Advances in
Biotechnology & Microbiology
ISSN 2474-7637

Juniper Publishers
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Research Article
Volume 8 Issue 3 - February 2018
DOI: 10.19080/AIBM.2018.08.555740

Adv Biotech & Micro

Quantitative Microbial Risk Assessment (Qmra) of Groundwater in Abonnema Community in Kalabari Kingdom, Rivers State, Nigeria

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Submission: November 16, 2017**; Published:** February 28, 2018

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Abstract

Groundwater aquifer pollution is of great public health concern due to the severity of the disease and infections associated with it. In this study, forty (40) groundwater samples were collected from five(5) different geo-referenced locations in Abonnema town in Rivers State, covering the rainy and dry seasons. Microbiological techniques for the water samples included MPN analyses, determination of pathogenic bacteria, total heterotrophic bacterial and fungal count. The result obtained was compared to the WHO and the Standards Organization of Nigeria (SON) values for drinking water. The water quality index (WQI), the disease burden (db) of the pathogens isolated and the Disability-Adjusted-Life Years(DALY) of each groundwater source were determined to ascertain the risk level of the groundwater in Abonnema.

The result obtained showed that the well samples gave high total coliform count (W2- 368/2200MPN/100ml), faecal coliform count (W1-36/43MPN/100ml), Vibrio count (W2-5E02/8E02Cfu/ml), *Salmonella* count (6E02/3E03Cfu/ml) and total fungal count (1.65E05/5.0E04Cfu/ml), while the boreholes gave high total heterotrophic bacterial count (2.4E06/3.23E06Cfu/ml). The QMRA as DALY showed that the well sources had high value (6.43E06/3.10E06) of the disease burden and probability of illness; the water quality index (WQI) rating for BH1 was medium (>50), all other sampled sources were rated as bad (<50). This work has provided a risk-based novel base-line data of some microbiological parameters such as total coliform, faecal coliform, *Salmonella sp., Vibrio* sp, total heterotrophic bacterial count (THBC) and total fungal count (TFC) for groundwater sources in Abonnema. The risk-based water quality rating revealed that the rural water sources require treatment for public health safety, human development and sustainability.

Keywords: QMRA; Disease burden; Ground water; Risk; Water quality index

Introduction

Groundwater is the water that is found in the aquifer, deep in empty spaces, in soils and cracks of rock under the ground. The value of groundwater lies not only in its widespread occurrence and availability but also in its consistent quality, which makes it an ideal source for different purposes especially for drinking [1]. Water is the most important basic element in nature and is essential for life sustenance. Adequate water supply is chemically, physically and microbiologically palatable and wholesome necessary for man's progress and health benefits [2]. In many parts of the world, the most important single source of water for the purpose of drinking is groundwater sources, used particularly in areas with limited or polluted surface water sources. In Nigeria, majority of the rural populace do not have access to potable water and therefore, depend on well, stream and river water for domestic use.

Abonnema is one of the major communities in Kalabari Kingdom experiencing rapid economic growth. But civilization and development had led to the construction of more modern toilet facilities and drilling of bore holes as source of water for domestic use within the area (Abonnema foundation.org). The land mass of the community is very small and definitely warrant some level of environmental pollution which is an important consideration that must not be overlooked. When siting these ground water sources as a result of the diverse health problems to the rural dwellers the location where it will be sited and its environs must be considered, due to possible organic (faecal) and inorganic pollution. These factors give rise to diverse health implications, thereby transmitting a lot of diseases and infection to their end users. These infectious diseases are transmitted by faecal-oral route, makes about one-sixth of the populace sick and kill a lot.

In developing countries, most sickness which affects humanity is lack of safe, clean, portable and wholesome water supply.

The bacterial qualities of groundwater, pipe borne water and other natural water supplies in Nigeria, meant to be ideal for consumption has been reported unsatisfactory, with coliform

counts exceeding the recommendation limits by WHO [3-5]. The reasons for elucidating certain important water quality assessment parameters may be attributed to the fact that such parameters should not be ignored [6]. Contaminated municipal water supply with faecal material must be seriously monitored quantitatively and routinely because of the inhabitants of the area and severity of health implications involved [7]. Water intended for drinking must not contain agents of waterborne diseases. The guideline for bacteriological quality is that *Escherichia coli* must not be detectable in any 100ml sample of water intended for drinking [8].

The incidence of water-related illness in Africa has escalated in recent times, according to World Health Organization, an estimated 4 billion cases of diarrhea and 2.2 million deaths occur annually. The major cause of these diseases can be implicated by consumption of unsafe water, due to the gradual deterioration of water quality resulting from the increase in human population and urbanization leading to severe water pollution. The bacterial qualities of groundwater, pipe borne water and other natural water supplies in Nigeria, have been reported to be unsatisfactory To create awareness on the need to properly treat water for consumption in the community to promote human health sustainability.Investigation of the ground water status in the rural community which is one of the main achievements of the Millennium Development Goal (MDG), assessing the potablity of the water that is free from disease producing microorganisms chemical substances that is dangerous to health and the risk associated with their consumption.

Materials and Method

Study area

Geographical description of sampled area: Abonnema is the capital of Akuku Toru Local Government Area, Rivers State, Nigeria, located within longitude 6° 46′10′′E and 6° 46′40′′E and latitude 4° 43′20′′N and 4°44′25′′N. The Community has a population of about 68,591 people, is about 67.4km away from Port Harcourt, the Capital city of Rivers State. It has a population of 68 591 people as one of the most populated communities in Kalabari kingdom. The time zone is given as; sunrise at 06:46 and sunset at 18:46. The weather exhibits a light thunderstorm rain, while the temperature is 22 °C/72 °F. The area experiences a North West wind of about 5.8km/h with a cloud which is broken at 500ft and a Cumulonimbus at 2000ft (Figure 1).

Water sample collection: The sampling was carried out during the raining (Aug-Oct) and dry season (Nov-Jan.), with a view to check the essence for seasonal variations of organic and inorganic pollutants in ground water in the area. Areas sampled include, Bob Manuel compound, George-will/Grandville compound and Briggs Compound. A total of 40 samples (two wells and three boreholes) were asceptically collected randomly from five different locations in Abonnema community using sterile sampling bottles and well covered in black cellophane bags. Environmentally unstable parameters such as pH and temperature were analysed in-situ. Results of such analysis were recorded in the field logbook and the samples were transported immediately to the laboratory for analyses in an ice packed cooler (Figure 2).

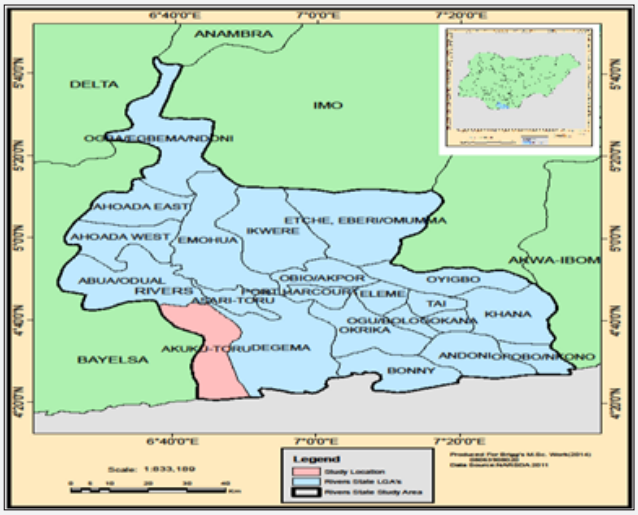

Figure 1: Rivers State Showing Study Location.

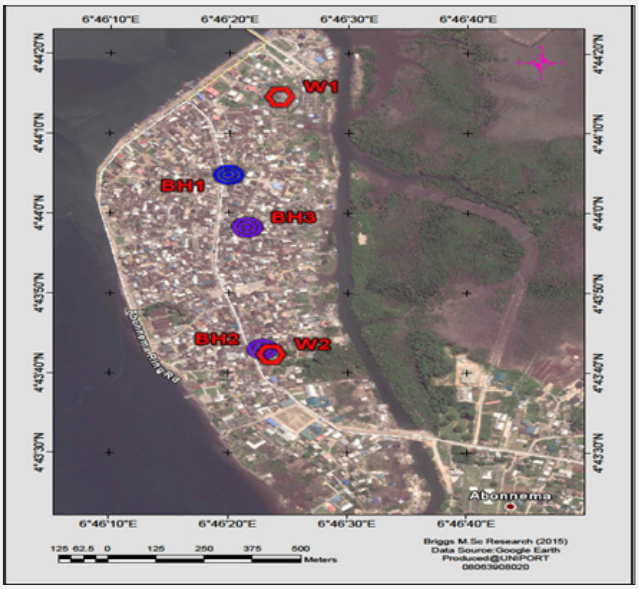

Figure 2: Abonnema Community Showing Sampled Boreholes & Wells.

Microbiological analysis

Estimation of total heterotrophic bacteria, fungi and their characterization

The spread plate method [9] was adopted for determination of total heterotrophic bacteria and fungi. About 0.1ml dilutions of the groundwater samples were aseptically transferred into sterile plates of standard plate count agar (SPCA) and potatoes dextrose agar (PDA). The inoculum was spread evenly on the surface of the agar plates in duplicates with a sterile glass spreader and allowed to dry. The plates were incubated at 37

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°C for 18 to 24h and observed for bacterial colonies; plates yielding 30-300 colonies were recorded and expressed as colony forming units per milliliter (cfu/ml). To sterile plates of potatoes dextrose agar, 0.1ml of the appropriate dilutions of the water samples were inoculated, incubated at 28+1 °C for 5-7 days and the fungal colonies were enumerated.

After incubation, culture plates were counted (Total Viable Count (TVC) and results calculated thus:

$$Cfu/ml = \frac{TVC\ x\ dilution\ factor}{Inoculum\ volume}$$

Isolation and identification of bacterial isolates from water samples was based on their cultural characteristics, cell morphology and biochemical reactions.

Enumeration of total coliform

The most probable Number (MPN) also called the multiple tube fermentation technique was used for coliform enumeration [2], of the water samples. The MPN procedures employed in this research are the Presumptive test, the Confirmed test and the Completed test as the last stage of the whole process.

Calculation of the Water Quality Index (WQI)

The National Sanitation Foundation Water Quality Index (WQI) procedure according to Brown [10] was used to calculate the WQI. Eight parameters analysed were considered for calculation of nitrate, pH, temperature, dissolved Oxygen, Biological Oxygen demand, turbidity and total dissolved solids based on the WQI proposed by NSF following the algorithm as given below:

Step 1:- Calculate the water quality parameter value.

Step 2:-Calculate quality value (Q value) from the value function graph using a calculator (http://www.water-research.net/waterqualityindex/index.html) for each parameter.

Step 3:- Multiply the Q value by weight factor to get the parameter sub-index. The arithmetic mean of the data was used to calculate the WQI. According to Heathcote [11], geometric mean is used for faecal coliform only.

Step 4:- Compute the WQI from the sub-index and weight factor by dividing the sum of the sub-index of parameters by the sum of weight factors for these paramaters

As $\Sigma(W_t)i = 1$ $\qquad W_i = \frac{(W_i)I}{\Sigma(W_t)i}$

For calculating WQI, the sub-index (SI) is first determined for each parameter given as $(SI)_i = q_i W_i$

Therefore $WQ_I = \sum q_i W_i$ as $\sum W_i = 1$

Quantitative Microbial Risk Assessment (QMRA) and Determination of the Disease Burden of the Ground Water Sources

The key component in undertaking a quantitative microbial risk assessment (QMRA) of pathogens is to define what level of disease burden could be ascribed to the specific agent, as expressed in Disability-Adjusted Life Years (DALY) [3]. The subsections below show how the disease burden for each of the identified pathogen was calculated. The disease burdens that result provide an indication of the burden associated with each pathogen and on the overall range of impacts expected across a population group.

The estimate of the years of life lost (YLL) from premature death (the mortality fraction) and years impaired (the morbidity fraction) for each pathogen was calculated using the average expectancy at birth for Nigeria, 52 years [12]. The mortality burden was based on average age of 2.5 years. DALYs are applied once pathogen numbers, dose response and exposures are determined; that is after completion of a QMRA.

Four (4) steps for QMRA [13];

1. Hazard identification: this involves using a reference pathogen hazard (pathogen numbers).
2. Dose response: establish the relationship between the dose of the reference pathogen and the likelihood of illness.
3. Exposure assessment: identify the population exposed to the hazard and the pathway, quantity and duration of exposure. This step includes assessment of the intended volume of water consumed.
4. Risk characterization: calculate the DALYs to determine if the water sources is of an acceptable risk.

Escherichia coli

Watery diarrhea and bloody diarrhea, the proportion of symptomatic cases was 53% and 47% respectively [14]. For this assessment, the severity weights for the different outcomes were taken from Havelaar & Melse [14]. The duration of watery and bloody diarrhea was 3.4 and 5.6 days respectively.

Vibrio spp.

Table 1: Simplified Risk Assessment Procedure (Adapted from WHO, 2004).

Escherichia coli		*Vibrio sp.*	*Salmonella sp.*
Raw Water Quality,	From Sampling		
Organism Per Litre			
Treatment Effect (PT)	Estimated(Calculated) removal of pathogen		

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Drinking Water Quality (CD)	CR X (1-PT)		
Consumption of unheated drin drinking water (V)	WHO (2003)		
ExExposure by drinking water, org organism per litre (€)	C_DX V		
Dose response (r)	Rose & Gerba [18], Haas et al. [19]	FAO/WHO (2005)	Regli et al. [20]
Risk of infection per day($P_{inf.d}$)		E X r	
Riskof infection per year ($P_{inf.y}$)		$P_{inf.Y}$ X 365	
Risk of diarrhoea disease given (P I ($P_{ll/inf}$)	Haas et al. [21]	Adagbada et al. [16]	Akinyemi et al. [17]
Risk of Diarrhoea disease (P_{ill})		($P_{inf.Y}$) X ($P_{ill/inf}$)	
Disease burden (db)			
Susceptibility fraction (fs)		From Study Area	
Disease Burden (DB)		P_{ill} X db X fs	

Vibrio sp.

Mild diarrhea and severe diarrhea, the proportion of symptomatic cases was 80% and 20% respectively (WHO, 2012). Adagbada (2012) reported a mortality rate of 4.1% cholera outbreak in Rivers State. The severity weights for the different outcomes were taken from Havelaar & Melse (2003). The duration of mild and severe diarrhea was 3 days and 15 days respectively (Table 1).

Salmonella sp.

The outcomes, gastroenteritis (64%), typhoid fever (35.5%), mortality rate for gastroenteritis (0.76%) and typhoid fever (0.26%) were reported by Akinyemi et al. (2012). The severity weights for the different outcomes were taken from Havelaar & Melse (2003). The duration of mild and severe diarrhea is 5 days each [5].

Statistical analysis

The analysis of variance (ANOVA) was used to compare the values of the results of the physicochemical analysis of the borehole water and well water samples to test for the level significance. The result of the total heterotrophic bacteria (THB) and coliform count between the bore hole a and well water samples from the different locations in the community was also compared using the same method.

Statistical analysis

The analysis of variance (ANOVA) was used to compare the values of the results of the physicochemical analysis of the borehole water and well water samples to test for the level significance. The results of the total heterotrophic bacteria (THB) and coliform count between the boreholes and well water samples from the different locations in the community was also compared using the same method to test for the level significance

Result

The results of the microbial analyses of the groundwater samples from Abonnema Town is given as follows (Figure 3-8):

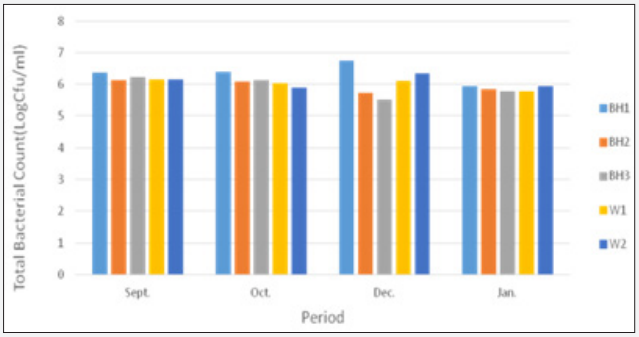

Figure 3 : Total Heterotrophic Bacterial Count.

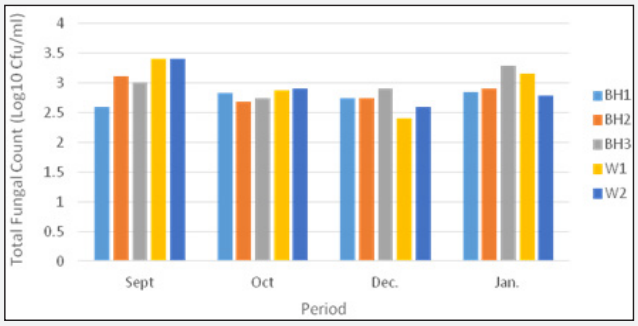

Figure 4 : Total Fungal Count.

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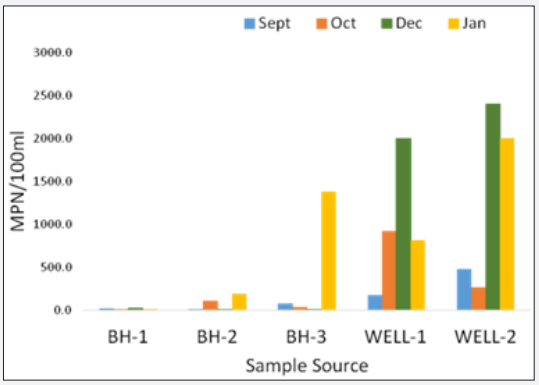

Figure 5 : Most Probable Number (MPN).

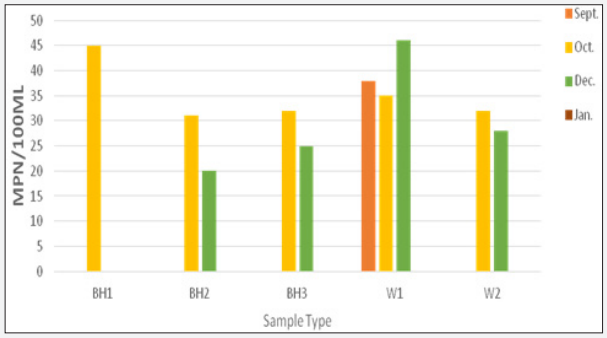

Figure 6 : Faecal Coliform Count.

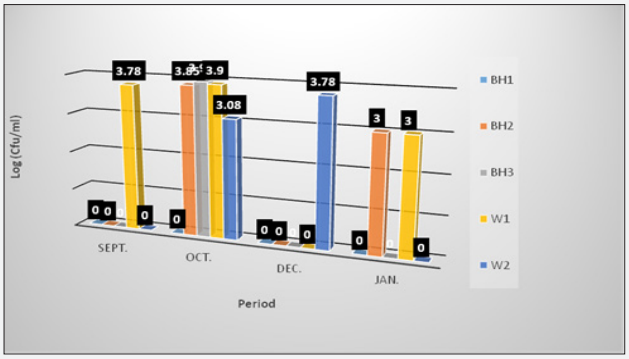

Figure 7 : *Salmonella* Count of Abonnema Community Ground-Water Sources.

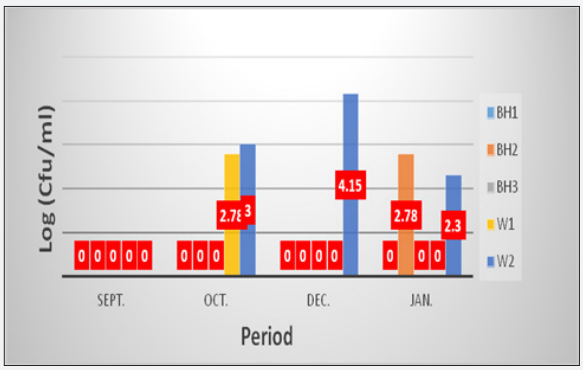

Figure 8 : *Vibrio* Count of Abonnema Community Ground-Water Sources.

Severity, duration and disease burden for pathogens of concern

The tables for the severity rate, duration and disease burden (Disability-Adjusted Life Years, DALY) of *Escherichia coli, Vibrio* and *Salmonella species* are given below. The results show that the disease burden per case for *Escherichia coli* is 0.35 DALY, for *Vibrio sp.* is 2.07 DALY while for *Salmonella sp.* is 0.53 DALY respectively (Table 2-9).

Table 2: Water Quality Indices (WQI) of Rural Water Sources.

Water Station	WQI Value	Classification
BH1	53	Medium
BH2	45	Bad
BH3	42	Bad
W1	46	Bad
W2	48	Bad

Table 3: Water Quality Index Legend.

Range	Quality
90-100	Excellent
70-90	Good
50-70	Medium
25-50	Bad
0-25	Very bad

Table 4: Seasonal Water Quality Index (WQI) of each water sources.

Water Station	Season	WQI Value	Classification
BH1	Wet	52.67	Medium
	Dry	53.62	Medium
BH2	Wet	47.23	Bad
	Dry	43.41	Bad
BH3	Wet	42.79	Bad
	Dry	41.92	Bad
W1	Wet	45.64	Bad
	Dry	45.86	Bad
W2	Wet	49	Bad
	Dry	46.78	Bad

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Table 5: Severity, duration and disease burden for pathogens of concern.

Pathogen	Outcomes	Severity	Duration	Disease Burden (DALY)
Escherichia coli	Watery diarrhoea	0.067	3.4 days	0.0006
	Bloody diarrhoea	0.39	5.6 days	0.006
	Death from diarrhoea	1	50.5 years	50.5
Vibrio sp.	Mild diarrhoea	0.067	3 days	0.0005
	Severe diarrhoea	0.23	5 days	0.0029
	Death from diarrhoea	1	50.5 years	50.5
Salmonella spp.	Gastroenteritis	0.23	5 days	0.0029
	Death from Gastroenteritis	1	52 years	52
	Typhoid fever	0.23	5 days	0.0029
	Death from Typhoid fever	1	52 years	52

Table 6: Disease burden for pathogens.

Pathogen	Outcomes	Disease Burden Per 1000 Symptomatic Cases		Disease Burden (DALY)
Escherichia coli	Watery diarrhoea	1000 X 53% (Watery diarrhoea) X 0.067 X 0.009	=	0.3
	Bloody diarrhoea	1000 X 47% (bloody diarrhoea) X 0.39 X 0.015	=	2.8
	Death from diarrhoea	1000 X 0.7% (death)X 50.5	=	353.5
	Total diarrhoea only		=	356.6
Vibrio spp.	Mild diarrhoea	1000 X 80% (mild diarrhoea) X 0.067 X 0.008	=	0.43
	Severe diarrhoea	1000 X 20% (severe diarrhoea) X 0.23 X 0.013	=	0.59
	Death from diarrhoea	1000 X 4.1% X 50.5	=	2070.5
	Total Death from diarrhoea only		=	2071.52
Salmonella spp.	Gastroenteritis	1000 X 64% X 0.23 X 0.013	=	1.93
	Death from Gastroenteritis	1000 X 0.76% x 52	=	395.2
	Total Death from Gastroenteritis		=	397.13
	Typhoid fever	1000 X 35.5% X 0.23 X 0.013	=	1.06
	Death from Typhoid fever	1000 X 0.26% X 52	=	135.2
	Total Deaths from Typhoid fever		=	136.26
	Total(Gastroenteritis and Typhoid fever)			532.33

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Table 7: Simplified Risk Assessment for *Escherichia coli*.

Assessment Model	BH1	BH2	BH3	W1	W2
Raw Water Quality, Organism Per Litre	11.25	21.5	23.5	39.75	28.75
Treatment Effect (PT)	0	0	0	0	0
Drinking Water Quality (CD)	1.13E+01	2.15E+01	2.35E+01	3.98E+01	2.88E+01
Consumption of unheated drinking water (V)	1	1	1	1	1
Exposure by drinking water, organism per litre (E)	1.13E+01	2.15E+01	2.35E+01	3.98E+01	2.88E+01
Dose response (r)	1.00E-03	1.00E-03	1.00E-03	1.00E-03	1.00E-03
Risk of infection per day ($P_{inf,d}$) (E X r)	1.13E-02	2.15E-02	2.35E-02	3.98E-02	2.88E-02
Risk of infection per year	4.106	7.848	8.578	14.509	10.494
Risk of diarrhoea disease given (P_{ill}/inf)	0.25	0.25	0.25	0.25	0.25
Risk of Diarrhoea disease (Pill)	1.0265	1.962	2.144	3.627	2.623
Disease burden (db)	0.35	0.35	0.35	0.35	0.35
Susceptibility fraction (f_s)	1	1	1	1	1
Disease Burden (DB)	0.359	0.687	0.75	1.269	0.918

Table 8: Simplified Risk Assessment for *Vibrio* sp.

Assessment Model	BH1	BH2	BH3	W1	W2
Raw Water Quality, Organism Per Litre	0	150	0	150	650
Treatment Effect (PT)	0	0	0	0	0
Drinking Water Quality (CD)	0	150	0	150	650
Consumption of unheated drinking water (V)	1	1	1	1	1
Exposure by drinking water, organism per litre (E)	0	150	0	150	650
Dose response (r)	1.00E+06	1.00E+06	1.00E+06	1.00E+06	1.00E+06
Risk of infection per day (Pinf,d) (E X r)	0	1.50E+08	0	1.50E+08	6.50E+08
Risk of infection per year	0	5.48E+08	0	5.48E+10	2.37E+11
Risk of diarrhoea disease given (P ill/inf)	1.31E-05	1.31E-05	1.31E-05	1.31E-05	1.31E-05
Risk of Diarrhoea disease (Pill)	0	7.17E+05	0	7.17E+05	3.11E+06
Disease burden (db)	2.07	2.07	2.07	2.07	2.07
Susceptibility fraction (fs)	1	1	1	1	1
Disease Burden (DB)	0	1.49E+06	0	1.49E+06	6.43E+06

Table 9: Simplified Risk Assessment for *Salmonella* sp.

Assessment Model	BH1	BH2	BH3	W1	W2
Raw Water Quality, Organism Per Litre	0	2000	2250	3750	1800
Treatment Effect (PT)	0	0	0	0	0
Drinking Water Quality (CD)	0	2000	2250	3750	1800
Consumption of unheated drinking water (V)	1	1	1	1	1
Exposure by drinking water, organism per litre (E)	0	2000	2250	3750	1800
Dose response (r)	2.36E+05	2.36E+05	2.36E+05	2.36E+05	2.36E+05
Risk of infection per day (Pinf,d) (E X r)	0	4.72E+08	5.31E+08	8.85E+08	4.25E+08
Risk of infection per year	0	1.72E+11	1.94E+11	3.23E+11	1.55E+11
Risk of diarrhoea disease given (P ill/inf)	4.50E-04	4.50E-04	4.50E-04	4.50E-04	4.50E-04
Risk of Diarrhoea disease (Pill)	0	7.74E+07	8.73E+07	1.45E+08	6.97E+07
Disease burden (db)	0.53	0.53	0.53	0.53	0.53
Susceptibility fraction (fs)	1	1	1	1	1
Disease Burden (DB)	0	4.10E+07	4.63E+07	7.69E+07	3.69E+07

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Discussion

Total heterotrophic bacteria count (THBC)

The total heterotrophic bacterial count varied from $2.35x10^9/2.45x10^9$Cfu/ml-$5.58x10^9/8.8x10^8$Cfu/ml for Bob Manuel (BH1). That for Briggs' compound (BH2) ranged from $1.36x10^9/1.2x10^9$Cfu/ml-$5.2x\ 10^8/7.3x10^8$Cfu/ml. That of George-will (BH3) ranged from $1.7x10^9/1.35x10^9$Cfu/ml-$3.35x10^8/5.85x\ 10^8$Cfu/ml. On the other hand for Bob Manuel's compound (Well 1), the total bacterial count ranged from $1.43x10^9/1.1x10^9$Cfu/ml-$1.31x10^9/5.85x10^8$ Cfu/ml, and that of Briggs' compound (Well 2) ranged from $1.43x10^9/7.85x10^8$Cfu/ml-$2.29x10^9/8.85x10^8$Cfu/ml. BH1 and W2 gave the highest count of the average bacterial count estimated in the dry season in this research.

Total fungi count (TFC)

The total fungi count for the samples treated for the two seasons varied from $4.0\ x10^7/6.75\ x10^7$Cfu/ml to $5.50x10^7/7.0x10^7$Cfu/ml for BH1, $1.3x10^7/4.75x10^7$Cfu/ml to $5.50x10^7/8.0x10^7$Cfu/ml for BH2, $7.0x\ 10^8/5.55x10^7$Cfu/ml to $8.0x10^7/1.9x10^8$Cfu/ml for BH3, $2.6x10^8/7.51x10^7$Cfu/ml to $2.50x10^7/1.4x\ 10^8$Cfu/ml for Well1 and $2.50x10^8/8.05x10^7$Cfu/ml to $4.0x10^7/6.0x10^7$Cfu/ml for Well2. The total Fungi count was highest for BH3 followed by W1 in the two seasons and least for BH1 and Well2.

Coliform analysis

Total coliform count: The MPN index was highest for the well samples for all the period of sampling but BH3 (1375MPN/100ml) was higher than W1 (815MPN/100ml) for the month of January, 2015. BH1 (<30MPN/100ml) gave very negligible MPN index in comparison with all the bore-holes. The coli form bacteria are best or the primary indicators of faecal pollution in water [22,23].

Faecal coliform count

The faecal coliform count was 0MPN/100ml for BH1 in September, December and January but 45MPN/100ml in October. This indicates less faecal pollution of that water supply. BH2 had faecal coliform count of 35MPN/100ml in January as the highest for the period of study and this indicates that the water supply is not safe for consumption. BH3 had 37MPN/100ml in January also as the highest value for the study period. The Well samples produced the highest values of 46MPN/100ml (Dec.) and 40MPN/100ml (Jan.) for W1 and 55MPN/100ml (Jan.) for W2. The Faecal coliform count was least in the Wet season and highest in the dry season. *Milkiyas et al*. [24] recorded a total coliform count of up to 35 MPN/100ml in tap and domestic water. The presence of faecal coliforms suggests the presence of human faecal contamination of these water sources which infers that treatment options should be considered for public health safety.

Coliform bacteria

The coliform bacteria are best and are the primary indicators of faecal pollution in water [22,23]. The results obtained reveal that it is very possible that the well sources had a poor sanitary condition than the boreholes. Coliform bacteria are widely found in nature and do not necessarily indicate faecal pollution [25]. The presence of coliform, however indicates the possibility of the presence of pathogenic microorganisms and further suggests the possibility of sewage contamination of the water source [26]. High coliform counts appear to be characteristic of rural ground water quality in Nigeria [27]. Certain *Enterobacteria* were repeatedly isolated compared to other genera found in the Groundwater Samples. Genera such as *Aeromonas, Vibrio* and *Salmonella* were less isolated as they had relatively negligible frequency of occurrence in the groundwater samples treated.

Escherichia coli was isolated once in the month of December in **BH1**, *Citrobacter diversus* (September), *Hafnia alvei* and *Enterobacter aerogenes* were isolated once in January. No *Salmonella* sp., *Shigella* sp., *Vibrio* sp. And *Klebsiella pneumonia* were isolated from the sample source **(BH1)** for the period sampled, but *Edwardsiella tarda* and *Aeromonas hydrophila* were found in October and *Serratia rubidoea* was found in January. In **BH2**, *Escherichia coli* was found in January, *Hafnia alvei* in October and January, *Salmonella* (October), *Vibrio* (January (2)), *Citrobacter diversus* (September), *Edwardsiella tarda* (October) and *Serratia marcenscens* (October and January). For **BH3**, *Escherichia coli* was found in January, *Hafnia alvei* in October and January, *Salmonella* sp. (October). No *Vibrio* sp. and *Shigella* sp. were found. For W1 there was Escherichia coli present in the month of September, October and January but none in December. *Citrobacter diversus* occurred in the month of December and January. No *Hafnia alvei* was found for all the period sampled. *Vibrio* spp. was found only in October for the two sampled period. There was *Salmonella* found. For Well2, *Escherichia coli* were found in September and January (2). There was *Citrobacter freundeii* in October, while *Hafnia alvei* was found in December. *Salmonella* was found in October, *Shigella* (none). There was *Vibrio* sp. in October and December. *Serratia liquefaciens* was found in January. This indicates that the occurrence of microorganisms in the water supplies is based on changing or differing intrinsic environmental condition.

The production of green metallic sheen (GMS) by coliforms such *Escherichia coli* is not peculiar to *E. coli* in that there were some isolates which did not produced GMS (BH2 (1)) (Jan) and was identified as *E coli*, but at the same time there were some isolates that produced GMS but the biochemical identification test(s) did not correspond to *E coli*. One of the isolates was identified to *Citrobacter diversus* (this was very amazing). The rate at which *E. coli* was identified was very low compared with the total coliform bacteria. This indicates less faecal pollution of the water supplies. The production of GMS depends on certain

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factors such as environmental conditions, percentage nutrient concentration and the nature of the strains (species) present in the water. Another school of thought for this possible deviation from the norm could be that a completely new or different strain of *E. coli* exists which lack the property (production of GMS).

Variation in gas production by coliforms: In the completed test, coliforms ferment (utilize) lactose sugar to produce acid and gas. This characteristic property varied greatly among coliform bacteria. For instance, *Citrobacter freundeii* was noted to produce more gas than *Enterobacter intermedius*. This indicates a variation in the ability of these microorganisms to utilize the sugar as their sole carbon source.

Reliability

To ascertain the reliability of the biochemical test(s), controls were provided as a guide in the experimental work. The control result served as an indicative proof of 90% reliability of the identification test (s) conducted.

Pathogenic bacteria

The result of plating the groundwater samples on *Salmonella-Shigella* agar gave about 40% of isolates of the genera *Salmonella* and *Shigella*. However, very negligible colony count was recorded for all sample type for the two seasons. Findings from plate readings and biochemical tests indicated the occurrence of Salmonella in the rural water supplies mostly for Bore-hole2, Bore hole3, Well1 and Well2. Bore hole1 showed no presence of Salmonella in the samples treated for the period of study. For all the samples in the two seasons there was less than 10% occurrence of Vibrio sp in the rural water supplies. *Vibrio* sp. found was suspected to be *Vibrio natriegens, Vibrio harveyi, Vibrio anguillarum, Vibrio furnissii* and *Vibrio cholerae*. The reliability of this result can be substantiated with the fact that there had been no reported case(s) of Cholera outbreak in the rural area caused by the Cholera bacteria Vibrio cholerae. Plates that indicated microbial growth had very negligible colony count for all samples treated in this research. However, an interesting microorganism isolated to note is *Hafnia alvei* known to be part of the human gut flora. Once thought to be a simple commensal of the gastrointestinal tract, there is increasing evidence to suggest *H. alvei* is a rare but significant bacterium that may contribute to opportunistic infections in humans [28]. Bacteraemia and respiratory tract infections are the leading extra intestinal manifestations of *H. alvei* as a pathogen [28].

Water and health: Much of the poor health in developing countries is largely due to lack of safe drinking water [16-18]. According to the World Health Organization, about 600 million cases of diarrhoea and 46,000,000 childhood deaths are reported per year because of contaminated water and lack of sanitation [30]. Surveillance of water quality to ensure safety is a vital public health function especially in developing countries [31]. Water-related disease remains one of the major health concerns in the world. Diarrhoeal diseases, which are largely derived from poor water and sanitation account for 2.4 million deaths each year and contribute over 73 million Disability Adjusted Life Years per annum [32]. On a global scale, diarrhoeal disease is placed sixth highest cause of mortality and third in the list of morbidity and it is estimated that 5.7% of the global disease burden is derived from poor water, sanitation and hygiene[33].

There was significant difference in the BOD for BH1, BH3 and W1 and there was significant difference in the pH for BH3 and W2. This work has provided a risk-based base-line data of some microbiological parameters such as total coliform, faecal coliform, *Salmonella sp., Vibrio sp.*, total heterotrophic bacterial count (THBC) and total fungal count (TFC) and physicochemical parameters for groundwater sources in Abonnema. The risk-based water quality rating revealed that the rural water sources require treatment for public health safety and human development of the community.

Conclusion

The result of this work has provided a novel base-line data of microbiological parameters such as total coliform, faecal coliform, *Salmonella sp., Shigella sp., Vibrio sp.*, total heterotrophic bacterial count (THBC) and total fungal count (TFC) for bore holes and well water sources in Abonnema community in Kalabari Kingdom, Rivers State. The results obtained showed there is need to improve our rural water supplies considering the total and faecal coliform counts and the water quality index for some of the rural water supplies, and this calls for necessary intervention in providing durable treatment measures for public health safety.

Safe Water

Water for human consumption should be free potentially harmful microorganisms for public health safety [21]. Safe water for the rural dwellers should be a major focus in grass root development initiative implementation. Almost one tenth of the global disease burden could be prevented by improving water supply, sanitation, hygiene and management of water resources. Better management of water resources to reduce the transmission of vector-borne diseases (such as viral diseases carried by mosquitoes) and to make water bodies safe for recreational and other users can save many lives and has extensive direct and indirect economic benefits, from the micro-level of households to the macro-perspective of national economies. The global importance of water, sanitation and hygiene for development, poverty reduction and health is reflected in the United Nations Millennium Declaration, in particular, its eight Millennium Development Goals, in the reports of the United Nations Commission on Sustainable Development and at many international fora [34].

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DOI: 10.19080/AIBM.2018.08.555740

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