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Impact of seasonal variations on the physicochemical and bacteriological parameters of spring water in Oji river LGA, Enugu State, Nigeria

Elochukwu Chidubem Sunday Okoye[1*], Samuel Chinedu Onuorah[2], Lydia Chidimma Okoye[1], Chioma Felicity Mbonu[2]
[1] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
[2] Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Abstract
This study was conducted to determine the impact of seasonal variations on the physicochemical and bacteriological parameters of spring water samples in Oji River Local Government Area of Enugu State, Nigeria. Water samples were collected in sterile plastic water containers at the point of discharge during the dry and wet seasons and analyzed using the standard methods for the examination of water and wastewater for physicochemical parameters. Bacteriological analyses were carried out using most probable number (MPN) method by multiple tube fermentation technique. The results showed that the colour (7-14.2 TCU); pH (7.18-8.4); temperature (24.6-28.1 ºC); electrical conductivity in wet season (128.5-285.6 µs/cm); total dissolved solids (114.7-401 mg/L); total suspended solids (101.8-423.6 mg/L); total hardness (58-255 mg/L); total acidity (3.55-19.5 mg/L); total alkalinity (9.2-47 mg/L); biological oxygen demand (1.70-3.28 mg/L); nitrate (0.73-2.23 mg/L); ammonium in wet season (0.18-0.34 mg/L); zinc (0.011-0.085 mg/L); mercury (0.001-0.005 mg/L); copper (0.002-1.008 mg/L) and iron (0.031-0.318 mg/L) were within the WHO standard for drinking water quality while the phosphate (0.14-1.08 mg/L), lead (0.013-0.098 mg/L) and ammonium in dry season (0.48-0.75 mg/L) levels were not. The total viable counts exceeded the 1.0 x 10^2 cfu/mL WHO standard for drinking water and the MPN counts for total and faecal coliforms of (8-13 MPN/100 mL) and (0-2 MPN/100 mL) respectively also exceeded the WHO standard slightly. The isolated organisms were identified to be *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Vibrio cholerae*. The presence of these pathogenic, indicator organisms in the water samples, as well as their physicochemical implications, render them unfit for drinking without adequate treatment. Liming and boiling treatment techniques are recommended.

Keywords: bacteriological parameters, dry season, Oji river LGA, physicochemical parameters, seasonal variation, spring water, wet season

Introduction

Water is one of the key elements for the survival of living things on earth, because it plays an indispensable role in the maintenance of life. The average water intake required by man per day is between 3.7 litres and 4.0 litres (15-18 cups) for male and 2.7 litres and 3.0 litres (11-14 cups) for female (Agah *et al*., 2018) [3]. Water supply and accessibility is goal no. 6 of the sustainable development goals (SDGs) and aims at ensuring environmental sustainability. Access to safe drinking water is critical to human health and development (Edbert *et al*., 2017) [15]. Water that is good for drinking is important to human physiology and man's continued existence depends so much on its availability.

Potable water is a fresh water body that is unpolluted, suitable for drinking, odourless and tasteless (Onuorah *et al*., 2020) [26]. Provision of potable water to the rural and urban population is necessary to prevent health hazards. Due to the inability of government to meet the ever increasing demand of water in Nigeria, people resort to groundwater sources of water such as borehole, shallow wells and natural spring water to meet their water needs. Spring water is a form of groundwater, which has its source in the aquifers of the underground pool of a water body (Onuorah *et al*., 2020) [26]. The inhabitants of Oji River LGA of Enugu State heavily depend on spring water sources for their water needs and there's no form of treatment before usage. Traditionally, groundwater had been considered the least water source to be contaminated by human or animal wastes, most especially ground water from the deep, confined aquifers (Atiku *et al*., 2018) [7]. It is assumed that water passing through rocks and soil, filters off most impurities and microbial contaminants, thereby leaving the consumers with little concern of groundwater contamination (Okoye *et al*., 2022) [25].

Pollution of groundwater occurs when much of undesirable or harmful substances find its way into the aquifers, exceeding the water's natural ability to remove the undesirable material, dilute it to a harmless concentration or convert it to harmless form (Edbert *et al*., 2017; Onuorah *et al*., 2020) [15, 26]. It's estimated that 80% of all illnesses is related to water and sanitation, and that 15% of all child deaths under the age of 5 years in developing countries results from diarrhoeal diseases (WHO, 2003; WHO, 2004). The presence of certain chemicals such as iron, copper, zinc, mercury, lead, cadmium, nitrate, nitrite, calcium and magnessium salts, phosphate and ammonium in high quantities can be detrimental to human health (Shittu *et al*., 2008) [30].

Physicochemical parameters are used to ascertain the quality of any water sample, especially drinking water and they are vital for monitoring water quality (APHA, 2017). These parameters such as the pH, temperature, electrical conductivity, turbidity, total dissolved solids, dissolved oxygen, biological oxygen demand, acidity, alkalinity, nitrate, chloride, etc can affect the drinking water quality if their values are in higher concentrations than safe limits set by the World Health Organization (WHO) and other regulatory bodies (Okoye *et al*., 2022) [25].

Bacterial contamination of drinking water is a major public health concern globally, because water can serve as a reservoir for many pathogenic organisms which can cause diseases (Suthar *et al*., 2009) [31]. Faecal pollution of drinking water may introduce a variety of intestinal pathogens, which may cause diseases from mild gastroenteritis to severe and sometimes fatal dysentery, diarrhoea, cholera, typhoid, giardiasis, hepatitis, etc (Wanda *et al*., 2006). Monitoring the bacterial quality of drinking water is done through laboratory testing for the presence of coliform bacteria which are used as indicator organisms. Potable water is any water sample that has complied with certain physical, chemical and microbiological standards, which are designed to ensure that the water is safe for drinking. Studies have been conducted to ascertain these parameters in varying drinking water sources in many parts of the country but none has been conducted on the spring water samples of Oji River LGA of Enugu State, Nigeria.

Spring water bodies in Oji River LGA are Obii, Omumo, Ogbanenu, Uruku, Ogba, Ngene-akpu, Onwii and Ovu, and are located in the five communities of the local government area. They serve as the major source of drinking water for the people, and also used for irrigation for agriculture, domestic and other purposes during the dry and wet seasons. This study will create awareness on the physicochemical and bacteriological parameters of the spring water within the LGA during both seasons, and recommends possible means of treatment of the water before use.

Materials and Methods

Study Area

This study was on the spring water sources in Oji River Local Government Area of Enugu State, Nigeria. Farming, petty trading, teaching and artisans are the major occupation of the people of the area. Oji River Local Government Area (LGA) is one of the seventeen LGAs in Enugu State of Nigeria. It is located at the Northeast part of the state, and covers an area of 451.8 km^2 with a total land mass of 7,161 km^2 of Enugu State. It has an estimated population of 173,800 with a population density of 347.8/km (Nigerian National Bureau of Statistics, website). The five sampling communities were Achi, Akpugoeze, Awlaw, Inyi and Ugwuoba communities. Its geographical coordinates are 6^{o}14’52” North, and 7^{o}30’85” East, and it borders Anambra and Abia States, Southeast, Nigeria. The LGA is characterized by tropical climate with distinct wet and dry seasons.

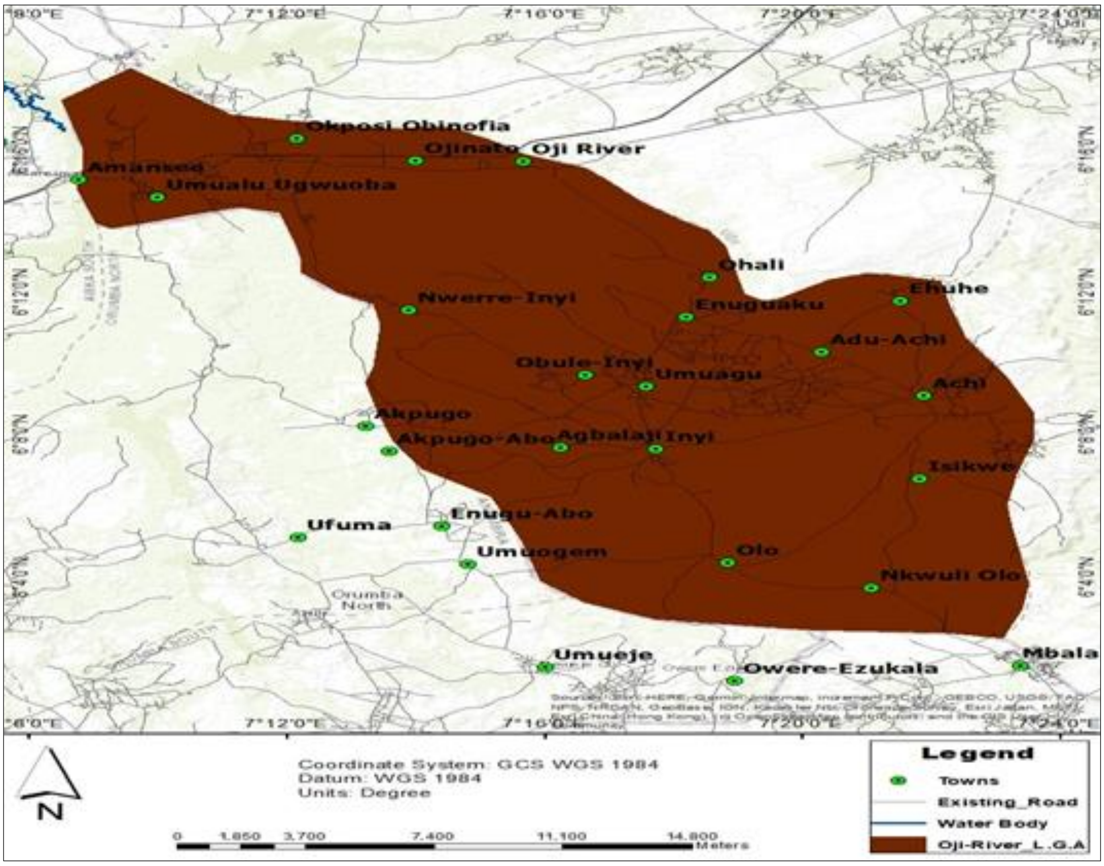

Fig 1: Map showing the sampling sites in Oji River LGA, Enugu State

Study Design

Two seasonal samplings were carried out between November, 2018 and October, 2019, and the period represented alternating seasons of dry (November, 2018 to March, 2019) and wet seasons (April to October, 2019) within the communities.

Sampling

Water samples were collected following standard sampling guidelines and methods as described by the American Public Health Association (2012). The samples were taken from the point of discharge at each location. Two sources of spring water each from Awlaw (Omumo spring water and Obii spring water, decoded as site 2 & site 3); Akpugoeze (Ogbanenu spring water and Ngene-akpu spring water, as site 4 & site 5); Inyi (Uruku spring water and Ogba spring water, as site 6 & site 7) and Ugwuoba (Ovu spring water and Onwii spring water, as site 8 & site 9) were sampled, while one was sampled from Achi (Iyi-agu spring water, decoded as site 1) for both seasons. Since sampling was done for 12 months, this brought the sample size to 45 in dry season (November, 2018 to March, 2019) and 63 in wet season (April to October, 2019) and 108 for both seasons.

Samples Collection

Water samples were collected in sterile plastic containers, which had been previously washed and rinsed thoroughly. At each collection point, these containers were rinsed with the water to be sampled. Samples for physicochemical analyses were taken to Graceland Research & Analytical Labortaory, Awka while samples for bacteriological analysis were transported to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University, Uli Anambra State, Nigeria.

Determination of Physicochemical Parameters

The physicochemical parameters were determined according to the procedures described by APHA (2017). Temperature, pH, colour, electrical conductivity, total dissolved solids, total suspended solids, total acidity, total alkalinity, total hardness, biochemical oxygen demand, nitrate, phosphate, ammonium, lead, iron, zinc, mercury, copper and cadmium were determined.

Bacteriological Analysis

Bacteriological assessments were carried out using the total viable counts and most probable number (MPN) method of Multiple Tube Fermentation technique. All the media used were prepared according to their manufacturer's specification and sterilized in the autoclave at 121 ºC for 15 minutes. All glass wares used were sterilized in the hot air oven at 160 ºC for 1hr. Total coliform counts were determined using MacConkey agar. Faecal coliform was determined using Eosin methylene blue medium via pour plate technique. The confirmations of the isolates were done using lactose broth at 44.5 ºC for 24hrs. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacterial isolates using keys provided in the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 2002) [20].

Statistical analysis and data management

Data was entered in a spreadsheet, checked and errors corrected before exporting to SDA 7.0 Statistical Software (WINKS). Comparison for variations between the sampling sites was done using two-way ANOVA. Paired *t*-test was used to analyze the seasonal variations between the dry and wet seasons. The results are presented in tables and graphs.

Results

Mean values of the physical parameters studied in the water samples during the dry and wet seasons

The mean values of the physical parameters studied in the water samples during the dry and wet seasons are presented in Tables 1 and 2 respectively. The data showed that pH, colour, TSS, and TDS were all within the maximum permissible limits of WHO standards for drinking water and the means ranged from 6.8-8.4 (pH); 7-14.2 TCU (colour); 101.08-423.6 mg/L (TSS) and 114.7- 401 mg/L (TDS). Differences between the sites showed that site 8 had the highest level of TSS (198.3-423.6 mg/L), while site 9 had the highest level of TDS (268.5-401 mg/L).

Temperature plays a critical role in the metabolic activities of organisms and also affects the solubility of oxygen in water (Gopakrushna, 2011; Kataria *et al*., 2011) [22]. Water temperature was within the limit in the wet season (24.6-26.6 ºC) and all the sites in the dry season except for site 2 (25.8-28.1 ºC). Analysis of variance indicated that there was no significant difference in the water temperature between the nine sites. However, seasonal variations showed that the temperature was significantly high in the dry season.

Electrical conductivity values in the wet season were all within the maximum permissible limit (128.5-285.6 µS/cm) whereas most sites (5 out of 9) in the dry season (408-535.8 µS/cm) were above the limit as set by WHO (2006) recommendation of <400 µS/cm. Differences in sites showed that water from site 9 had the highest EC and was significantly different ($P < 0.05$) from those at the other sites. Paired comparison using *t* test showed that EC was significantly high ($P < 0.05$) in the dry season.

Table 1: Mean values of the physical parameters studied in the spring water samples of Oji River LGA during the dry season

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site6	Site 7	Site 8	Site 9	Range	WHO Limits
pH	7.7	7.3	7.21	7.58	8.2	7.18	8.3	8.4	8.16	7.18–8.4	6.5 – 8.5
Colour (TCU)	9.2	7.5	8.1	8.7	7.6	7.9	8.5	8.3	7.0	7.0–9.2	15 (colour less)
Temp. (ºC)	27	28.1	26.8	25.8	26.5	27	26	27	26.8	25.8–28.1	27
EC (µS/cm)	320	408	357.55	415.08	433.17	395.6	387.89	478	535.8	320–535.8	400
TSS (mg/L)	101.08	121	231	192.7	181.5	136.3	178.76	198.34	185.4	101.8–231	500
TDS (mg/L)	358.5	331	298	311	285	315	365	387	401	311–401	500

Table 2: Mean values of the physical parameters studied in the spring water samples of Oji River LGA during the wet season

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site6	Site 7	Site 8	Site 9	Range	WHO Limits
pH	6.9	7.47	8.13	6.8	7.4	7.8	7.2	8.3	8.0	6.8–8.3	6.5 – 8.5
Colour (TCU)	14.2	12.8	12	12.2	11.5	12	13.2	12.5	10	10–14.2	15 (color less)
Temp. (ºC)	25.1	26.6	24.9	25	25.07	24.6	25.5	25.2	25.3	24.6–26.6	27
EC(µS/cm)	134.95	145.18	154.6	181.5	187.05	128.5	141.87	215.6	285.6	128.5–285.6	400
TSS(mg/L)	324.8	387.52	344	298.51	311.90	327	322.55	423.6	401.5	298.51-423.6	500
TDS(mg/L)	234	261.9	184.8	185.6	201	114.7	199.85	214	268.5	114.7–268	500

Mean values of the chemical parameters examined in the water samples during the dry and wet seasons

The mean values of the chemical parameters examined in the water samples during the dry and wet seasons are presented in Tables 3 and 4 respectively. The data showed that the values of total acidity (TAci) and total alkalinity (TAli) were all within the maximum permissible limit as set by WHO (2006) to be 100mg/L. Total acidity values were high in the wet season, and the means ranged from 8.02-19.5 mg/L than in the dry season of 3.55-8.06 mg/L. Total alkalinity was also higher in the wet season than in the dry season, with 13-47 mg/L and 9.2-33.78 mg/L respectively. Site 6 had the highest values of total acidity for the dry and wet seasons as 8.06 mg/L and 19.5 mg/L respectively. Similarly, site 9 also had the highest values of total alkalinity as 33.78 mg/L and 47 mg/L for dry and wet seasons respectively. Differences between the sites showed that site 1 had the lowest total alkalinity levels of 9.2 mg/L and 13 mg/L for dry and wet seasons respectively. Also, site 1 had the lowest total acidity levels of 3.28 mg/L and 8.02 mg/L for the dry and wet seasons respectively. Paired comparison using *t* test indicated that TAci and TAli were significantly high ($p < 0.001$) in the wet season.

The total hardness (TH) levels, measured by its calcium and magnesium content of carbonates, showed that they were within the maximum permissible limit of 100–300 mg/L as set by the WHO (2006). The total hardness ranged from 58 – 255 mg/L in all the sites. Site differences showed that water from site 9 had the highest TH values, followed by water from site 6 and they were significantly different (p<0.001) from those at the other sites. Paired comparison using *t* test showed that TH was significantly high (p <0.001) in the wet season than the dry season. Total hardness levels also showed that site 1 had the least value. The degree of hardness in drinking water is important for its aesthetic and acceptability by consumers, and also for economic considerations and wellbeing of the people.

All the BOD_5 values (1.70-3.28 mg/L) were within the permissible limit as set by the WHO (2006). Site 7 recorded the highest average level of 5.31 mg/L for both seasons, followed by site 1 (5.30 mg/L). Analysis of variance showed that there was no significant difference (p = 0.208) in the BOD_5 levels between the different sites. Paired comparison using *t* test showed that BOD_5 values were not significantly high in the dry season.

Nitrate ranged between 0.73-2.23 mg/L and they were all within the maximum permissible limits. Site 8 had the highest level of 2.23 mg/L while site 6 had the least value of 0.73 mg/L. Seasonal variations between the sites showed that the levels of nitrate were significantly high in the wet season than dry season. The nitrate level in the dry season ranged from 0.73-1.08 mg/L and 1.25-2.23 mg/L in the wet season.

For phosphate, the levels ranged from 0.14-1.08 mg/L and they were all above the maximum permissible limit. **Site 2** had the highest level of phosphate with 1.08 mg/L while site 3 had the least value of 0.14 mg/L. Comparison between the sampling sites showed insignificant differences in the levels of phosphates between the sampling sites. Seasonal variations between the nine sites indicated that there were increased levels of phosphates in the wet season than the dry season. Also, the seasonal variations showed that the phosphate level was significantly high in the wet season.

The ammonia levels between the sites ranged from 0.18-0.75 mg/L. The levels in the wet season were all within the maximum permissible limit, whereas the levels in the dry season were all above the permissible limit (0.52-0.75 mg/L) except for site 2 with a value of 0.48 mg/L. Comparisons between the sampling sites showed insignificant differences in the levels of ammonia between the sampling sites. Seasonal variations between the nine sites indicated that there were increased levels of ammonia in the dry season than the wet season. Also, the seasonal variations showed the ammonia level was significantly high in the dry season.

Table 3: Mean values of the chemical parameters examined in the spring water samples of Oji River LGA during the dry season

Parameter (mg/L)	Site 1	Site 2	Site3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Range	WHO Limits
Total acidity	4.96	3.55	4.05	5.35	7.45	8.06	4.32	6.50	7.65	3.55–8.06	100
Total alkalinity	9.2	14.5	13.0	15.5	21.8	24.5	29.10	30.5	33.78	9.2–33.78	100
Total hardness	58	75	105	135.5	125	165	140	161	210	58–210	100–300
BOD	3.20	2.90	2.85	2.95	2.80	3.10	3.28	2.65	3.05	2.65–3.28	<5.0
Nitrate	1.05	0.89	0.99	1.04	0.85	0.73	0.76	0.86	1.08	0.73–1.08	<45
Phosphate	0.26	0.15	0.14	0.16	0.20	0.22	0.18	0.15	0.25	0.14–0.26	<0.1
Ammonium	0.75	0.48	0.52	0.60	0.72	0.66	0.64	0.59	0.62	0.48–0.75	<0.5

Table 4: Mean values of the chemical parameters examined in the spring water samples of Oji River LGA during the wet season

Parameter (mg/L)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Range	WHO Limits
Total acidity	8.02	12.5	11.05	13.0	13.5	19.5	8.35	9.8	15.85	8.02–19.5	100
Total alkalinity	13	21.8	19.8	24	30.5	36.7	41.5	44.6	47	13–47	100
Total hardness	85	125	145	189	175	215	185	190	255	85–255	100–300
BOD	2.10	1.85	1.82	1.70	1.80	1.90	2.03	1.95	2.25	1.70–2.25	<5.0
Nitrate	2.10	1.83	1.95	2.02	2.06	1.27	1.58	2.23	2.15	1.25–2.23	<45
Phosphate	0.92	1.08	0.86	0.94	0.66	0.65	0.70	0.55	0.72	0.55–1.08	<0.1
Ammonium	0.22	0.21	0.20	0.18	0.24	0.34	0.18	0.22	0.19	0.18–0.34	<0.5

Mean values of heavy metals parameters examined in the spring water samples during the dry and wet seasons

The mean values of heavy metals parameters examined in the spring water samples during the dry and wet seasons are presented in Tables 5 and 6 respectively. Some elements were not detected or were below detection limit especially during the dry season. The levels of zinc, mercury and copper were all within the maximum permissible limit, with zinc levels ranging from 0.011-0.085 mg/L; mercury: 0.001-0.005 mg/L; and copper: 0.002-1.008 mg/L. Copper in most sites was not detected in the dry season except for site 5, site 8 and site 1. In almost all the sites, cadmium was not detected or was below detection limit (<0.001 mg/L), except at site 7 where it was recorded at 0.032 and 0.010 mg/L for wet and dry seasons respectively.

Iron levels were all within the maximum permissible limit, except at site 1 during the dry season where it was recorded at 0.318 mg/L, above <0.3 mg/L limit. Lead levels were all above the maximum permissible limit of 0.01 and they ranged from 0.013 mg/L in site 4 during the dry season to 0.098 mg/L in site 1 during the wet season. When compared by sites, site 1 had significantly high levels of iron and lead than the other eight sites. Seasonal variations showed that the levels of heavy metals were significantly high in the dry season except for lead which had higher values in all the sites in the wet season.

Table 5: Mean values of heavy metals in water sampled at the nine study sites of Oji River LGA during the dry season

Parameter (mg/L)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Range	WHO Limits
Zinc	0.085	0.071	0.068	0.059	0.046	0.038	0.061	0.039	0.028	0.028–0.085	<5.0
Iron	0.318	0.292	0.219	0.167	0.145	0.176	0.213	0.171	0.162	0.167–0.318	<0.3
Lead	0.028	0.013	BDL	0.013	0.018	0.023	0.045	BDL	0.018	BDL– 0.045	<0.01
Cadmium	BDL	0.003	ND	0.002	ND	ND	0.010	ND	BDL	ND– 0.010	<0.003
Mercury	ND	0.002	ND	BDL	0.002	ND	ND	0.001	ND	ND–0.002	0.006
Copper	0.002	ND	ND	ND	0.085	ND	ND	0.023	BDL	ND–0.085	<2.0

ND: Not detected. BDL – Below Detection Limit (<0.001 mg/L)

Table 6: Mean values of heavy metals in water sampled at the nine study sites of Oji River LGA during the wet season

Parameter (mg/L)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Range	WHO Limits
Zinc	0.028	0.016	0.019	0.016	0.013	0.020	0.018	0.026	0.011	0.011–0.028	<5.0
Iron	0.211	0.118	0.093	0.059	0.117	0.122	0.018	0.031	0.041	0.031–0.	<0.3
Lead	0.098	BDL	BDL	0.015	0.071	0.076	0.081	BDL	0.095	BDL–0.098	<0.01
Cadmium	0.002	0.021	0.002	0.010	0.001	0.002	0.032	0.002	0.003	0.001–0.032	<0.003
Mercury	0.005	0.004	0.003	0.001	0.004	0.003	ND	0.004	0.002	ND–0.005	0.006
Copper	0.056	0.087	0.095	0.56	0.83	0.67	1.008	0.84	1.005	0.056–1.008	<2.0

ND: Not detected; BDL: Below Detection Limit (<0.001 mg/L)

Mean values of bacteriological parameters in spring water samples during the dry and wet seasons

The mean values of the total viable counts, total and faecal coliform counts in the water sampled during the dry and wet seasons are presented in Tables 7 and 8 respectively. The total viable counts ranged from 1.72 x 10^2 to 4.8 x 10^5 CFU/mL and are above the WHO standard of 1.0 x 10^2 CFU/mL of drinking water. Seasonal variations showed that the values of total viable counts were higher in wet season (2.3 x 10^2 to 4.8 x 10^5 CFU/mL) (Table 8) than dry season (1.72 x 10^2 to 2.4 x 10^4 CFU/mL) (Table 7). When compared by sites, site 9 had the highest value of total viable count (Table 8) while site 3 had the least value (Table 7).

Out of the 108 samples of spring water in Oji River LGA, 80 samples tested positive for coliforms, which represented a contamination rate of 74%. These values ranged from 8-13 MPN/100 mL and are slightly above the WHO standard of zero (0) MPN/100 mL of drinking water. Also, 14 samples out of the 80 samples of positive coliforms tested positive for faecal coliforms and ranged between 1-2 MPN/100 mL. Seasonal variations showed that there were more coliform bacteria during the wet season (Table 8) than dry season (Table 7). When compared by sites, the results also showed that site 9 had highest counts of total and faecal coliforms (Table 8) while site 7 had the least counts (Table 7).

Table 7: Mean seasonal prevalence of total coliform, faecal coliform and total viable count from the nine study sites in Oji River LGA during the dry season

Sampling sites	Total coliform (MPN/100mL)	Faecal coliform (MPN/100mL)	Total viable count (CFU/mL)
Site 1	11	1	1.10 x 10^3
Site 2	11	0	2.13 x 10^2
Site 3	9	0	1.72 x 10^2
Site 4	10	1	2.4 x 10^4
Site 5	9	0	1.15 x 10^4
Site 6	9	0	1.8 x 10^3
Site 7	8	0	1.6 x 10^2
Site 8	10	1	3.1 x 10^3
Site 9	9	0	2.2 x 10^2
Range	8–11	0–1	1.72 x 10^2 – 2.4 x 10^4
WHO Standard	10	0 (zero)	1.0 x 10^2

Table 8: Mean seasonal prevalence of total coliform, faecal coliform and total viable count from the nine study sites in Oji River LGA during the wet season

Sampling sites	Total coliform (MPN/100mL)	Faecal coliform (MPN/100mL)	Total viable count (CFU/mL)
Site 1	12	2	3.5 x 10^4
Site 2	11	2	4.1 x 10^3
Site 3	10	1	2.3 x 10^2
Site 4	11	1	2.9 x 10^3

Site 5	10	1	4.8 x 10^2
Site 6	10	1	1.82 x 10^3
Site 7	9	0	1.3 x 10^2
Site 8	11	1	3.2 x 10^3
Site 9	13	2	4.8 x 10^5
Range	9–13	0–2	2.3 x 10^2 – 4.8 x 10^5
WHO Standard	10	0 (zero)	1.0 x 10^2

The morphological characteristics of different bacterial isolates identified in the spring water sources sampled in this study are presented in Table 9. The bacterial isolates included *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae* and *Staphylococcus aureus*. Their mean occurrence is presented in Table 10 and it showed that *Salmonella* spp. is the most dominant bacterial genera (100%), followed by *Vibrio cholerae* (88.9%), while *Shigella* spp. and *Escherichia coli* had the same percentage occurrence of 77.8%. The least dominant bacterial isolate was *Saphylococcus aureus* (55.6%). Therefore, *Salmonella* spp. is the most public health pathogenic bacterial isolate from the spring water samples. The results also showed that sites 1, 4, 8 and 9 had all the bacterial isolates (Table 10).

Table 9: Morphological characteristics and identification of the bacterial isolates on selective culture media

Isolates	Colony characteristics	Culture media
Escherichia coli	Circular, low convex, with entire margin, mucoid, opaque, rod-shaped, pinkish glistering medium colonies with metallic sheen raised margin and entire edge	EMB
Salmonella spp.	Medium pale-pink colonies with black centers, flat, raised margin and entire margin	SSA
Shigella spp.	Medium pink-red, translucent colonies with 1–2 mm diameter and no black centers	XLD
Vibrio cholerae	Large yellow, circular colonies with flat elevation, entire edge and 2–3 mm in diameter	TCBS
Staphylococcus aureus	Medium clumps of yellow, pigmented, circular colonies with smooth, low convex and entire margin, and moist surface in 2–3 mm diameter.	MSA

Table 10: Mean occurrence of bacterial isolates in the spring water samples of different study sites

Isolates	Study sites								
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**	**Site 9**
Escherichia coli	+	+	+	+	–	+	–	+	+
Salmonella spp.	+	+	+	+	+	+	+	+	+
Shigella spp.	+	+	+	+	–	+	–	+	+
Vibrio cholerae	+	+	+	+	+	+	–	+	+
Staphylococcus aureus	+	+	–	+	–	–	–	+	+

Discussion

The pH of a substance is used to express the intensity of the acid and alkaline condition of such substance (Chadne, 2014) [11]. The pH of all the sampled water was within the WHO limit (6.5-8.5). During the dry season, the pH in all sites was above neutral, 7 and alkaline (7.3, 7.58, 7.7, 8.16, 8.3 and 8.4), whereas during the wet season, some sites recorded below the neutral (6.8, 6.9) and acidic. The result agreed with the report of Ukpong and Peter (2012) [33] on the physicochemical parameters in drinking water in Ibeno Local Government Area of Akwa Ibom State. The alkaline nature of the water source especially during the dry season can be attributed to a number of factors such as the presence of inorganic acids from decaying vegetation (Ukpong and Peter, 2012; Paschke *et al*., 2001) [33, 27], dissolved carbon dioxide and the dissolution of carbonate salts (Onuorah *et al*., 2020) [26]. Water in a pure state is neither acidic nor basic.

The temperature values during both seasons were within the WHO limit of 27°C (Tables 1 and 2) except for site 2 which had 28.1 °C during the dry season. The result agreed with the findings of Onuorah *et al*. (2020) [26] on Iyifeyi stream of Ugwobi Abbi, Uzo-Uwani LGA of Enugu State and that of Akubuenyi *et al*. (2013) [4] on some of the sources of water in Calabar, Nigeria. The values of temperature were found to be higher during the dry season than wet season. Jaji *et al*. (2007) [21] also reported increased levels of temperature on water quality assessment of Ogun River, Nigeria during the dry season.

Colour in drinking water is usually due to the presence of organic matter (primarily humic and fulvic acids) associated with the humus fraction of soil or the presence of iron and other metals, either as natural impurities or as corrosion products (Bernard and Ayeni, 2012) [10]. Water in its pure state has a slight blue colour that becomes a deeper blue as the thickness of the observed sample increases, though relatively small quantities of water appear to be colourless. The blue colour of water is an intrinsic property and is caused by selective absorption and scattering of white light. The colour of the spring water sources investigated during both seasons (Tables 1 and 2) did not exceed the recommended limit as set by the WHO (2006) and there was no significant seasonal variation in colour. This agreed with the reports of Onuorah *et al*. (2020) [26] on Iyifeyi stream, Ugwobi-Abbi, Enugu State Nigeria and Krishna *et al*. (2011) [23] on Kaveri River, India.

Electrical conductivity is a measure of the capacity of a water sample to conduct electric current as well as the relative level of total dissolved salts in the water (Gopakrushna, 2011; Chadne, 2014) [11]. The electrical conductivity of the spring water during wet season were within the WHO recommended limit (Table 2), whereas most sites exceeded the recommended limit during the dry season. Similar results were obtained by Udousoro and Umoren (2014) [32] in their assessment of surface and ground water quality. Higher values of EC during the dry season may be attributed to concentrated dissolved salts as a result of human activities (Chindo *et al*., 2013) [13].

Total Dissolved Solids (TDS) is a measure of both anions and cations in a water body. Total dissolved solids in ground and surface water could come from natural and or anthropogenic sources such as agricultural runoff, industrial effluents, sewage and the chemicals used in the treatment of water (Edet *et al*., 2012; Saana *et al*., 2016) [16, 28]. The TDS during both seasons were within the WHO recommended limit (Tables 1 and 2). The result also agreed with the reports of Onuorah *et al*. (2020) [26] and Udousoro and Umoren (2014) [32] on surface and groundwater qualities.

Total Suspended Solids (TSS) in water affect the aesthetic appeals of drinking water and even bathing water. Water that is high in TSS is more of an aesthetic than a health hazard (Gupta *et al*., 2017) [19]. TSS is a precursor to turbidity due to silt and organic matter (Essumang *et al*., 2011; Shawai *et al*., 2018) [17, 29]. The values obtained in both seasons were within the WHO recommended limit (Tables 1 and 2) and agreed with the results of Shittu *et al*. (2008) [30] on the physicochemical and bacteriological analyses of water used for drinking, and that of Edet *et al*. (2012) [16] on the bacteriological and physicochemical quality of stream water in Nduetong Oku, Uyo, Akwa Ibom State, Nigeria.

The acidity and alkalinity of water are its quantitative capacity to react with strong base and acid respectively. Strong mineral acids and weak acids such as carbonic and acetic as well as hydrolyzing salts such as aluminium or iron sulphates may contribute to measured acidity. Similarly, strong base such as salts of calcium or magnesium carbonates can contribute to measured alkalinity (Chandra *et al*., 2011) [12]. There were no significant seasonal variations in the total acidity and alkalinity obtained during both seasons. This result agreed with Krishna *et al*. (2011) [23] who observed no significant seasonal variation in the physicochemical parameters studied in Kaveri River, India. The values obtained during both seasons were within the WHO range for potable water (Tables 3 and 4).

Hardness of the water is the property that decreases the lather formation of soap and increases scale formation in hot-water heaters and low-pressure boiler at high levels. Total hardness is mainly due to calcium and magnesium salts (Gopakrushna, 2011; Chadne, 2014) [11] and is derived from limestone or industrial effluents. The value for total hardness in this study did not exceed the WHO recommended limit (Tables 3 and 4) for both seasons. The values obtained in this study were similar to those reported by Onuorah *et al*. (2020) [26] for stream water used for drinking purposes and Essumang *et al*. (2011) [17] for ground water. The spring water samples can be classified as soft water based on WHO (2004) classification.

Biochemical oxygen demand indicates the amount of organic waste present in water. BOD_5 values obtained in this study during both seasons were within the maximum limit of 5.0 mg/L (Tables 3 and 4) as set by the WHO (2006). However, BOD_5 levels were higher in the wet season than dry season and this can be attributed to eutrophication of nutrients into the water source during the season. This agreed with the reports of Ukpong and Peter (2012) [33] on the physicochemical analysis of drinking water and Edet *et al*. (2012) [16] on the physicochemical analysis of stream waters in Nduetong Oku Community, Uyo.

Nitrate is the most highly oxidized form of nitrogen compounds and is commonly present in surface and ground water samples. It is the final product of the biochemical oxidation of ammonia (Chindo *et al*., 2013) [13]. High nitrate concentration in drinking water has been widely reported to have detrimental effects on pregnant women and babies less than six months old (Akubuenyi *et al*., 2013; Longe and Balogun, 2010) [4] and could be a source of eutrophication in the receiving water. The values obtained for nitrate in this study were within the WHO limit for drinking water (Tables 3 and 4). The results agreed with Chadne (2014) [11] who reported values that were within the WHO limit and observed seasonal variations in physicochemical parameters of the water samples investigated.

The phosphate levels were above the WHO limit for both seasons, whereas the ammonium levels were above the standard for dry season only (Tables 3 and 4). Phosphate levels were higher in the wet season (0.55-1.08 mg/L) than dry season (0.14-0.26 mg/L). This can be attributed to the usage of artificial fertilizers, made of phosphate, nitrate and potassium, in the cultivation of bitter leaf and *ugu* leaf around the sites. The residues of fertilizers and other pesticides can permeate the soil particles and rock layers during the wet season, thereby polluting the aquifers of the spring water. The values of phosphate agreed with the report of Gopalkrushna (2011) on the assessment of physicochemical status of groundwater samples in Akot city where the phosphate levels were all found to be above the standard limit. Similarly, Akubuenyi *et al*. (2013) [4] reported that phosphate levels in major sources of drinking water in Calabar metropolis, Nigeria were above the standard limit as set by the WHO (2006).

The values obtained for the heavy metals during both seasons were within the WHO limit except for lead which had higher values than the recommended limit. Heavy metals were not detected in many sites and some sites had metals below detection limit (BDL). BDL means there's presence of such metal in the water sample but could not be measured. Heavy metals are needed at low levels for enzymatic activities as catalysts. Their presence in the spring water samples can be through weathering of rocks and leaching. The values obtained for zinc, copper, iron and lead in this study agreed with the reports of Onuorah *et al*. (2020) [26], who investigated the heavy metals parameters in Iyifeyi stream of Ugwobi-Abbi, Enugu State, Nigeria. Similarly, the values obtained for mercury and cadmium agreed with the report of Ukpong and Peter (2012) [33], on the heavy metals parameters of drinking water in Ibeno Local Government Area of Akwa Ibom State. Cadmium, mercury and copper were not detected in most sites during the dry season and this agreed with the report of Kataria *et al*. (2011) [22] on the study of physicochemical parameters of drinking water of Bhopal City. High levels of these metals may be hazardous to human health (Akubuenyi *et al*., 2013) [4].

The total viable counts (TVCs) during both seasons (1.72 x 10^2 to 4.8 x 10^5 CFU/mL) exceeded the WHO limit of 1.0 x 10^2 CFU/100 mL. The values agreed with the report of Eboh *et al*. (2017) on the microbiological quality of borehole water samples in Amai kingdom, Delta State, Nigeria where the total viable counts were found to be above the WHO limit. Similarly, Suthar *et al*. (2009) [31] also reported elevated levels of total bacterial counts in the drinking water samples of Northern Rajasthan, India which exceeded the WHO limits. Seasonal variations showed that there were higher levels of TVCs during the wet season than dry season. This could be attributed to high loads of organic matter deposits from eutrophication of nutrients during rainfalls in wet season. Adeiza *et al*. (2018) also reported higher levels of TVCs in the groundwater sources of drinking water in Kurnar Asabe Quarters of Kano metropolis in the wet season.

The coliform counts in most study sites especially during the dry season exceeded the WHO standard. Faecal coliform was not found in most sites during the dry season but was found at all sites during the wet season. All the study sites recorded presence of coliform bacteria which were higher in the wet season than dry season. This could be attributed to organic deposits, predominantly from human and animal sewerage as well as high suspended solid matter. Allamin *et al*. (2015) [5] also reported presence of faecal coliform in the drinking water samples of Kaduna metropolis, Northwest Nigeria.

Similarly, Ayandele *et al*. (2015) [8] also reported coliform bacteria in all the borehole water samples of Mosimi, Ogun State, Nigeria. The occurrence of these bacterial genera is in agreement with the works of Edet *et al*. (2012) [16] in Nduetong-Oku community, Uyo Akwa Ibom, Nigeria; Ayandele *et al*. (2015) [8] in Mosimi area of Ogun State, Nigeria; Eboh *et al*. (2017) in Amai kingdom, Ukwuani LGA of Delta State, Nigeria; Atiku *et al*. (2018) [7] in Abuja, Nigeria and Adeiza *et al*. (2018) in Kano metropolis, Northwest Nigeria, though their counts were less compared to the reports from above authors. This is an indication that the spring water samples of Oji River LGA are safer for drinking compared with aforementioned towns and states of Nigeria.

Conclusion and Recommendations

The quality of the spring water samples investigated was acceptable in terms of some of the physicochemical parameters, but the bacteriological qualities failed to meet the WHO standard. Some physicochemical parameters did not comply with the WHO standard, for example lead and phosphate levels for both dry and wet seasons; electrical conductivity for dry season, etc. The total viable counts and coliform counts also exceeded the WHO standard. There was no significant variation in the physicochemical parameters of the spring water samples during the dry and wet seasons, but there was significant variation in the bacteriological parameters investigated. The spring water samples should be subjected to simple water treatment methods such as boiling, flocculation, liming, etc before consumption. There should be regular programmes to educate the villagers on the need for personal hygiene before and after fetching water from the spring water source. Local authorities in the communities should ban activities like washing of clothes, motorcycles and tricycles near the spring water. Also, Government and non-governmental organizations should render assistance by providing potable water supply to the residents of Oji River Local Government Area.

Conflict of interest

The authors declare there is no conflict of interest.

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