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Determination of Microbiological Load in Periwinkle (*Tympanotonos fuscatus*), Water and Sediment of Azubie/Woji Creek, Port Harcourt, Nigeria

Peremobowei Beldin Kpikpi
Department of Fisheries and Aquatic Environment, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria

ABSTRACT

Background and Objective: Microbiological loads are influenced by a variety of natural and anthropogenic factors in an environment. These characteristics have a bearing on the ecosystem's distribution, production and microbial community, as well as its overall integrity. This study was carried out to determine the microbiological load in Periwinkle (*T. fuscatus*), water and sediment of Azubie/Woji Creek, Port Harcourt, Rivers State, Nigeria. **Materials and Methods:** Periwinkle, sediment and water samples were collected from three locations for six months from January to June, 2021, along Azubie/Woji Creek Port Harcourt, Rivers State, Nigeria. Samples were analyzed for microbiological analysis following standard methods. **Results:** Microbiological load mean values range between total fungal count (TFC) 0.64×10^{5} CFU g^{-1} (periwinkle) and mean values hydrocarbon utilizing bacteria count (HUBC) 78.42×10^{5} CFU g^{-1} (sediment) while hydrocarbon utilizing fungal counts (HUFC) range between (1.32×10^{5} CFU g^{-1}) periwinkle and (90.92 01×10^{5} CFU g^{-1}) in water, fecal coliform count (FCC) range from (0.90×10^{5} CFU g^{-1}) periwinkle to (109×10^{5} CFU g^{-1}) in water and total *Vibrio* count (VC) range from (0.94×10^{5} CFU g^{-1}) periwinkle to (9.03×10^{5} CFU g^{-1}) water. In the study, twelve genera of bacteria and four genera of fungi were identified. The species were *Escherichia coli*, *Bacillus* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus aureus*, *Enterobacter aeroens*, *Pseudomonas* sp., *Klebsiella* sp., *Serrata* sp., *Shigella* sp., *Salmonella* sp. and *Vibrio* (bacteria) while *Aspergillus* sp., *Penicillium*, *Rhizopus* and *Fusarium* sp. (fungi). **Conclusion:** The high feasible bacteria count indicated that the river was contaminated. To lessen the risky consequences on the locals who depend on the biota for survival, there is a desire for sensitization and even monitoring of the Azubie/Woji Creek.

KEYWORDS

Water, sediment, microbial load, Azubie/Woji Creek, Port Harcourt

INTRODUCTION

The examination of surface water offers evidence of the water quality status and its suitability for human use[1]. Due to the lack of natural soil protection and filtering, as well as the very short trips between the occurrence of contamination and water usage, surface water bodies are thought to become more vulnerable to fecal contamination than groundwater aquifers[2].

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Sediment has historically been thought to be an important and dynamic element of aquatic ecosystems, with ecological and environmental shifts that have been employed as environmental indicators for contaminant assessment[3] and serve as both pollutant sinks and sources for the line carriers and water column beneath[4]. They are lifetime mats for aquatic life.

Microbial load is the quantity and category of microorganisms contaminating an entity or organism when exhibited in a favorable condition. In recent times, most Nigerian rivers serve as storage areas for various solid wastes and untreated wastewater, contaminating the aquatic ecosystem. Industrial institutions, domestic residences, agricultural operations, abattoirs, municipal trash, gasoline stations, garages and health centers all produce this waste[5]. Microbial species such (as *Salmonella* sp., *Escherichia coli* and *Campylobacter* sp.) breed well due to limiting chemical factors in water reservoirs receiving effluents. Ikpesu and Ariyo[6] reported that biotas' biggest bioburden is due to the nearby human population absorbing excessive industrial and home garbage. Thus, microbial load transmission within biota niches may ascend via the food chain, potentially exposing benthic and pelagic fauna consumers to health and fitness risks. Aquatic assets are influenced by their surroundings, therefore water becomes insecure for life both in water and land as a result of the pathogen, which may occasionally restore the natural surroundings and also prevent additional contamination[7]. The causes of bacteria in the river environment remain clearance of human left-over and municipal wastewater over sewage and drainage ditches systems resulting in the distribution of pathogens[8].

The Azubie/Woji Creek is a tidal brackish water linking several creeks located within Port Harcourt Local Government Area of River State, Nigeria. The creek is owned to various human activities such as catching fish, defecation, paddling, bathing and swimming, commercial shipping (boating), laundering, interference to industrial facilities, trading illegal crude oil refined goods and uncontrolled garbage disposal are all popular practices along the creek. Consequently, these anthropogenic developments have contributed to the deliberate or unintended discharge of organic and inorganic pollutants into the adjacent water bodies, which might have had a harmful influence on the ecosystem. This study aims to investigate the microbiological load of Azubie/Woji Creek, to isolate, identified potential pathogens and assess the level of contamination.

MATERIALS AND METHODS

Study area: The study was carried out between the hours of 10.00 am and 1:30 pm from January to June in Azubie/Woji Creek, Port Harcourt, 2021. It is a tidal watercourse that empties into the Bonny Estuary via multiple branching tributaries with latitude 4.80727"E and longitude 7.05582"N (Fig. 1). The creek's anthropogenic impacts include factories near the beach, ship repairs and constructions, dredging, boating, illicit crude oil product trading, an abattoir, open latrines along the riverbank and the Port Harcourt veterinary park.

Samples collection: The species were considered for their year-round availability. Twenty samples of the specimen were collected once monthly. The periwinkles (*T. fuscatus*) were handpicked from the creek silt in shallow water and preserved in a sterile plastic container, while water samples were taken in sterile plastic containers that had been rinsed three times with the same creek water samples and were transported to the laboratory for microbiological analysis. At the laboratory, to eliminate dirt, debris and surface pollutants, the periwinkle samples were thoroughly washed and rinsed with a normal saline solution. A sterilized sterile needle was used to remove the edible sections of the periwinkle from the shell. At several sampling stations along the creek's length, sediment samples were taken in triplicate. The specimens were sealed in sterile containers, labeled and delivered to the lab for microbiological testing.

Microbial analysis: Total heterotrophic bacteria count (THBC) was determined by taking samples from each site. Samples were prepared by diluting and a portion of each was placed on a nutrient agar medium for THB isolation, with 50 g mL^{-1} nystatin added to prevent fungus growth. Plates were incubated at 30°C for 24 hrs before the colonies were counted. Microscopic techniques (Gram staining) and biochemical assays were used to describe the bacterial isolates[9]. The agar plates were stirred (clockwise and anti-clockwise) before being solidified and tented at 37°C for 24-48 hrs (bacteria) and 3-5 days (fungi). The colonies that emerged were enumerated and expressed as colony-forming units per gram of material.

The most probable number (MPN) approach was used to assess coliform bacteria in water samples from the creek. Responses to the MPN technique and thermos-accepting coliform bacteria MPN index/100 mL of every water sample were obtained at twice the normal strength. MacConkey Broth for 10 mL of the sample and single strength MacConkey Broth for 1 mL and 0.1 mL of the sample. The test for the estimation of coliforms involves the following steps: Presumptive, confirmatory and completed test. It was performed as described by Ja'afaru *et al.*[9]. Total coliforms count (TCC) was determined on pre-poured, surface-dried MacConkey agar and incubated at 37°C for 24 hrs.

Total fecal coliform count (TFCC), 1 mL of dilution 101 of material inoculated on surface-dried paper yielded Eosin Methylene Blue Agar at 44.5 for 24 hrs. For each sample, the most likely value methodology (MPN) was used with test tubes of 10, 1 and 0.1 mL with double or single strength[10].

Utilizing the spread-plate method, *Vibrio* count (VC) was determined in duplicates on surface-dried Thiosulphate-Citrate Bile-Salt Sucrose (TCBS) agar and plates were incubated at 37°C for 24 hrs. Colony-Forming Units (CFUs) were counted for representative colonies (30-300) and bacterial isolates were identified using cultural, morphological and biochemical features. Total fungal count (TFC) was inoculated over solidified Sabouraud's dextrose agar and incubated aliquots of decimal dilutions with 0.1 mL at 27±2°C for 1-4 days. Randomly selected healthy representative colonies were smeared, transplanted for purification and stored in the refrigerator at 2-4°C.

Statistical analysis: The data were concise for each bacteria count using descriptive statistics. Statistical differences between factors were analyzed using a two-way analysis of variance with a Tukey's confidence limit of ($p<0.05$) using Minitab (23.0 version).

RESULTS AND DISCUSSION

Total fungal counts (TFC) observed were lowest with a mean value ranging from 645000 CFU g^{-1} (periwinkle) to highest in the sediment with mean values 4356700 CFU g^{-1} (Fig. 1). Hydrocarbon utilizing bacteria count (HUBC) was recorded as lowest in periwinkle with a value of 5628300 CFU g^{-1} and high in the sediment with a mean value of 784200 CFU g^{-1} (Fig. 2). Total heterotrophic bacterial count (THBC) was observed lowest in periwinkle with a mean value of 666200 CFU g^{-1} and highest in the sediment with mean values of 6452000 CFU g^{-1} (Fig. 3). Though, there were significant ($p<0.05$) variations in the biota. According to Mourinño-Pérez *et al.*[11], the prevalence of hydrocarbon-disintegrating bacteria in the river could induce commensality or supportive quality degradation in the aquatic ecosystem. The increased total heterotrophic bacterial count (THBC), hydrocarbon utilizing bacteria count (HUBC) and total fungal counts (TFC) in the sediment were due to temperature, nitrate and phosphate from washing, fecal deposits, waste dump site in the river. The result tied with the report by Maki *et al.*[12], that photo-oxidation boosted the biodegradability of petroleum hydrocarbons by improving their bioavailability, which stimulates the microbial population.

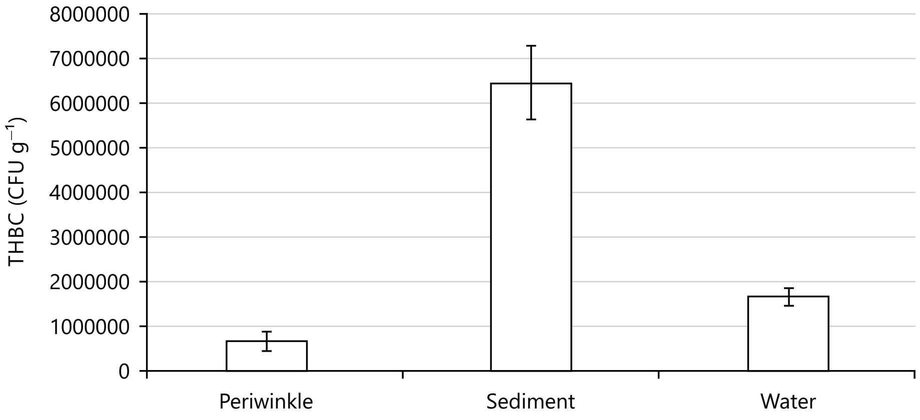

Fig. 1: Total heterotrophic bacterial count (THBC) loads in the biota of Azubie/Woji Creek

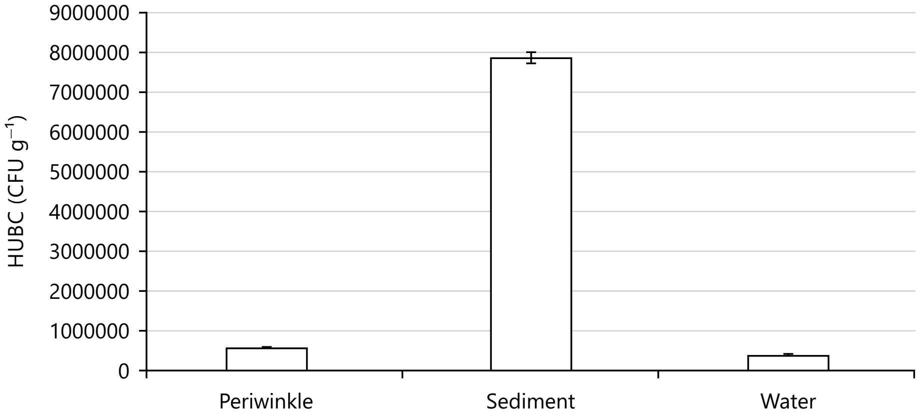

Fig. 2: Hydrocarbon utilizing bacteria count (HUBC) loads in the biota of Azubie/Woji Creek

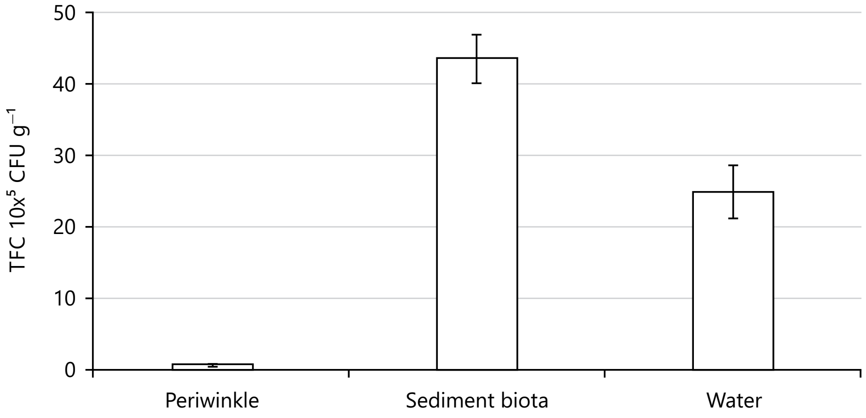

Fig. 3: Total fungal counts (TFC) loads in the biota of Azubie/Woji Creek

Hydrocarbon utilizing fungal counts (HUFC) was low in periwinkle and high in the water with mean values ranging between 132000 and 9092000 CFU g^{-1} (Fig. 4). Faecal coliform count (FCC) was significantly lower in periwinkle 90000 CFU g^{-1} as against the highest value of 1090000 CFU g^{-1} in water (Fig. 5), while *Vibrio* count (VC) had a low concentration in periwinkle and higher concentration in the water with a mean value ranging between 94400 and 903300 CFU g^{-1} (Fig. 6). The result indicated the presence of suspended or particulate matter rich in organic constituents which may serve as nutrient sources in the surface water. Amadi *et al.*[13] noted that the presence of bacteria in the aquatic environment is a function of the growth

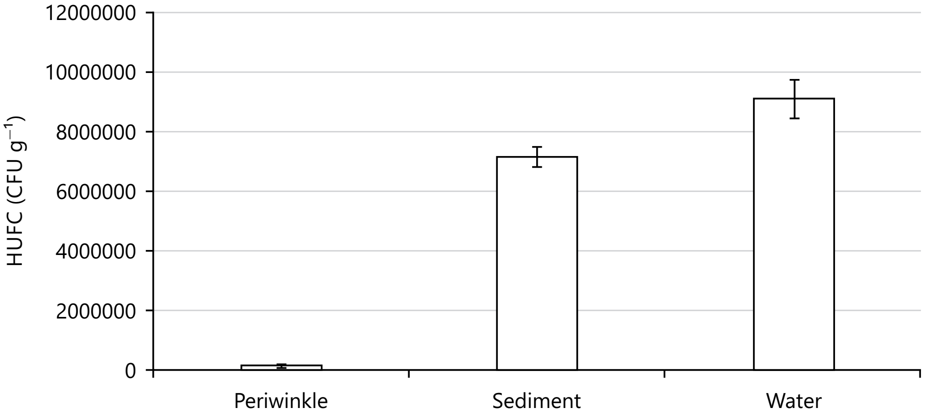

Fig. 4: Hydrocarbon utilizing fungal counts (HUFC) loads in the biota of Azubie/Woji Creek

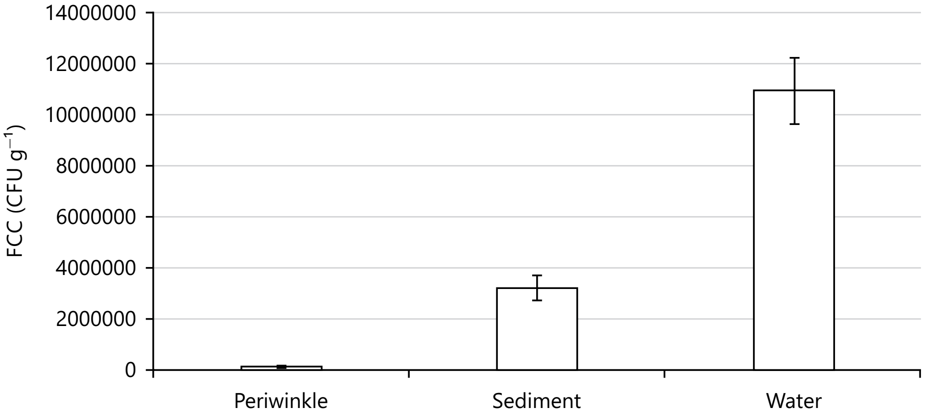

Fig. 5: Faecal coliform count (FCC) loads in the biota of Azubie/Woji Creek

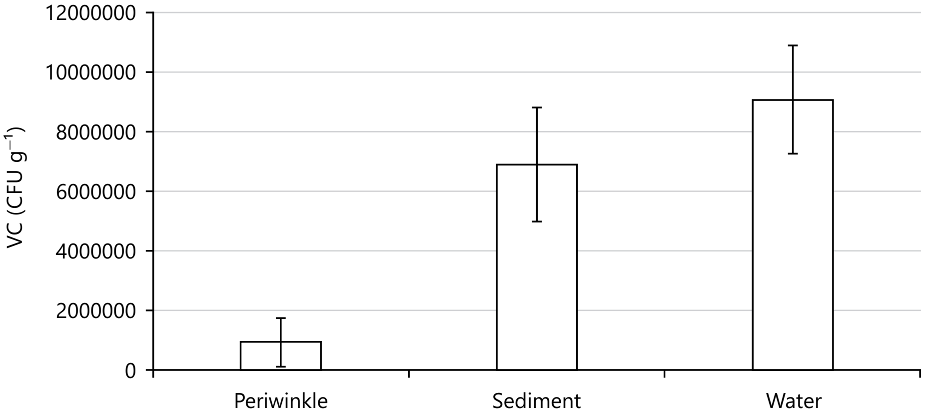

Fig. 6: *Vibrio* count (VC) loads in the biota of Azubie/Woji Creek

of the human population, the treatment of municipal wastewater services for sewage disposal, drainage and the presence of dangerous substances in the atmosphere. The incidences of coliforms could be because, surface water is a major recipient of the different influx of wastes as the first aqua reservoir. However, the number of bacteria in organisms cannot be greater than in water. This result disagreed with the report by Sroczynska *et al.*[14], who recorded a higher bacterial load in oyster *Crassostrea thizophorae* tissues than in water. This result was in line with the assertion by Jahan *et al.*[8], who reported that the

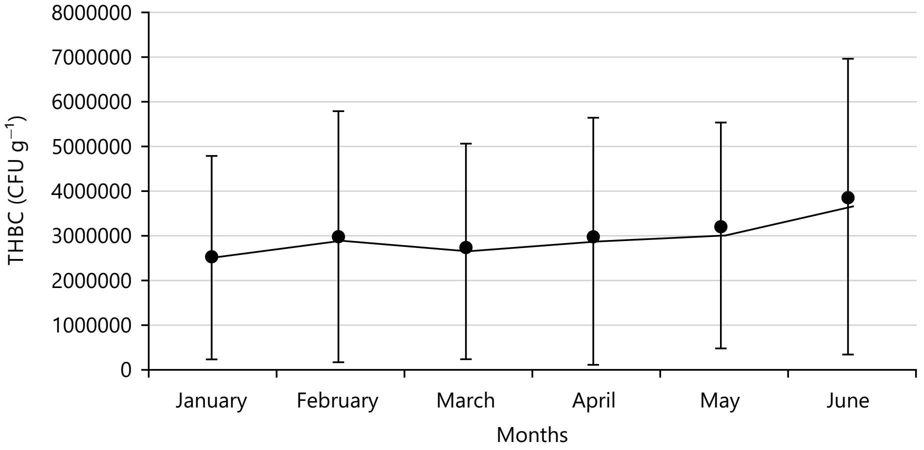

Fig. 7: Monthly variation of THBC in the biota of Azubie/Woji Creek

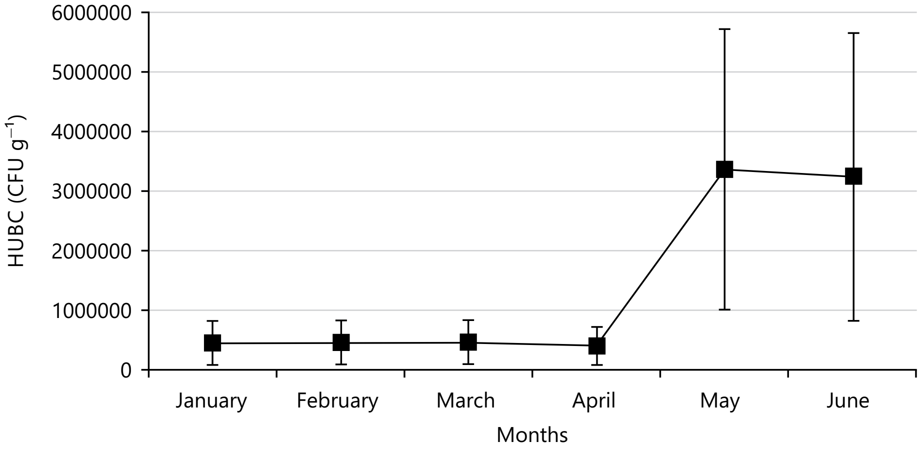

Fig. 8: Monthly variation of HUBC in the biota of Azubie/Woji Creek

transmission of waterborne infections may be linked to open defecation, discharge of industrial and home wastes, soil erosion, washing facilities and other factors. This result was also owned by Winfield *et al.*[15], which reported that excessive human activities might lead to an increase in organic matter, resulting in a high microbial load in the water.

Total heterotrophic bacterial count (THBC) concentration was particularly low in the month of January and high in the month of June (Fig. 7). Hydrocarbon utilizing bacteria count (HUBC) was low in the month of January and high in May following weighty contamination of crude oil products when the activities of illegal refinery operations were high (Fig. 8). The reason for higher counts of hydrocarbon-using bacteria and fungi in June could be linked to crude oil from illegal local refinery wastes from runoff around the environment and water tidal currents, which may have distributed hydrocarbon substance combined with other active ingredients that may enhance favorable conditions for hydrocarbon-using bacteria and fungi. This report supports the findings by Donderski and Kalwasińska[16], who opined that the proportion of planktonic bacteria in Chemyskie Lake was significantly greater during the wet season than it was during the dry season.

The Hydrocarbon utilizing fungal counts (HUFC) concentration was observed low in the month of January and highest in the month of February with no significant differences ($p>0.05$) (Fig. 9). Total fungal counts (TFC) had the least concentration in the month of May and the highest concentration in the month of

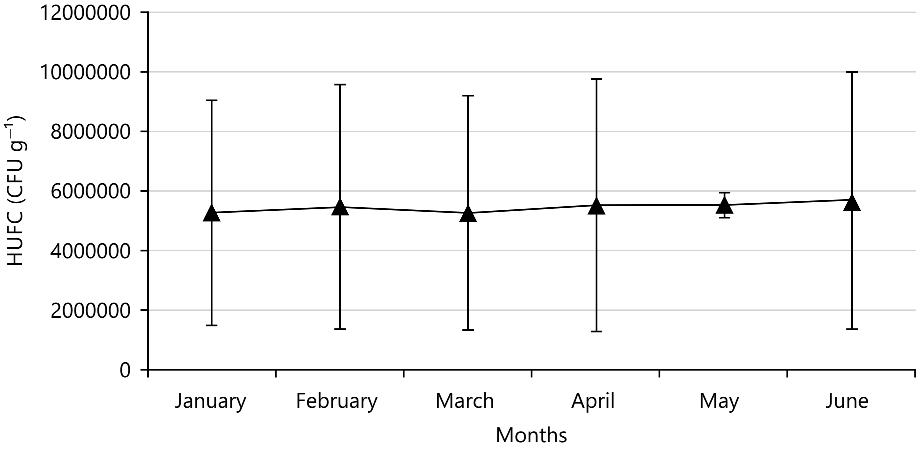

Fig. 9: Monthly variation of HUFC in the biota of Azubie/Woji Creek

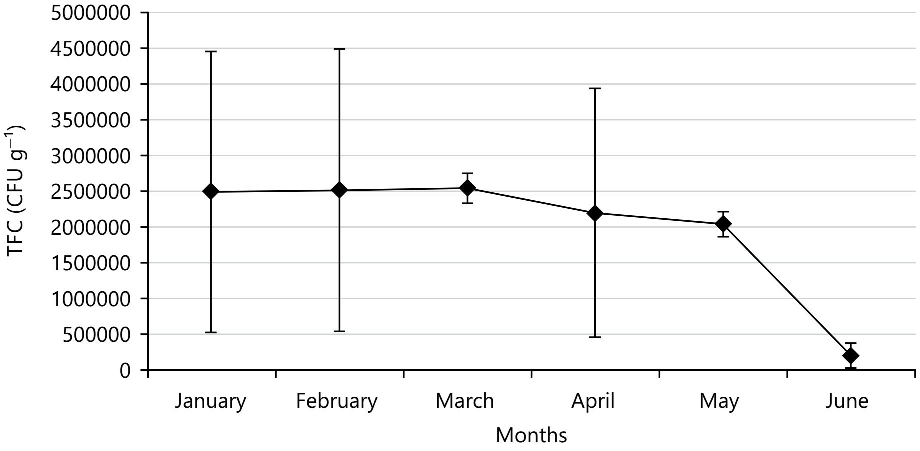

Fig. 10: Monthly variation of TFC in the biota of Azubie/Woji Creek

March (Fig. 10). The observed fecal coliform count (FCC) was low in the month of March and high in the month of June. The FCC concentration in the study decreases as the month progresses, though a very sharp peak was noticed in April (Fig. 11), while *Vibrio* count (VC) bacteria showed an increased trend between the month of January and June (Fig. 12). The study could be due to the anthropogenic activities captivating place of the study area as coliforms are pointers of fecal uncleanness. The study tie with is similar to the finding by Ikpesu and Ariyo[6] where the highest number of bacteria were accounted for in March and February.

Correlations were found between TFC and HUBC in sediment (Fig. 13). While *Salmonella* sp., showed no correlation in the study but *E. coli* exhibited a positive correlation with water, whereas *Pseudomonas* sp. and *E. coli* had a similar relationship with periwinkle species (Fig. 14). Ogbonna *et al.*[17] opined that microbes are everywhere, decomposing organic substances, bioaccumulating chemicals and recycling components biogeochemical. The result indicated high input of human activities, such as bathing and feces by the native population, along with waste from abbatoir dump sites, are both common occurrences in the area. Thus, the species' feet serve as a good breeding ground for proliferation. Consequently, the present study was in line with the findings by Edun *et al.*[18], shellfish are filter feeders able to absorb numerous contaminants and accumulate high concentrations of pathogens from their surroundings.

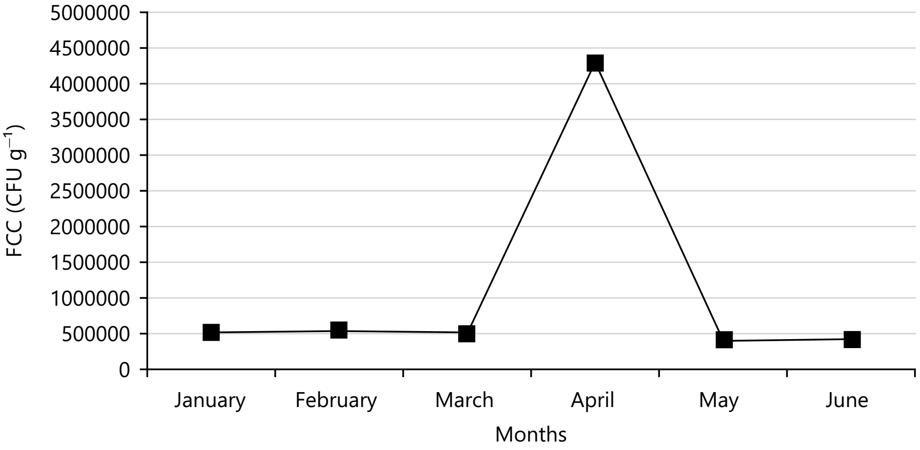

Fig. 11: Monthly variation of FCC in the biota of Azubie/Woji Creek

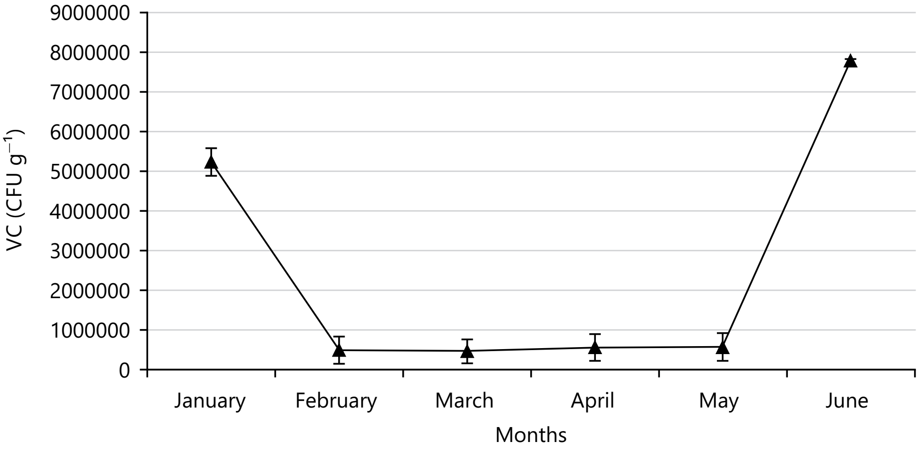

Fig. 12: Monthly variation of VC in the biota of Azubi/Woji Creek

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 $\times 10^5$ CFU g^{-1})

Manmade activities spot extremum significant impact on the amount of bacterial and fungi weight in the aquatic environments which humans depend on for a living. Consequently, surface water aids the transmission of pathogens to aquatic life and different compartments in the aquatic environment. In contrast, samples of periwinkle, sediment and water showed higher amounts of ongoing anthropogenic activity. The present study stress that the indicator species *Escherichia coli*, *Bacillus* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus aureus*, *Enterobacter aeroens*, *Pseudomonas* sp., *Klebsiella* sp., *Serrata* sp., *Shigella* sp., *Salmonella* sp. and *Vibrio* (Bacteria) while *Aspergillus* sp., *Penicillium*, *Rhizopus* and *Fusarium* sp. (fungi) recorded may be implicated to cause sicknesses corresponding to typhoid fever, diarrhea, cholera, dysentery, pneumonia, ring worm and sometimes smallpox outbreak as a result of bacteria and fungi infections to the inhabitants of the catchment area and other water linked contributors. However, Azubie/Woji Creek should be checked regularly to avoid contamination with bacteria and fungi and subsequent passage to aquatic life. Residents should collaborate closely with the government to adhere to sanitary measures such as environmental cleanliness, removal of open toilet pipes and construction along the riverbanks. Quality check organizations should make sure that the residents in the catchment closely adhere to these rules. Therefore, the differences between the results of this study and other findings on the microbial load from various sites may be due to nutrients along with the creek's fundamental physicochemical characteristics.

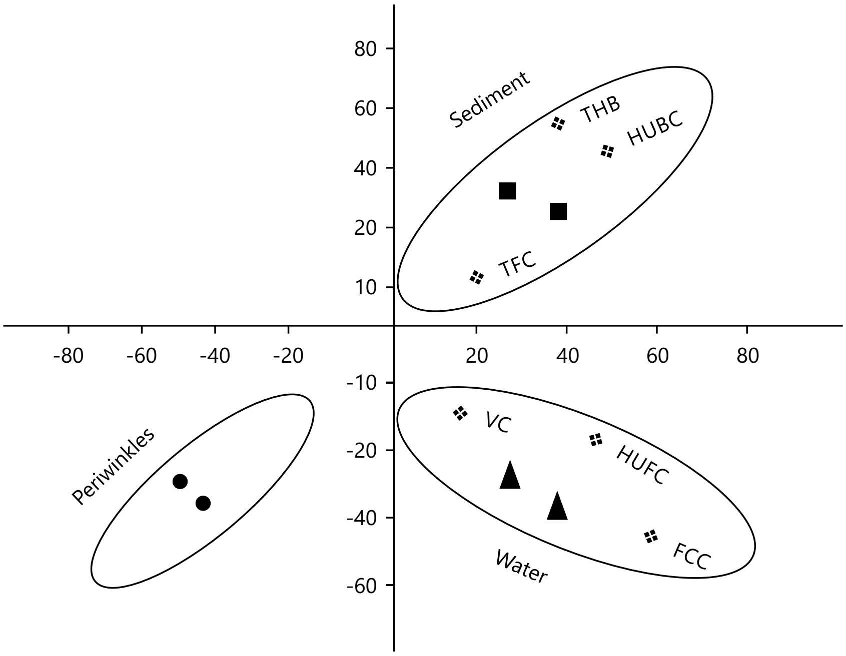

Fig. 13: Biplot of principal canonical correlation diagram showing the relationship between microbial load and the biota of Azubie/Woji Creek. Samples from the biota are differentiated with triangles, circles and rectangles of the Azubie/Woji Creek. Microbial loads are encircled to show association relationships

THBC: Total heterotrophic bacterial count, HUBC: Hydrocarbon utilizing bacteria count, TFC: Total fungal counts, HUFC: Hydrocarbon utilizing fungal counts, FCC: Fecal coliform count, VC: *Vibrio* count (all in ×10^5 CFU g^{-1})

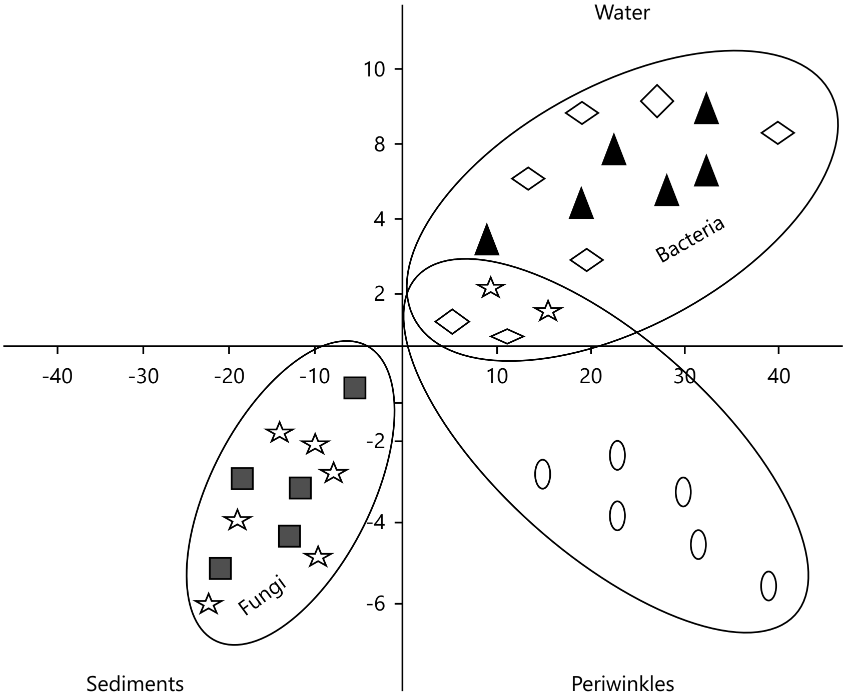

Fig. 14: Biplot of principal canonical relationships showing microbes isolate and the biota Azubie/Woji Creek. The shapes “stars and oval” indicates the different pathogen with regards to their microbial isolates. Samples from the biota are differentiated with triangles, circle and rectangles of the Azubie/Woji Creek. Shapes are encircled to show association relationships. Sediments: *Aspergillus* sp., *Fusarium* sp., Water: *Escherichia coli*, *Proteus* sp., *Streptococcus* sp., *Enterobacter aeroens*, *Shigella* sp., *Salmonella* sp., while Periwinkles and water: *Bacillus* sp., *Penicillium*, *Rhizopus*, *Enterococcoci*

CONCLUSION

This study reveals that Azubie/Woji Creek was impacted by a variety of anthropogenic factors which may become a prolific source of microbial assortment and thus water and sediment take a dynamic form in self-purification. The study found that there was significant variation between periwinkle, water and sediment. This depicts various levels of domestic and anthropogenic activities along the water body. The present study revealed that uptake by aquatic organisms can never be greater than contaminants left in the surrounding environments. More so, the presence of total fecal coliforms and pathogens in the creek underlines the tall magnitude of the contamination issue.

SIGNIFICANCE STATEMENT

The present study discovered that sediment and the water medium had more contaminants than the periwinkle. The study also unveiled that aquatic organisms pick up contaminants from the environment that are health-implicating. However, during uptake, bioconcentration can never be greater than the receiving source (environment) even though it exceeded the threshold limit. It only calls for alarm about public health. This study will help researchers to divulge critical proactive measures to sensitize the public on the effect of untreated, indiscriminate waste disposal and control measures for the benefit of mankind.

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