NJB, Volume 32(1), June, 2019

CHEMICAL EVALUATION OF EFFLUENT FROM A PHARMACEUTICAL INDUSTRY USING *TYMPANOTONUS FUSCATUS*

***Edeghagba, B.O. and Badru, T. I.**

Environmental Biology Unit, Department of Biological Science, Yaba College of Technology, Yaba, Nigeria

Received 20th January, 2018; accepted 23rd February, 2019

ABSTRACT

The study was conducted to evaluate the effect of pharmaceutical effluent from an industry located at Sango Otta, Ogun State on *Tympanotonos fuscatus*. It was used to assess the possible impacts of the effluent on aquatic life using periwinkle (*Tympanotonus fuscatus).* Samples of the pharmaceutical effluent were collected several times at the company's discharge point via an outlet immediately after production . Physico-chemical characteristics, twelve (12) in all, and five (5) heavy metal contents were analysed and determined. The heavy metals included Fe, Pb, Zn, Mn and Hg using Atomic Absorption Spectrophotometer (AAS- Unican 919 series). Exposure of the pharmaceutical effluent at different concentrations of toxicant (0, 5, 10, 15, 20 and 25 ml/L) to test organisms was done in replicates. The mean mortality of the periwinkles increased with concentration and time. Consequently, the 96 hr LC_{50} value obtained for the effluent was 4.92 ml/L at the mean lethal time (MLT 50). The pH value was 5.1; EC, 187 uS cm^{-1}; DO, 2.4 mg/L; BOD, 80 mg/L; NO_3^-, 3.15 mg/L and Cl^-, 100 mg/L. The level of some heavy metals was above the internationally recommended maximum contaminant level of WHO and USEPA, except for mercury which was not detected. Wastewaters discharged into water bodies do ultimately pose a serious threat to human and routine functioning of ecosystem. Regular and proper treatment of effluents among others before disposal to safeguard the environment is suggested.

Keywords: Heavy metals, pharmaceutical effluent, toxicity, periwinkle (*Tympanotonus fuscatus*), Lagos Lagoon.

INTRODUCTION

Pharmaceutical industry represents a range of industries with operation and processes as diverse as its product. Hence effluent coming from pharmaceutical industries vary from industry to industry. Wastewater is generally evaluated in terms of temperature, pH, TSS, BOD, COD, Oil and grease, chlorides and sulphates. Pharmaceutical industries are among the major contributors to industrial waste. Their effluents when wrongly handled and disposed off endanger both human and environmental health. They contain heavy metals which are non-biodegradable. Pharmaceutical manufacturing companies in the country and hence increased pharmaceutical waste contain substantial amount of heavy metals. These effluents which are usually discharged into the environment when improperly handled and disposed, affect both human health and the environment (Osaigbovo and Orhue, 2006; Ayodele *et al.*, 1996; Anetor *et al.*, 1999).

The uncontrollable growing use of pharmaceutical products now constitutes a new challenge. Most pharmaceutical effluents are known to contain varying concentrations of organic compounds and total solids including heavy metals. Heavy metals such as lead, mercury, cadmium, nickel, chromium and other toxic organic chemicals or phenolic compounds discharged from pharmaceutical industries are known to affect the surface and ground waters (Foess and Ericson, 1980).

*Author for Correspondence

Larsson *et al.* (2007) showed that the effluent from a treatment plant receiving water from approximately 90 Indian bulk drug manufacturers contained very high levels (up to several milligrams per litre) of a variety of drugs. These findings were later confirmed by *Fick et al*. (2009) who also showed that the surface, ground and drinking water in the region had become highly polluted with residual drugs.

The periwinkles are estuarine snails which are found in the inter-tidal area of the mangrove edges and surfaces and could be hand-picked (Dambo, 1993). They inhabit the quiet waters where the substratum is rich in decaying organic matter and muddy (Jamabo and Chinda, 2010). Dekae (1987) stated that the factors that affect their distribution in the coastal areas of West Africa include salinity, water depth, currents and nature of bottom deposits. The periwinkle flesh is edible and also used as bait by fisher folks. They are rich in protein (about 21%), vitamins and minerals (Egonmwan, 1980).

This work was carried out to evaluate the impact of pharmaceutical effluent on aquatic life using periwinkles, *Tympanotonus fuscatus,* as a test organism.

MATERIALS AND METHODS

Chemicals

The effluent used for this study was collected from Fermex Mayer Pharmaceutical Company, located at Sango Otta, Ogun State.

Instrumentation

The metals in the wastewater samples were determined by spectrophotometry method using Atomic Absorption Spectrophotometer (AAS- Unicam 919 series) with air-acetylene flame. The AAS was standardized using calibrated standard solutions of each metal at their respective wave-lengths.

Sampling

The test organisms used in this study were collected from the University of Lagos Lagoon front using Global Positioning System (GPS) with location N 06^{o} 31’ 008’, E 003^{o} 24’27’. They were kept in a fresh polyethylene bag with decayed mud which served as substrate for them. The animals were transported to the laboratory where they were acclimatized for four days under laboratory conditions. The purpose of acclimatization was to make them get adapted to the new environment. Lagoon water (dilution water) was collected into a fresh 25-litre keg. The effluent was collected immediately after production at the discharge point of the company using five litre (5L) kegs from the outlet channeled out via a pipe.

Sample preparation

The physico-chemical parameters in the wastewater samples were determined by colorimetric/spectrophotometry using HACH 2500 spectrophotometer with appropriate reagent in the pillows. The reagents in the pillows and other necessary reagents were added to the aliquot quantity of the wastewater sample (10 or 25 ml). The reaction was allowed to reach completion according to the reaction period of each parameter. The concentration of each parameter was read directly at a specific wavelength in nanometres.

Assessment of quantal response

Test organisms were considered dead if there was no movement or response to stimulus even when prodded with a fresh glass rod or sharp object. Mortality assessments were carried out at 24 hr,72 hr and 96 hr under non-static renewal bioassay technique (ASTM,1990).

Statistical analysis

Data obtained from study involving dose-response (mortality) for single action of the effluent were analysed using probit (Finney, 1971). The index of toxicity measurement derived from this analysis includes:

LC_{50} at the concentration of 15 ml/L=4.92 ml/L;

Using student T-test the p-value was = 0.14;

95% confidence limit =6.64;

5% confidence limit = 3.36;

Remark = not significant

RESULTS AND DISCUSSION

Heavy metals

The data for analysis heavy metals and physico-chemical characteristics of the pharmaceutical effluents are presented in Tables 1 and 2, respectively. Figure 1 shows the probit analysis of acute toxicity testing with pharmaceutical effluent. In this study, the level of iron (Fe) detected in the wastewater was 0.79 mg/L higher than WHO (1993) and US-EPA (1998) acceptable level of 0.3 mg/L. The level of manganese (Mn) was observed to be 0.28 mg/L which was within WHO permissible limits of 0.5 mg/L but slightly higher than US EPA standard of 0.10 mg/L. Also, lead (Pb) was detected at a level of 0.014 mg/L, within the acceptable limits of WHO and US EPA standards of 0.01 and 0.05mg/L, respectively (Table 1). Similarly, the zinc level detected in this study was 4.51 mg/L, which was higher than the acceptable limits of WHO (3.00mg/L) but within the permissible limits of US EPA (5.00 mg/L); this corroborates the report of Tariq *et al*. (2006). The heavy metal, mercury (Hg), was not detected in the wastewater sample. Heavy metal pollution is an ever increasing problem of our oceans, lakes and rivers. Incidence of heavy metal accumulation in fish, oysters, sediments and other components of aquatic ecosystems have been reported globally (Lokhande *et al.*, 2011).

Temperature

Table 2 shows, that the temperature value of the effluent was 27.4^0C, lower than US EPA standard of 40^0C for discharge of wastewater into streams and, therefore, acceptable, though it may fluctuate depending on the dilution rate. According to Govindaswamy *et al*. (2007), temperature is one of the most influential factors on water quality.

Table 1A: Levels of heavy metals detected in pharmaceutical effluent

PARAMETERS	FERMEX EFFLUENT	WHO (1993) LIMIT	USEPA (1998)
Fe (mg/L)	0.79	0.30	0.30
Pb (mg/L)	0.014	0.01	0.05
Zn (mg/L)	4.51	3.0	5.00
Mn (mg/L)	0.28	0.5	0.10
Hg (mg/L)	ND	0.001	-

ND = Not detected

Table 1B: Determination of physico-chemical characteristics of pharmaceutical effluent

PARAMETERS	FERMEX EFFLUENT	WHO (1993) LIMIT
Temperature(^{0}C)	27.4	40
pH	5.1	6.49-8.49
Conductivity (uScm^{-1})	187.0	749.64
Nitrate (mg/L)	3.15	50
Sulphate (mg/L)	8.0	500
Phosphate (mg/L)	0.26	-
Chloride (mg/L)]	100	250
Dissolved Oxygen (mg/L)	2.4	4
Detergents (mg/L)	0.35	-
Oil and Grease (mg/L)	0.008	-
BOD (mg/L)	80	50
COD (mg/L)	170	-

DO: dissolved oxygen, BOD: biological oxygen demand, COD: chemical oxygen demand

NJB, Volume 32(1), June, 2019 Edeghagba, B.O. and Badru, T. I.

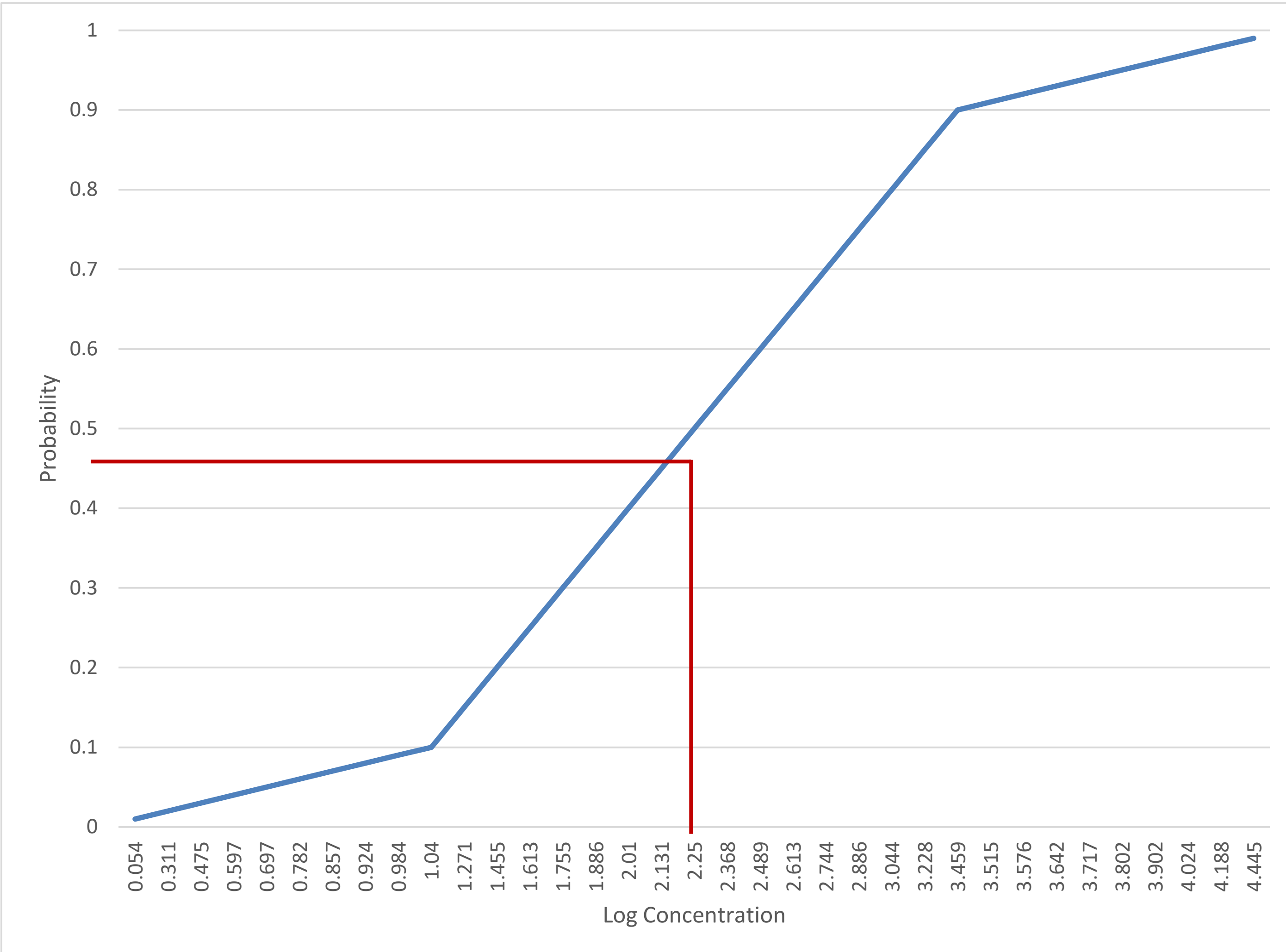

Figure 1: Probit analysis of acute toxicity with pharmaceutical effluent

pH

The pH value recorded in this study was 5.1 (Table 2), which was slightly acidic and lower than the tolerance limits of WHO (6.5-8.5) and US EPA (6.0-8.5) for wastewater discharge. Low pH values in a river, for example, impair recreational uses of water and affect aquatic life.

Electrical conductivity

Conductivity of water is a useful indicator of its salinity or total salt content. The conductivity obtained was 187.0 µScm^{-1}, which was lower than the WHO guideline limits of 250 µScm-^{1} for the discharge of wastewater through channel into stream. The conductivity is within permissible limits and the water can be used for irrigation purposes.

DO, BOD, COD

Wastewater discharge from sewage and industries are a major component of water pollution, contributing to oxygen demand and nutrient loading of the water bodies. This promotes toxic algal blooms and leads to a destabilized aquatic ecosystem (Morrison *et al.*, 2001; DWAF and WRC, 1995). The levels of BOD and COD detected were 80.0mg/l and 170.0mg/, respectively. There were no threshold values indicated by WHO (1993); however, when compared with US EPA (1998) standards, the BOD value was above the acceptable limit while the COD was within the allowable level for discharge. The DO is a measure of the degree of pollution by organic matter and the self purication capacity of the water body. The standard for sustaining aquatic life is stipulated at 5mg/L, a concentration below this value adversely affects aquatic biological life, while concentration below 2 mg/L may lead to death for most fishes (Chapman, 1997). The value of DO obtained in this work (2.4 mg/L) is moderately acceptable. The findings of this study showed a low DO value (2.4 mg/L) and a high BOD value (80 mg/L). When BOD levels are high, dissolved oxygen levels decrease because the oxygen that is available in the water is used by the bacteria.

Inorganic nutrients

All the inorganic nutrients like nitrate (3.15 mg/L), sulphate (8.0 mg/L) and chloride (100 mg/L) were significantly lower and within the regulatory limits of WHO (1993), except for phosphate (0.26 mg/L),which could not be ascertained as no threshold value by WHO was available (Table 2). High nitrate concentrations are frequently encountered in treated wastewater, as a result of ammonium nitrogen. Their presence in wastewater could also contribute to eutrophication effects, particularly in freshwater (OECD, 1982). Phosphate is an essential nutrient for living organisms and exists in water bodies in both dissolved and particulate form. In general, it limits the nutrient for algal growth and controls primary productivity (Harsha *et al*., 2006).

Acute toxicity

The lethal median dose (LC_{50}) determination examines the relationship between concentration and the most extreme response effect (death). The more potent or toxic the chemical, the lower the LC_{50} and the smaller the concentration needed to cause death. Findings from the acute toxicity test carried out in this study showed that the 96 hr LC_{50} value obtained for the effluent was 4. /L (Figure 1) at the mean lethal time ((MLT 50). Thus, the pharmaceutical effluent was found to be toxic to *Tympanotonus fuscatus.* Generally, the mean mortality of the periwinkles increased with concentration and time. The results obtained gave a toxicity factor of 0.84 compared to the toxicity factor of 0.78 reported by Chukwu and Lawal (2010), implying that the 96 hr LC_{50} concentration was more toxic to the test organism.

CONCLUSION

The characteristics of effluent used in this study varied and the heavy metal concentration was in the increasing order of Zn>Fe>Mn>Pb>Hg. Fe and Zn levels were observed to be higher than WHO permissible limits while Pb and Mn were within the standard range. Similarly, the heavy metals, Pb and Zn, were lower in value; Fe and Mn were higher than US EPA permissible limits. The biological oxygen demand was above the US EPA permissible limit. The pH was slightly acidic and conformed to the international standards. However, other parameters were in conformity with acceptable limits. The pharmaceutical effluent was found to be toxic to the test organism.

REFERENCES

Chapman, D. (1997). Water Quality Assessment. A Guide to the use of Biota, Sediments and Water in Environmental Monitoring, E & FN Spon, London. *Available at: http//Hydrology and Water Quality of Lake Merced.html.*

Chillers, J. and Henrik, A. (1996). Effect of contaminated water. *Global Water Hazards* (2nd Ed.). Department of Water Affairs and Forestry, Pretoria (1) 25: 25-27.

Chukwu, L.O. and Lawal, O.S. (2010). Joint action response and haematological profile of *Macrobrachium v ollenhoevenii* (Herklots, 1857) Exposed to Binary Mixtures of Spent lubricant oil and Dispersant. *Research Journal of Environmental Sciences*. 4: 115 – 126.

Dambo, W.B. (1993). Tolerance of the Periwinkle *P. aurita* and *T. fuscatus* to refined oils. *Journal of Enviromental Pollution*, 79: 293 – 296.

Deekae, S.N. (1987). The ecological distribution of mangrove mollusks in the Bonny-New calabar Rivers System of Niger Delta. M.Sc. Thesis. University of Port Harcourt, Choba :158p.

Egonmwan, R.T. (1980). On the biology of *Tympanotonus fuscatus var. odula* (Gastropoda: Prosobranchia:potamididae). *Proceedings of the 8th International Malacological* Congress, Budapest.

Fick, J., Soderstrom, H., Lindberg, R.H., Phan, C., Tysklind, M. and Larsson, D.G. (2009). Contamination of surface, ground and drinking water from pharmaceutical production. *Journal of Environmental Toxicology Chemistry*, 28 (12): 2522-2527.

Finney, D.J. (1971). Probit Analysis, 3rd Edition, Cambridge University Press, London. 330p.

Foess, G.W. and Ericson, W.A. (1980). The trend of the future water wastes. *Journal of Environmental Chemistry and Ecotomology*, 3(2):21-27.

Govindasamy, C., Kannan, L. and Jayapaul, A. (2000). Seasonal variation in physico-chemical properties and primary production in the coastal water biotopes of Coromandel Coast, *Indian Journal of Environmental Biology*, 21:1-7.

Harsha, T.S., Yamakanamardi, S.M. and Mahadevswamy, M. (2006). Temporal variation in the abundance of heterotropic bacteria in the river Cauvery and its downstream tributaries. *Indian Journal of Microbiology*, 46(3):249-257.

Jamabo, N. and Chinda, A. (2010). Aspects of the Ecology of *Tympanotonus fuscatus var. fuscatus* (Linnaeus, 1758) in the Mangrove Swamps of the Upper Bonny River, Niger Delta, Nigeria. *Current Research Journal of Biological Sciences*, 2 (1): 42-47.

Larson, D.G.J., Pedro, C. and Paxeus, N. ((2007). Effluent from drug manufacturers contains extremely high levels of pharmaceuticals. *Journal of Hazardous Materials,* 148 (3): 751-755.

Lokhande, R.S., Singare, P.U. and Pimple, D.S. (2011) . Quantification Study of Toxic Heavy Metals Pollutants in Sediment Samples Collected from Kasardi River Flowing along the Taloja Industrial Area of Mumbai, India. *The New York Science Journal,* 4(9): 66-71.

Morrison, G. O., Fatoki, O. S. and Ekberg, A. (2001). Assessment of the impact of point source pollution from the Keiskammahoek sewage treatment plant on the Keiskamma River. 27: 475-480.

Murdoch, T., Cheo, M. and O'Laughlin, K. (2001). Streamkeeper's Field Guide:Watershed Inventory and Stream Monitoring Methods. Adopt-A-Stream Foundation, Everett,WA,USA, 297p.

Nickles, M. (1950). Mollusquetestaces marine de la cote occidenteled'atrique. *Manuels Quests Africains*, 2: 1-269.

Odum, E.P. (1977). *Fundamentals of Ecology*. W. B. Saunders Coy., Philadelphia.

OECD, (1982). Eutrophication of waters: Monitoring, Assessment and Control. Technical Report, *Organization for Economic Co-operation and Development*, Paris.

Tariq, M., Ali, M. and Shah, Z. (2006). Characteristics of industrial effluents and their possible impacts on quality of underground water. *Journal of Soil and Environment,* 25(1):64-69.

US EPA (1998). United States Environmental Protection Agency. Standards for drinking water. Washington, D.C.

WHO International Programme on Chemical Safety (IPCS), (1993). *Biomarkers and risk assessment: concepts and principles. Environmental Health Criteria*, World Health Organization, Geneva.155p.