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Occurrence of Acetaminophen, Amoxicillin, Diclofenac and Methylparaben in Lagos and Ologe Lagoons, Lagos, Nigeria

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ABSTRACT: The occurrence of acetaminophen, amoxicillin, diclofenac and methylparaben in Lagos and Ologe Lagoons was investigated by random sampling of the water bodies during rainy and dry seasons. Samples analysis was carried out using high performance liquid chromatography. For the two seasons, the mean environmental concentrations of acetaminophen in Lagos and Ologe Lagoons were 0.075 µg/L and 1.233 µg/L respectively. The mean environmental concentration of amoxicillin in Lagos Lagoon was 0.008 µg/L while 0.003 µg/L was the mean value in Ologe Lagoon. Diclofenac occurrence in Lagos Lagoon had a mean value of 0.136 µg/L while 0.519 µg/L was recorded in Ologe Lagoon. The environmental concentration of methylparaben in Lagos Lagoon was 0.453 µg/L while its means concentration in Ologe Lagoon was 0.089 µg/L. From the results, the environmental concentrations of acetaminophen and diclofenac in Ologe Lagoon were higher than their level of occurrence in Lagos Lagoon. However, the levels of occurrence of amoxicillin and methylparaben in Lagos Lagoon were higher than their environmental concentrations in Ologe Lagoon. Methylparaben was the most frequently detected in the water bodies with 87.5% detection frequency. This was followed by diclofenac with 75%. Also, 50% of the water samples analysed contained acetaminophen while amoxicillin had the least (37.5%) frequency of occurrence. The study established that there are active pharmaceuticals compounds and excipients present in the Nigerian aquatic environment which are micropollutants of emerging environmental concerns and of significant ecological risk. Therefore, there is need for environmental regulators to make concerted efforts towards environmental monitoring of this group of pollutants.

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Acetaminophen, diclofenac, amoxicillin and methylparaben represent thousands of active pharmaceutical compounds and excipients produced and used in different parts of the world. During the last several years many studies have shown pharmaceutical compounds to be present in measurable concentrations in the aquatic environment (Daughton and Ternes, 2000; Kummerer, 2001; Heberer, 2002). Thus, pharmaceuticals have emerged as a major group of environmental contaminants. However, a global-scale analysis of the presence of 203 pharmaceutical compounds across 41 countries indicated there was inadequate information about the occurrence of pharmaceuticals in surface waters in Africa (Hughes *et al.,* 2013). Globally, the presence of pharmaceuticals and personal care products in environmental media, especially in the aquatic environment and their biological effects on non-target organisms had generated sustained interest. Lately, the European Union adopted a watch-list of potential priority substances, which includes pharmaceuticals and personal care products that should be monitored to ascertain their environmental risk (Barbosa *et al.,* 2016). Pharmaceutical and personal care products hardly consist only of active pharmaceutical ingredient(s) (APIs). In most cases, a product consists of one or more APIs and inert materials, commonly referred to as excipients. Conventionally defined as pharmacologically inactive compounds, excipients are components of pharmaceutical preparations added to aid manufacture, administration or absorption of drugs (Nishath *et al.,* 2011). The substantial production and pervasive use of pharmaceuticals and personal care products make their occurrence in environmental media inevitable (Corcoran *et al*., 2010). They are continuously released into the environment in very large amounts, either through industrial effluent discharge, disposal of expired products or through excretion of used drugs. Effluents from the production of pharmaceuticals have been a major source of pharmaceuticals in the aquatic environment because they are not totally removed through conventional wastewater treatment techniques (Osorio *et al*., 2016). Painkillers are the most detected pharmaceutical compounds investigated in water environment globally, which account for 31% of records, and this is

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followed by antibiotics (Hughes *et al.,* 2013). Amoxicillin is an antibiotic with widespread use, which is excreted unchanged in urine and faeces (Elizalde-Velázquez *et al.,* 2016); and whose presence in the environment has also been broadly studied. Parabens, in general, have extensive use as antibacterial preservatives in food, pharmaceuticals and personal care products. As a result, these pharmaceutically inactive additives are likely to be released into the environment along with the active pharmaceutical substances. In Nigeria, the presence of acetaminophen, diclofenac and amoxicillin in groundwater and surface water samples had been confirmed by Olaitan *et al.* (2014). Lagos and Ologe Lagoons are two important surface water bodies in Lagos State, which serve as habitats for a wide range of aquatic organisms. Notably, the anthropogenic influences on Lagos Lagoon include pharmaceutical industries (Oguguah *et al.*, 2018) while Ologe Lagoon is proximate to pharmaceutical industries located within the Agbara Industrial Estate. Therefore, the essence of this study was to determine the environmental occurrence of some painkillers and antibiotics commonly used in Nigeria. In addition, the presence of methylparaben, which has common use as an incipient in pharmaceutical products, was also investigated. The presence of pharmaceuticals in surface water presents some levels of risk to aquatic organisms. Most of the pharmaceuticals are biologically active and are characterised by persistence and bioaccumulation potential, posing a threat to the ecosystem and human health (Juliano and Magrini, 2017). Therefore, the aim of this study was to determine the level of occurrence of acetaminophen, amoxicillin, diclofenac and methylparaben in Lagos Lagoon and Ologe Lagoon.

MATERIALS AND METHODS

Study Area and Water Sampling: This study involved initial surface water sampling to determine levels of PPCPs in Lagos and Ologe Lagoons being brackish and freshwater lagoons respectively. Specifically, water sampling was carried out following the methods of Oketola *et al.* (2006), which was followed by laboratory analysis. Amber glass bottles pre-rinsed with distilled water were used for sample collection. The samples were placed in a cooler with ice packs and delivered to the laboratory within 24 hours. Samples were immediately pre-treated and stored in a refrigerator (− 20 °C) until analysis, which was carried out within one week from the date of sampling. The rainy season sampling was carried out in August 2015 while the dry season sampling was carried out in February 2016. Each of the two surface water bodies was divided into two zones which were selected based on nearness to human settlements and anthropogenic activities, industrial facilities and sewage disposal. Lagos Lagoon was sampled at Bariga waterfront and Iddo while surface water samples were also collected from Ologe Lagoon near the discharge point of Agbara Estate Wastewater Treatment Plant and at a location 200 metre downstream. The selection of the two water bodies investigated water bodies was based on their distinct characteristics such as the freshwater, brackish and sources of PPCPs.

Table 1: Description of sampling locations and rationale for selection

Water Body	Locations / Geographical coordinates	Rationale for Selection	Description of Sampling Points
Ologe Lagoon	6° 30.014'N, 3° 6.196'E (near effluent discharge point) 6° 29.757'N, 3° 6.147'E (500 metres away from effluent discharge point)	Ologe is a freshwater lagoon located near a major industrial estate. Active ingredients of pharmaceuticals are likely to be released mainly from point source.	Agbara Industrial Estate hosts a significant number of pharmaceutical companies with a central wastewater treatment plant for industrial effluent and sewage treatment.
Lagos Lagoon	6° 31'40.18"N, 3° 24'5.37"E (Bariga) 6° 28'9.47"N, 3° 23'6.65"E (Iddo)	Lagos Lagoon is brackish in nature, and it receives a wide range of pollutants from diverse sources.	Two locations sampled (Bariga waterfront at Abule Eledu are characterised by open defecation and discharge of sewage into the lagoon, especially Iddo.

Solid Phase Extraction: Solid Phase Extraction (SPE) was used to extract acetaminophen, diclofenac, amoxicillin and methylparaben from water samples. The SPE cartridges were purchased from SiliCycle Inc., Quebec Canada. Extraction of the analytes followed the procedures of Olaitan *et al.* (2014). The extraction was carried out in batches using a vacuum SPE manifold, which accommodated twelve C18 cartridges. Each of the cartridges was preconditioned using 5 ml of Acetonitrile. Thereafter, 500 ml of each of the filtrates from the water samples were loaded on the cartridge. The extracted target analytes adsorbed to the sorbents were desorbed with acidified methanol. These compounds were slowly reduced to 100 µL under the flow of N_2 and then brought to 1 ml by adding 900 µL of aqueous high-performance liquid chromatography mobile phase (Cahill *et al.,* 2004, Olaitan *et al.,* 2014).

High Pressure Liquid Chromatograph: Analysis of water samples using high-performance liquid chromatography (HPLC) was carried out according to the method described by Olaitan *et al.* (2014). For each of the pharmaceutical compounds, a 200 µg/ml concentration stock solution was prepared using their respective standards to obtain a calibration plot. From the stock solution, 20, 10, 5, 2 and 1 µg/ml concentrations were also made by serial dilution. Analyses of the four extracted compounds were carried out using an Agilent 1100 LC System with a UV detector. The analytes were separated based on their respective chromatographic conditions. All chemicals and reagents were of analytical grade supplied by Sigma-Aldrich. They include HPLC grades of methanol, acetonitrile, triflouroacetic acid (TFA), standard paracetamol (BP), diclofenac sodium (BP), amoxicillin (BP) and methylparaben. Measurements of the analytes were in microgram per litre (ug/L).

RESULTS AND DISCUSSION

Rainy season: The laboratory analysis of water samples collected during the rainy season confirmed the presence of diclofenac, amoxicillin, and methylparaben in Lagos and Ologe Lagoons. Acetaminophen was not detected in any of the sampling locations in Lagos Lagoon during the rainy season while it was detected at the 2 sampling points in Ologe Lagoon with environmental concentrations of 3.588 µg/L and 1.340 µg/L for the effluent receiving point and at 500 metres away from the receiving point respectively. The mean concentration of acetaminophen in the lagoons during the rainy season was 1.23 µg/L. Amoxicillin was detected in the water sample collected at Bariga waterfront of the Lagos Lagoon with a concentration of 0.011 µg/L. In Ologe Lagoon, amoxicillin concentration of 0.013 µg/L was detected 500 metres away from the effluent receiving point of the Agbara Estate Sewage Treatment Plant. The mean environmental concentration of the investigated antibiotic compound was 0.01 µg/L. Diclofenac and methylparaben were detected in the four water samples collected from Lagos and Ologe Lagoons. The concentrations of diclofenac ranged from 0.01 µg/L to 2.067 µg/L with a mean concentration of 0.65 µg/L while methylparaben ranged from 0.052 µg/L to 1.66 µg/L with a mean concentration of 0.47 µg/L (Table 2).

Table 2: Environmental concentrations of investigated compounds in rainy season

	Lagos Lagoon		Ologe Lagoon		Mean ± SD
PPCPs	Point A	Point B	Point A	Point B	
Acetaminophen (µg/L)	ND	ND	3.588	1.340	1.23 ± 0.00
Amoxicillin (µg/L)	0.011	ND	ND	0.013	0.01 ± 0.01
Diclofenac (µg/L)	0.256	0.267	0.010	2.067	0.65 ± 0.95
Methylparaben (µg/L)	1.660	0.052	0.132	0.044	0.47 ± 0.79

ND – Not Detected

Dry Season: The dry season study shows that acetaminophen, amoxicillin and diclofenac were detected in Lagos Lagoon and none of these three substances were detected in Ologe Lagoon. However, methylparaben was detected in the water samples collected from Ologe Lagoon having occurrence levels of 0.088 µg/L and 0.092 µg/L for Points A and B respectively. In Lagos Lagoon, acetaminophen was detected in Bariga and Iddo axes of the Lagos Lagoon at the environmental concentrations of 0.232 µg/L and 0.068 µg/L respectively. The mean environmental concentration of the analgesic compound in dry season was 0.08 µg/L. Amoxicillin was detected only in the water sample collected from Iddo axis of the lagoon at 0.02 µg/L with a mean value of 0.01 µg/L across the two lagoons. The environmental concentrations of diclofenac at the two sampling stations of Lagos Lagoon was 0.01 µg/L. Meanwhile, the compound was not detected in Ologe Lagoon during the dry season study. Methylparaben was detected only in the water sample collected at the Bariga waterfront (0.096 µg/L) of the Lagos Lagoon while the compound was detected at the two sampling stations in Ologe Lagoon. The levels of methylparaben occurrence in Ologe Lagoon were 0.088 µg/L and 0.092 µg/L. (Table 3).

Table 3: Environmental concentrations of investigated compounds in dry season

	Lagos Lagoon		Ologe Lagoon		Mean ± SD
PPCPs	Point A	Point B	Point A	Point B	
Acetaminophen (µg/L)	0.232	0.068	ND	ND	0.08 ± 0.11
Amoxicillin (µg/L)	ND	0.02	ND	ND	0.01 ± 0.01
Diclofenac (µg/L)	0.010	0.011	ND	ND	0.01 ± 0.01
Methylparaben (µg/L)	0.096	ND	0.088	0.092	0.07 ± 0.05

ND – Not Detected

Occurrence Level and Detection Frequency of Investigated Compounds: The environmental concentrations of acetaminophen and diclofenac in Ologe Lagoon were higher than their level of occurrence in Lagos Lagoon.

However, the levels of occurrence of amoxicillin and methylparaben in Lagos Lagoon were higher than their environmental concentrations in Ologe Lagoon. Amoxicillin had the least environmental concentrations of the four compounds investigated across the two water bodies (Figure 1). Methylparaben was the most frequently detected compound in the water samples with a detection frequency of 87.5%. This was followed by diclofenac with 75% detection frequency. Acetaminophen was detected in 50% of the total samples analysed while amoxicillin had the least (37.5%) frequency of occurrence (Figure 2).

Generally, the study established that generally PPCPs are present in Lagos and Ologe Lagoons, and these pollutants must have been released from diverse point and non-point sources into the surface waters. Specifically, the study has been able to confirm that acetaminophen; amoxicillin, diclofenac, and methylparaben occur in the water bodies in Nigeria.

Acetaminophen, amoxicillin and diclofenac were not detected in Ologe Lagoon during the dry season study while methylparaben was detected in all the sampling locations in rainy and dry seasons. Ologe Lagoon had more painkillers (acetaminophen and diclofenac) in rainy season than Lagos Lagoon.

The high level of these PPCPs in Ologe Lagoon may be attributed to the presence of some pharmaceutical companies in Agbara Estate, which is close to Ologe Lagoon - the major receiving water body for wastewaters from the estate. The high detection frequency of methylparaben indicates its widespread use in a broad range of PPCPs, which are released into the environment through point and non-point sources.

In addition, the high frequencies of occurrence of diclofenac (75%) and acetaminophen (50%) are indication of their common use as over-the-counter painkillers. This is in line with the submissions of Hughes *et al.* (2013), Al-Kaf *et al.* (2017) and Cardoso-Vera *et al.* (2017).

However, the low detection frequency of the antibiotic amoxicillin could be attributed to its short half-life and susceptibility to rapid biodegradation in the environment (Lamm *et al.,* 2009). The maximum concentration of acetaminophen detected in this study was 3.588 µg/L detected in a water sample collected from Ologe Lagoon, which was lower than the 23.3 µg/L detected in surface water samples reported by Gašo-Sokač *et al.* (2017). However, in a study carried out in Ogun State, the highest concentration of acetaminophen detected in surface water samples collected from an irrigation canal was 1.52 µg/L. Also, the highest concentration of diclofenac detected in water sample during this study was approximately 2.07 µg/L while Olaitan *et al*. (2014) recorded 57.16 µg/L in a water sample. In Czech rivers environmental concentration of diclofenac as high as 20 µg/L was reported by Praskova *et al.* (2014) and up to 510 µg/L has been reported by Gašo-Sokač *et al.* (2017). However, lower concentration (0.18 µg/L) of diclofenac was detected in Volturno River, Italy by Rocco *et al.* (2011).

The maximum concentration of methylparaben in this study was 1.45 µg/L, which was lower than the values reported for Mogi Guaçu River, Sao Paolo, Brazil (8 µg/L) and 79.6 µg/L detected in Colorado River, California, USA (Galinaro *et al.,* 2015). The highest amoxicillin concentration recorded during this study was 0.02 µg/L. This is comparatively close to the concentrations of amoxicillin detected in Iran, which were 0.018 µg/L and 0.03 µg/L in Kan River and Firozabad Ditch Tehran Province respectively (Mirzaei *et al.,* 2017). Conversely, amoxicillin concentration as high as 0.245 µg/L was detected in surface water samples in the United Kingdom and 0.2 µg/L was reported to have been detected in surface waters in Australia and Spain (Elizalde-Velázquez *et al.,* 2016).

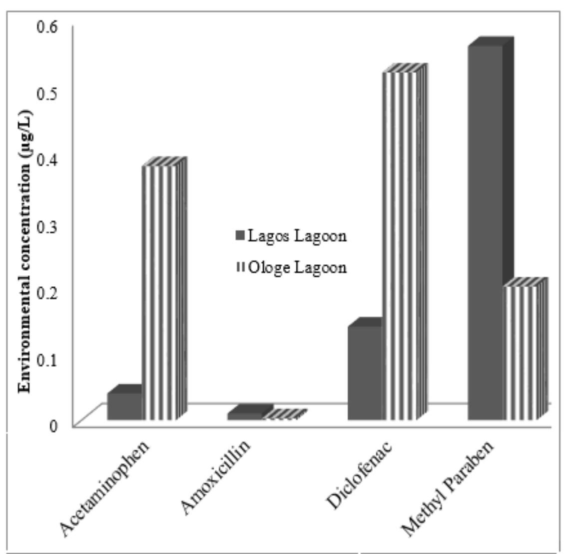

Fig 1: Variations in levels of occurrence of investigated PPCPs in Lagos and Ologe Lagoons

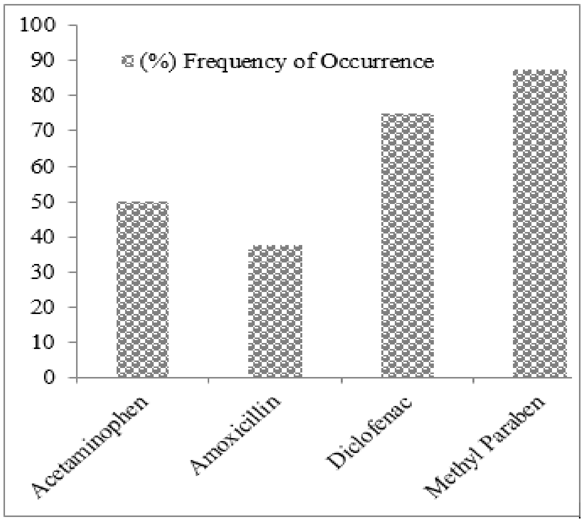

Fig 2: Frequency of occurrence (%) of investigated compounds in the study area

The concentrations of acetaminophen and diclofenac which are capable of causing acute toxicological effects in some aquatic organisms were much higher than their environmental concentrations. For example, the 96 h LC_{50} of acetaminophen and diclofenac for juveniles of *Clarias gariepinus* were 1283.6 mg/L and 2.6 mg/L respectively (Folarin *et al*., 2018). However, prolonged exposure to these micropollutants at environmental concentrations is capable of chronic biological effects on algae (Osorio *et al*., 2016) and some biological effects such as oxidative stress in many non-target aquatic organisms (Mehinto *et al*., 2010). The nephrotoxic potential of diclofenac after a period of chronic exposure to aquatic organism was described by Revai and Harmos (1999). Tubular necrosis in the kidney of rainbow trout was observed and reported in concentrations ranging from 7 to 15 µg/L of diclofenac while hyperplasia and fusion of the villi in the fish intestine were also detected in concentrations above 1 µg/L. The kidney of fish exposed to diclofenac showed hyaline droplet degeneration of the tubular epithelial cells while interstitial nephritis and necrosis of the pillar cells causing damage to the capillary wall within the secondary lamellae were equally observed (Schwaiger *et al.,* 2004).

The occurrence of antibiotics such as amoxicillin in the aquatic environment may affect the microbial community. Apart from adverse effects such as a possible initial decline in a microbial community exposed to antibiotics (Kümmerer, 2009); development of drug resistance in pathogens has equally been linked with the presence of antibiotics in the aquatic environment (Martinez, 2009; Maal-Bared *et al.,* 2013; Larsson, 2014). As a result, the presence of these emerging pollutants in the water environment and possible exposure of non-target organisms to these pollutants are of increasing environmental and health concerns.

Conclusion: The findings of this study provide further evidence of the occurrence of active pharmaceutical compounds and excipients in the aquatic environment. The study shows that methylparaben, acetaminophen and diclofenac have high detection frequency. Although the environmental concentrations of the compounds were lower than their lethal concentrations for some aquatic organisms, prolonged exposure of the aquatic life to pollutants even at trace concentration has been known to cause adverse biological effects. The presence of these micropollutants with substantial toxicological potentials in water bodies poses a grave ecological threat to a wide range of aquatic organisms. As a result, adequate public enlightenment on elimination or minimisation of these emerging pollutants sources is essential for the protection of life below water.

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