**AJPSP August 15, 2017**

# A Screening for Selected Human Pharmaceuticals in Water Using SPE-HPLC, Ogun State, Nigeria

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## *ABSTRACT*

Pharmaceuticals are a structurally diverse class of emerging contaminants that have been detected throughout the world as trace contaminants in the water environment. The study is aimed at determining the occurrence and quantification of diclofenac, paracetamol, ibuprofen, ciprofloxacin, sulphadoxine and amodiaquine in well-water, tap-water and river-water. The study is conducted around a hospital environment, in Ogun State, Nigeria, using SPE and HPLC analysis. Water samples were collected from tap-water, well-water and river-water around Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State. Samples were extracted using solid phase extraction technique and further analyzed using High Performance Liquid Chromatography. The tap-water water samples contained paracetamol, ibuprofen, diclofenac, ciprofloxacin and sulphadoxine in concentrations of 0.306ng/ml, 3.738ng/ml, 0.138ng/ml, 0.44ng/ml and 1.012ng/ml respectively. The well-water samples contained paracetamol, ibuprofen, sulphadoxine and amodiaquine in concentrations of 0.152ng/ml, 5.078ng/ml, 1.008ng/ml and 0.01892ng/ml while the river-water samples were found to contain paracetamol, ibuprofen and sulphadoxine in concentration 0.192ng/ml, 3.042ng/ml and 1.294ng/ml respectively. The results confirm pharmaceuticals contamination indeed occurred in the water samples collected, which further supports previous studies around the world. Of significant importance, is the detection of sulphadoxine and amodiaquine waste, which to the best of our knowledge have not been detected elsewhere in the world. Effective water treatment plants that can conveniently remove pharmaceuticals in water is warranted, thus, preserving life and ecosystem at large.

***Keywords:***

Pharmaceuticals, Water, Environment, Contaminants

## *Introduction:*

Until recently, so much time was spent on drug discovery with little or no time expended at ascertaining the fate of these drugs after use. Recently, increasing global concerns over the public health impacts attributed to environmental pollution efforts are now being garnered towards the search of pollutants that cause health hazards in humans, animals, and the ecosystem at large. Some of these pollutants are pharmaceuticals and personal care products (PPCP). While considerable effort has been made in developed countries, African countries seem to be lagging in the prevention of pharmaceutical waste environmental contamination (1).

Pharmaceutical products contain active ingredients that have been designed to have pharmacological effects and confer significant benefits to society. They however, become our enemies when these products find their way into the environment and cause some immediate or long-term damages to microorganisms, plants, animals, man and the eco-system at large.

While the potential side effects on human and animal health arising from direct treatment have been widely documented, only recently has the implications of the occurrence, fate and effects of such medicines on the environment have been considered (2). A range of pharmaceuticals, including hormones, antibiotics, NSAIDS, antidepressants and antifungal agents have been detected in soils, surface water and ground water (3, 4, 5, 6, 7). The occurrence of pharmaceuticals in the environment and the water cycle at trace levels (in the range of nanograms to low micrograms per liter) has been widely discussed and still being published in the literature.

Concentrations of pharmaceuticals in surface waters, groundwater and partially treated water are typically less than 0.1 µg/L (or 100 ng/L), and concentrations in treated water are generally below 0.05 µg/L (or 50 ng/L) (8). The increase in detection is largely attributable to the advances in analytical techniques and instrumentation. This research is aimed at detecting the presence of analgesics (paracetamol, ibuprofen and diclofenac), antibiotic (ciprofloxacin) and antimalarials (sulphadozine and amodiaquine) in water.

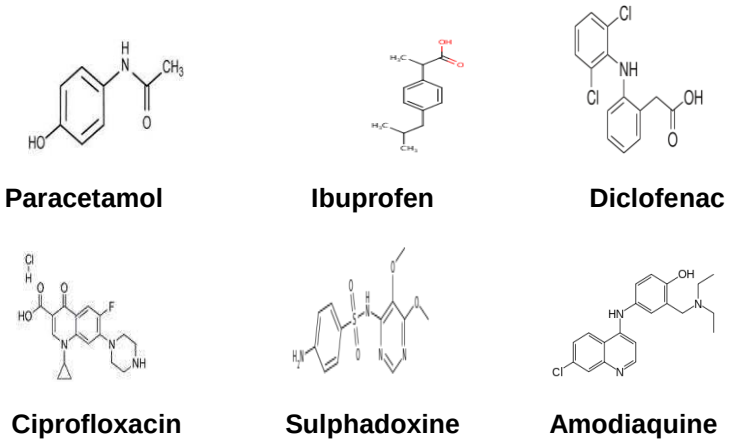


***Figure 1:*** ***Chemical Structures of the Studied Compounds***

## *METHODOLOGY:*

### CHEMICALS

All chemicals, reagents were of analytical grade, highest purity and obtained from *Fischer Scientific* UK. They included methanol HPLC grade, acetonitrile HPLC grade, triflouroacetic acid (TFA) HPLC grade. Standard paracetamol (BP), ibuprofen (BP), diclofenac (BP), ciprofloxacin powders (BP), sulphadoxine (BP) and amodiaquine were supplied by Sigma-Aldrich (Steinheim, Germany). Solid Phase Extraction Cartridges i.e. C18, Si-Cyano, C8-(12ml, 2g) were purchased from SiliCycleInc, Quecbec Canada.

### SAMPLE COLLECTION

Water samples were collected in triplicate from three different sources (tap-water, river-water and well-water) at a healthcare institution that has been in operation for over 25 years in Ogun State, Nigeria. The sampling was carried out between December 2013 and January 2014 using coherent protocols and procedures designed to obtain a representative water sample. Water samples were collected into pre-cleaned amber glass-bottles. Samples were analyzed within 36-hours of collection. To minimize contamination of samples. Use of personal care items such as insect repellents, colognes, and perfumes, caffeinated products, pharmaceuticals and tobacco were discouraged during sample collection and processing (9).

### SAMPLE PREPARATION

The first step in sample preparation was to subject water samples collected to a pre-filtration process by passing the sample through a 0.45-ųm glass fiber filter. The filtrates were respectively collected into a clean container. To further minimize contamination of the samples. Use of personal care items such as insect repellents, colognes, perfumes as well as the use of caffeinated products, pharmaceuticals and tobacco were avoided during this process.

### SPE EXTRACTION

Solid-phase extraction (SPE) procedures were employed to extract the target analytes from the aqueous samples. Water, 5mls, and 5mls of 10% methanol were measured and poured into each cartridge to activate the sorbents (C18, C8, Cyano). Water was added to promote the adsorption of the analytes onto the sorbents. Water samples, 500mls each, were loaded into the cartridge at a rate of 10ml/min. The rate at which each of the water samples was applied was controlled. Methanol 10mls (10%) was used as the wash solvent which was poured into the cartridge to remove sample constituent that were less retained on the sorbent than the analyte of interest. Methanol 5mls, (100%) which was of high eluting strength was poured into the cartridge, precisely controlled at a rate of 2ml/min to ensure reproducible result.

### PREPARATION OF STOCK SOLUTION OF STANDARD

A 200 µg/ml concentration stock solution was prepared for each of the pharmaceuticals using their respective standards. From the stock solution, 50µg/ml, 20gµ/ml, 10µg/ml, 5µg/ml and 1µg/ml concentrations were also made using serial dilution.

### HPLC ANALYSIS

Analyses of the six extracted compounds were quantitatively carried out using, a Reversed Phase Agilent 1100 LC System. The analytes were separated with their respective chromatographic conditions as stated below (Table 1:

***Table 1:*** **Chromatographic conditions of the pharmaceuticals.**

| | SP | MP | FR | UvDW | IV | RT |
|---|---|---|---|---|---|---|
| **IBUPROFEN** | YMC C18 (100 x 4.6 mm, 5$\mu$ | 0.1% TFA: ACN (40:60) | 1.0ml/min | 248nm | 10 µl | 3.8minutes |
| **DICLOFENAC** | YMC C18 (100 x 4.6 mm, 5$\mu$ | MeO**H** (100%) | 0.5ml/min | 283nm | 10 µl | 3.5minutes |
| **PARACETAMOL** | YMC C18 (100 x 4.6 mm, 5$\mu$ | $NaH_2PO_4$: ACN (65:35) | 0.8ml/min | 260nm | 10 µl | 2.5minutes |
| **CIPROFLOXACINE HCL** | YMC C18 (100 x 4.6 mm, 5$\mu$ | 0.1% TFA:ACN (80:20) | 1.0ml/min | 278nm | 10 ml | 3.2minutes |
| **SULPHADOXINE** | YMC C18 (100 x 4.6 mm, 5$\mu$ | 0.1% TFA:ACN (70:30) | 1.0ml/min | 278nm | 10 µl | 3.8minutes |
| **AMODIAQUINE** | YMC C18 (100 x 4.6 mm, 5$\mu$ | 0.1% TFA:MeO**H** (10:90) | 1.0ml/min | 341nm | 10 µl | 2.0minutes |

***Key****: SP-Stationary Phase, MP-Mobile Phase, FR-Flow Rate, UvDW-UV Detector Wavelength, IV-Injector Volume, RT-Run Time, TFA-Tetrafluoroacetic Acid, MeOH-Methanol, $NaH_2PO_4$- Sodium Diydro**g**en-P**h**osp**h**ate, ACN-Acetonitrile*

## *Results:*

Calibration curves were obtained using standard concentrations of the standards for all the six pharmaceuticals. The six calibration curves were all linear with a correlation coefficient ranging from 0.9882-0.995. All water samples analysed largely contained the pharmaceuticals in varying concentrations.

*Table 2* below, provides a summary of results obtained in this study. Further illustrations of the distribution of the pharmaceuticals in the water samples are shown in *Figures 2* to *10* below.

***Table 2:*** **Average concentration of pharmaceutical water samples**

| Pharmaceutical | Water samples | Water source | Concentration(ng/ml) |
|---|---|---|---|
| **Paracetamol** | A | Tap-water | 0.306 |
| | B | Well | 0.152 |
| | C | River | 0.192 |
| **Ibuprofen** | A | Tap-water | 3.738 |
| | B | Well | 5.078 |
| | C | River | 3.042 |
| **Diclofenac** | A | Tap-water | 0.138 |
| | B | Well | NOT DETECTED |
| | C | River | NOT DETECTED |
| **Ciprofloxacin** | A | Tap-water | 0.44 |
| | B | Well | NOT DETECTED |
| | C | River | NOT DETECTED |
| **Sulphadoxine** | A | Tap-water | 1.012 |
| | B | Well | 1.008 |
| | C | River | 1.294 |
| **Amodiaquine** | A | Tap-water | NOT DETECTED |
| | B | Well | 0.01892 |
| | C | River | NOT DETECTED |

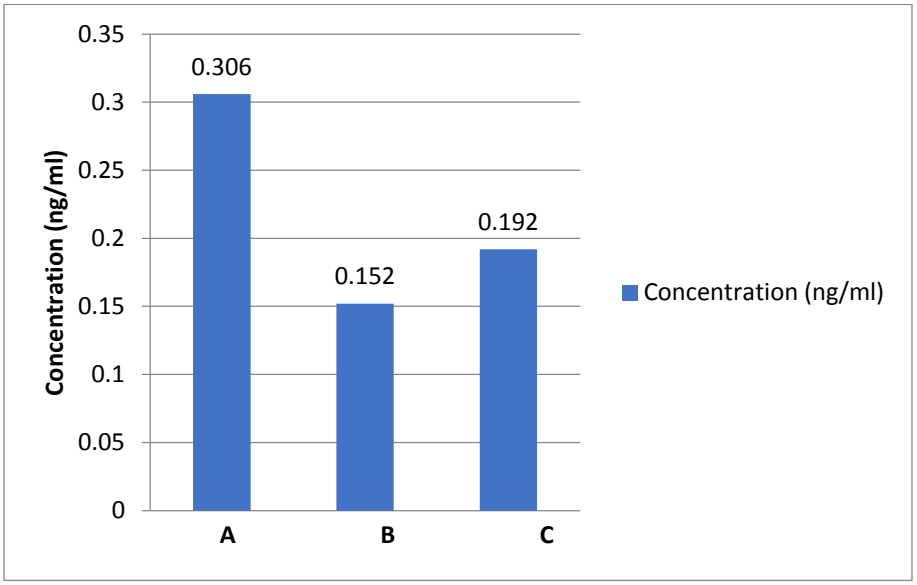


***Figure 2:*** ***Concentration of paracetamol in each of the water samples***

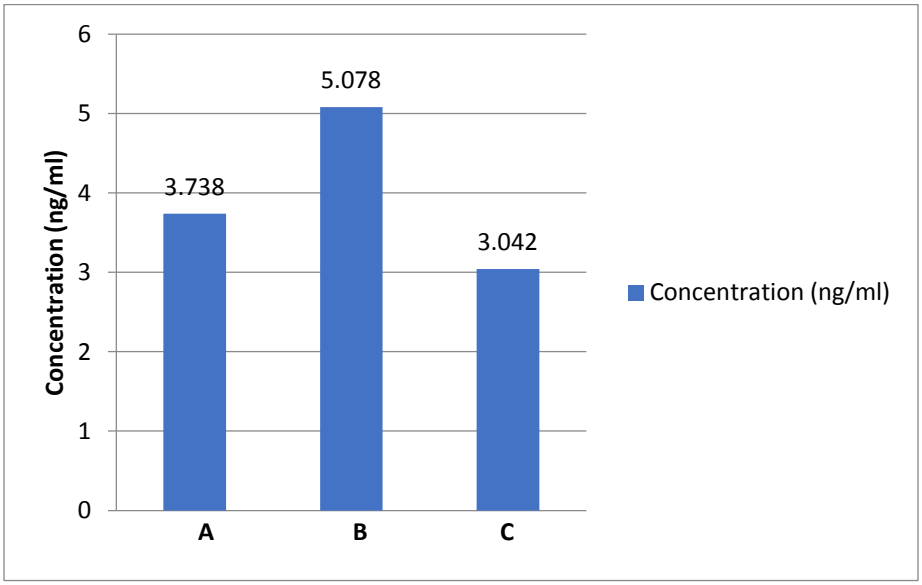


***Figure 3:*** ***Concentration of ibuprofen in each of the water samples***

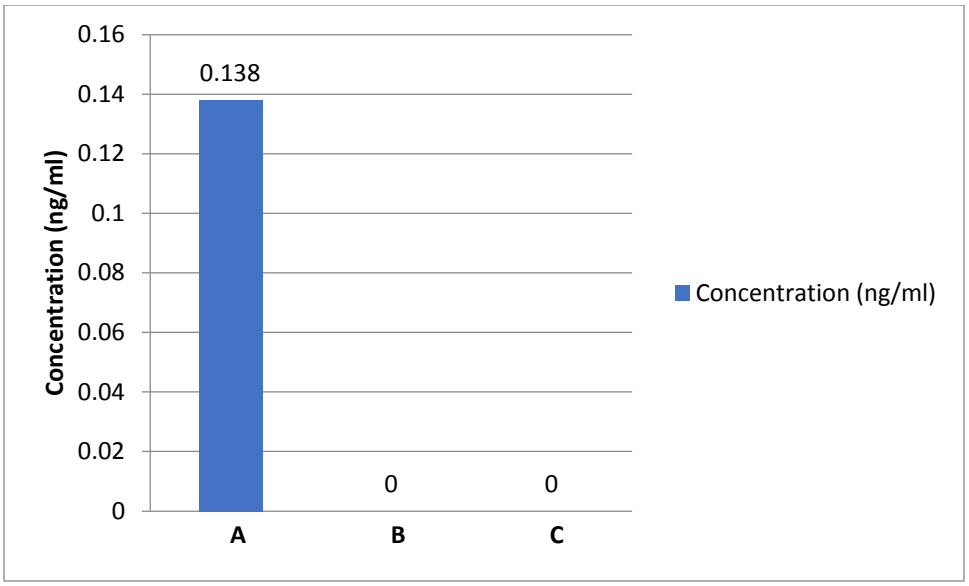


***Figure 4:*** ***Concentration of diclofenac in each of the water samples***

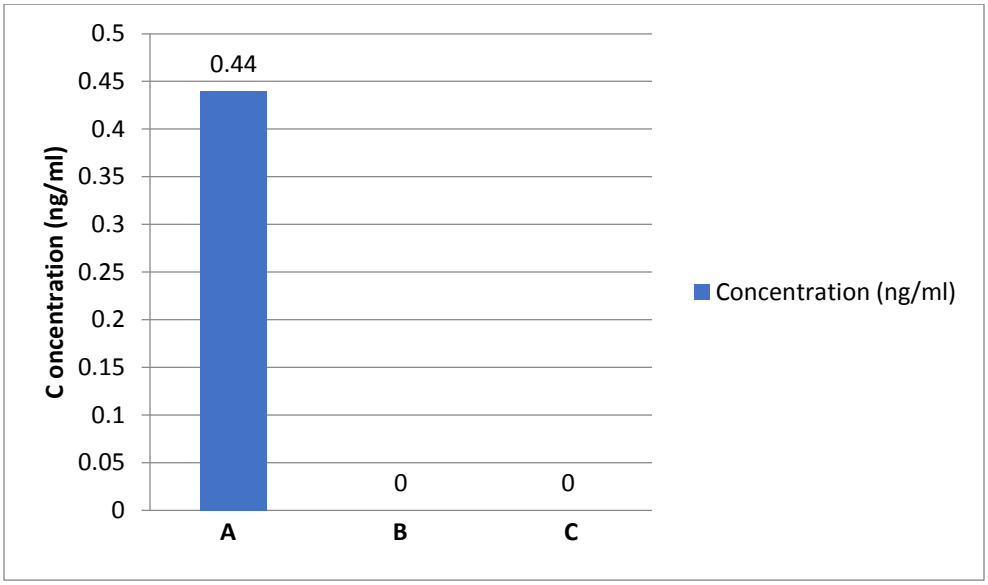


***Figure 5:*** ***Concentration of ciprofloxacin in each of the water samples***

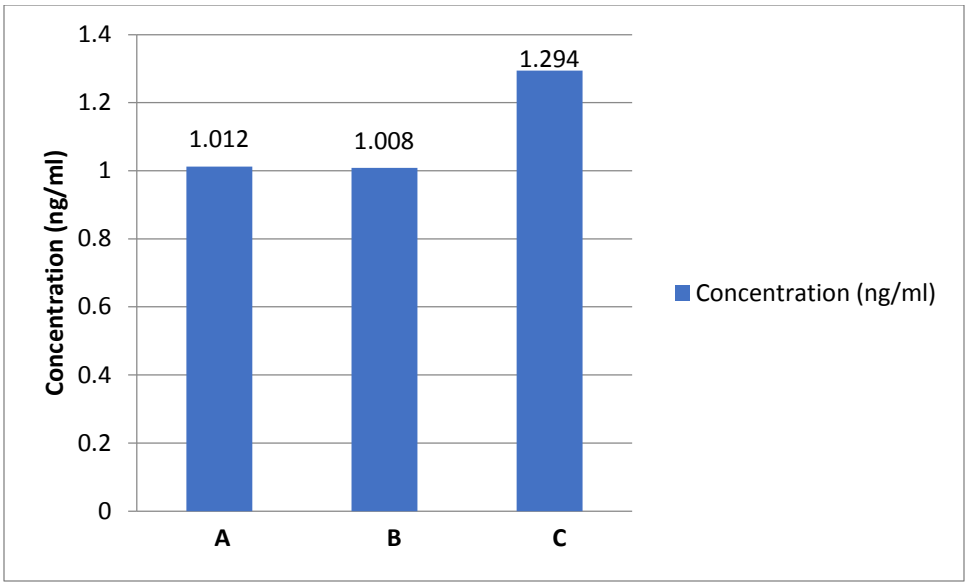


***Figure 6:*** ***Concentration of sulphadoxine in each of the water samples***

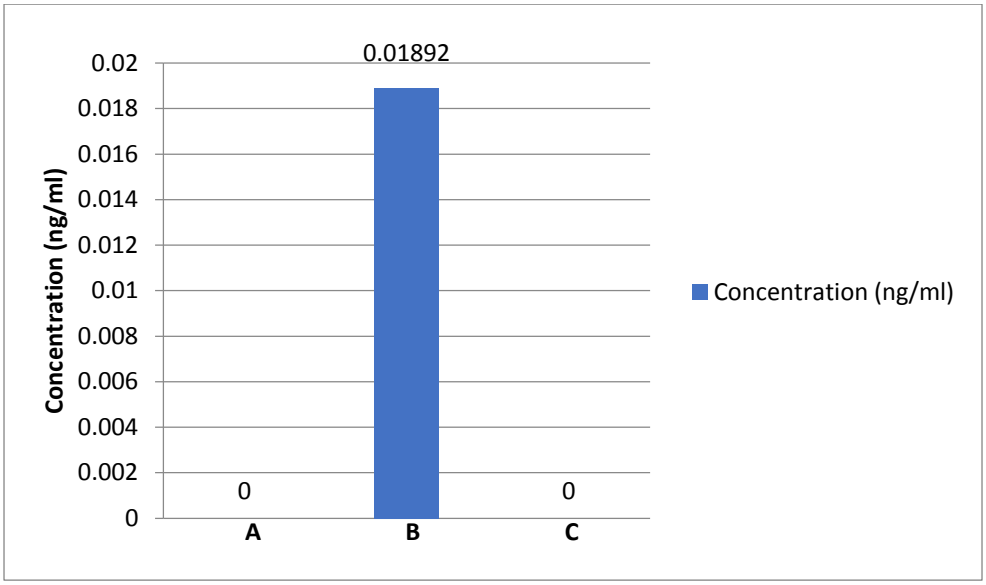


***Figure 7:*** ***Concentration of amodiaquine in each of the water samples***

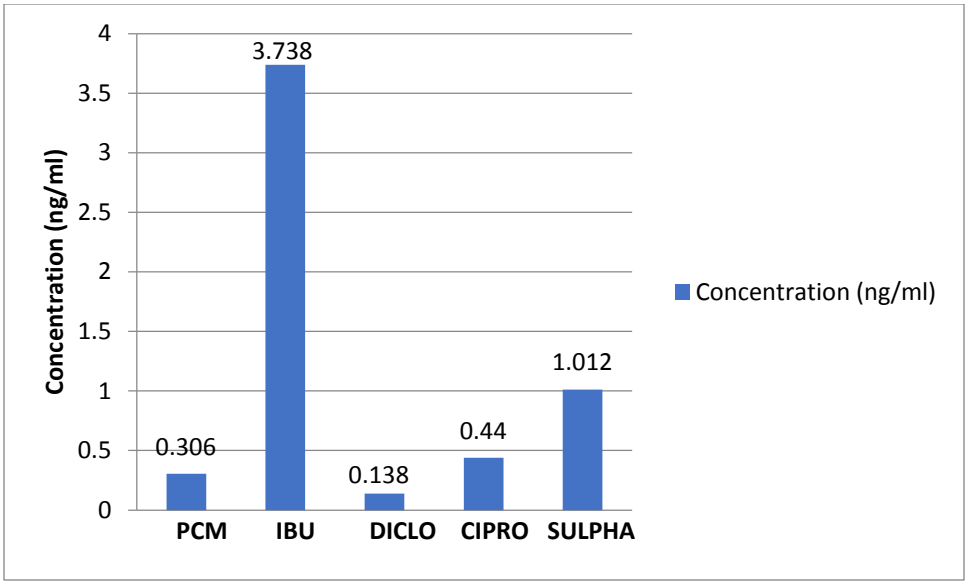


***Figure 8:*** ***Concentration of pharmaceuticals found in borehole water sample***

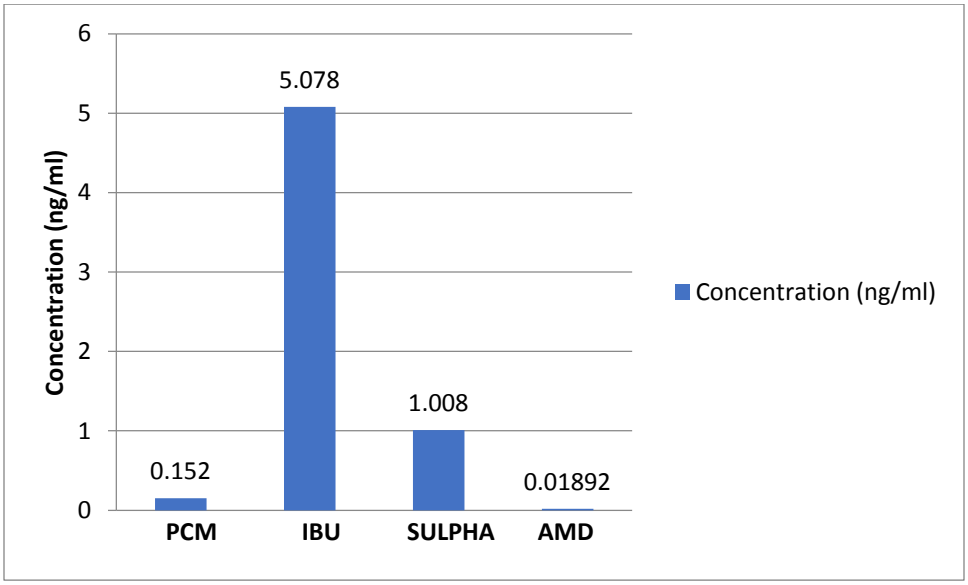


***Figure 9:*** ***Concentration of pharmaceuticals found in well-water sample***

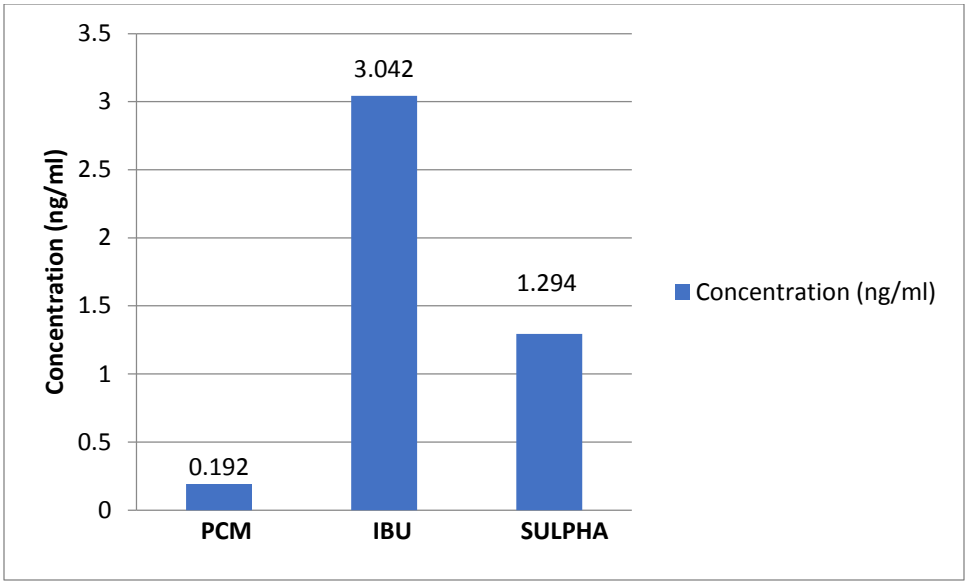


***Figure 10:*** *Concentration of pharmaceuticals found in river-water sample*

## *Discussion:*

The distribution of each pharmaceutical analyzed in the water samples is shown in *Figures 2* to *7*.

Paracetamol was detected in all water samples (tap-water, well-water, and river-water) with concentrations of 0.306ng/ml, 0.152ng/ml and 0.192ng/ml respectively as shown in *Figure 2*. *Figure 3* shows the distribution of Ibuprofen in each water sample with concentrations of 3.738ng/ml, 5.078ng/ml and 3.042ng/ml in tap-water, well and river-water samples respectively. Diclofenac and ciprofloxacin were detected only in tap-water water sample with concentrations of 0.138ng/ml and 0.44ng/ml respectively as seen in *Figures 4* and *5*. Sulphadoxine was detected in all water samples (tap-water, well-water, river-water) with concentrations of 1.012ng/ml, 1.008ng/ml and 1.294ng/ml respectively as shown in *Figure 6*. Amodiaquine was detected in well-water only, at a concentration of 0.01892ng/ml as demonstrated in *Figure 7*. In *sample A* (tap-water), ibuprofen was observed to have the highest individual concentration of 3.738ng/ml, with other drug concentrations observed to be as follows: sulphadoxine 1.012ng/ml, ciprofloxacin 0.44ng/ml, paracetamol 0.306ng/ml, and diclofenac 0.138ng/ml. *Figure 8* demonstrates distribution of the concentrations of five pharmaceuticals that were detected in the borehole sample. In *sample B* (well-water), the pharmaceutical with the highest individual concentration was ibuprofen with a concentration of 5.078ng/ml, with other drug concentrations as follows: sulphadoxine 1.008ng/ml, paracetamol 0.152ng/ml, and amodiaquine 0.01892ng/ml, as demonstrated in *Figure 9*. In *sample C* (river-water), three pharmaceuticals were found in the sample, these included: Paracetamol, ibuprofen and sulphadoxine. Ibuprofen was observed to have the highest concentration at 3.042ng/ml, with the other drug concentrations as follows: sulphadoxine 1.294ng/ml, and paracetamol 0.192ng/ml. Paracetamol, ibuprofen and sulphadoxine were detected in all the water samples tested. Ibuprofen was observed to have the

highest concentration in all the water samples that were analyzed with concentrations of 3.738ng/ml, 5.078ng/ml and 3.042ng/ml in tap-water water, well-water and river-water respectively. The average concentration of paracetamol detected in all water samples analyzed was 0.2167ng/ml, average concentration for ibuprofen in all water samples was 3.9526ng/ml, while the average concentration for sulphadoxine was 1.1046ng/ml.

The presence of paracetamol, diclofenac, ibuprofen and ciprofloxacin in water samples from Sango Ota, Ogun State, Nigeria is in line with an earlier study in this environment which showed concentrations of µg/ml (6). In a similar previous research by Kolpin *et al*. (6), most concentrations recorded exceeded 1 µg/ml. Chronic exposure to diclofenac can impair renal functions in fish. The kidney has also been found to be a target organ of diclofenac toxicity in many organisms such as birds, mice and humans (6,9,10,11). The exposure of activated sludge microbes to 5-500 µg/L of diclofenac, ibuprofen, can lead to a shift in the community structure and inhibit the growth of bacteria (genus *Nitrospira*) that play a key role in nitrification during wastewater treatment (12). These, may in turn, reduce the removal efficiency of NSAIDs in wastewaters. Laboratory-based experiments showed that ibuprofen was fully mineralized by microbes present in activated sludge but only after addition of lactose as another carbon source, a mechanism known as co-metabolism (13). The effective diclofenac concentration for chronic fish toxicity was in range of wastewater concentrations. Diclofenac residues and renal disease were reproduced experimentally in oriental white-backed vultures by direct oral exposure and through feeding vultures the remains of diclofenac-treated livestock (9).

The anti-inflammatory compounds, ibuprofen (up to 93 ng/l) and diclofenac (up to 261 ng/l) were among the most frequently detected. In a survey conducted by the United States Geological Survey. Ibuprofen was detected at a maximum concentration of 1.0 ug/l (0.20 ug/l median concentration, 0.018 ug/l reporting level) at a 9.5% frequency in 84 submitted water samples from a network of 139 US stream sampling sites across 30 states during the period of 1999 to 2000 (4). Ciprofloxacin, for example, was detected in concentrations between 0.7 and 124.5 µg/L in hospital effluent.

Information about the effects of the active substances on organisms in aquatic and terrestrial environments is increasing but still too little. Effects on fish, daphnia, algae, and bacteria have been demonstrated using low concentrations in long-term tests. Ciprofloxacin, for example, was found in concentrations between 0.7 and 124.5 µg/L in hospital effluent (14). Bacteria resistance to antibiotics has been observed in the aquatic environment (15).

The links between the presence of antimicrobials and the favoring of resistant bacteria as well as the transfer of resistance at concentrations as low as those found for antimicrobials in the environment have not yet been established. This could also explain the resistance in antimalarial-therapy facing this part of Africa. The result from this research further supports observations and conclusions of a number of previous studies outside Nigeria that suggests pharmaceutical compounds are present in water which include the presence of ibuprofen in Somes river, Romania, in concentrations of 300-10000ng/l (16); ibuprofen and diclofenac detected in Pearl river, South China, in concentrations of 17-685ng/l (17); ibuprofen and paracetamol detected in Nairobi river, Kenya, in concentrations of 10 -30µg/l (18).

## *Conclusion:*

This research demonstrates water samples (tap-water, well-water and river-water) obtained from Olabisi Onabanjo University Teaching Hospital Sagamu, Ogun state contain six pharmaceutically active ingredients. These include, paracetamol, ibuprofen, diclofenac, ciprofloxacin, sulphadoxine, amodiaquine in varying low concentrations. Due to low concentrations of these pharmaceuticals, their health impact on humans may be minimal. The accumulation of these pharmaceutical agents over time can pose some harmful effects such as antimicrobial resistance, toxicity in humans, as well as aquatic toxicity. Further investigations of pharmaceuticals in African waters is therefore warranted.

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