, elevation, edges and surface texture. Cultural characteristics of the bacterial isolates were recorded while the following biochemical tests (gram's reaction, catalase test, motility test and oxygen-relation, spore staining, indole production, coagulase test, and starch hydrolysis) were carried out for identification according to Bergey's manual determination bacteriology (Cowan and Steel, 1977).

Multiple Tube Method for Faecal Coliforms

72g and 36g of Marconkey lactose broth was dispersed in a litre of sterile distilled water to make a double and single strength respectively, with 10ml of this broth dispersed in 15 different set of screw capped bottle (both double and single strength). Each of the bottles contained an inverted Durham's tubes to collect gas (if produced), and were autoclave at 121^{o}C for 15 minutes and allowed to cool at room temperature. 5 tubes of both single and double strength lactose broth with 0.1ml and 10ml of the sample respectively were inoculated. This was repeated with 1ml sample for the single strength. The 15 tubes were inoculated at 37^{o}C for 24-48 hours for acid and gas production respectively. Acid produced was indicated by color change from purple-blue to colorless, while the gas produced was collected in the Durham's tubes. A control experiment was set up with non-inoculated bottles containing equal volumes of the medium.

Results

Physicochemical Parameters of Water

The physicochemical parameters of the coastal waters of Ondo State varied slightly in the two stations as shown in figure 2.During the study period, temperature varied from 27.31^{o}C to 30.47 ^{o}C, with the minimum and maximum value recorded in Ayetoro both in July and June respectively. Turbidity range between 0.01-0.04 NTU in the two stations.The electrical conductivity recorded in this study ranged

between 1.77 and 9.3 (µhom's/cm), with the highest value recorded in Bijimi, and lowest in Ayetoro. The water pH was slightly acidic and ranged between 4.77-6.04. The quantity of mean dissolved oxygen ranged from 7.33-9.52 mg/L. Maximum value of DO was observed at Bijimi in the month of July while the minimum value was observed at Ayetoro in the month of May. BOD values were varied from 2.5mg/l to 4.22mg/l. Salinity values were ranged from 9.4ppt to 10.18ppt. Maximum salinity value was recorded in Bijimi in the month of July and minimum in Ayetoro in the month of May. COD range between 13.26mg/l to 13.76mg/l.

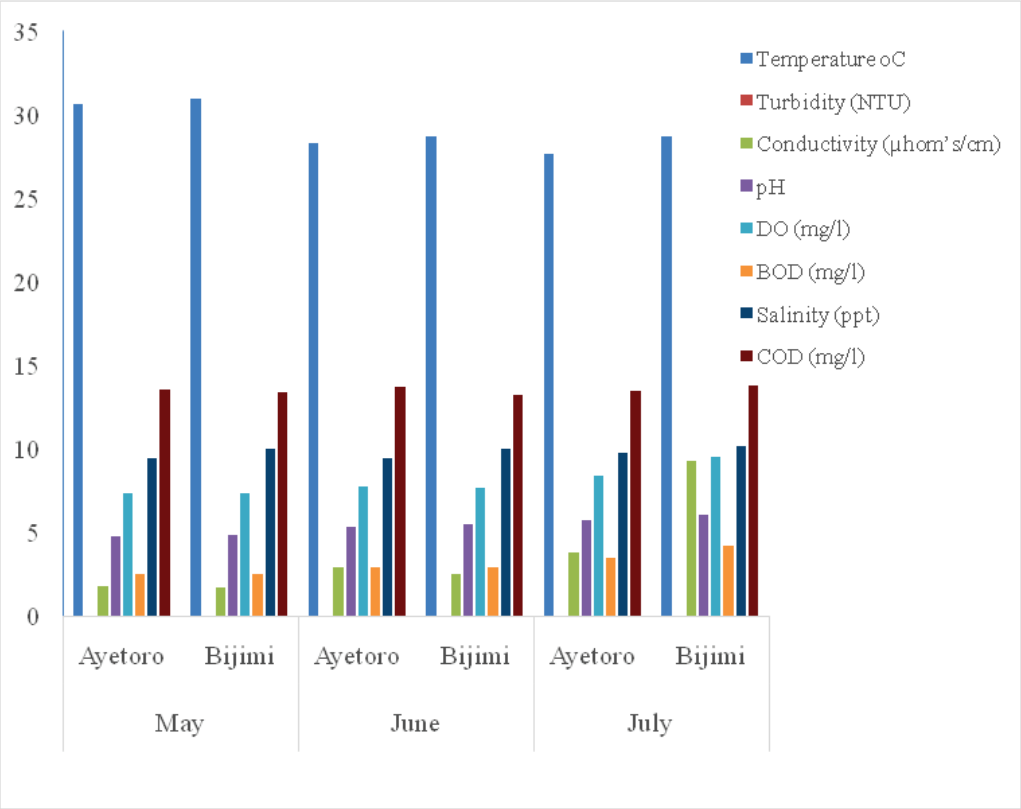

Figure 2: Physicochemical parameters of Ayetoro and Bijimi coastal waters

Microbial Load in Water

The microbial load on the two water bodies is shown in figure 3. The results obtained during the study period for THBC ranged between 128±1.00-323±3.00 in Bijimi, and 240±3.00-650±2.00 in Ayetoro. TCC ranged between 25±3.00-64±4.00 in Bijimi, and 27±1.00-30±2.00 in Ayetoro. The TSSC ranged obtained in Bijimi was 16±0.00-18±1.00, while in Ayetoro it ranges between 16±4.00-19±2.00. TVC was not detected in both waters, while TFC ranged from 5±0.00-7±0.00 in Bijimi, and 6±3.00-8±1.00 in Ayetoro. ECC range obtained in Bijimi was between 11±1.00-12±3.00, and 15±0.00-25±3.00 in Ayetoro.

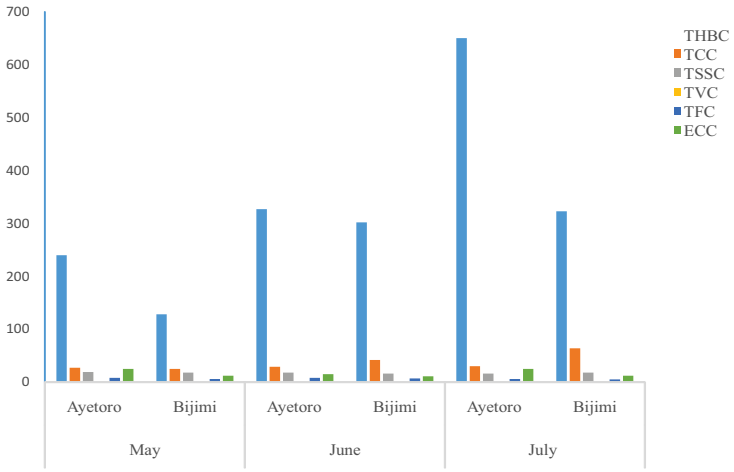

Figure 3: Microbial load on the water bodies

Microbial Load in Sediments

The microbial load in sediments of Ayetoro and Bijimi water bodies is shown in figure 4. The results obtained during the study period for THBC ranged between 66±1.00-101±2.00 and 108±1.00-210±3.00 for Bijimi and Ayetoro respectively. TCC ranged between 27±2.00-42±1.00 for Bijimi, and 24±1.00-36±1.00 for Ayetoro. TSSC ranged obtained in Bijimi was 19±2.00-21±1.00, while in Ayetoro it ranges between 18±0.00-21±2.00. TVC which was detected in both water, range between 10±0.00-16±3.00 and 12±0.00-16±1.00 in Bijimi and Ayetoro respectively.TFC for Bijimi ranged between15±0.00-60±1.00, while Ayetoro had a range of 15±3.00-55±3.00. ECC ranged obtained in Bijimi was between 14±0.00-28±0.00, and 14±2.00-28±1.00 in Ayetoro.

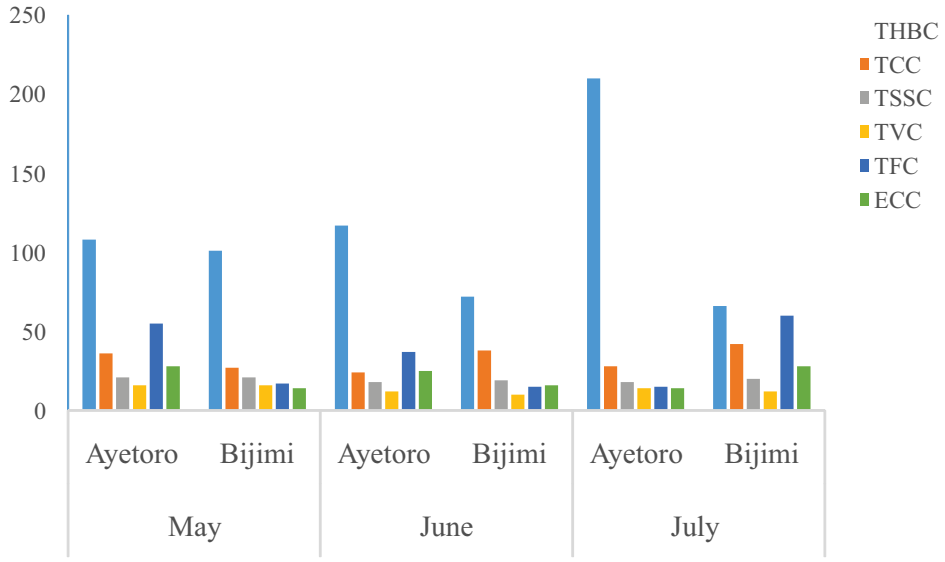

Figure 4: Microbial load in Ayetoro and Bijimi sediments

Microbial load in *F. notialis*and *N. hastatus*found in Bijimi and Ayetoro

Figures 5 and 6 shows the microbial load content on *F.notialis* and *N.hastatus* of the water bodies. The results obtained during the study period for THBC ranged from 110-216 for both species in Ayetoro and 103-114 in Bijimi. TCC ranged between 27-53 in Ayetoro, while that of Bijimi ranged between 23-42. TSSC in Ayetoro ranged between 27-33, while in Bijimi it ranges between 19-23. TVC was not detected in both species in any of the stations. Results obtained for TFC range between 9-57 in Ayetoro, and 52-62 in Bijimi, while the range of ECC in Ayetoro and Bijimi was 15-21, and 24-32 respectively.

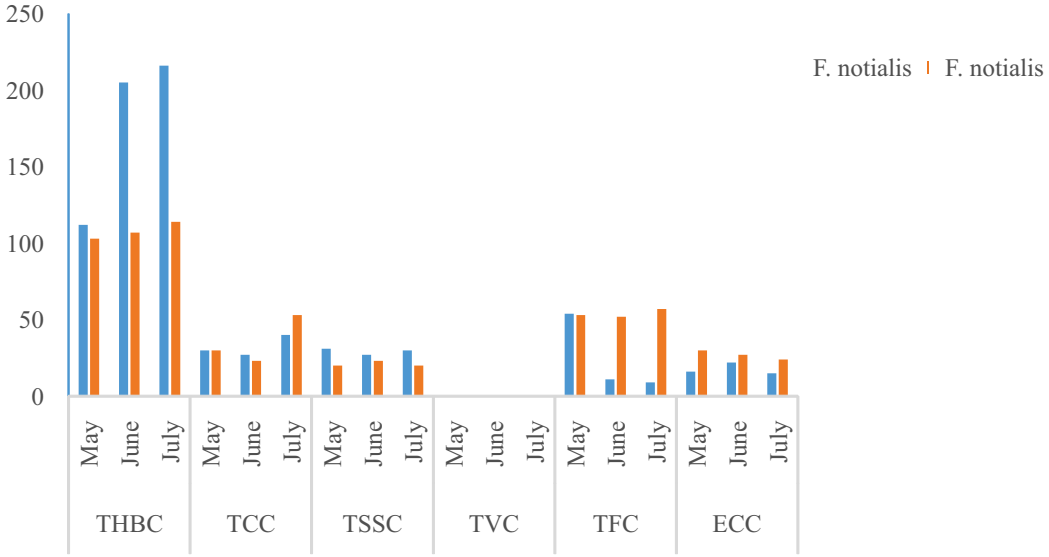

Figure 5: Microbial load in *F.notialis* found in Ayetoro and Bijimi waters

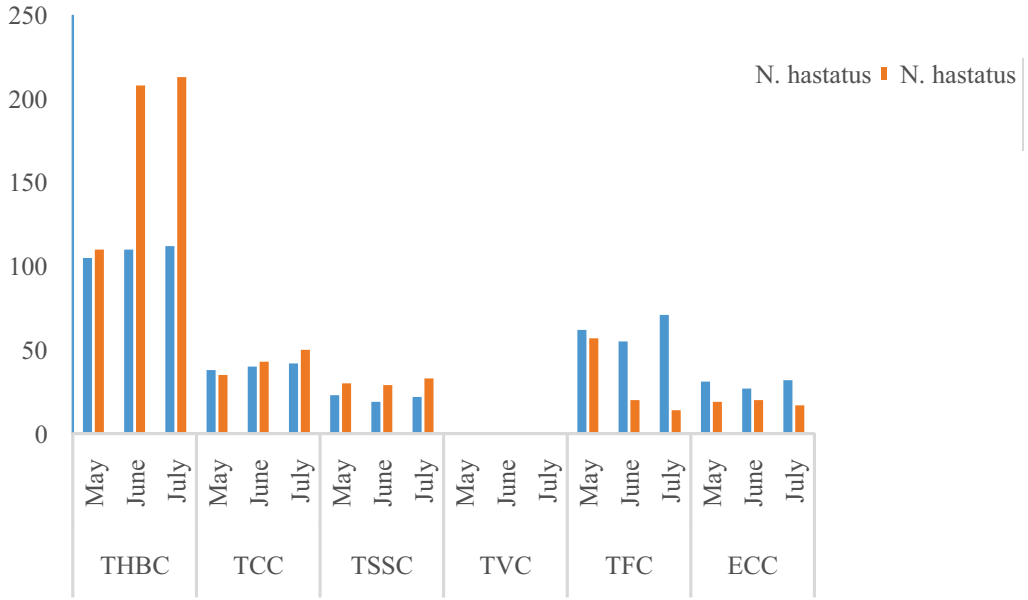

Figure 6: Microbial load in *N. hastatus* found in Ayetoro and Bijimi waters

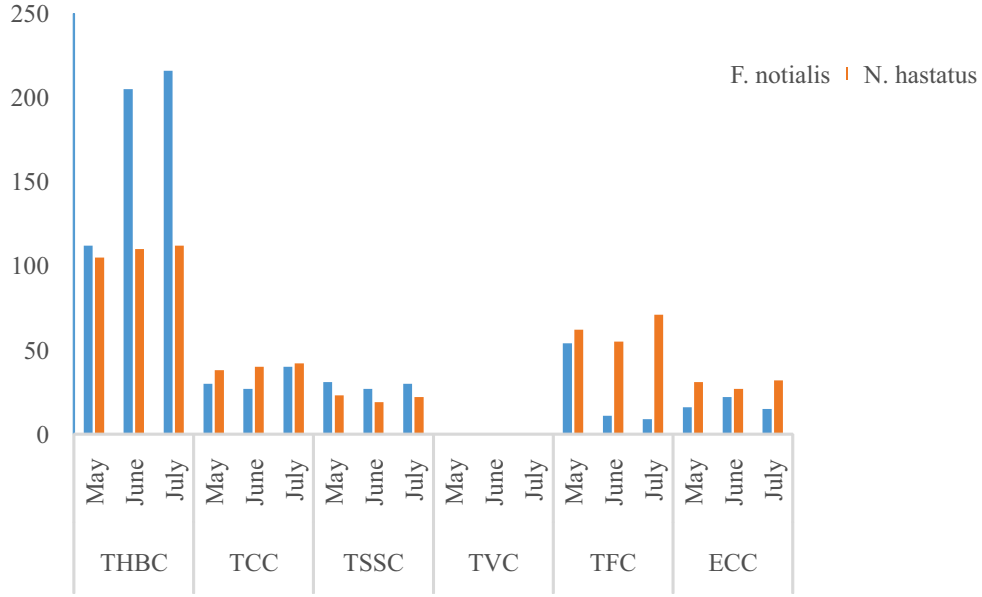

Figure 7: Microbial loads in *F.notialis* and *N. hastatus* found in Ayetoro coastal water

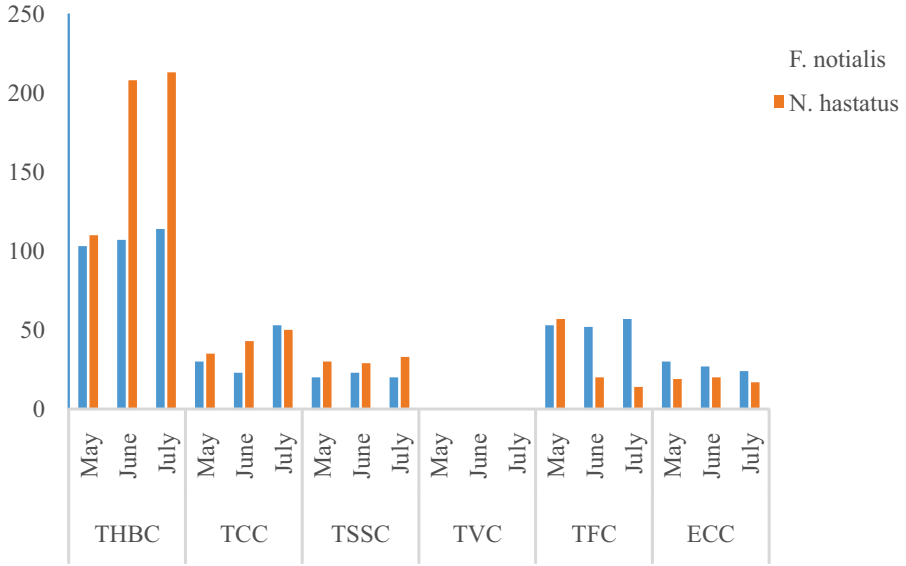

Figure 8: Microbial loads in *F.notialis* and *N. hastatus* found in Bijimi coastal water

Correlation Analysis of Water Physicochemical Parameters and Microbial Loads in water

The Pearson correlation analysis between water physicochemical parameters and heavy metals in the water is shown in table 1. The correlation values showed that salinity was positively correlated with TCC, while TVC showed no significance on the physicochemical properties.

Table 1: Correlation coefficients between microbial loads in water and physicochemical parameters in water collected in Ondo State Coastal waters from May-July 2014.

Temp °C 1	Turb (NTU)	Conductivity (µhom's/cm)	pH	DO (mg/l)	BOD (mg/l)	Salinity (ppt)	COD (mg/l)	THBC*w*	TCC*w*	TSSC *w*	TVC *w*	TFC *w*	ECC *w*
0.891*	1												
-0.245	-0.533	1											
-0.762	-0.902*	0.692	1										
-0.486	-0.764	0.943**	0.868*	1									
-0.604	-0.844*	0.900*	0.915*	0.987**	1								
0.478	0.447	0.021	-0.559	-0.239	-0.261	1							
-0.094	0.000	0.291	0.049	0.108	0.159	0.632	1						
0.267	-0.103	0.251	-0.041	0.218	0.192	0.455	0.006	1					
0.128	0.053	0.266	-0.271	0.090	0.081	0.859*	0.510	0.059	1				
0.097	0.040	-0.519	-0.118	-0.378	-0.324	-1.620	-0.255	-0.359	-0.061	1			
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1		
0.164	-0.136	-0.037	0.164	0.090	0.079	-0.274	-0.433	-0.121	-0.401	0.670**	NS	1	
0.258	-0.157	0.472	0.063	0.411	0.358	0.432	0.000	0.511*	-0.443	0.123	NS	0.255	1

Significant levels are indicated by * at $p < 0.05$, ** at $p<0.01$.
NS: Not Significant

Correlation Analysis of Microbial Load in Water and Sediments

The Pearson correlation analysis between microbial load in water and sediments is shown in Table 2. The results showed a strong positive correlation coefficient of THBC in sediments with THBC and ECC in water ($P < 0.01$), and TCC in sediments with TCC in water ($P < 0.01$). TVC in sediments was positively correlated with TSSC and ECC in water ($P < 0.05$). In contrast, THBC in sediment with TCC in water, TSSC in sediments and THBC in water, and TVC in sediment and TCC in water at ($P < 0.05$),were negatively correlated.

Table 2: Correlation coefficients between microbial load of sediments and water collected in Ondo State Coastal waters from May-July 2014

	THBC*s*	TCC*s*	TSSC*s*	TVC*s*	TFC*s*	ECC*s*	THBC*w*	TCC*w*	TSSC*w*	TVC*w*	TFC*w*	ECC*w*
THBC*s*												
TCC*s*	-0.570*											
TSSC*s*	-0.316	0.376										
TVC*s*	0.264	-0.273	0.23									
TFC*s*	-0.404	0.493*	0.263	0.082								
ECC*s*	-0.379	0.404	0.21	-0.066	0.961**							
THBC*w*	0.781**	-0.132	-0.498*	-0.167	-0.232	-0.196						
TCC*w*	-0.512*	0.748**	-0.018	-0.525*	0.453	0.377	0.059					
TSSC*w*	-0.225	-0.066	-0.023	0.507*	0.46	0.369	-0.359	-0.061				
TVC*w*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
TFC*w*	0	-0.314	-0.363	0.256	0.05	0.119	-0.121	-0.401	0.670**	NS		
ECC*w*	0.707**	-0.197	-0.178	0.479*	0.107	0.094	0.511*	-0.443	00.123	NS	0.255	

Significant levels are indicated by * at $p < 0.05$, ** at $p<0.01$.

Discussion

The physicochemical parameters of water obtained in this study were within the tolerable range when compared with FEPA (1991) limit. The pH value obtained was slightly acidic, and the acidic nature of the water is capable of steeming the pH of the respective water bodies thereby, destabilizing fundamental properties. Wang *et al.,*(2002) corroborate the fact that metabolic activities of aquatic organisms are dependent on the pH values, and changes in pH will influence microbial growth and behavior, and affect bacteria interaction with the surroundings.Hariharan *et al.,*(2010) reports that biochemical reactions of aquatic organisms are temperature dependent. Therefore, increase in temperature of the water body will promote chemical reactions in the water. Effects such as bad odour and taste will result due to non-solubility of gases such as oxygen.

The highest microbial load in water was found in Ayetoro and may be attributed to the increased human population and activities that exist there. Akpor and Muchie(2011) recently reported that water containing high organic matter require high microbial load to enhance degradation of solid wastes in such water. The occurrence of coliforms from sampled water in these locations could be an evidence of faecal contamination of the water as observed during the study period and this agreed with the report of Aluyi*et al.,*(2006) who attributed high faecal load to human activities within an area.

The high level of heterotrophic bacteria counts in water, sediments and organisms may be attributed to the fact that they are the major agent shaping the organic composition of the ocean. Its distribution, diversity and activities in the ecosystem are controlled by various hydro-biological factors and nutrient levels present in the aquatic environment, and largely depends on changes in water temperature, salinity and physicochemical parameters. It could also be attributed to the rain water flow which brings huge quantities of pathogens in marine waters as observed by (Mahalakshmi *et al.,* 2011).

The high level of organic matter present in sediment may also be a reason for promoting the survival of the entire pathogens especially Vibrios which was not detected in water and organisms. Although, the probability and existence of Vibrios in coastal waters is majorly from contaminants that exist near the surface of polluted areas, they are often more pronounced in sediments as their habitat decreased from the source of pollutants.

Sediment properties, such as texture, viscosity and permeability are factors that influence the efficiency of microbial dispersion, the survival and accumulation of bacteria. It is thought likely that many bacteria (up to 75%) in the water column are located on particulates. Gerba and McLeod, (1976)corroborate the fact that sediments when compared to seawater, contains a greater content of organic matter and aids in the longer survival of *E. coli*. All these could be related to the increased penetration depth of oxygen and enriched nutrient content of the sediment. Thus, it can be ascertained that sediments provides the more conducive ecological niche for the survival of pathogens and was similar to the report of (Agbabiaka and Oyeyiola, 2012).

The microbial load in *N.hastatus* and *F. notialis* of Ayetoro was observed to be higher than that of Bijimi, and this may be attributed to the increased human activities and settlements in the area. TVC was not detected in any of the species as it was not found in the water bodies, and is supported with the fact that the incidence of microorganism and other shell fish depends on the quality of water from which the animals are obtained. Though shell fish are benthic organisms, their microbial accumulation is influenced by several abiotic and biotic parameters such as food acquisition capability, stage of gonadal development, and size/weight etc. These natural variables may influence observed variations in bio-accumulated microbial load.

The organisms isolated from these shrimps; Salmonella, Ecsherichia and Shigella are very important bacteria that are of public health implications. Organisms like Escherichia coli have been associated with infantile diarrhea while Salmonella causes paratyphoid in humans (Nester 1995). Though shrimps are cheap source of proteins, it has the tendency of harboring pathogenic microorganisms especially those relevant to human health due to the poor sanitary condition of the water bodies where these animals are cultivated and this agrees with the findings of Adebayo-tayo*et al.,*(2006).

Conclusion

The coastal waters of Ondo state and adjacent water bodies are increasingly used for waste disposal which has led to the changes in the natural characteristics of the coastal ecosystem. Untreated sewage discharges, agricultural waste, industrial effluents, human and animal excreta etc, releases high load of pathogenic bacteria into the water bodies and aquatic organisms, thereby making potential threat to human health who

consume them.It can be concluded that Ayetoro is the most polluted site characterized by the high rate of anthropogenic activities and human settlement. This is alarming as its leads to quality deterioration of coastal resources which pose a human health hazard and subsequent economic loss. Necessary attention and proper treatment should be given to the area, with the establishment of sanitary requirements so as to reduce the pollution load and protect the coastal livelihood.

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