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ASSESSMENT OF THE LEVEL OF SELECTED PHARMACEUTICALS IN WATER AROUND HOSPITAL ENVIRONMENTS IN OGUN STATE, NIGERIA

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ABSTRACT

Indiscriminate disposal of pharmaceuticals through sewage, on-land and water-ways could contribute to water contamination in and around the hospital environment. This study determined the levels of Paracetamol, Metronidazole, Sulfamethoxazole and Trimethoprim in groundwater from three selected hospitals and their environs in Ogun State, Nigeria. Physicochemical properties were determined using standard analytical procedures while, solid phase extraction was employed for pre-concentration of the samples and quantification of the selected pharmaceuticals were determined using High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) detector. The result of physicochemical properties are as follows: pH ≤ 7.58, electrical conductivities ≤ 1720.05 µs/cm, total dissolved solids ≤ 808.40 mg/L, salinities ≤ 0.86 mg/L, chloride levels ≤ 509.43 mg/L and total hardness ≤ 8.50 mg/L. The pharmaceutical concentrations in the water samples (mg/L) are; Paracetamol ≤ 2.610, Metronidazole ≤ 0.331, Sulfamethoxazole ≤ 0.007 and Trimethoprim ≤ 0.058. The physicochemical properties of some of the samples did not conform to the permissible limits for potable water. Furthermore, though trace concentrations of the pharmaceuticals were found to be present, but level of some were higher than the WHO permissible limits for drinking water. Therefore, cautions needs to be taken in long-term consumption of these water as it could lead to drug resistance and other health-related adverse conditions.

Keywords: *Paracetamol, Metronidazole, Sulfamethoxazole, Trimethoprim, HPLC-UV*

INTRODUCTION

Improved water quality is germane to achieving Sustainable Development Goal (SDG) and this can be threatened by unregulated and indiscriminate disposal of pharmaceuticals, pesticides and wastes into the environment, especially water bodies. Pharmaceuticals are meant for prevention and treatment of diseases can contaminants the environment if improperly disposed. As the world population increases so is the drug production and usage, number of community pharmacy stores, hospital facilities and other healthcare channels have also increased over the years. This has consequently increased the amount of drug wastes (directly or indirectly) released into the environment. Asides from the benefits of pharmaceuticals in treatment of illnesses, a number of pharmaceuticals have been reported to possess undesired adverse effects on living organisms and their immediate environment (Chanti and Durga, 2015).

Studies have shown that pharmaceuticals indiscriminately disposed may eventually decompose and could find their way into the water-bodies, be it surface or underground water, though the quantity are mostly trace, but could accumulate overtime. Individuals have been advised by several guidelines to dispose drugs properly, in order to reduce this contamination (Muhammad *et al.*, 2020).

Pharmaceuticals can be released into the environment through, some unused or expired drug are usually thrown on ground or dustbin, through

effluents and sludges, as parent compounds, metabolites and as transformation products, which could be washed by rainfall from one point to the other, leading to accumulation overtime into the water bodies and contamination of surface water, groundwater and even drinking water (Martinez, 2009, Grover *et al.,* 2011). Trace quantities of pharmaceuticals have been reported in drinking water, waste water from pharmaceutical industries (Bexfield et al., 2019) which ranges from anti-malarial, antibiotics, analgesics and other categories of drugs as a result of indiscriminate disposal, unethical manufacturing processes to mention a few (World Health Organization, 2012). This unintentional consumption of contaminated water containing different classes of drugs could lead to drug resistance and a progressive damage to different organs of the body eventually. As beneficial as they are in terms of their therapeutic advantage, they also contribute to chemical pollution of the environment (Nair Abhilash, 2012). common side effects of short term intake of paracetamol are nausea and abdominal pain, but overdose could lead to liver damage (Okokon *et al.,*2015).

High levels of pharmaceuticals have been reported in aquatic environment; Balakrishna *et al.*(2017) reported high levels of Trimethoprim and Sulfamethoxazole (antibiotics), Ibuprofen and Acetaminophen (analgesics), Caffeine (stimulant), Carbamazepine (antipsychoactive), Triclocarban and Triclosan (antimicrobials) which are frequently found in higher concentrations in Indian waste water treatment plants, Olatunde et al., (2014) detected high levels of Diclofenac, Chloroquine, Paracetamol and Ciprofloxacin in water samples in Sango-Ota of Ogun state, Nigeria. Babatunde *et al,* (2014) detected high level of Chloroquine and Metronidazole in groundwater in Ikeja area of Lagos state.

The detection of pharmaceuticals in drinking water is of concern because unintentional exposure to some of these compounds could result in adverse effects even at low doses, including endocrine disruption and antibiotic resistance (Fuhrman et al., 2015; Schaider et al., 2014). There are also reports of increasing resistant to antibiotics drugs by man, which could result from over-ingestion of these drugs even in contaminated water (Spellberg, 2008). Hospital serves as centre of generation for most drug prescriptions; there is a pressing need to investigate the levels of pharmaceuticals in water around hospital arena from time to time, so as to provide recommendations on proper methods of drug disposal. The aim of this study is to evaluate the levels of pharmaceuticals in bore-hole water in and around hospital environments in Ogun State, Nigeria.

MATERIALS AND METHODS

Description of Study Areas

Three hospitals were selected for this study, all located in Ogun State. Site selection is done on the basis of their proximities and number of patients that normally attend the hospital. The first site had samples labelled "**A**", a university health centre is located here in Alabata, Abeokuta, Ogun State with a student population of over nineteen thousand. The primary function of this facility is to provide healthcare needs for the students and staffs. Site "**B**" is a private tertiary hospital situated in Ilishan-Remo, Ogun state, Nigeria. The facility provides healthcare services, for both in and out-patients, and also medical research and training. The third site had samples labelled "**C**", a tertiary hospital located along Olabisi Onabanjo way, Idi-Aba, Abeokuta, Ogun State, Nigeria. This hospital was established in 1983, provides healthcare for in and out-patients across the country. Sample codes and their sampling points are presented on Table 1.

Collection of Samples

Water samples from borehole-tap were collected from the chosen locations. Three samples in duplicates were collected from each of the sampling points in one litre pre-cleaned amber bottle, after rinsing the bottle thrice with the water to be collected in. The tap water was allowed to run for two minutes before collecting in the bottle, so as to flush-out the sediments around the tap-mouth and to ensure a steady temperature. The samples were instantly labelled and put in a cooler filled with ice-packs, after which they were refrigerated at 4 ^{0}C prior to analysis.

Table 1: Description of sampling points

Samples	Locations
A1, B1, F1	Hospital premise
A2, B2, F2	50 metres from the outside of the hospital premise
A3, B3, F3	150 metres from the outside of the hospital premise

Laboratory Analysis

Physicochemical Analysis

The physical and chemical analyses of these samples were carried out distinctively with the standard methods. The physicochemical properties of the samples were investigated using standard methods and this includes; odour, chloride ion, hardness, pH, conductivity, salinity and total dissolved solids. pH and electrical conductivity (EC) of each sample was determined using the pH meter Consort C3010. This determination was achieved using the human sense of smell and sight for colour detection. Determination of Hardness and Chloride ion were carried out using titrimetry method with EDTA and Silver Nitrate respectively as standards.

Standards and Chemicals

All pharmaceutical standards Trimethoprim, Metronidazole, Sulfamethoxazole and Paracetamol, with purity degree ≥ 98%, were purchased from Sigma-Aldrich (Lagos). . Reagent used were, HPLC-grade Methanol, HPLC -grade water, acetonitile (ACN), and ethylenediamine tetraacetic acid (EDTA). Stock solutions were prepared by dissolving the pharmaceutical standard in acetonitrile using a 100 ml standard volumetric flask. Thereafter, working solutions of 50, 25, 12.5 and 6.25 µg/ml were prepared by serial dilution and were later introduce into the instrument for quantification. The concentrations of Trimethoprim, Metronidazole, Sulfamethoxazole and Paracetamol in the prepared standards were determined using Technologies 1200 series HPLC system with UV-detector for the calibration of the instrument. The chromatogram of some of the standards is presented in Figure 1.

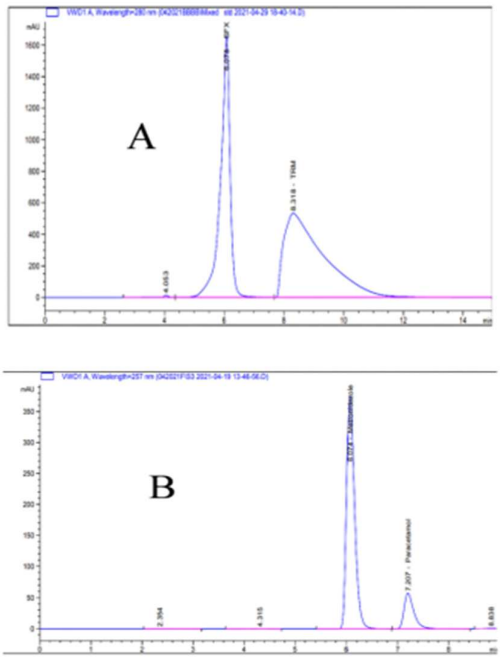

Figure 1: Chromatogram of Standards
(a) Paracetamol and Metronidazole
(b) Sulfamethoxazole and Trimethoprim

Determination of the concentration of pharmaceuticals in the collected samples

The concentration of Trimethoprim, Metronidazole, Sulfamethoxazole and Paracetamol in the collected samples after extraction and clean-up using the procedure of ----- was determined using Technologies 1200 series HPLC system with UV-detector. Separation was achieved under the following instrumental conditions summarized in Table 2

Table 2: HPLC Instrumental Conditions

HPLC Conditions	Paracetamol/ Metronidazole	Sulfamethoxazole/ Trimethoprim
HPLC Type	Agilent 1200 series	Agilent 1200 series
Column	Hypersil C-18, reversed phase	Hypersil C-18, reversed phase
Dimension	250 X 4.0 mm	250 X 4.0 mm
Mobile Phase	0.1% Formic acid + Methanol HPLC grade (75 : 25) v/v	0.1% Formic acid + Acetonitrile HPLC grade (60 : 40) v/v
Detection wavelength	257nm	280nm
Flow rate	0.5 ml/ min	0.6 ml/ min
Mode of elution	Isocratic	Isocratic

RESULTS AND DISCUSSION

The result of the physicochemical properties of the sampled water collected at different locations is presented in Table 3. The results of the concentrations of the selected pharmaceuticals are presented in Table 4, Figures 2-6.

Table 3: Result of Physicochemical Properties of the Samples

	A1	A2	A3	B1	B2	B3	F1	F2	F3	WHO (STDs)
pH	7.58 ±	6.72 ±	7.05 ±	5.82 ±	5.91 ±	6.43 ±	5.78 ±	6.72 ±	7.43 ±	
(µs/cm)	0.04	0.03	0.04	0.02	0.02	0.03	0.02	0.03	0.04	6.5-8.5
CONDUCT		1720.0		1324.5	242.6		255.8	437.4	1239.0	
IVITY	1636.02	5 ±	1171.0	0± 1.3	0 ±	367.10	0 ±	0±	8 ±	1500.0
(mg/L)	± 1.60	1.70	1± 1.10	0	0.20	± 0.30	0.25	0.43	1.20	0
					116.4		125.3	214.3		
TDS	736.20	808.40	550.37	622.52	5 ±	176.21	4 ±	3 ±	569.94	
(mg/L)	± 0.70	± 0.80	± 0.50	± 0.60	0.10	± 0.10	0.10	0.20	± 0.50	500.00
SALINITY	0.816 ±	0.816 ±	0.577 ±	0.152 ±	0.116	0.165 ±	0.122	0.209	0.715 ±	
(mg/L)	0.08	0.08	0.05	0.01	± 0.01	0.01	± 0.01	± 0.02	0.07	1.000
					86.63			121.5		
CHLORID	482.80	509.43	342.39	90.53 ±	2 ±	100.52	88.5 ±	7 ±	423.04	
E (mg/L)	± 0.40	± 0.50	± 0.30	0.09	0.08	± 0.10	0.08	0.10	± 0.40	250
	8.50 ±	7.52 ±	6.05 ±	3.52 ±	2.85 ±	3.06 ±	3.15 ±	3.75 ±	6.25	
TH(mg/L)	0.08	0.07	0.06	0.03	0.02	0.03	0.03	0.03	±0.06	600

*TDS= Total Dissolved Solids *TH= Total Hardness

Physiochemical Properties

The pH of all the water samples from all the locations falls within the permissible limits range of 6.5 – 8.5 for potable water as recommended by the WHO (2011) except samples **B1**, **B2**, **B3** and **F1** with values 5.87, 5.91, 6.43 and 5.78 respectively this indicates high level of acidity. Consumption of acidic water may lead to demineralization of tooth enamel and cause dental decay (Osobamiro et al., 2023) and could make the water to be corrosive for consumption overtime (WHO, 1996), further treatments are necessary to make this water safe for drinking.

The EC is the measure of the amount of dissolved inorganic substance and all the samples fall within the permissible level except samples **A1** and **A2** with values 1636.02 and 1720.05 µs/cm respectively. These high EC values could be traced to the presence of high concentrations of dissolved inorganic and polar organic substances in the sampled water. Samples **A1**, **A2**, **A3**, **B1** and **F3** had TDS greater than 500 mg/l, which is the maximum permitted level recommended by the Nigerian standard for drinking water quality (Standard Organization of Nigeria, 2015). Consuming water with high TDS could be harmful to people with kidney or heart-related disease conditions and may also trigger constipation overtime (Sasikaran *et al*, 2012). The salinity of the samples were generally below 1 mg/l, but samples **A1** and **A2**, still recorded highest salinities 0.816 and 0.861 mg/l respectively, which was expected, because they both had the highest EC values, as salinity is directly proportional to EC (Sasikaran *et al*, 2012).

The chloride levels 482.8, 509.43, 342.39 and 423.04 mg/l of samples **A1**, **A2**, **A3** and **F3** respectively were above the permissible level of 250 mg/l. Though analyses of chloride concentrations in rivers done by Al-Khateeb (2014) reported organic sewage as a contributing factor, but chemical weathering of rocks which would release different ions (Sasikaran *et al*, 2012), would also be a likely causative factor in this study. Water with high chloride levels has an unpleasant salty taste and can cause nausea, throat irritation and stomach irritation (Al-Khateeb, 2014). The total hardness of all the samples reported were below the maximum limit of 600 mg/l for drinking water, though samples **A1**, **A2**, **F3** and **A3** recorded the highest values of 8.50, 7.52, 6.25 and 6.05 mg/l respectively, while **B2** had the lowest value at 2.85 mg/l.

Table 4: Results of concentrations of Pharmaceuticals detected in water samples

SAMPLES	A1	B1	F1	A2	B2	F2	A3	B3	F3
Metronidazole	0.085 ± 0.001	BDL	BDL	BDL	BDL	0.102±0	BDL	BDL	0.331±0
Paracetamol	0.190 ± 0.010	0.040± 0.001	BDL	0.168 ± 0.010	2.610 ± 0.200	7.04 ± 0.700	BDL	BDL	0.264 ± 0.200
Sulfamethoxazole	BDL	0.004 ± 0.001	0.005 ± 0.001	0.004	BDL	0.007 ± 0.001	0.004 ± 0.001	0.007 ± 0.001	BDL
Trimethoprim	0.038 ± 0.030	0.016 ± 0.010	BDL	BDL	0.006 ±0.001	0.058 ± 0.050	0.018 ± 0.010	0.009 ±0.001	BDL

***BDL= Below Detection Limits**

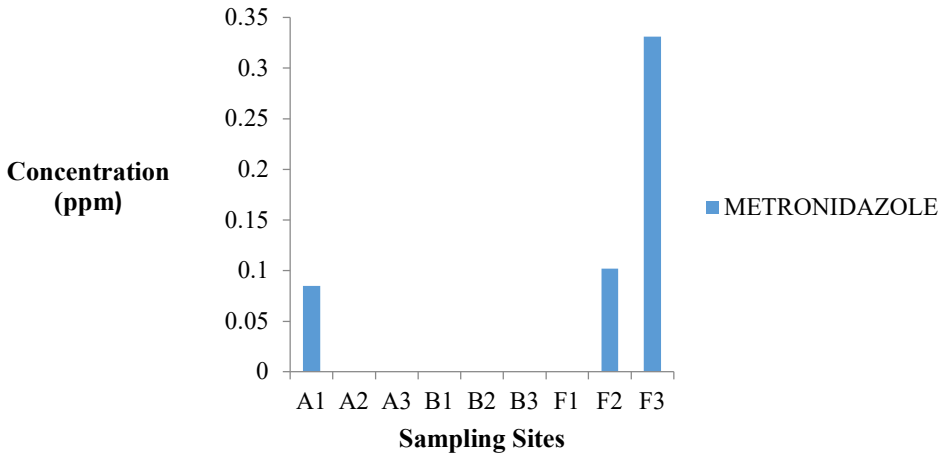

Figure 2: Comparison of the levels of Metronidazole detected in all sampling sites.

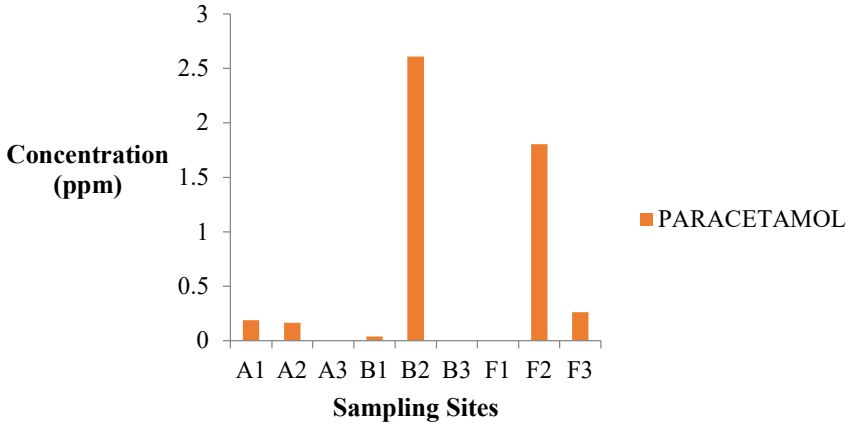

Figure 3: Comparison of the levels of Paracetamol detected in all sampling sites.

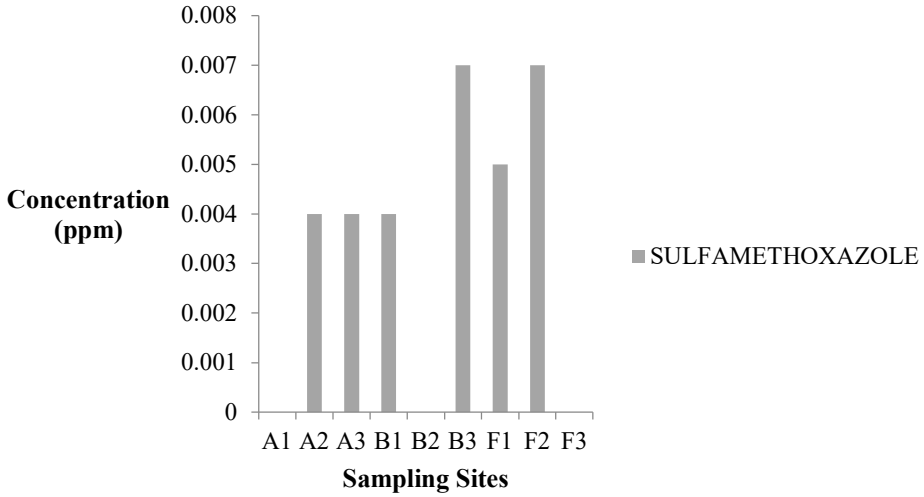

Figure 4: Comparison of the levels of Sulfamethoxazole detected in all sampling sites.

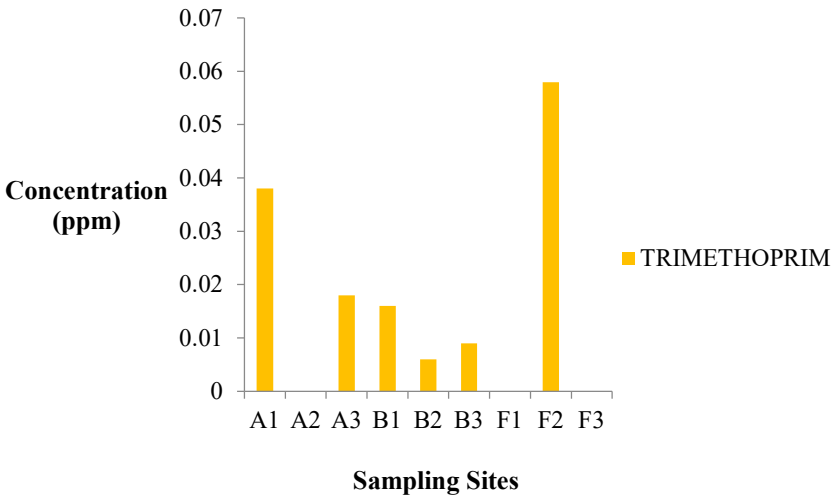

Figure 5: Comparison of the levels of Trimethoprim detected in all sampling sites.

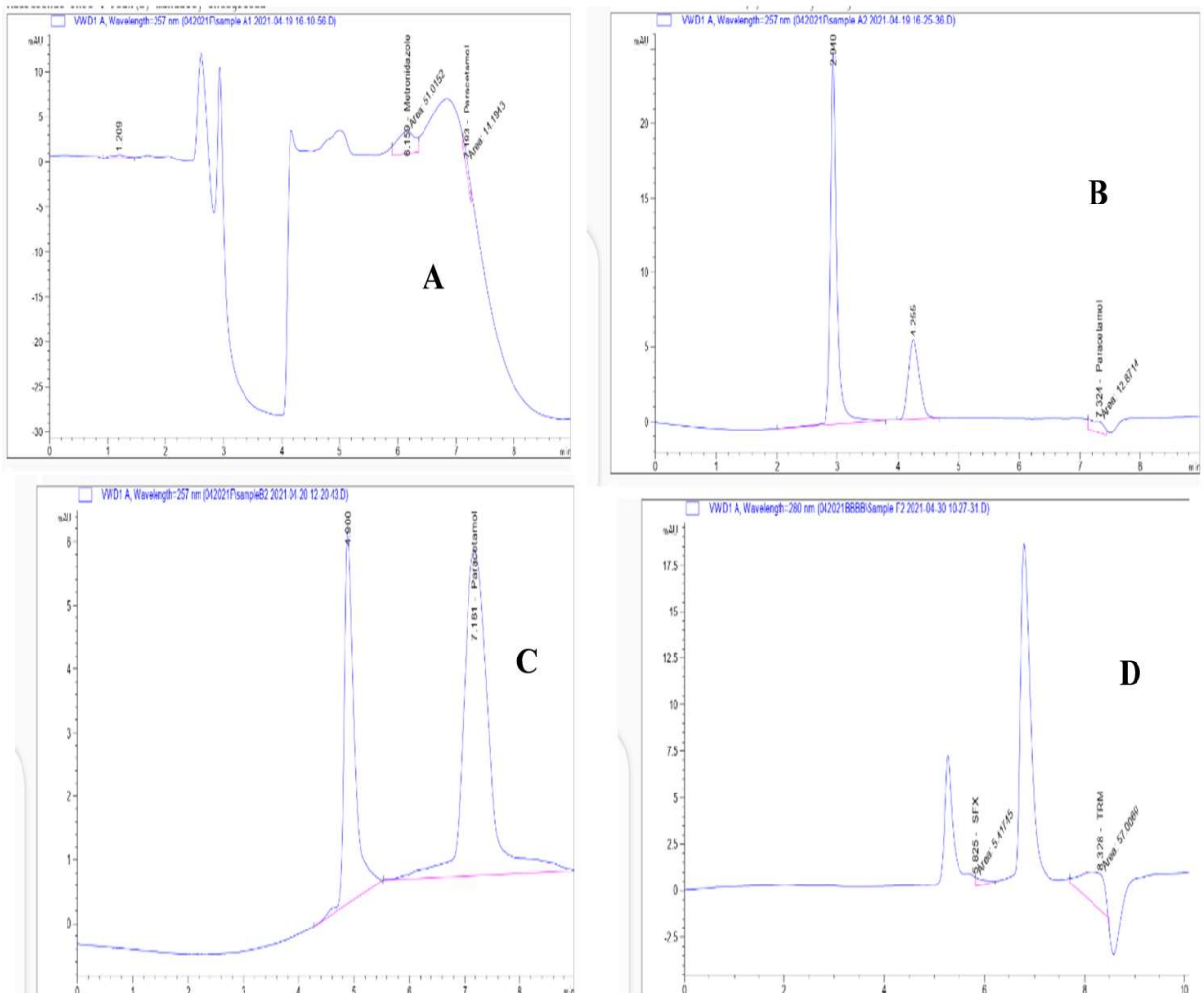

Figure 6: A, B, and C are Chromatogram of samples A1, A2 and B2 for Metronidazole and Paracetamol while D is Chromatogram of sample F2 for Sulfamethoxazole and Trimethoprim.

RESULTS OF QUANTITATIVE DETERMINATION OF PHARMACEUTICALS IN THE SAMPLED WATER

The results of the concentrations of selected pharmaceuticals as presented in Table 4 and Figures 2-6 indicated that almost all of these pharmaceuticals are detected in all the sampling sites.

Metronidazole was not detected in all sampled water except samples **A1**, **F2** and **F3** in concentrations 0.085, 0.102 and 0.331 mg/l respectively sample **F3** having the highest value which may be due to site **F** being a Federal medical centre with many in and out patient compared to site **A** and **B** but was not detected in other samples. The drug is an antibiotic used to treat wide range of bacterial infections in different parts of the body (Rossi, 2013). It could be administered in oral or intravenous dosage forms for patients, which also explains why it is one of the most dispensed antibiotic drugs in the hospital, for both in and out patients. Babatunde *et al.* (2014) had also reported concentration of 0.05 mg/l of Metronidazole in one of the analysed groundwater samples in Ikeja area of Lagos state, Nigeria.

Paracetamol, a common analgesic was detected in higher concentrations in all the samples analyzed except samples **A3**, **B3**, and **F1.** The highest concentration was found in sample **B2** and the lowest in **B1**. Paracetamol was found in all the samples collected at 50 m from outside of the hospitals which could be traced to the higher number of patients visiting the hospital and invariably higher consumption of drugs, as it has also been established as one of the most prescribed drugs for most ailments (Oshikoya and Ojo, 2007). Paracetamol is indicated for fever and conditions like aches and colds (Okonkon *et al.*, 2015), it could also be in intravenous or oral forms. An average concentration of 2.566 µg/l of Paracetamol in surface and underground water in Sango, Ogun State, Nigeria had also been reported by Olatunde *et al.* (2014).

Sulfamethoxazole was detected in all samples except **A1, B2** and **F3**, while Trimethoprim was detected in all samples except **A2**, **F1** and **F3**. These drugs are mostly manufactured and marketed as a combination drug of Sulfamethoxazole and Trimethoprim, an antibiotic, and commonly used for treatment of bacterial infections in the ear, respiratory tract, skin and other parts of the body (Tsadik *et al.*, 2015). It could also be deduced that they were both present in samples **A3**, **B1**, **B3** and **F2**, owing to their combination formulation usage and consumption. Carla *et al.* (2010) had also reported Sulfamethoxazole and Trimethoprim average concentrations of 0.028 and 0.007 mg/L respectively in hospital effluents in Brazil.

The concentration trend of the pharmaceuticals in this study varied for the three sites; this could be linked to obvious reasons which include, but not limited to different ailments being treated across the three sites, leading to some drugs prescribed and used more than others. Different forms of the drugs used; some in tablet, syrup or fluid forms, which determines their solubility levels and the degree at which they can easily permeate the underground water-bodies. Variation in the detected levels of studied pharmaceuticals may also be due to the enlightenment level of patients and healthcare-givers in these three sites on proper drug disposal method differ.

All in all, the concentration of pharmaceuticals detected in water in this study were trace quantities and not sufficient enough to exert immediate adverse effects on humans. Pressing factors such as amount of rainfall and wind, number of drug users in and around the hospitals, efficiency of hospital wastewater remediation, degree of leaching and also the extent of indiscriminate disposal after drug-use were suggested to have caused the variations and distributions of the amount of pharmaceuticals

detected, but considering the accepted level of pharmaceuticals in surface and underground water by the WHO (2012) not to exceed 0.0001 mg/l, the pharmaceuticals detected were above this permissible value. Long term consumption of such contaminated water may lead to accumulation of these pharmaceuticals in the body system, which could cause drug and antimicrobial resistance among other effects. The WHO has iterated antimicrobial resistance as one of the global health threats challenging humanity (WHO, 2020).

CONCLUSION

This study evaluated the physicochemical parameters of borehole-running water in hospital environs in Ogun state, Nigeria. The pH, electrical conductivities, total dissolved solids and chloride levels of some of the samples did not conform to the permissible limits for potable water. Furthermore, SPE-HPLC technique was employed for the detection of Metronidazole, Paracetamol, Sulfamethoxazole and Trimethoprim in the water, though trace concentrations of these pharmaceuticals were found to be present, but they were higher than the WHO permissible limits for drinking water. It can therefore be concluded that this water is not safe for consumption; it could lead to drug resistance and other health-related adverse conditions in humans' overtime.

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