



Biomonitoring of the Physicochemical Variables, *Vibrio* and *Salmonella* Pathogens from Raw Shrimp in Selected Rivers in Southern Region Nigeria

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Abstract

This study was designed to reveal the physicochemical and microbial variables of raw shrimps and their habitat. Water and raw shrimp samples were obtained from different surface water bodies in Delta, Bayelsa, and Rivers States Nigeria from June 2016 to May 2017 and analysed using standard analytical and culture-based methods for their physicochemical and microbial diversity respectively. Analytical Profile Index (API 20NE) was used to secure the identity of Vibrio and Salmonella isolates. The mean physicochemical variables values range as follows: pH (7.73±0.47 - 7.82±0.53), turbidity (179.66±9.07 - 181.79±5.92 NTU) and COD (83.66±6.75 - 86.63±6.61 mgL⁻¹). The mean population cell density from the surface water range as follows: total heterotrophic population (6.3×10⁹±21.83 - 7.5×10⁹±48.86 CFU/mL), Vibrio species (2.0×10³±6.02 - 2.1×10³±13.00 CFU/mL), Salmonella species (1.8×10⁴±5.60 - 3.8×10⁴±27.02 CFU/mL), total coliform count (1.0×10⁴±4.54 - 2.5×10⁴±10.44 CFU/mL). The mean population cell density from the raw shrimp range as follows: total heterotrophic population (3.3×10⁶±12.11 - 8.1×10⁶±31.49 CFU/mL), Vibrio species (1.4×10²±7.72 - 2.7×10²±15.91 CFU/mL), Salmonella species (1.7×10³±7.73 - 4.6×10³±33.98 CFU/mL), total coliform count (9.8×10²±3.52 - 1.1×10³±5.36 CFU/mL). Significant correlation of the physicochemical variables on the population cell density of the bacteria isolated from both the water bodies and the raw shrimps were observed both at 0.05 and 0.01 levels in Delta, Bayelsa and Rivers State. The findings demonstrated that the shrimps associated with coliforms, vibrios and Salmonella give an insight on the sanitary condition of the harvesting period, thus call for public and environmental health control measures.

1. Introduction

The aquatic milieu usually contains substances that can be toxic, deliberately or accidentally introduced into the aquatic environment. The implication of such pollutants impairs the quality of water and makes it not suitable for aquatic life [1]. In southern region of Nigeria, surface waters pollution by crude oil, other solid and liquid wastes is common within the last decades rendering most of the surface water undesirable for human use [2]. Such toxic discharge into the aquatic environment is not solely accompanied within the food chain via bioaccumulation, but might also either limit the density and biodiversity of species or result in the formation of elevated microbial

count [3]. Pollution of specific surface waters can be associated to sewage/effluent or industrial processes disposal.

It was previously thought that shrimps in open waters were safe generally as a result of the paucity of epidemiological data of shrimp-borne ailments. Contrarily, current studies in areas of sea-food, water management and environmental pollution propose otherwise [4]. Shrimp customarily exist at the top of the food chain and can bio-accumulate significant amounts of pollutants in their organs and muscle [5]. Pathogenic microorganisms have been reported to be responsible for lots of diseases in both cultured and wild seafood and may vary from opportunistic to primary pathogens [6, 7]. Shrimps may also harbour pathogens or indigenous probiotics inside or on its body after it has been exposed to polluted water or contaminated food [8], but findings from disease-laden shrimps have limitation due to inadequate understanding of abundant exchanges that exist between shrimp hosts and their pathogens. Literature have revealed isolation of *Vibrio*, *Salmonella* and other bacterial species from shrimps and other sea-food in different polluted water bodies [7,9].

The south-southern district of Nigeria encompasses six states situated at the point where the Y-tail of the river Niger links the Atlantic Ocean via the Gulf of Guinea. States from south-southern Nigeria (Cross-River, Akwa-Ibom, Bayelsa, Rivers, Edo, and Delta) constitutes an economically significant part of the economic backbone of the economy: oil; and the biggest coastal and freshwater regions in the country Nigeria. Additionally gas and oil equally add other crucial resources, with prospective enormous investment opportunities in agriculture and tourism. The widespread incidence of *Vibrio* and *Salmonella* species in the marine environment permits the bacteria to use susceptible seafood to cause and transmit seafood-borne gastroenteritis [10]. Human pathogenic *Vibrio* and *Salmonella*, are of particular concern since they are accountable for ailments in humans which include cholera, seafood-borne gastroenteritis, wound infections, septicaemia, food intoxication and food poisoning in immunocompromised individuals in underdeveloped and developed countries, particularly in poorly sanitized regions [11, 12].

Shrimps are seafood with good market demand in Nigeria aquaculture industry. Contaminations by pathogenic bacteria in retail seafood in developing countries suggest the need for suitable measures for consumer protection. Therefore, monitoring the occurrence of *Vibrio* and *Salmonella* species by reliable techniques in Nigerian shrimps is of great importance and will provide vital information to consumers on the quality, food safety standard and policies on food security. Prior to this research, the microbiological quality of raw shrimps and the water source, as well as the physico-chemical variables, has not been properly established; hence, their impact on public health is not ascertained. This paper is therefore essential to enumerate and identify *Vibrio* and *Salmonella* species from the raw shrimps and water source from selected rivers in the south-south region in Nigeria.

2. Methodology

2.1 Study Area

Selected rivers where shrimps are harvested prior to transportation to open markets where they are sold were evaluated in the present study. The rivers were selected based on accessibility due to security reasons and catchment areas where shrimps are harvested from nearby rivers. Rivers evaluated in this study and their coordinates include Abonnema River (4°43'60"N 6°46'19"E), Boguma River (4°44'N 6°52"E), Choba River (4°53'26"N 6°54'12"E) and Ogu Creek River (4° 41' 13.79" N 7° 09' 16.80" E) situated in Rivers State; Burutu River (5.3510°N 5.5066°E), Idegehele River (5°45'N 5°43'E), Okerenkoko River (5.6261°N 5.3910°E), Ogulagha River (5.3512°N 5.3427°E), Escravos River (5.6115°N 5.1913°E), Ode Istekiri River (5.4724° N 5.6148°E), Ogbe

Ijoh River (5.6027°N 5.3888°E) and Omadino River (5.6333°N 5.6501°E) situated in Delta State; Brass River (4.3078°N 6.2456°E), Okpoma River (4.3200°N 6.3120°E), Okoroma River (4.5152°N, 6.2164°E), Ogboinbiri River (4.8265°N 5.9659°E) and Peremabiri River (4°38'35"N 6°4'50"E) located in Bayelsa State, Nigeria. All river sampled is characterized by industrial and anthropogenic activities.

2.2 Sample Collection

A total of 612 water samples from 17 different rivers from three states (Delta, Bayelsa and Rivers States) were assessed from June 2016 to May 2017. Water samples were obtained with a 2L sterilized plastic containers. During sampling, the containers were rinsed three times with sample water before collecting the samples from sampling points in the respective location. An average of 40g each of 612 newly harvested raw shrimp samples from 17 different riversides from the three states were also obtained from three different shrimp farmers respectively into sterile polythene bags. The samples were protected from direct sunlight and transported in a cooler box containing ice packs to the Applied Microbial Processes & Environmental Health Research Group laboratory for analyses. All samples were analysed within 24-48 h of sample collection depending on proximity.

2.3 Physicochemical Analyses

The physicochemical parameters of water quality comprising of temperature, pH, turbidity, conductivity, phosphate (PO₄), nitrate (NO₃⁻), nitrite (NO₂⁻) ammonia (NH₃), dissolved oxygen (DO) and chemical oxygen demand (COD) were assessed. Temperature, pH and turbidity were measured using a mercury thermometer, pH meter (HI 2210) and portable 2100P Hach turbidimeter respectively. Phosphate (PO₄), nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonia (NH₃) were assessed using the standard method [13]. COD was determined using the SpectroQuant Nova 60 COD cell test (Merck).

2.4 Isolation and Enumeration of the Bacterial Isolates

Water samples were serially diluted (10¹-10⁸) with 100 µL aseptically transferred via spread plate technique to Petri dishes containing the appropriate selective and non-selective media. Tryptic soy agar (Merck, Germany) was used for heterotrophic bacteria, chromocult coliform agar (Merck, Germany) was used in the coliform count, thiosulphate citrate bile salt sucrose (TCBS) agar (Lancashire, UK) was used for *Vibrio* spp. and hektoen enteric agar (Lancashire, UK) was used for *Salmonella* species. TCBS, hektoen enteric agar, nutrient agar and chromocult coliform agar were incubated at 37°C for 18-24 h. After incubation, the total colony counts were expressed in colony forming units per millilitre (CFU/mL). Twenty-five (25) grams of shrimp samples were weighted and placed in a sterile homogenizer bag containing 225 mL of alkaline peptone water with 2% NaCl, pH 8.5. The samples were homogenized for 1 min using a stomacher (Bagmixer400W, Interscience, St Nom, France). A hundred microliters (100 µL) of each pre-enriched homogenates was spread plated with appropriate sample dilution (1:10, 1:100, 1:1000, 1:10000 and 1:100000) for the enumeration of total heterotrophic bacterial count, presumptive *Vibrio* spp., and *Salmonella* spp. in respective samples. TCBS, hektoen enteric agar, nutrient agar and chromocult coliform agar were incubated at 37°C for 18-24 h. After incubation, the total colony counts were expressed in colony forming units per gram (CFU/g).

2.5 Biochemical Characterization of the Presumptive *Vibrio* and *Salmonella* species

The presumptive isolates were then subjected to Gram reaction 3% KOH test. All isolates were characterized using API 20 NE. The strips were thereafter read and the final identification made using API lab plus software (bioMerieux, Marcy l'Etoile, France).

2.6 Statistical Analysis

The experimental data were analysed using IBM SPSS software version 21.0. Statistical analysis was performed in order to determine if there will be any significant difference in the distribution of heterotrophic, *Vibrio* and *Salmonella* count from water and raw shrimps in the respective states using the Analysis of Variance (One Way ANOVA). Correlation analysis was also carried out to determine the effect of one variable on another. The significance level was set at a *p*-value of <0.05.

3. Results and Discussion

3.1 Results

3.1.1 Physicochemical Parameters of the River Water

The pH of the river water examined in Bayelsa state ranged from 6.89 to 8.97 with an average pH of 7.73 ± 0.47 . In Delta state, the pH ranged from 6.99 to 8.99 with an average pH of 7.82 ± 0.53 . While in Rivers state, the pH ranged from 6.98 to 8.98 with an average pH of 7.77 ± 0.47 . The DO of the river water examined in Bayelsa state ranged from 2.22 to 2.96 mgL^{-1} with an average DO of $2.51 \pm 0.49 \text{ mgL}^{-1}$. In Delta state, the DO ranged from 2.06 to 2.93 mgL^{-1} with an average DO of $2.48 \pm 0.64 \text{ mgL}^{-1}$. While in Rivers state, the DO ranged from 2.18 to 2.97 mgL^{-1} with an average DO of $2.54 \pm 0.19 \text{ mgL}^{-1}$ (Table 1).

3.1.2 Microbial Population Densities from the River Water

In Bayelsa state, the total heterotrophic bacterial ranged from 2.0×10^9 to 1.5×10^{10} cfu/ml; *Vibrio* count ranged from 5.0×10^2 to 3.6×10^3 cfu/ml; *Salmonella* count ranged from 6.0×10^3 to 3.2×10^4 cfu/ml; coliform count ranged from 1.1×10^4 to 5.5×10^4 cfu/ml. In Rivers state, the total heterotrophic bacterial ranged from 2.3×10^9 to 1.5×10^{10} cfu/ml; *Vibrio* count ranged from 1.0×10^2 to 5.9×10^3 cfu/ml; *Salmonella* count ranged from 6.0×10^3 to 1.1×10^5 cfu/ml; coliform count ranged from 3.0×10^3 to 2.9×10^4 cfu/ml (Table 2).

3.1.3 Microbial Population Density from the Raw Shrimps

In Delta state, the total heterotrophic bacterial ranged from 1.5×10^6 to 1.7×10^7 cfu/g; *Vibrio* count ranged from 5.0×10^1 to 7.6×10^2 cfu/g; *Salmonella* count ranged from 6.0×10^2 to 7.8×10^3 cfu/g; coliform count ranged from 3.0×10^2 to 4.4×10^3 cfu/g. In Rivers state, the total heterotrophic bacterial ranged from 3.4×10^6 to 1.7×10^7 cfu/g with; *Vibrio* count ranged from 5.0×10^1 to 7.7×10^2 cfu/g; *Salmonella* count ranged from 8.0×10^2 to 1.4×10^4 cfu/g; coliform count ranged from 3.0×10^2 to 1.7×10^3 cfu/g (Table 3).

Table 1: Physico-chemical qualities of the river water from Bayelsa, Delta and Rivers States

Variable	Bayelsa State			Delta State			Rivers State			P-value	Recommended Standards
	Min	Max	(Mean ±SD)	Min	Max	(Mean ± SD)	Min	Max	(Mean ± SD)		
Temperature (°C)	25.2	30.2	27.42±1.08 ^a	25.8	30.7	27.82±0.98 ^a	25.2	30.2	27.72±1.08 ^a	0.606	25°C, WHO
pH	6.89	8.97	7.73±0.47 ^a	6.99	8.99	7.82±0.53 ^a	6.98	8.98	7.77±0.47 ^a	0.434	6.5-8.5, NIS
Turbidity (NTU)	160	201	181.66±9.09 ^b	165	198	181.79±5.92 ^b	160	201	179.66±9.07 ^a	0.019	5 NTU, NIS
COD (mgL ⁻¹)	74	99	83.66±6.75 ^a	74	100	86.63±6.61 ^c	74	99	84.66±6.75 ^b	0.009	30 mg/L, WHO
DO (mgL ⁻¹)	2.22	2.96	2.51±0.49 ^b	2.06	2.93	2.48±0.64 ^a	2.18	2.97	2.54±0.19 ^c	0.007	6 mg/L, WHO
Ammonia (NH ₃ (mgL ⁻¹))	26.72	36.34	32.11±2.82 ^b	26.08	35.9	29.79±2.76 ^a	26.15	36.12	30.11±2.82 ^{ab}	0.021	35 mg/L, WHO
Conductivity (µScm ⁻¹)	315	467	378.85±40.21 ^a	323	493	404.92±38.11 ^a	323	486	397.85±40.21 ^a	0.123	1000 µS/cm, NIS
Phosphate (PO ₄) (mgL ⁻¹)	27.95	40.43	33.00±3.42 ^a	28.09	39.98	33.03±3.17 ^a	27.03	40.12	33.01±3.64 ^a	0.749	0.1 mg/L, USEPA
Nitrite (NO ₃ ⁻) (mgL ⁻¹)	36.33	47.17	42.01±2.17 ^b	35.12	46.67	40.68±2.44 ^a	35.12	46.67	40.03±3.07 ^a	0.028	1 mg/L, USEPA
Nitrate (NO ₂ ⁻) (mgL ⁻¹)	37.73	48.23	46.28±2.94 ^b	36.48	47.02	40.99±2.82 ^a	36.52	47.21	41.27±2.82 ^a	0.045	10 mg/L, USEPA

Legend: Values are means of triplicate ± Standard deviations (SD) of readings from June 2016 to May 2017. Means with the same letter across rows are not significantly different ($p > 0.05$). WHO: World Health Organization; NIS: Nigerian Industrial Standards; USEPA: United States Environmental Protection Agency

Table 2: Presumptive heterotrophic, *Vibrio* and *Salmonella* counts from the river water (CFU/mL)

Biological Variables	Bayelsa State			Delta State			Rivers State			P-value
	Min	Max	(Mean ± SD)	Min	Max	(Mean ± SD)	Min	Max	(Mean ± SD)	
Total heterotrophic bacterial	2.0×10 ⁹	1.5×10 ¹⁰	6.3×10 ⁹ ±21.83 ^a	1.3×10 ⁹	1.6×10 ¹⁰	7.5×10 ⁹ ±48.86 ^b	2.3×10 ⁹	1.5×10 ¹⁰	7.2×10 ⁹ ±32.89 ^b	0.008
<i>Vibrio</i> species	5.0×10 ²	3.6×10 ³	2.0×10 ³ ±6.02 ^a	2.0×10 ²	5.7×10 ³	2.1×10 ³ ±13.00 ^a	1.0×10 ²	5.9×10 ³	2.0×10 ³ ±13.35 ^a	0.173
<i>Salmonella</i> species	6.0×10 ³	3.2×10 ⁴	1.8×10 ⁴ ±5.60 ^a	3.0×10 ³	6.8×10 ⁴	2.4×10 ⁴ ±15.44 ^b	6.0×10 ³	1.1×10 ⁵	3.8×10 ⁴ ±27.02 ^c	0.000
Total coliform count	1.1×10 ⁴	5.5×10 ⁴	2.5×10 ⁴ ±10.44 ^c	3.0×10 ³	2.9×10 ⁴	1.5×10 ⁴ ±5.43 ^b	3.0×10 ³	2.9×10 ⁴	1.0×10 ⁴ ±4.54 ^a	0.000

Legend: Values are means of triplicate ± Standard deviations (SD) of readings from June 2016 to May 2017. Means with the same letter across rows are not significantly different ($p > 0.05$).

Table 3: Presumptive heterotrophic, *Vibrio* and *Salmonella* counts from the raw shrimp sample (CFU/g)

Biological Variable	Bayelsa State			Delta State			Rivers State			P-value
	Min	Max	(Mean ± SD)	Min	Max	(Mean ± SD)	Min	Max	(Mean ± SD)	
Total heterotrophic bacterial	1.2×10 ⁶	7.0×10 ⁶	3.3×10 ⁶ ±12.11 ^a	1.5×10 ⁶	1.7×10 ⁷	7.4×10 ⁶ ±48.76 ^b	3.4×10 ⁶	1.7×10 ⁷	8.1×10 ⁶ ±31.49 ^b	0.000
<i>Vibrio</i> species	2.0×10 ¹	4.0×10 ²	1.4×10 ² ±7.72 ^a	5.0×10 ¹	7.6×10 ²	2.6×10 ² ±14.56 ^b	5.0×10 ¹	7.7×10 ²	2.7×10 ² ±15.91 ^b	0.000
<i>Salmonella</i> species	5.0×10 ²	4.5×10 ³	1.7×10 ³ ±7.73 ^a	6.0×10 ²	7.8×10 ³	2.8×10 ³ ±17.59 ^b	8.0×10 ²	1.4×10 ⁴	4.6×10 ³ ±33.98 ^c	0.000
Total coliform count	1.0×10 ²	1.9×10 ³	9.8×10 ² ±3.52 ^a	3.0×10 ²	4.4×10 ³	1.1×10 ³ ±5.36 ^b	3.0×10 ²	1.7×10 ³	1.0×10 ³ ±3.13 ^a	0.000

Legend: Values are means of triplicate ± Standard deviations (SD) of readings from June 2016 to May 2017. Means with the same letter across rows are not significantly different (p > 0.05).

3.1.4 Correlation of the Physicochemical and Microbial Population Density

The correlation of the physicochemical variables and microbial population densities from the water sources and the raw shrimps in Bayelsa state is presented in Table 4. COD significantly and correlates DO ($r=-0.402$), NH_3 ($r=0.329$), EC ($r=0.530$), PO_4 ($r=0.339$), NO_3^- ($r=0.232$), NO_2^- ($r=0.307$), TWHB ($r=0.215$), VRS ($r=0.193$), TCR ($r=0.218$) at the 0.01 level and TRHB ($r=0.148$) at 0.05 level. TWHB significantly and positively correlates TRHB ($r=0.223$), VRS ($r=0.239$), SRS ($r=0.271$), while it negatively by strongly correlates TCW ($r=-0.197$) at the 0.01 level.

The correlation of the physicochemical variables and microbial population densities from the water sources and the raw shrimps in Delta state is presented in Table 5. The pH significantly and correlates NTU ($r=0.323$), COD ($r=0.412$), DO ($r=-0.409$), NH_3 ($r=0.364$), EC ($r=0.365$), PO_4 ($r=0.365$), NO_3^- ($r=0.427$), NO_2^- ($r=0.538$), SWS ($r=0.176$), and SRS ($r=0.190$) at the 0.01 level and TCW ($r=0.123$) at 0.05 level. VWS significantly and positively correlates SWS ($r=0.653$), TRHB ($r=0.223$), VRS ($r=0.867$), SRS ($r=0.613$), TCR ($r=0.539$) while it negatively but strongly correlates TCW ($r=-0.237$) at the 0.01 level.

The correlation of the physicochemical variables and microbial population densities from the water sources and the raw shrimps in Rivers state is presented in Table 6. DO significantly and correlates NH_3 ($r=-0.700$), EC ($r=-0.706$), PO_4 ($r=-0.777$), NO_3^- ($r=-0.760$), NO_2^- ($r=-0.760$), VWS ($r=-0.258$), SWS ($r=-0.218$), VRS ($r=-0.310$), SRS ($r=-0.221$), TCW ($r=-0.272$), and TCR ($r=-0.259$) at the 0.01 level; while TWHB ($r=-0.177$) was correlated at 0.05 level. TWHB significantly and positively correlates VWS ($r=0.695$), SWS ($r=0.904$), TRHB ($r=0.990$), VRS ($r=0.691$), SRS ($r=0.871$), and TCW ($r=0.259$) at the 0.01 level.

Table 4: Correlation matrix on the physicochemical variables and population densities from the water sources and the raw shrimps from Bayelsa State

	Temp	pH	NTU	COD	DO	NH ₃	EC	PO ₄	NO ₃ ⁻	NO ₂ ⁻	TWHB	VWS	SWS	TRHB	VRS	SRS	TCW	TCR
Temp	1.000																	
pH	0.021	1.000																
NTU	-0.001	0.117	1.000															
COD	0.025	0.198**	0.279**	1.000														
DO	0.059	-0.086	-0.194**	-0.402**	1.000													
NH ₃	0.144	0.039	0.357**	0.329**	-0.315**	1.000												
EC	-0.024	0.252**	0.297**	0.530**	-0.273**	0.234**	1.000											
PO ₄	0.063	0.149*	0.320**	0.339**	-0.365**	0.296**	0.451**	1.000										
NO ₃ ⁻	0.190*	-0.020	-0.119	0.232**	-0.144	0.100	-0.059	0.180*	1.000									
NO ₂ ⁻	0.144	0.013	0.384**	0.307**	-0.226**	0.454**	0.229**	0.460**	0.159*	1.000								
TWHB	0.079	0.045	0.160*	0.215**	-0.299**	0.205*	0.041	0.306**	0.109	0.294**	1.000							
VWS	0.094	0.003	0.024	-0.036	-0.085	0.025	0.082	0.072	0.003	0.007	0.014	1.000						
SWS	0.124	0.079	0.021	0.025	-0.087	-0.123	0.043	0.011	0.003	0.083	0.056	0.047	1.000					
TRHB	0.108	0.145	0.215**	0.148*	0.047	0.142	0.132	0.260**	0.192**	0.074	0.223**	0.024	-0.187*	1.000				
VRS	0.093	0.019	0.102	0.193**	-0.128	-0.086	0.132	0.160*	0.025	-0.052	0.239**	0.195**	0.156*	0.193**	1.000			
SRS	-0.007	-0.218**	0.111	-0.004	0.059	-0.060	-0.069	0.107	0.060	0.084	0.271**	0.228**	0.028	0.016	0.532**	1.000		
TCW	0.122	-0.032	-0.024	-0.068	-0.120	-0.003	-0.050	-0.049	-0.092	0.009	-0.197**	-0.115	0.377**	-0.216**	-0.236**	-0.266**	1.000	
TCR	0.107	0.091	0.263**	0.218**	-0.123	0.191*	0.197**	0.017	0.099	0.086	-0.004	0.048	0.034	0.345**	0.029	-0.114	0.064	1.000

Legend: Temp: Temperature, NTU: Turbidity, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, NH₃: Ammonia, EC: Electrical conductivity, NO₃⁻: Nitrite, NO₂⁻: Nitrate, PO₄: Phosphate, TWHB: Total heterotrophic bacterial counts from the water samples, VWS: Total *Vibrio* count from the water samples, SWS: Total *Salmonella* count from the water samples, TRHB: Total heterotrophic bacterial counts from the raw shrimp samples, VRS: Total *Vibrio* counts from the raw shrimp samples, SRS: Total *Salmonella* counts from the raw shrimp samples, TCW: Total coliform count on the river samples, TCR: Total coliform count on the raw shrimp samples **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Table 5: Correlation matrix on the physicochemical variables and population densities from the water sources and the raw shrimps from Delta State

	Temp	pH	NTU	COD	DO	NH ₃	EC	PO ₄	NO ₃ ⁻	NO ₂ ⁻	TWHB	VWS	SWS	TRHB	VRS	SRS	TCW	TCR
Temp	1.000																	
pH	0.457**	1.000																
NTU	0.353**	0.323**	1.000															
COD	0.333**	0.412**	0.340**	1.000														
DO	0.388**	-0.409**	-0.270**	-0.508**	1.000													
NH ₃	0.311**	0.364**	0.288**	0.422**	-0.459**	1.000												
EC	0.359**	0.365**	0.254**	0.596**	-0.427**	0.494**	1.000											
PO ₄	0.386**	0.365**	0.351**	0.356**	-0.541**	0.569**	0.538**	1.000										
NO ₃ ⁻	0.436**	0.427**	0.284**	0.514**	-0.429**	0.449**	0.410**	0.461**	1.000									
NO ₂ ⁻	0.408**	0.538**	0.280**	0.462**	-0.478**	0.583**	0.387**	0.413**	0.442**	1.000								
TWHB	0.258**	0.077	0.123*	0.071	-0.041	-0.221**	-0.048	-0.118*	0.225**	0.149*	1.000							
VWS	0.038	0.084	0.056	0.107	-0.089	0.043	0.172**	0.194**	0.123*	0.299**	0.072	1.000						
SWS	0.040	0.176**	0.111	0.089	-0.128*	-0.193**	0.130*	-0.014	0.135*	0.180**	0.252**	0.653**	1.000					
TRHB	0.240**	0.105	0.049	0.102	-0.091	-0.222**	-0.002	-0.091	0.269**	0.162**	0.859**	0.223**	0.437**	1.000				
VRS	0.040	0.104	0.064	0.175**	0.004	0.008	0.205**	0.128*	0.135*	0.316**	0.240**	0.867**	0.778**	0.416**	1.000			
SRS	0.070	0.190**	0.110	0.173**	-0.059	-0.129*	0.180**	0.027	0.182**	0.233**	0.308**	0.613**	0.916**	0.502**	0.782**	1.000		
TCW	0.148*	0.123*	0.065	0.027	-0.259**	-0.088	0.035	0.020	0.191**	0.013	0.207**	-0.237**	-0.108	0.114	-0.243**	-0.102	1.000	
TCR	-0.122*	-0.030	-0.130*	0.105	-0.028	-0.185**	0.069	-0.004	0.035	0.077	0.099	0.539**	0.535**	0.163**	0.608**	0.529**	-0.143*	1.000

Legend: Temp: Temperature, NTU: Turbidity, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, NH₃: Ammonia, EC: Electrical conductivity, NO₃⁻: Nitrite, NO₂⁻: Nitrate, PO₄: Phosphate, TWHB: Total heterotrophic bacterial counts from the water samples, VWS: Total *Vibrio* count from the water samples, SWS: Total *Salmonella* count from the water samples, TRHB: Total heterotrophic bacterial counts from the raw shrimp samples, VRS: Total *Vibrio* counts from the raw shrimp samples, SRS: Total *Salmonella* counts from the raw shrimp samples, TCW: Total coliform count on the river samples, TCR: Total coliform count on the raw shrimp samples **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Table 6: Correlation matrix on the physicochemical variables and population densities from the water sources and the raw shrimps from Rivers State

	Temp	pH	NTU	COD	DO	NH ₃	EC	PO ₄	NO ₃ ⁻	NO ₂ ⁻	TWHB	VWS	SWS	TRHB	VRS	SRS	TCW	TCR	
Temp	1.000																		
pH	0.379**	1.000																	
NTU	0.573**	0.612**	1.000																
COD	0.543**	0.605**	0.745**	1.000															
DO	0.557**	-0.622**	-0.664**	0.717**	1.000														
NH ₃	0.567**	0.524**	0.729**	0.702**	-0.700**	1.000													
EC	0.524**	0.611**	0.677**	0.748**	-0.706**	0.683**	1.000												
PO ₄	0.636**	0.672**	0.757**	0.875**	-0.777**	0.756**	0.852**	1.000											
NO ₃ ⁻	0.617**	0.689**	0.803**	0.803**	-0.760**	0.738**	0.785**	0.887**	1.000										
NO ₂ ⁻	0.623**	0.690**	0.850**	0.818**	-0.760**	0.836**	0.852**	0.879**	0.882**	1.000									
TWHB	0.120	0.398**	0.165*	0.161	-0.177*	-0.053	0.325**	0.247**	0.244**	0.199*	1.000								
VWS	0.227**	0.278**	0.003	0.096	-0.258**	-0.040	0.271**	0.226**	0.099	0.112	0.695**	1.000							
SWS	0.135	0.351**	0.108	0.123	-0.218**	-0.062	0.351**	0.230**	0.198*	0.194*	0.904**	0.799**	1.000						
TRHB	0.105	0.395**	0.138	0.138	-0.159	-0.085	0.303**	0.224**	0.212*	0.178*	0.990**	0.700**	0.900**	1.000					
VRS	0.290**	0.340**	0.109	0.189*	-0.310**	-0.029	0.325**	0.298**	0.170*	0.194*	0.691**	0.936**	0.794**	0.704**	1.000				
SRS	0.135	0.303**	0.076	0.154	-0.221**	-0.113	0.312**	0.245**	0.190*	0.142	0.871**	0.840**	0.965**	0.869**	0.826**	1.000			
TCW	0.180*	0.153	0.315**	0.240**	-0.272**	0.108	0.337**	0.252**	0.226**	0.288**	0.259**	0.209*	0.332**	0.247**	0.326**	0.300**	1.000		
TCR	-0.131	-0.210*	-0.423**	-0.337**	-0.259**	-0.332**	-0.281**	-0.253**	-0.359**	-0.362**	-0.013	0.181*	0.034	-0.009	0.204*	0.055	-0.273**	1.000	

Legend: Temp: Temperature, NTU: Turbidity, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, NH₃: Ammonia, EC: Electrical conductivity, NO₃⁻: Nitrite, NO₂⁻: Nitrate, PO₄: Phosphate, TWHB: Total heterotrophic bacterial counts from the water samples, VWS: Total *Vibrio* count from the water samples, SWS: Total *Salmonella* count from the water samples, TRHB: Total heterotrophic bacterial counts from the raw shrimp samples, VRS: Total *Vibrio* counts from the raw shrimp samples, SRS: Total *Salmonella* counts from the raw shrimp samples, TCW: Total coliform count on the river samples, TCR: Total coliform count on the raw shrimp samples **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

3.2 Discussion

Understanding the physicochemical variables of significant chemical substitutes is a requisite of the alternative assessment procedure. The intrinsic hazard of a chemical which includes its inherent capacity to alter the normal biotic processes coupled with its physical hazard and ecological fate whether breaking down or persistence are assessed via its intrinsic physicochemical characteristics and the system with which it interacts. The present study has revealed the physicochemical properties and microbial pathogens of raw shrimps in the southern region of Nigeria. Water quality depends on a number of factors including organization and appropriately designed and constructed fronts that have a reliable water supply. Temperature is important for water quality management and responsible for changes of physicochemical variables of water. Increase in temperature generally translates to an increase in the rate of chemical reactions [14]. Temperature values obtained from rivers in this study were within recommended guidelines. The pH has a significant impact on chemical and biological succession in the water body [15]. The pH values in this study were within the recommended standard limit. The concentration of dissolved oxygen from the river water was significantly low compared to the recommended standard. Natural purification procedures of stream require adequate oxygen levels to provide for aerobic life forms [16]. The low level of dissolved oxygen observed in this study could be as a result of the high agricultural, industrial and waste materials released in untreated form into these water bodies. This also explains why a negative correlation exists between the dissolved oxygen (DO) and water nutrient and quality parameters. Electrical conductivity (EC) of water interprets the number of dissolved solids in the water which could be as a result of significantly high concentration of contaminants in surface waters. However, the EC values in this study were lower than the recommended standard. Bioavailable nutrient forms such as inorganic nitrogen (nitrite, ammonia and nitrate) and phosphorus are crucial factors affecting water quality [17]. They undertake a key role in surface water eutrophication. Orthophosphates can quickly be assimilated by plants and usually have a larger eutrophication influence when compared to nitrogen. Unlike others, ammonia is very toxic to seafood when it occurs in its un-ionized form (temperature dependent and pH), irrespective of concentrations [18].

High levels of nutrients and phosphorus observed in this study have been reported to increase eutrophication and thus deplete the DO concentration of the rivers. Nutrient pollution in Rivers showed extremely high values which could be ascribed to agricultural overflow [19]. In this study, the elevated values of the nutrient can also be attributed to sources of pollutant such as oil bunkering and other related activities as observed during field investigation. In addition, the connection of untreated waste to the receiving watershed and the continued runoff of neighbouring faecal waste into the neighbouring river could contribute to the high levels of nutrients and phosphate observed. Pesticide and fertilizer use for agricultural activities was also observed. Chemical oxygen demand (COD) helps to predict requirements of oxygen during the oxidation of inorganic chemicals and breakdown of organic matter. Where COD level is higher than the recommended standard, the water is regarded as polluted [20]. The COD values from this study were higher than the permissible limit by World Health Organization [21]. This appreciable increase in COD could likely result from industrial activities. In a study by Koji *et al.* [22] reveals that water temperature varied from $25.4 \pm 0.2^\circ\text{C}$ to $29.9 \pm 0.4^\circ\text{C}$; conductivity varied from $178.1 \pm 30.1 \mu\text{S}/\text{cm}$ to $375.0 \pm 122.7 \mu\text{S}/\text{cm}$; pH varied from $6.36 \pm 0.12\text{UC}$ to $7.79 \pm 0.06\text{UC}$, and turbidity values ranged from $26.6 \pm 5.5 \text{NTU}$ to $65.8 \pm 18.8 \text{NTU}$. These values vary significantly from the findings in this study. Water samples assessed by Lyimo *et al.* [23] contained $>100 \text{CFU}/100 \text{ml}$ coliforms which were somewhat similar to our findings. Aerobic bacteria density and coliform density of river water by Omeji *et al.* [24] range from 4.7×10^6 to $8.6 \times 10^6 \text{CFU}/\text{mL}$.

and 3.6×10^4 to 5.4×10^4 CFU/mL respectively, which was higher compared to the findings from this study. Any distress emanated from the release of untreated sewage, toxin as well as diverse wastes into an environment may predispose such habitat to bio-accumulative action of pollutants.

Water-related challenges of shrimps continue to be one of the global health problems, studies that bacteria are common flora of frozen fish and fish related products during packaging have been reported. It is estimated that 80% of all illnesses are intertwined to use of water of poor bacteriological quality [25]. More so, the bacterial contamination of shrimps is a serious threat to aquaculture systems that may result in severe damage and mortality rate in Nigeria coupled with large economic losses among shrimp farmers. In a study conducted by Ayandiran and Dahunsi [5] from indigenous *Clarias* species revealed the highest bacterial count to be $6.02 \log_{10}$ CFU/mL while the lowest was $2.00 \log_{10}$ CFU/mL. Adewoye and Lateef [26] also reported $5.00 \log_{10}$ CFU/mL for bacterial density in some contaminated water bodies in Nigeria exposed to human, agricultural and industrial wastes. Omogbai and Obichi [27] reported the total viable *Vibrio* count in unprocessed seafood samples in Lagos State, Nigeria ranged from 3.30 to $5.80 \log_{10}$ CFU/g. A study by Chakma *et al.* [28] reported that fresh Nappi (Fish-Paste) had a maximum heterotrophic bacterial count (1.43×10^{10} CFU/g), total coliform (7.75×10^9 CFU/g), *Salmonella* spp. ($2.6 \pm 0.10 \times 10^7$ CFU/g) and *Vibrio* spp. ($3.35 \pm 0.25 \times 10^6$ CFU/g) from Chaufaldandi market, Bangladesh. These are somewhat similar to the findings in this study. The high density of coliform recorded from surface water samples in this study depicts the occurrence of faecal contamination. The proliferation of microorganisms in water is exceedingly influenced by factors of environmental concern. As such, the bacterial density and physicochemical characteristics of river water are imperative. This explains why correlations exist between the bacterial density and physicochemical variables in this study. Rural and urban centres are non-point routes of faecal bacteria due to rodents, wild animals, avian faeces and domestic animals. The coliform bacteria according to Cabral [29] may pollute recreational and surface waters in the majority of developing countries. All surface water sources screened in this study exceeded the WHO standard for total coliform.

Rivers owing to nearness to human habitat ease spread of salmonellosis to humans. *Salmonella* species with varying densities have been recovered from different surface rivers [23, 24, 30]. Waterborne ailments connected with *Salmonella* spp. are usually common as a result of increased faecal contamination of water bodies (Levantesi *et al.*, 2012). The density of *Salmonella* spp. has been reported to be high in surface water during frequent disturbances (Walters *et al.*, 2007). The occurrence of this disease-causing bacterium of faecal origin poses serious threats to human and environmental health. Ekwanzala *et al.* [30] reported the mean counts of *Salmonella* spp. in water to range from 0 to $2.98 \log_{10}$ CFU/100 mL with a mean density of $2.60 \log_{10}$ CFU/100 mL which was in line with the findings from this study. Many relative factors which contribute to this incidence include inadequate supplies of clean water, increased urbanization, increased regional movement and antibiotic resistance.

Shrimps are subjected to risk culminating in contamination from different sources during harvesting, marketing until it reaches the final consumers. The main sources of shrimp contamination are water, soil, sewage, workers and equipments. Such contamination may render the product unfit for human consumption resulting in significant economic losses or public health hazard to the consumer. The abundance of *Vibrio* cells associated with raw shrimps in the coastal waters of Cameroon varied between 10^3 and 1.6×10^5 CFU/g from the shrimp, and from 10^3 to 1.4×10^4 CFU/mL in the coastal water [22] Seafood by Omeji *et al.*

[24] had aerobic bacteria density and coliform density which ranged from 7.20×10^4 to 3.20×10^5 and 2.40×10^4 to 3.12×10^5 , respectively. These are significantly higher than the findings in this study. Yang *et al.* (2015) reported a most probable number per gram (MPN/g) of 0.3-10, with one sample exceeding 110 MPN/g which was somewhat similar to the *Salmonella* density in this study. The mean *Salmonella* density in this study varied across the states. *Salmonella* spp., was reported as the most frequent cause of outbreaks connected with crustaceans from 1998 to 2004. Among species of crustacean, shrimp were reported as the most internationally traded, economically important seafood commodity, and the most predominantly aquaculture-raised seafood imported to the United States [31]. Pathogenic *Salmonella* spp., are accountable for serious foodborne ailments and can be brought into the shrimp production chain via inadequate hygiene or handling or in conjunction with polluted water. Microbiological safety of shrimp is a concern for industries, consumers and monitoring agencies globally. Shrimp, a significant category of food in international trade often exported to numerous countries, can act as a route for *Salmonella* dissemination throughout the shrimp production chain. Concerns regarding the inadequate usage of antibiotics in aquaculture can also result in increased isolation of antibiotic-resistant and multidrug-resistant serovars of *Salmonella* [32, 33].

Koji *et al.* [22] reported that the dynamics of *Vibrio* cells showed that suspended solids and rainfalls could also influence the abundance of vibrios associated with shrimps. Inversely, turbidity had negative effect on the variation of the concentration of *Vibrio* and *Salmonella* cells associated with raw shrimps. The water temperature and pH provided little additional information on the development of the concentration of *Vibrio* cells associated with fresh shrimp [22]. This is associated to the findings observed in Rivers state but deviates from those observed in Bayelsa and Delta state respectively. Biologically, *Vibrio* and *Salmonella* species exhibit a significant role in cleavage of biological matter mainly of anthropogenic origin in mineral elements, ejecting dissolved carbon dioxide while assimilating dissolved oxygen in the aquatic milieu [34].

It is evident that the development of shrimps in surface water increases the survival of *Vibrio* when the environmental conditions are becoming difficult for a certain period of the year [35]. Numerous diseases can be attributed to water polluted with faeces washed into rivers as indicators of faecal pollution. More so, surface water is at risk of contamination as revealed by the significant level of recurrently detected coliform bacterial [36]. The concentration of *V. parahaemolyticus* was higher in shrimp samples than in water samples in the work reported by Pilakka and Ranjeet [37]. However, the reverse was the case in this study which could as a result of the enumeration of the entire vibrios and difference in methodology. Shrimps that are caught from rivers for human consumption are infected with several types of microorganisms due to many factors related to the environment especially polluted water which enhance microbial proliferation. In addition, methods of catch, onboard handling of the fish and vessel sanitation processing and storage condition play a significant role in the health status of shrimps.

4. Conclusion

This study offers insights into the effects of anthropogenic activities on the water quality and bacterial diversity of the river studied. Contamination of shrimps with coliform bacteria may give a clear indication of poor sanitary condition exposure of shrimps during harvesting till it reaches the markets resulting in both public health hazard and economic losses. The most significant risk to human health is as a result of consumption of raw, undercooked /insufficiently processed shrimp or shrimp products. Shrimp are exposed to the risk of

contamination with different pathogens from polluted water environment and during harvest, transportation and marketing. Such contamination may render these shrimps unfit for consumption perhaps even harmful to humans that consume them. *Vibrio* and *Salmonella* pathogens associated with freshwater shrimps in the southern region of Nigeria is a risk for consumer health.

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