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Microbiological and Biophysiological Examination of Spring Water Sources From Orlu, Imo State

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Abstract
Biophysical quality of "Ngwu", " Iyi Okwu" and "Oyi Ogidi" spring water sources in Orlu zone, Imo State Nigeria was studied. Water samples were collected from these sources using sterile plastic containers during dry and rainy seasons. Samples were transported to the laboratory immediately after collection for analysis using standard microbiological methods. These samples were analysed for total heterotrophic bacterial count, total coliform count, total fungi count, total salmonella, shigella count and total vibrio count using membrane filtration method. Additionally, samples were analysed for physicochemical parameters using standard methods. Results showed that during the raining season, total heterotrophic bacteria, total coliform, total faecal coliform, total *salmonella / shigella* and total *vibrio* counts range respectively between 84-145 cells /100ml, 13-41cells/100ml, 5-8cells /100ml, 3-6cells/100ml and 1-2cells/100ml. during dry season, counts of 39-81cells/100ml, 7-13cells/100ml, 4-8cells/100ml, 15-32cells/100ml and 2-7cells/100ml were respectively obtained for total heterotrophic bacterial, total coliform, total feacal coliform, total *salmonella / shigella* and total *vibrio*. Bacterial identification test revealed presence of the following bacteria general "Ngwu" source both during raining and dry seasons; *Escherichia coli*, *Pseudomonas species*, *salmonella sp*, *enterobacter sp*. And *vibrio sp*. Similar bacteria general were isolated "Iyi Okwu" and "Iyi Ogidi" during both seasons except *Enterobacter sp* and *Pseudomonas sp*, respectively. In the same vein, *Aspergillus sp* and *Mucus sp*, are fungal genera isolates from spring water samples analysed during both dry and rainy seasons. Physicochemical analysis revealed that values were within WHO permissible limit. In view of the above results, samples have acceptable physical and chemical qualities but rather require bacteriological treatments before consumption.
Key words; springwater, physicochemical, bacteriological treatments, heterotrophic bacterial, membrane filtration

INTRODUCTION

Water is essential to the existence of all living organisms, but in spite of its importance, it is being threatened by the exponential growth of human population and demand for more water of high quality both for domestic and economic use (Okechukwu *et al.* 2012). Population increase has also resulted in increased pressure on water resources of the

developed and developing countries.(Adekunle *et al*; 2007) These pressure involve the contamination from domestics, industrial and agricultural wastes, direct effects caused by climate change and other ecological disturbances (Saka *et al*. 2013). Population growth pattern suggests that these pressures can only increase without adequate and approximate interventions (Adekunle *et al*; 2007). Without adequate interventions, water borne diseases pandemic or epidemic can happen and increase dramatically (Ford and Colwell, 1996). The major concern of people in developing countries is that of obtaining clean water.

In Africa and Asia, most of the large cities utilize surface water but many millions of people in peri-urban communities and rural areas are dependent on groundwater (Obiri-Danso *et al*. 2009). These age long spring sources have remained untapped by any water processing company, government-powered town water suppliers and yet to be researched upon by Scientists-Microbiologists, Biochemists, Chemists, Geologists, Environmentalists and Biotechnologists alike. There is need therefore, to provide baseline information about these spring sites; "Ngwu, Iyi Okwu and Iyi Ogidi"; upon which redemptive projects can be hinged.

This work will help to confirm and standardize the quality of these water sources. In order to achieve this aim, this work will, enumerate and isolate bacteria present in the spring water and also determine physicochemical quality of the spring water .The recommendation will lead to the development of these areas so as to make potable water easily accessible, bring tourist attraction, revenue generation as well as job creation.

MATERIALS AND METHODS

Study Area

Orlu zone is located on longitude 7.03889^{o}E and latitude 5.79639^{o}N in the South Eastern part of Nigeria. It is in the humid tropical climatic region and is characterised by two distinct rainy and dry seasons. The rainy season is experienced in the months of April to October, while the dry season is experienced in the months of November to March every year.(Adekunle *et al*; 2007)

The Sampling Sites

Ngwu spring water site also known as Iyi Umugara is found in Nkwerre Local Government Area, in Orlu Senatorial Zone of Imo State. The spring water is accessed by

a stairway leading down to the valley of the hill and is tapped directly from the exuding source with a plastic pipe.

Iyi Okwu spring is located in Okwuepe Village, Ebenese Ihioma Autonomous Community in Orlu L.G.A of Imo State. It is at the foot of a hill with a steep slope. It is not built and it drains from a stone forming a pond, from where water is scooped with a cup or bowl. Iyi Ogidi spring is located at Ofeahia Amaifeke Autonomous Community in Orlu L.G.A of Imo State. The water is accessed at the valley of a hill. The environmental condition is very cool in these areas on account of green vegetation and tall trees.

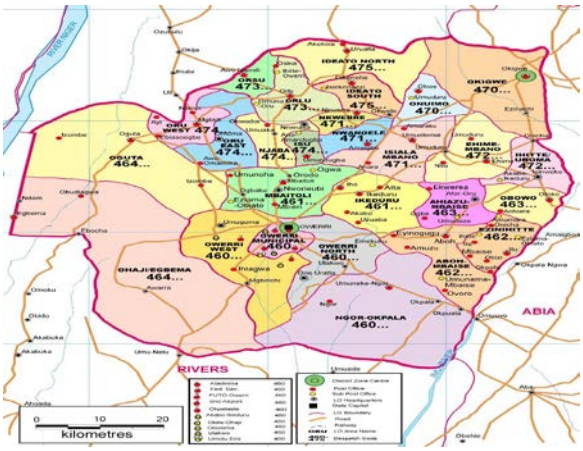

Fig. 2.1 Map of Imo State

Sample Collection

Water samples for the physicochemical and microbiological analysis were aseptically collected in sterile two-litre polyvinyl chloride (PVC) plastic bottles from the point people take water from. The sample bottles were completely filled and tightly closed to prevent loss of dissolved gases. The samples were collected during the dry season in the month of February and during the rainy season, in the month of October 2015, at 9:00am, 12:00pm and 3:00pm respectively. A composite sample was constituted from the triplicate samples. The samples were labeled accordingly with codes SW1 – Ngwu Spring, SW2 – Iyi Okwu Spring and SW3 – Iyi Ogidi Spring, and transported in iced chest to the laboratory for analysis within 24hrs.

Laboratory Analysis

The physical and biological parameters of water are separately determined using standard method of American Public Health Association (APHA, 1998) in order to ascertain the biophysical properties of the water. The following physiochemical analysis was done Temperature, Total Soilds Chemical Oxygen Demand (COD) and Nitrate was determined by cadmium reduction method using H183200 multiparameter bench photometer at a wave length of 525nm. Phosphate was determined by Amino Acid methods using H183200 multiparameter bench photometer at a wavelength of 525mm. Determination of Total Hardness in Water, Determination of Colour of Water Sample, Total alkalinity (carbonate), and Heavy Metal Analysis were determined using Atomic Absorption Spectrophotometer. Protocol and procedures of APHA was adopted in the aforementioned tests.

Microbiological Analysis of Water Sample

Isolation and Enumeration of organism

A measure of 10ml of water sample was diluted in 90ml of sterile distilled water, followed by serial dilution. Then, the serial diluents were aseptically inoculated onto different plates of melted sterile medium after cooling to 45^0C and glass spreader was used to spread the inoculum.

Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS) was used for the selective isolation of Vibrio cholerae and other enteropathogenic vibrios. Salmonella/Shigella Agar (SSA) was used for the isolation of Salmonella and Shigella spp. MacConkey agar was used for enumeration of total coliform Total heterotrophic bacteria was determined on nutrient agar according to procedure described by National Committee for Clinical Laboratory Standards. (2001) as modified by Okonko *et al*; 2008. All plates were prepared in duplicates and inoculated. The plates were thereafter incubated at 37^oC for 24 – 48hours.

Fungi isolation was done on Sabouraud Dextrose Agar. The plates were prepared in duplicates and inoculated. The plates were incubated for 3-5 days at room temperature.

In each case the plates with colonies ranging between 30 and 300 were selected and counted. Colonies were taken from the plates and subcultured onto freshly prepared media for isolation (pure culture) of the respective organism.

Identification of Bacterial Isolates

After the period of incubation, bacterial isolates were identified using colonial and cellular features of isolates, followed by biochemical tests. Tests performed are catalase, methyl red, voges proskauer (Mr-Vp), coagulase, indole, motility, gram stain, etc. as described by Chessbrough 2005.

Identification of Mould Isolates

Moulds were identified macroscopically by considering their cellular morphology and cultural characteristics which include the following: The colonial (cultural) characteristics: size, surface appearance, texture and colour of the colony. The vegetative mycelium: presence or absence of cross-wall (septum) and diameter of hyphae. The sexual and asexual reproduction structures, e.g. sporangia, conidial heads, zygospores and arthrospores.

Isolates were also identified microscopically using the method of National Committee for Clinical Laboratory Standards (2001) With a pair of dissecting needles, a portion of the growth to be examined was picked and placed on a microscope slide in a drop of lactophenol cotton blue and it was teased out (carefully) with a dissecting needle, and it was covered with a cover slip and care was taken to exclude air bubbles. Then the wet preparation was examined microscopically using low power objective (x10) and then with a high power objective (x40) for closer examination.

RESULTS

Bacterial Counts of the Samples Analysed

Bacterial counts of the spring water samples analysed as shown in Table 1 revealed that total heterotrophic bacterial counts ranged from 84cells/100ml – 145cells/100ml, total coliform counts were 13cells/100ml - 41cells/100ml, total faecal coliform counts 5cells/100ml - 8cells/100ml, total *Salmonella/Shigella* counts 3cells/100ml - 6cells/100ml and total *Vibrio counts* 1cells/100ml - 2cells/100ml during the rainy season while during the dry season total heterotrophic bacterial counts ranged from 52cells/100ml - 81cells/100ml, total coliform counts were 1cells/100ml - 13cells/100ml, total faecal coliform counts 4cells/100ml - 8cells/100ml, total *Salmonella/Shigella* counts 15cells/100ml - 32cells/100ml and total *Vibrio counts* 2cells/100ml - 7cells/100ml.

Table 1 Bacterial Count of Spring Water Samples Analysed.

Sample Code	Season	Bacterial Counts (No of cells/100ml of the sample)				
		THBC	TCC	TFC	TSSC	TVC
SW1	**Rainy**	145	13	5	6	2
SW2		84	20	6	3	1
SW3		103	41	8	4	2
	Dry					
SW1		81	10	8	15	2
SW2		39	7	4	32	3
SW3		52	13	8	16	7

Key
THBC =Total Heterotrophic Bacteria Count, TCC =Total Coliform Count, TFCC = Total Faecal Coliform Count, TSSC = Total *Salmonella shigella* Count, TVC = Total Vibrio Count, SW1 = Ngwu Water Sample, SW2 = Iyi Okwu Water Sample, SW3 = Iyi Ogidi Water Sample

Biochemical Identification of the Bacterial Isolates

The Biochemical tests performed on the pure culture of bacteria isolated showed that four genera of bacteria were isolated in all during both seasons viz; *Escherichia coli*, *Pseudomonas* sp, *Salmonella* sp, *Vibrio* sp and *Enterobacter* sp. These are shown in Table 2

Table 2 Result of biochemical analysis

Sampling sites	Period	
	Rain	Dry
Ngwu	*E. coli* *Pseudononas sp.* *Samonella sp.* *Enterococcus sp.*	*E. coli* *Pseudomonas sp.* *Salmonella* *Enterococcus sp*

	Vibro sp.	*Vibro sp.*
Okwu	*E. coli* *Pseudomonas sp.* *Samonella sp.* *Vibro sp.*	*E. coli* *Pseudomonas sp.* *Salmonella sp* *Vibro sp*
Ogidi	*E. coli* *Enterococcus sp.* *Vibro sp.*	*E. coli* *Psudomonas sp.* *Enterococcus sp*

Fungal Count of the Samples Analysed

The fungal count of the spring water samples analysed as shown in Table 3 ranged from 1 – 2cells/100ml in the rainy season and 1 – 3cells/100ml during the dry season.

Table 3 Fungal Count of Spring Water Samples Analysed

Sample Code	Season	TFC (No. of cells/100ml)
SW1	**Rainy**	1
SW2		2
SW3		1
	Dry	
SW1		1
SW2		3
SW3		2

Key
TFC = Total Fungal Count, SW1 = Ngwu Water Sample, SW2 =Iyi Okwu Water Sample, SW3 = Iyi Ogidi Water Sample

Identification of Fungal Isolates

The spring water samples had mould but no yeast. The result of identification of mould isolates is shown in Table 4. From the result, *Aspergillus spp* and *Mucor spp* were isolated from the samples of the three spring sites during both seasons.

Table 4 Identification of Mould Isolates of the Samples from the three spring water sites

S/N	Macroscopic Identification	Microscopic Identification	Probable Organism
R1	Black and raised colonies	Septate hyphae, conidia with elongated hyphae	*Aspergillus spp*
R2	White coloured growth which turned grayish-brown with aging	Non-septate hyphae and spores	*Mucor spp*
D1	Black and raised colonies	Septate hyphae, conidia with elongated hyphae	*Aspergillus spp*
D2	White coloured growth which turned grayish-brown with aging	Non-septate hyphae and spores	*Mucor spp*

Key: R = Rainy season isolates, **D =** Dry season isolates

Physicochemical Parameters of the Samples Analysed

The result of the physiochemical parameters of the water samples analysed during the rainy and dry seasons are shown in Tables 5.

Table 5 Results of Physicochemical Analyses of Samples during Rainy and Dry Seasons

Parameter with Unit	SW1		SW2		SW3		WHO Permissible Limit
	Rainy	Dry	Rainy	Dry	Rainy	Dry	
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Odour	Un	Un	Un	Un	Un	Un	Un
Colour (pcu)	10	10	13	13	16	14	5.0
Temperature (^{o}C)	28.6	29.0	24	25	26.5	7	-
pH	5.8	6.0	6.5	6.4	6.5	6.6	6.5-8.5
EC (µs/cm)	50.6	60.4	148	148	520	520	500-1250
TDS (mg/l)	25.8	30.1	100	99	21.67	21.65	<600
Nitrate(mg/l)	19.8	19.9	8	7.5	23	20	40

COD(mg/l)	2.1	2.8	6.70	6.70	6.57	6.55	15
Total Chloride (mg/l)	18.2	20.0	3	3	48	49	250
Sulphate(mg/l)	5	4	1	1	3.43	3.42	100
Total Alkalinity (mg/l)	232	235	148	147	100	100	500
Total Hardness (mg/l)	0.800	0.800	150	149	15.42	15.43	200
Bi-carbonate (mg/l)	132	133	38	38	270	271	380
Dissolved oxygen (mg/l)	6.6	6.7	4.3	4.4	3.2	3.1	4
Copper(mg/l)	0.365	0.365	0.249	0.249	0.216	0.216	1.0
Iron(mg/l)	0.038	0.038	0.08	0.08	0.04	0.04	0.3
Manganese(mg/l)	0.023	0.023	0.10	0.10	0.12	0.12	0.50
Zinc(mg/l)	0.062	0.062	0.10	0.10	0.03	0.03	0.50
Chromium(mg/l)	0.045	0.045	0.04	0.04	0.033	0.033	0.05
Arsenic(mg/l)	0.000	0.000	0.00	0.00	0.00	0.00	0.10
Mercury(mg/l)	0.034	0.034	0.02	0.02	0.08	0.08	0.05
Lead(mg/l)	0.044	0.044	0.002	0.002	0.02	0.02	0.05
Nickel(mg/l)	0.027	0.027	0.039	0.039	0.039	0.039	0.07

WHO (2011)

Key:

SW1 = Ngwu Water Sample SW2 = Okwu Water Sample

SW3 = Iyi Ogidi Water Sample WHO = World Health Organisation

Un = Unobjectionable

Discussion

This study revealed the microbiological and physicochemical parameters of spring water in Orlu Zone, Imo State. The total bacterial count of the samples analysed during the two sampling periods as shown in Table 1 were generally high, exceeding the limit of 1cell/100ml which is the standard limit for heterotrophic bacterial count for drinking water (EPA, 2002). The high total heterotrophic count is an indication of high organic and dissolved salt in water. The primary sources of these bacteria are animals and human wastes as a result of surface runoff, pasture and other land area where animal waste are deposited.

However, during the rainy season, high heterotrophic bacterial populations were recorded more than the dry season period. This is consistent with the findings of many researchers (Radhika *et al.*, 2004). They attributed the high population of heterotrophic bacteria during the rainy season to discharge and washing of organic matter into water bodies. For spring water as observed in this study, the water table rises during the rainy season, hence more water will flow out from the discharge point, while during the dry season, the ground water table will recede thereby a low rate of flow and hence low level of contamination with organic matter (Nouyang *et al.*, 2009). Also, the samples failed short

of the WHO guidelines for Coliforms in drinking water which is 0cfu/100ml. The samples recorded a high number of counts in coli-form bacteria (Total and Faecal Coliforms) during the rainy season. This is due to discharge of organic matter into the water. The high coliform count indicates that human faecal material contaminated the water due to human activities (Nouyang *et al.* 2009). The contamination of Ngwu of which the water flows from above human height may be due to pit latrines of dwellers around the hill area. Due to inadequate provision of tap water in the area, this water is used for cleaning activities such as bathing, washing of clothes, etc. All these activities lead to the discharge of wastes into the spring water which will culminate in the proliferation of coliforms.

The *Salmonella/Shigella* and *vibrio* count during the sampling period was higher during the dry season. This could be as a result of increased human activities during the dry season at the springs. Taulo *et al.* (2008) in their study revealed that due to frequent human and animal contact with springs, it leaves a negative impact on the microbial counts of springs which fall outside WHO standard. This is in conformity with this study.

The bacterial genera isolated from the samples analysed as shown in Tables 2 indicated that five bacterial genera were isolated which include *Escherichia spp, Pseudomonas spp., Salmonella spp., Enterobacter spp.* and *Vibrio spp.*

These organisms are potential pathogens that cause varying degrees of illness to man. *Escherichia spp* is a well established index of faecal contamination, *Enterobacter spp* isolated from the water is an example of non faecal coliform and can be found in vegetation and soil which serve as a source by which the pathogen enters the water. *Salmonella spp* and *Pseudomonas spp,* though not indicator organisms are also a threat to health status of people that utilise the springs as their only source of water.

Salmonella spp has been implicated in enteric fever and salmonellosis. *Escherichia coli* causes acute gastro-enteritis mostly in infants. *Enterobacter spp* is an important opportunistic pathogen that is responsible for bacteriaemia and urinary tract infection. *Vibrio spp* is responsible for cholera, spread by contaminated water or food ingestion. *Pseudomonas spp* is a significant human pathogen and has been seriously implicated in respiratory infections, urinary tract infection, etc. (Uwaezuoke and Dozie, 2007). The presence of these organisms, as seen in this study, is an indication that the water from the

springs fell short of the standard required for untreated water as stipulated by WHO (2000).

The presence of mould can cause water contamination and serious health hazards. Different kinds of moulds such as *Aspergillus* when present in water, are usually allergic and toxigenic (Hageskal *et al.* 2006). This fungi are not only accountable for the adverse effect on health, but also cause taste and odour problems in drinking water (Dogget, 2000).

The physicochemical parameters of the spring water samples during the rainy and dry seasons are shown in Table 3.7. The temperature of the water samples whose range are; 28.6^{o}C-24^{o}C and 29^{o}C -25^{o}C during the rainy and dry season respectively is believed to have been influenced by the intensity of sunlight.

The pH of the water samples fall within the WHO permissible limit of 6.5-8.5 except for sample SW1 during the rainy season whose pH is slightly acidic. This could be due to wash off from area of the spring containing calcite and dolomite minerals (Paka and Rao, 2000).

The Electrical Conductivity of the water samples was found to be below WHO standard, except for sample SW3 during the both rainy and dry seasons with a value of 520 (μs/cm) which falls within the WHO standard. This suggests that the spring water is not salty (Agatemor and Agatemor, 2010). The total dissolved solid chloride and sulphate, of the samples is low and below the acceptable limits which is a good indication that the sample does not contain elements that could be dangerous to human health.

Higher alkalinity value above 100mg/l has been considered nutritionally rich (Srivastava, 2002). Although the value recorded by the samples during the rainy and dry season are below the WHO permissible limits. On the basis of this, the samples are considered to be eutrophic. This further suggests that organic matter is directly or indirectly introduced into the springs by individuals fetching the water for washing of clothes and other uses. The level of nitrate in the water samples is low generally. The WHO standard is 40mg/l and above this limit, may cause cyanosis disease or blue baby syndrome in infants less than three months (EPA, 2006).

The COD of the samples is low if not, it will contain greater number of microbes. COD measures the capacity of microbes to consume oxygen during the decomposition of

inorganic chemicals such as nitrate and ammonia. However, the COD of the samples are normal. This makes them to be regarded as potable for domestic uses.

The level of bicarbonate in the water supply is low. This makes the water soft water. Bicarbonate has no effect on human health in water, but it can cause hardness problem. The dissolved oxygen during the rainy season for samples SW1 and SW2 are higher than the WHO limits except for SW3 which is slightly low and same thing is applicable during the dry season. This will lead to an increase in the microbial population in the samples. However, all heavy metals analysed during the two sampling periods fall below the WHO limits. There were no significant seasonal variations across the heavy metals. However, during the rainy and dry seasons the level of Lead (0.044) in sample SW1 is closer to the WHO limit (0.05). This may be caused by run-off water from residential area or waste from domestic use of chemicals like paints, etc. This high level of Lead in sample SW1 for the two seasons may cause health problems like cancer, anaemia, etc.

The Copper levels of the samples in the sampling periods were good. The range of Copper during the periods was 0.365 – 0.249. These water samples are almost free of Copper contaminants and this makes them good for drinking and also for domestic uses.

Conclusion

Spring water over time has been regarded as the purest source of water until studies like this began to reveal the true situation. Nevertheless, these natural sources of water, if harnessed, can bring a lot of improvement in the lives of the people that have access to them. With the growing human population, the development and use of natural spring to provide water supply in as many areas that it can be found will go a long way in combating the problem of availability of portable water to the citizens. There is need also to maintain good quality of the supply. This study has shown that the sampled sources have acceptable physical and chemical qualities but require bacteriological treatment before consumption.

The people who don’t have free access to boreholes or pipe-borne water suffer to get water from these sites, especially Iyiokwu and Iyiogidi, due to the steep undeveloped valley and hill they descend and ascend in a bid to get water. This leads to the miserly use of water and consequent spread of diseases. Therefore the sources of the spring waters

should be protected by housing it and passing PVC pipes within the rock/hill cavities and collecting the water into sedimentation tanks, where bacteriological treatment can be done. This will as well, prevent continuous contamination from anthropogenic sources observed in this study.

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