Oladimeji-Salami J. A. et al Toxicology Digest Vol. 4 (1): 94 – 107 (2019)

Toxicology Digest

Ecotoxicological effects of pharmaceutical waste in selected water bodies in Lagos state, Nigeria on *Amietophrynus regularis* tadpoles

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Abstract

The discharge of pharmaceutical effluents into nearby water bodies could constitute hazard to the environment because they contain toxic substances detrimental to health. Over 50% of pharmaceutical companies in Nigeria are domiciled in Lagos state, Nigeria. This study investigated the presence of selected pharmaceuticals in some water bodies in proximity to pharmaceutical companies within Lagos State and toxicological effects of pollution. Water samples were collected from three water bodies in Lagos environment located close to pharmaceutical companies: their physicochemical parameters were assessed and selected pharmaceutical residues were quantified using HPLC/UV detector. Acute toxicological effects of the contaminated water samples were assessed in the common African toad (*Amietophrynus regularis*). The results revealed the presence of levofloxacin (0.19 µg/ml), chloroquine (1.21 µg/ml), acetaminophen (7.52 µg/ml), metformin (4.83 µg/ml) and ibuprofen (3.27 µg/ml) in the water samples. Dissolved oxygen values were consistently low in all the locations sampled (0.90-2.58 mg/L) and elevated COD and BOD levels. In the 96 h acute toxicity study of water samples A, B and C, mortality occurred in tadpoles over a period of 96 h, LC_{50} values of were 33.5 %, 42.17 % and 37.58 % respectively. Results obtained showed that avoidance response was highest in sample B and lowest in sample C. The presence of pharmaceuticals in the water bodies located close to pharmaceutical companies may pose adverse consequences on the environment. There is therefore a need for the treatment of pharmaceutical waste-water before discharging into the environment.

***Keywords**: Pollution, Pharmaceuticals, Toxicity, Tadpoles*

Introduction

The continued increase in the human population has created an equivalent increase in the need for the Earth's inadequate freshwater supply (Byrne *et al*., 2016). Thus, the integrity of the Earth's water resources has become an important environmental issue. Pharmaceuticals are defined as prescription, over the counter and veterinary therapeutic drugs used to prevent or treat human and animal diseases (USGS, 2002; Ebele *et al*., 2017). The use of pharmaceuticals is ever increasing and they have been described as emergent pollutants (Diaz-Cruz *et al*., 2003; Zhang *et al*., 2016). Emerging contaminants are defined as "any synthetic or naturally occurring chemical or any micro-organism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological or human health effects" (Smith, 2014). Pharmaceuticals do not need to persist in the environment to cause deleterious effects as they are continually released into the environment, mainly from industrial processes and dumping of unused pharmaceutical products (Bowe, 2009; Wilcox, 2013).

In the past three decades, the presence of pharmaceuticals in the environment has gained increasing attention. There has been a renewed interest in the potential environmental impact of pharmaceuticals due to the improvement of detection methods. A number of studies have measured the levels of pharmaceuticals in surface water, groundwater, and drinking water in Europe, United States of America, China, South Africa and Nigeria (Liu and Wong, 2013; Loos *et al*., 2013; Agunbiade and Moodley, 2014; Kostich *et al*., 2014; Olaitan *et al*., 2014; Olarinmoye *et al*., 2016). Aus der Beek *et al*., (2016) revealed that over six hundred different pharmaceutical substances or their transformation products have been detected in the environment of many countries covering all continents and have been found to be above the detection limits of the different analytical methods employed. It has been shown in the literature that pharmaceuticals can enter the environment via water, sewage, manure, animal carcasses, dispersing through the food chain to produce adverse effects (Kuster and Adler, 2014) which could affect humans and other animal species. Pharmaceuticals are designed to alter physiology at low doses and so can be potent even at low doses (Arnold *et al*., 2013).

In Nigeria, several studies have expounded on the probable impact of pharmaceuticals in our environment (Ngwuluka *et al*.,

2011; Awodele *et al*., 2016; Ebele *et al*., 2017). Olatunde *et al*., (2014) reported the presence of pharmaceuticals in surface and groundwater samples collected from an irrigation canal and several wells in a pharmaceutical industrial area of Sango Ota, Ogun State, Nigeria. Furthermore, Olarinmoye *et al.,* (2016) also reported the presence of pharmaceutical residues in wastewater-impacted surface waters and sewage sludge. The toxicity of pharmaceutical effluents has also been assessed by several researchers who showed that these effluents were toxic to exposed organisms and potentially genotoxic (Bakare *et al*., 2009; Obasi *et al*., 2014; Adeoye *et al*., 2015; Awodele *et al*., 2016). However, there is a shortage of information showing a cause-effect relationship between pharmaceuticals at concentrations present in the water sources and adverse effects. More studies of pharmaceuticals are required to characterize their environmental presence in developing countries, as there are insufficient data in Africa on possible impact in our environment.

The continuous discharge of pharmaceutical effluents into nearby water bodies could constitute hazard to the environment because they contain toxic substances detrimental to health. Over 50% of pharmaceutical companies in Nigeria are domiciled in Lagos state, Nigeria (Ngwuluka *et al*., 2011). Hence, there may be an increase in the number of pharmaceutical compounds present in this environment. This study investigated the presence of selected pharmaceuticals in some water bodies in proximity to pharmaceutical companies within Lagos State and potential acute toxicological effects.

Materials and Methods

Chemicals

Acetic acid, hydrochloric acid, ethanol, methanol, formic acid, sulphuric acid, acetonitrile, trifluoroacetic acid and 0.2 M NaOH were obtained from Fischer Scientific (Leicestershire, UK). Metformin, amoxicillin trihydrate, ciprofloxacin, levofloxacin, artesunate, chloroquine, diclofenac, acetaminophen, salicylic acid and ibuprofen (BP) standards were supplied by Sigma-Aldrich (Steinheim, Germany). Solid phase extraction cartridges (C18) were purchased from Cole-Parmer Instrument Company, Illinois, USA.

Study Area and Sample Collection

This present study was carried out in Lagos Mainland, Lagos State (Lat 6.4531° N, Long 3.3958° E) in South West Nigeria. The State is bounded on the North and East by Ogun State, in the West by

Republic of Benin and South by the Atlantic Ocean. It has a total water area of 171.68 km2 with a population of about 17.5 million (Lagos State Government. Digest of Statistics, 2013). To determine the environmental extent of these pharmaceuticals in susceptible streams, selection criteria for the locations surveyed was mainly proximity to pharmaceutical manufacturing concerns. Water samples were collected in triplicates from three different locations (Iganmu, Isolo and Festac). They include a community Rivers and wastewater discharge areas, all in Lagos State, Nigeria.

The sampling was carried out using coherent protocols and procedures designed to obtain a representative water sample using standard depth and width integrating techniques (Olatunde *et al*., 2014). At each site of collection, a composite water sample was collected into pre-cleaned amber glass-bottles which were placed in coolers, chilled and maintained at 4 °C and then transferred to the laboratory for analysis. Amber coloured bottles were used in order to avoid the breakdown of light-sensitive chemical compounds that may be present in these water samples. Samples were analysed within 36 h of collection. The use of personal care items, caffeinated products and pharmaceuticals were avoided during sample collection and processing to minimize contamination of samples (Kolpin *et al*., 2002; Olatunde *et al*., 2014).

Determination of Physico-chemical Characteristics and Heavy Metals

The raw water samples were analysed for standard physico-chemical properties, including pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total solids (TS) according to the method prescribed by the American Public Health Association (APHA, 1998). The levels of metals such as manganese, lead, cadmium, nickel and chromium in the water samples were determined using Flame Unicam 969 atomic absorption spectrometer (FAAS) at the Federal Institute of Industrial Research, Oshodi laboratory in Lagos State, Nigeria as described by Akaahan *et al*., (2015).

Quantification of Pharmaceutical Compounds

Each of the water samples collected was subjected to a pre-filtration process by passing the sample through a 0.45 µm glass fibre filter. The filtrates were collected into a clean container Pre-

concentration of the filtered water samples was achieved using the four Solid-Phase-Extraction (SPE) techniques, namely conditioning, loading of water sample, washing and elution. Analyses of the extracted compounds were quantitatively carried out using a Reversed-Phase Agilent 1100 HPLC System with UV detector (Olatunde *et al*., 2014).

Animal Exposure and Experimental Design

Water samples were collected from selected water bodies within Lagos Mainland; Iganmu (A), Isolo (B) and Festac (C) were removed from storage in the fridge (4°C) and allowed to adjust to room temperature until it was approximately equal to those of the water in the bioassay tanks (about 26 °C). Three hundred and sixty tadpoles (5 days old) of the Common African toad (*Amietophrynus regularis*, Family Bufonidae) procured from the Zoological Garden of Department of Zoology, University of Lagos were divided into three with one hundred and twenty fish designated for each sampling location. The animals were divided into four groups with thirty fish in each group. The animals were exposed to 0 %, 25 %, 50 % and 100 % respectively for 96 hours. Mortality assessments were carried out every 24 hrs over a 96 hrs period. Bioassay conditions were same as for acclimatization. LC_{50} was calculated per water sample. The LC_{50} value in percent was converted to toxic units (Von der Ohe and de Zwart, 2013). Abnormal avoidance response was tested by gently prodding with a glass rod at an interval of 24 hours over a 96-hour period (Ezemonye and Ilechie, 2007).

Data Analysis

Results were expressed as Mean ± Standard Error of Mean. Student's t test was used to determine differences between groups. Levels of statistical significance were determined by analysis of variance (ANOVA), using GraphPad Prism 6.0 software and p values < 0.05 were considered significant. Multiple bar charts were also used for the pictorial representation of results obtained.

Results

Physicochemical Analysis

Results of physicochemical and heavy metals analyses are shown in Table 1. pH was within permissible range at all the locations sampled. Dissolved oxygen values fluctuated between 1.85±0.05 and 2.58±0.05 mg/L. Dissolved oxygen values were lower than the NESREA standard in all of the sampling locations (0.90±0.05 and 2.58±0.05 mg/L). Biochemical Oxygen Demand was highest at sample A

(154.25±0.02 mg/L) and lowest at sample C (143.15±0.01 mg5/L). Chemical Oxygen Demand fluctuated between 256.86 and 287.49 mg/L. BOD and COD values at all the locations sampled were higher than NESREA standard. The concentrations of heavy metals nickel and lead were above recommended levels.

Table 1: Physicochemical Parameters of the Water Samples.

Location	A	B	C	NESREA	WHO
TS (mg/L)	1873±6.00	66.67±3.33	455.00±2.00	NS	NS
TSS (mg/L)	1099.80±0.31	42.57±0.17	432.40±0.40	NS	NS
TDS (mg/L)	673.73±0.27	24.10±0.40	22.60±0.10	2000	NS
Temp (°C)	28.03±0.03	27.57±0.03	28.00±0.01	27	20-33
pH @ 20 °C	7.50±0.01	7.80±0.01	7.30±0.01	6-9	6-8
DO (mg/L)	2.58±0.05	1.85±0.05[a,b]	0.93±0.03 [a,b]	<2	<2
COD (mg/L)	256.86±0.01	266.15±0.01	287.49±0.01	80	80
BOD (mg/L)	154.25±0.02	153.83±0.01	143.15±0.01	50	15
Manganese (Mn) mg/L	0.064±0.01	0.395±0.01	0.235±0.01	0.30	3
Lead (Pb) mg/L	0.062±0.01	0.019±0.013	0.025±0.013	2.00	0.01
Cadmium (Cd) mg/L	0.014±0.01	0.01±0.01	ND	NS	0.013
Nickel (Ni) mg/L	0.03±0.01	0.01±0.01	0.01±0.01	0.30	0.02
Chromium (Cr) mg/L	0.02±0.01	0.05±0.01	0.04±0.01	0.13	0.05

Values are expressed as mean ± S.E.M. *p < 0.05. ND: Not detected. NS: Not stated. [a]National Environmental Standards and Regulations Enforcement Agency (2009) Nigeria, maximum permissible limits for wastewater discharge. [b]World Health Organization (2002) Guidelines for drinking water recommendation.

Quantitative Analysis

Five pharmaceuticals namely levofloxacin, ibuprofen, acetaminophen, metformin and chloroquine were found in the water samples at concentrations shown in Table 2. At least four pharmaceuticals were detected in all the locations. Levofloxacin had the lowest frequency (33.33 %).

Table 2: Concentration of Pharmaceuticals in Surface Water Samples in Lagos State

Analytes	**Concentration (µg/ml)**		
	A	B	C
Levofloxacin	0.19 ± 0.01	ND	ND
Chloroquine	1.21 ± 0.21	1.81 ± 0.27	2.65 ± 0.01
Acetaminophen	12.65 ± 0.17	5.09 ± 0.05	7.52 ± 0.11
Metformin	4.83 ± 0.01	8.20 ± 0.12	6.46 ± 0.03
Ibuprofen	3.27 ± 0.02	1.40 ± 0.03	4.86 ± 0.02

Values are expressed as mean ± S.E.M. ND: Not detected.

Acute Toxicity Testing

Acute exposure to the water samples resulted in a concentration dependent increase in mortality and abnormal avoidance response in tadpoles (Figures 1 and 2). Tadpoles in the control group were active and healthy throughout the exposure period. Results obtained showed that avoidance response was highest in B and lowest in C (Figure 1). The tadpoles exposed to varying concentrations of water samples B and C at for 96 h recorded mortality in all the concentrations. In the 96 hrs acute toxicity study of samples A, B and C, mortality occurred in tadpoles over a period of 96 h, LC_{50} values of water samples A, B and C were extrapolated to be 2.98, 2.37 and 2.66 toxic units respectively (Figure 2).

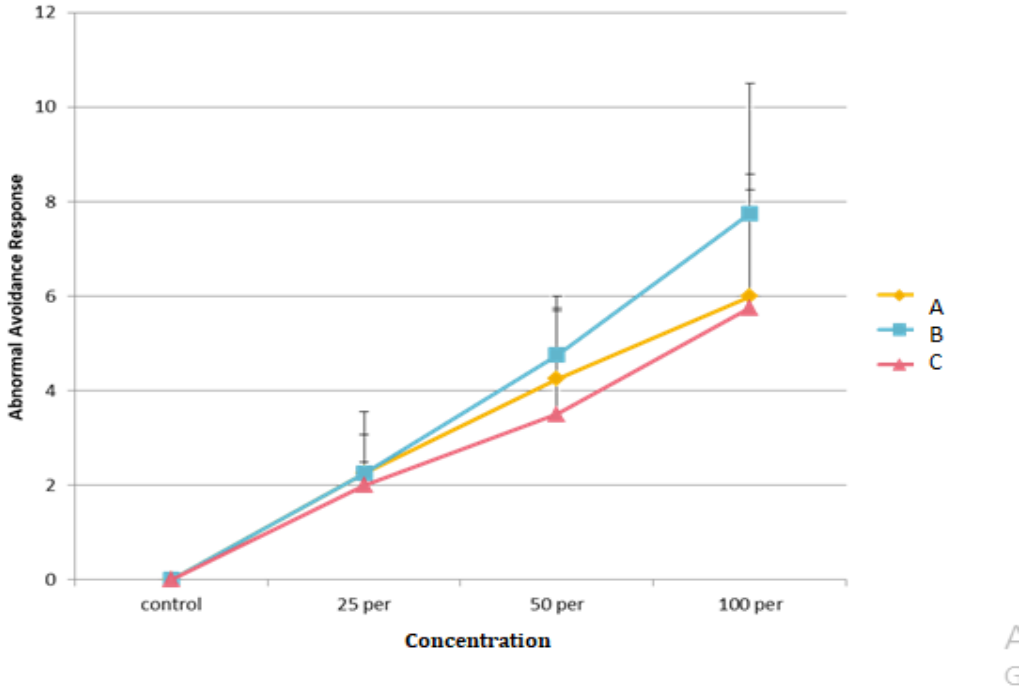
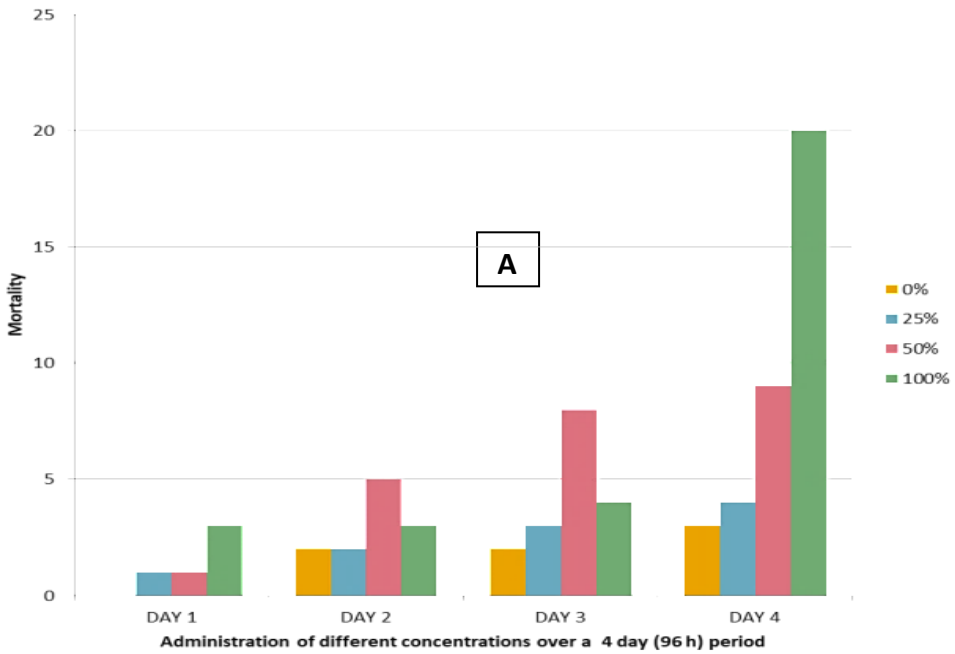


Figure 1: Mean abnormal avoidance response of tadpoles exposed to different concentrations of water samples A, B and C.



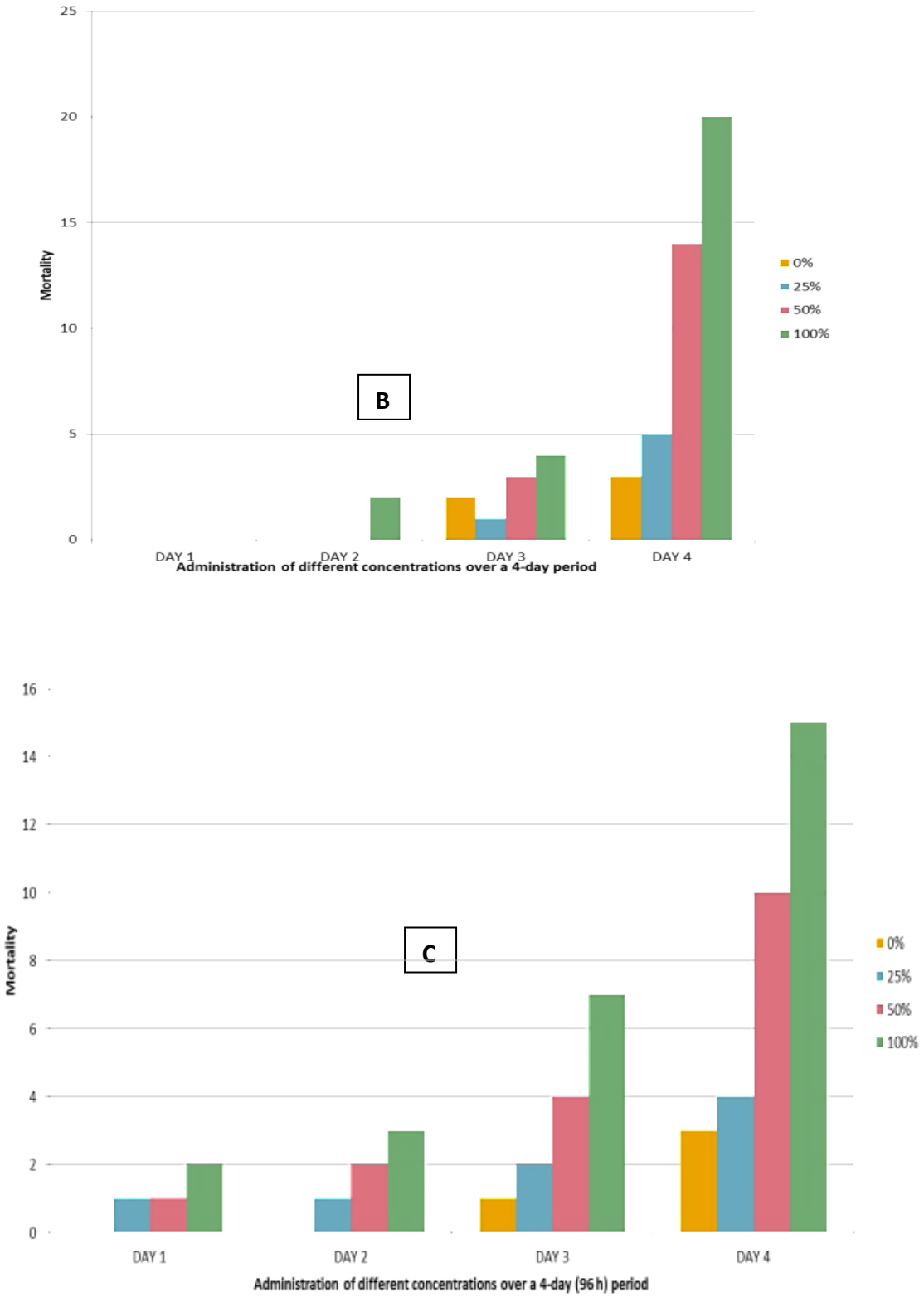


Figure 2: Mean mortality recorded in tadpoles over the 96 h exposure to water samples A, B and C.

Discussion

Over the last decade, a link between the natural and human-impacted environments and the growing public health challenges has been established. In areas subjected to strong anthropogenic forces, water plays a key role in the transfer of pollutants. The collection, treatment and delivery of wastewater to the environment are of major interest. Given the limited sources of water available for human use, continued contamination of the aquatic environment may pose a significant human health hazard. Consequently, there is a need to constantly monitor water sources as these surface waters and groundwater are interconnected, in order to protect human health and preserve the integrity of the aquatic environment (Lohdip, 2013). Pharmaceutical effluents are waste materials which have been generated from manufacturing processes of pharmaceutical companies. The discharge of pharmaceutical effluents into water bodies could constitute biohazard to man and other living organisms in the environment because they contain toxic substances detrimental to health (Bakare *et al*., 2003).

In this study, after pre-concentration and quantification using the HPLC, water samples analysed were found to contain: metformin (antidiabetic drug), acetaminophen and ibuprofen (analgesics), levofloxacin (antibiotic) and chloroquine (anti-malarial). The concentrations of most of the pharmaceuticals detected were comparable with concentrations identified by other researchers in other developing countries (Olatunde *et al*., 2014; Miraji *et al*., 2016; Ngumba *et al*., 2016). However, as more scientific evidence points towards the ecotoxic potential of pharmaceuticals occurring as mixture even at sub-therapeutic levels, it is still a cause for concern.

The toxic unit (TU) approach (Von der Ohe and de Zwart, 2013) was used to estimate the potential toxicity of test samples. Samples testing positive for toxicity had TU values > 1.0 (Harbi *et al*., 2017). In this study, the water samples had toxic units >1 in tadpoles which indicate that the water samples were toxic to test organisms. Furthermore, other factors such as BOD, COD and DO could also responsible for mortalities in the tadpoles as they were beyond their prescribed standards (Cardoso *et al*., 2014). Abnormal avoidance response of tadpoles in the treatment concentration showed a relationship when compared with mortality such that many tadpoles that displayed abnormal avoidance responses died the subsequent day. Fluctuation in avoidance response has been understood to precede

death (Ezemonye and Ilechie, 2007; Ezemonye and Tongo, 2009). This indicates that behavioural fluctuations resulting from exposure were a more profound measure of toxicity than death (Brodie Jr and Formanowicz Jr, 1983; Ezemonye and Ilechie, 2007). Pollutants are believed to be a possible culprit in the decline of amphibian populations, specifically through their effects on amphibian behaviours relating to foraging and predator-prey interactions in the breeding habitat (Relyea, 2010). Sub-lethal concentrations of contaminants have been shown to have possible indirect or community level effects on exposed animals (Hayden *et al*., 2015). Intoxicated tadpoles have demonstrated maladaptive behaviours, which may make them more vulnerable to predatory attacks by inability to utilize refugia in the presence of a predator, and spent more time in refugia in the absence of a predator, a behaviour that decreases feeding opportunities, potentially limiting growth (Reeves *et al*., 2011).

The occurrence of pharmaceuticals in these water bodies represents a potential threat to the environment since they are designed to exert a pharmacological effect even at low doses (Ebele *et al*., 2017). Presence of these drugs at sub-therapeutic doses can result in resistance e.g. antimicrobials (Andersson and Hughes, 2015). Although this study has brought to the fore possible ecotoxic potentials of pharmaceuticals in the water bodies analysed, there is still need for further studies to assess their effects on higher animals as well as possible bio-accumulator effects particularly as these water bodies are also a source of food and livelihood to people living within the state.

Conclusion

The levels of selected pharmaceutical analytes and acute effects on tadpoles of *Amietophrynus regularis* were reported. Data obtained from this study revealed the presence of pharmaceuticals in the samples collected from surface water. Acute exposure of tadpoles to the water samples resulted in mortality and abnormal avoidance response. This may present adverse consequences on the aquatic ecosystem. Therefore, there is a need for the treatment of pharmaceutical wastewater before discharging into nearby water bodies.

References

Adeoye, G. O., Alimba, C. G. and Oyeleke, O. B. (2015). The genotoxicity and systemic toxicity of a pharmaceutical effluent in wistar rats may involve oxidative

stress induction. Toxicology Reports, 2: 1265-1272.

Agunbiade, F. O. and Moodley, B. (2014). Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. Environmental Monitoring and Assessment, 186(11): 7273-7291.

Akaahan, T.J.A., Olabanji, F.M. and Azua, E.T. (2015). Studies on contamination of surface waters of river Benue with trace elements at Makurdi, Benue State, Nigeria. Journal of Environmental Chemistry and Ecotoxicology, 7(5), 49-55.

American Public Health Association (APHA), American Water Works Association and Water Environment Federation (1998). Standard Methods for the Examination of Water and Wastewater 20th Edition. United Book Press, Inc., Baltimore, Maryland 3111, 3-13.

Andersson, D.I. and Hughes, D. (2014). Microbiological effects of sublethal levels of antibiotics. Nature Reviews Microbiology, 12(7), 465.

Arnold, K. E., Brown, A. R., Ankley, G. T. and Sumpter, J. P. (2014). Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philosophical Transactions of the Royal Society: Biological Sciences, 369(1656): 20130569- 20130580.

Aus der Beek, T., Weber, F. A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A. and Küster, A. (2016). Pharmaceuticals in the environment--Global occurrences and perspectives. Environmental Toxicology and Chemistry, 35(4): 823-35.

Awodele, O., Adewoye, A. A. and Oparah, A. C. (2016). Assessment of medical waste management in seven hospitals in Lagos, Nigeria. BMC Public Health, 16(1): 1-11.

Bakare, A. A., Okunola, A. A., Adetunji, O. A. and Jenmi, H. B. (2009). Genotoxicity assessment of a pharmaceutical effluent using four bioassays. Genetics and Molecular Biology, 32(2), 373–381.Miraji *et al*., 2016

Bakare, A. A., Okunola, A. A., Adetunji, O. A. and Jenmi, H. B. (2009). Genotoxicity assessment of a pharmaceutical effluent using four bioassays. Genetics and Molecular Biology, 32(2): 373–381.

Bowe, L. (2009). Investigating Emergent Contaminants: Pharmaceutical impacts and possible solutions. Retrieved 22nd November, 2017 from www.mass.gov/eea/docs/dep/toxics/stypes/ec-bowe.doc.

Brodie Jr, E.D. and Formanowicz Jr, D.R. (1983). Prey size preference of predators: differential vulnerability of larval anurans. Herpetologica, 67-75.

Byrne, J. A., Fernández-Ibáñez, P. and Sharma, P. K. (2016). Water Scarcity in Developing Regions. In Sustainable Water Management, Chen David (Ed.), CRC press, Boca Raton, 1, chapter 9, 227-248.

Cardoso, O., Porcher, J. M. and Sanchez, W. (2014). Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: review of evidence and need for knowledge. Chemosphere, 115, 20-30.

Ebele, A. J., Abdallah, M. A. E. and Harrad, S. (2017). Pharmaceuticals

and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminants, 3(1), 1-16.

Ezemonye, L. I. N. and Ilechie, I. (2007). Acute and chronic effects of organophosphate pesticides (Basudin) to amphibian tadpoles (*Ptychadena bibroni*). African Journal of Biotechnology, 6(13), 1554-1558.

Ezemonye, L.I.N. and Tongo, I. (2009). Lethal and sublethal effects of atrazine to amphibian larvae. Jordan J Biol Sci, 2(1), 29-36.

Harbi, K., Makridis, P., Koukoumis, C., Papadionysiou, M., Vgenis, T., Kornaros, M., Ntaikou, I., Giokas, S. and Dailianis, S. (2017). Evaluation of a battery of marine species-based bioassays against raw and treated municipal wastewaters. Journal of Hazardous Materials, 321, 537-546.

Hayden, M.T., Reeves, M.K., Holyoak, M., Perdue, M., King, A.L. and Tobin, S.C. (2015). Thrice as easy to catch! Copper and temperature modulate predator-prey interactions in larval dragonflies and anurans. Ecosphere, 6(4), 1-17.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance. Environmental Science and Technology, 36(6), 1202-1211.

Kostich, M. S., Batt, A. L. and Lazorchak, J. M. (2014). Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environmental Pollution, 184: 354-359.

Küster, A. and Adler, N. (2014). Pharmaceuticals in the environment: scientific evidence of risks and its regulation. Philosophical Transactions of the Royal Society: Biological Sciences, 369(1656): 20130587.

Liu, J. L. and Wong, M. H. (2013). Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environment International, 59: 208-224.

Lohdip, Y. N. (2013). River and Stream Water Quality Monitoring in North Central Zone-Niger: Challenges and Solutions. Proceedings of the 1st Regional Workshop organized by National Water Capacity Building Network, North Central Regional Centre (NWRCBNet-NC), University of Ilorin held 3rd and 4th December, 2013.

Loos, R., Carvalho, R., António, D. C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L. and Jarosova, B. (2013). EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Research, 47(17): 6475-6487.

Ngumba, E., Gachanja, A. and Tuhkanen, T. (2016). Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. Science of the Total Environment, 539, 206-213.

Ngwuluka, N. C., Ochekpe, N. A. and Odumosu, P. O. (2011). An assessment of pharmaceutical waste management in some Nigerian pharmaceutical industries. African

Journal of Biotechnology, 10(54), 11259-11264.

Obasi, A. I., Amaeze, N. H. and Osoko, D. D. (2014). Microbiological and Toxicological Assessment of Pharmaceutical Wastewater from the Lagos Megacity, Nigeria. Chinese Journal of Biology, 2014(1): 1-1.

Olaitan, O. J., Anyakora, C., Bamiro, T. and Tella, A. T. (2014). Determination of pharmaceutical compounds in surface and underground water by solid phase extraction-liquid chromatography. Journal of Environmental Chemistry and Ecotoxicology, 6(3): 20-26.

Olarinmoye, O., Bakare, A., Ugwumba, O. and Hein, A. (2016). Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria. Journal of Environmental Chemistry and Ecotoxicology, 8(3): 14-24.

Olatunde, O. J., Olatunbosun, O., Olatunde, A., Anyakora, C. and Christopher, K. (2014). Occurrence of Selected Veterinary Pharmaceuticals in Water from a Fish Pond Settlement in Ogun State, Nigeria. International Journal of Environmental Monitoring and Analysis, 2(4), 226-230.

Relyea, R. A. (2010). Multiple stressors and indirect food web effects of contaminants on herptofauna. In Ecotoxicology of amphibians and reptiles (Second edition), Sparling D., Linder G., Bishop C. and Krest S. (Eds). Society of Environmental Toxicology and Chemistry Press, Pensacola, Florida, USA, 75–486.

Reeves, M. K., Perdue M., Blakemore G. D., Rinella D. J. and Holyoak M. (2011). Twice as easy to catch? A toxicant and a predator cue cause additive reductions in larval amphibian activity. Ecosphere 2: 72.

Smith Jr, J. S. (2014). Presence and fate of pharmaceuticals in the environment and in drinking water. Pharmaceutical Accumulation in the Environment: Prevention, Control, Health Effects, and Economic Impact, Ed. Goldstein W. E., CRC press, Taylor and Francis group, London, Chapter 3, 21-42.

United States Geological Survey (USGS) (2002). Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in US Streams. USGS Fact Sheet FS-027-02.

Von der Ohe, P. C. and de Zwart, D. (2013). Toxic Units (TU) Indicators. In: Encyclopaedia of Aquatic Ecotoxicology. Férard, JF and Blaise C. (Eds.) Springer, Dordrecht, Netherlands, 1161 – 1170.

Wilcox E (2013). Pharmaceuticals in the environment: review of current disposal practices for medications and the influence of public perception on environmental risks. Environmental Assessment. Accessed 12th November, 2017.

Zhang, Z., Wang, B., Yuan, H., Ma, R., Huang, J., Deng, S., Wang, Y. and Yu, G. (2016). Discharge inventory of pharmaceuticals and personal care products in Beijing, China. Emerging Contaminants, 2(3): 148-156.