FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 6 No. 2, April, 2022, pp 236 - 240
DOI: https://doi.org/10.33003/fjs-2022-0602-947

POLYCYCLIC AROMATIC HYDROCARBON METABOLITES AND ANTIOXIDANT STRESS MARKERS IN *LUMBRICUS TERRESTRIS* FROM AREAS AROUND KOLO CREEK, NIGER DELTA, NIGERIA

[1]Ejovi Osioma and [*2]Godswill Okeoghene Tesi

[1]Department of Biochemistry, Faculty of Science, Federal University Otuoke, Bayelsa State, Nigeria.
[2]Department of Chemical Sciences, University of Africa, Toru-Orua, Bayelsa State, Nigeria.

*Corresponding authors' email: godswillinfodesk@yahoo.com

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are contaminants of interest due to their known toxic effects. PAHs may interfere with physiological and biochemical activities of soil organisms like Lumbricus terrestris. PAHs metabolites have been employed in the assessment of external PAHs exposure and metabolic activation. Thus, this study investigates PAHs metabolites and antioxidant stress markers in Lumbricus terrestris which is a recognized bioindicator of environmental pollution. Samples of Lumbricus terrestris were collected from four sampling sites of Emeyal, Imiringi, Elebele and Otuasega around Kolo Creek, Niger Delta, Nigeria. The PAH metabolites and other biochemical parameters were determined using standard laboratory procedures. The results showed that the concentrations of the ∑5-OH PAHs in the Lumbricus terrestris ranged from 0.55 to 16.6 mg/kg. On the average, the concentrations of the PAHs metabolites followed the order: 9-OH Fluorene (Flu) > 2-OH Napthalene (Nap) > 1-OH Pyrene (Pyr) > 3-OH Phenathrene (Phen) > 3-OH Benzo(a)pyrene (Bap). The antioxidative stress markers which comprises of superoxide dismutase, catalase, glutathione peroxidise and glutathione s-transferase activities and concentrations of malondialdehyde and reduced glutathione including the activities of acetylcholinesterase and lactate dehydrogenase in the Lumbricus terrestris varied significantly among the sampling sites. The data obtained from this study suggests that Lumbricus terrestris were exposed to PAHs and changes in oxidative stress markers confirm PAHs in the study area. Thus, PAH metabolites and oxidative stress markers in Lumbricus terrestris could be employed as biomarkers for exposure to polycyclic aromatic hydrocarbon and be effectively employed in the biological monitoring of PAHs.

Keywords: PAH metabolites, oxidative stress markers, Lumbricus terrestris, acetylcholinestrase, Kolo Creek

INTRODUCTION

The exploration of crude oil takes place in the marine ecological environment and the scope of oil exploration activities and its associated environmental consequences have been increasing over the years in the Niger Delta area of Nigeria. Soil, plants, animals and water resources are negatively affected when some quantity of petroleum and its products due to accidental, transportation or operational mishap are introduced or released into the environment during exploration (Iniaghe *et al*., 2013; Onokare *et al*., 2022). Heavy metals, total hydrocarbons and petrogenic polyaromatic hydrocarbons (PAHs) are the major contaminants associated with petroleum exploration activities.

Polycyclic aromatic hydrocarbons have been recognised as environmental chemical pollutants (Nasr *et al*., 2010) and of great importance due to their documented carcinogenicity, known toxic and consequences of bioaccumulation in animals (Tesi *et al*., 2016; Iwegbue *et al*., 2020; Emoyan *et al*., 2021). Like other pollutants such as heavy metals, PAHs may interfere with physiological and biochemical activities of soil animals like earthworm by inducing an elevation in reactive oxygen species (ROS) giving rise to an excess of free radicals in the cells (oxidative stress) or altering their biochemical processes (Chen *et al*., 2011; Silva *et al*., 2013). The exposure of an organism to PAHs can be assessed by measuring the concentration of PAHs in their tissues. However, Varanasi *et al*. (1989) reported that fish sample caught at highly PAH polluted site often showed only trace levels in tissues, due to its capacity to easily metabolize PAHs into more polar and easily excretable forms, hence the determination of PAH metabolites is an alternative method to assess the uptake of PAHs. Valuable information may be obtained on bioavailability and toxicological effect of PAHs on the terrestrial environment by the measurement of the concentration of PAH metabolites with biomarkers of oxidative stress. Such analysis of PAH metabolites can be employed in the biological surveillance of animal exposure to PAH.

The importance of evaluating pollution impact in soil ecosystem has been acknowledged and attention has grown regarding the application of biomarkers using earthworms as sentinel organism for ecotoxicological testing of industrial chemicals (Calisi *et al*., 2011; Dhiman and Pant, 2022). *Lumbricus terrestris* belongs to the Anecic worms, function as an aerator, grinder, crusher, chemical degrader and biological catalyst, acclimatize in soil which is contaminated with an elevated concentrations of PAHs, break down PAHs into simpler molecule and efficient accumulator of pollutants from soil (Dendooven *et al*., 2011; Zeb *et al*., 2020).

Kolo Creek has played host to oil exploration and exploitation activities since the 1950s and is a major recipient of effluents from petroleum exploration activities. High concentration of total hydrocarbon content, polycyclic aromatic hydrocarbon (PAHs), presence of Cu, Pb, Cd, Ni, have been reported in Kolo creek (Inengite *et al*., 2010; Olowu *et al*., 2010; Aghoghovwia and Chijoke, 2012; Ebenezer and Eremasi, 2012).Yaguo *et al*. (2021), reported high concentration of total petroleum hydrocarbon in the Tilapia fish from Kolo Creek. However, there is scarcity of data about PAH metabolites and antioxidative stress markers in earthworm tissue from Kolo Creek hence, this present research was undertaken to determine the concentration of PAH metabolites [2- hydroxyl naphthalene (2-OH Nap), 9 – hydroxyl fluorene (9 – OH Flu), 3 – hydroxyl phenanthrene

(3 – OH Phen), 1 – hydroxyl pyrene (1 –OH Pyr), 3 –hydroxyl benzo – a-pyrene (3-OH BaP)] and antioxidative stress markers (superoxide dismutase, catalase, glutathione peroxidase, glutathione s-transferase, reduced glutathione, lactate dehydrogenase, malondialdehyde and acetylcholinesterase in *Lumbricus terrestris* harvested from areas around Kolo Creek.

MATERIALS AND METHODS

Description of Study Area and Sampling Sites

Kolo Creek is a non – tidal fresh water which transverses through several communities including Emeyal, Otuasega, Imiringi, Oruma, Amurukani, Kolo1, Kolo 2 etc (Ineginte *et al*., 2010). This area of approximately 695 km^2 with a population of about 179,926 has been urbanized and industrialized due to crude oil exploration activities. The oil and gas facility is operated by the Shell Petroleum Development Company (SPDC). Inhabitants of Kolo Creek area are engaged in fishing on a subsistence and commercial level. Other economic activities carried out around Kolo Creek include dredging, boating and farming of food crops like plantain, cassava and potato. The sampling sites includes four (4) different communities (Emeyal, Imiringi, Elebele and Otuasega) of the Kolo Creek in Bayelsa State, Nigeria.

Collection of *Lumbricus terrestris*

Samples of *Lumbricus terrestris* were collected from four (4) different locations in each site around Kolo Creek, Bayelsa State, Nigeria using a spade and forceps to dig beneath the earth. Earthworms were handpicked into sterile plastic containers and labelled appropriately. The samples were collected at 1m intervals at each site in a given location. Sites were designated as follows; Site A= Emeyal, Site B= Imiringi, Site C= Elebele, Site D= Otuasega. For each site, four determinations, eight worms were pooled together per location (i.e., n = 4).

Determination of Polycyclic Aromatic Hydrocarbon Metabolites in *Lumbricus terrestris*

An extraction solvent of n – hexane and methylene chloride in ratio 3:1 was prepared. 10 mg of the well –mixed sample was measured into a solvent rinsed beaker and dry wet with anhydrous sodium sulphate until particles are loosed. 30 mL of the extraction solvent was then added to the sample and spiked with ortho –terphenyl, shaken in a vortex mixer for 5 minutes and sonicated for 10 minutes at 70º C. Extract was filtered through a glass wool and anhydrous sodium sulphate after which it transferred to a Teflon – lined screw cap vial for analysis. Sample was analysed for polycyclic aromatic hydrocarbons (PAHs) metabolites using the Gas Chromatography coupled with mass selection detector.

Preparation of Tissue Supernatant of *Lumbricus terrestris*

Lumbricus terrestris (0.5 g) was homogenized in ice cold 2mL phosphate buffer (pH 7.2). The homogenates were centrifuged in 4000 rpm for 10 min. The supernatant was decanted into a 2 mL sterilized plain container and used for the biochemical analysis.

Biochemical Analysis

Superoxide dismutase activity was determined according to the method of Misra and Fridovich (1972) while catalase activity was assayed according to the method of Kaplan and Groves (1972). The concentration of malondialdeyde was measured as described by Buege and Aust (1978). Reduced glutathione was estimated in the soft tissue supernatant of *Lumbricus terrestris* using the method of Ellman (1959). The methods of Habig *et al*. (1974) and Moin (1986) were used to evaluate the activities of glutathione s- transferase and glutathione peroxidise respectively. The determination of acetylcholinesterase was carried out by the method of Ellman *et al*. (1961) while the activity of lactate dehydrogenase was determined as prescribed by Amador *et al.* (1963). Detailed descriptions of all laboratory methods employed were previously reported by Osioma and Ejoh (2021).

Statistical Analysis

Data obtained were represented as mean ± SD (n = 4). One – way Analysis of Variance (ANOVA) was employed to establish significant differences of results while Duncan's Multiple Range Test (DMRT) was used to compare the groups means which were considered significant when $p < 0.05$. SPSS version 25 was used to perform the statistical analysis (SPSS, Inc – Chicago, Illinois, USA).

RESULTS

Table 1: Concentration (mg/kg) of polycyclic aromatic hydrocarbon metabolites in *Lumbricus terrestris* from experimental Sites

PAH Metabolites	A	B	C	D
2-OH Nap	0.15	0.05	0.15	3.75
9-OH Flu	0.50	0.30	0.05	4.85
3-OH Phe	0.35	0.15	0.25	2.95
1-OH Pyr	0.20	0.10	0.05	3.55
3-OH BaP	0.30	0.45	0.05	1.50
∑5 OH-PAHs	1.50	1.05	0.55	16.6

2- OH Nap = 2 –hydroxyl naphthalene; 9-OH Flu = 9- hydroxyl fluorene; 3-OH Phe = 3 –hydroxyl phenanthrene; 1 –OH Pyr = 1 – hydroxyl pyrene; 3-OH BaP = 3- hydroxyl benzo –a-pyrene.
A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Table 1 indicated that *Lumbricus terrestris* collected from Otusega had the highest concentrations of PAH metabolites. Table 1 also revealed that *Lumbricu sterrestris* from sites A, B and C had individual PAH metabolites concentrations of less than one (< 1).

Table 2: Biochemical parameters in *Lumbricus terrestris* from experimental sites

Parameters	A	B	C	D
Superoxide dismutase (Unit/g tissue)	30.30 ± 2.22[a]	33.02 ± 0.79[b]	33.38 ± 1.44[b]	32.35 ± 0.46[ab]
Catalase (Unit/g tissue)	37.37 ± 2.92[a]	47.91 ± 3.31[b]	50.57 ± 3.59[b]	43.97 ± 1.66[ab]
Malondialdehyde (Unit/g of wet tissue)	0.380 ± 0.14[a]	0.886 ± 0.18[b]	0.720 ± 0.13[c]	0.205 ± 0.10[d]
Reduced glutathione (Unit/mg protein of wet tissue)	25.62 ± 2.63[a]	39.35 ± 1.76[b]	28.64 ± 1.45[c]	25.20 ± 0.67[a]
Glutathione s- transferase (nmol/mg protein/min)	70.92 ± 7.98[a]	61.29 ± 8.80[a]	34.32 ± 2.66[b]	41.82 ±4.55[b]
Glutathione peroxidase (nmol/mg protein/min)	35.27 ± 3.50[a]	37.37 ± 3.51[a]	19.75 ± 1.45[b]	24.45 ± 2.53[c]
Acetylcholinesterase (nmol/mg protein/min)	19.99 ± 2.41[a]	15.05 ± 1.11[b]	17.77±0.99[a]	14.67 ±1.23[b]
Lactate dehydrogenase (IU/L)	434.96 ± 18.72[a]	487.83 ± 18.84[b]	459.44 ± 18.78[a]	452.89 ± 14.87[a]

Values are expressed as means ± SD; n = 4. Means with different superscript letters on the same row differ significantly at p < 0.05.
A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Table 2 indicated that superoxide dismutatse (SOD) and catalase (CAT) activities were elevated ($p < 0.05$) in *L. terrestris* from site B, C & D as compared to those from site A. The lipid peroxidation indices expressed as the concentration of malondialdehyde (MDA) was elevated ($p < 0.05$) in *L. terrestris* from site B as compared with those from sites A, C and D respectively. Table 2 also showed that the concentration of GSH was elevated ($p < 0.05$) in *L. terrestris* from site B as compared to earthworms from sites A, C & D. *L. terrestris* from sites A and D had comparable ($p > 0.05$) GSH contents. Comparable ($p > 0.05$) GST activity was expressed by *L. terrestris* from site A and B, although, higher ($p < 0.05$) than the activities of GST in earthworms from sites C and D. Table 2 also revealed an elevated ($p < 0.05$) GPx activities in *L. terrestris* from site A and B as compared with those from site C and D.

The activities of acetylcholinesterase were elevated ($p < 0.05$) in *L. terrestris* from site A and C as compared with those of site B and D according to Table 2. An elevated ($p < 0.05$) lactate dehydrogenase (LDH) was also observed in *L. terrestris* from site B as compared with *L. terrestris* from sites A, C & D which have comparable ($p > 0.05$) LDH activities.

DISCUSSION

In this study, the concentrations of the ∑5-OH PAHs in the *Lumbricus terrestris* from the selected area ranged from 1.50 to 16.6 mg/kg. The concentrations of PAHs metabolites in the *Lumbricus terrestris* varied significantly ($p < 0.05$) within each sampling site and among the sampling sites. The significant variation observed may be due to the varied bioaccumulation tendency of the individual *Lumbricus terrestris* and the rate of metabolism of PAHs in the *Lumbricus terrestris*. On the average, the concentrations of PAHs metabolites was in the order of 9-OH Flu > 2-OH Nap > 1-OH Pyr> 3-OH Phe> 3-OH Bap. The above results point to the fact that earthworms in experimental sites were exposed to PAHs since PAH metabolites can be correlates with the levels of exposure (Barra, *et al*., 2001; Jacob and Seidel, 2002; Fragoso *et al*., 2006).

PAHs like other pollutant may induce an increase in reactive oxygen species (ROS) in earthworms, these ROS if not checked by antioxidants in the animal's system could lead to oxidative stress – in which antioxidant defense system in the cells are inadequate to maintain the levels of ROS below a toxic concentration (Kolakowsta and Bartosz, 2010). SOD and CAT activities in *Lumbricus terrestris* from site B and C were higher ($p < 0.05$) than that of site A. SOD catalyzes the disproportionation of superoxide radical producing H_2O_2 which is removed by catalase or glutathione peroxidase. Lipid peroxidation indices expressed as malondialdehyde concentration was elevated in worms from sites B and C. The decrease MDA level observed in earthworms from site D could be as a result of the increase in the concentration of PAHs in that location as expressed by the concentrations of PAH metabolites.

Glutathione peroxidase (GPx) is a selenoenzyme that functions to detoxify peroxides to their corresponding alcohols in the mitochondria and sometimes in the cytosol (Cichoski *et al*., 2012). *L. terrestris* from site B exhibited highest GPx activities with an elevated level of reduced glutathione (GSH). This could mean increased capacity to scavenge hydrogen peroxide coupled with the fact that GPx uses GSH as an electron donor to regenerate the reduced form of the selenocysteine. High glutathione s- transferase (GST) activity observed in *Lumbricus terrestris* from site A and B could mean increased PAHs biotransformation process by *L. terrestris*. GST, catalyses the conjugation of electrophilic compounds with GSH (Maity *et al*., 2018).The activity of lactate dehydrogenase (LDH) is related to tissue damage and hypoxia and has been reported to be increased under condition of oxidative stress (Javanovic *et al*., 2010). Significant ($p < 0.05$) increase in LDH activity from *L. terrestris* in site B may not be unconnected with stress condition. A reduced acetylcholinesterase ($p < 0.05$) activity was observed in *L. terrestris* from site B and D in this study, which may be indications that *L. terrestris* are exposed to pollutants capable of inhibiting acetylcholinesterase.

CONCLUSION

The data obtained in this study suggests that *Lumbricus terrestris* from the experimental sites are exposed to PAHs and *L. terrestris*. The observed changes in oxidative stress markers and activity of lactate dehydrogenase may confirm the presence of contaminants or stressors in the experimental sites. Thus, PAH metabolites together with oxidative stress markers in earthworm could be employed as biomarkers for

exposure to polycyclic aromatic hydrocarbon contaminated environment and be effectively employed in the biological monitoring of PAHs.

REFERENCES

Aghoghovwia, O.A. and Chijoke, A. (2012). Total hydrocarbon (THC) of the lower Kolo Creek in Otuogidi, Bayelsa State, Nigeria. *J. Environ. Ext*., 10: 36 – 38

Amador, E., Dorfman, L. E. and Wacker, W.E.C. (1963) Serum lactate dehydrogenase: an analytical assessment of current assay. *Clin Chem. 9:391-399.*

Barra, R., Sanchez –Hernility, J.C., Orrego, R., Parra, O and Gavilan, J.F. (2001). Bioavailability of PAHs in the Biobio river (Chile): MFO activity and biliary fluorescence in juvenile *Oncorhynchus mykiss.Chemosphere*, 45 (4-5): 439 - 444.

Buege, J.A. and Aust, S.D. (1978) Microsomal lipid peroxidation. MethodsEnzymol. *52:* 302- 305.

Calisi, A., Lionetto, M.G. and Schettino, T. (2011). Biomarker response in the earthworm *Lumbricus terrestris* exposed to chemical pollutants. *Science of the Total Environment*, 409(20): 4456-4464. DOI: 10.1016/j.scitotenv.2011.06.058

Chen, C., Zhou, Q., Liu, S. and Xiu, Z. (2011). Acute toxicity, biochemical and gene expression responses of the earthworm *Eisenia fetida* exposed to polycyclic musks. *Chemosphere* 8:1147 – 1154.

Cichoski, A. J., Rotta, R.B., Scheuermann, G., Cunha Junior, A. and Barin, J.S. (2012). Investigation of glutathione peroxidase activity in chicken meat under different experimental conditions. *Cienc. Tecnol. Aliment, Campinas*, 32(4): 661 – 667.

Dendooven, L., Alvarez – Bernal, D. and Contreras – Ramos, S.M. (2011). Earthworms, a means to accelerate removal of hydrocarbons (PAHs) from soil: A mini – review. *Pedobiologia* 54: S187 – S192.

Dhiman, V. and Pant, D. (2022). Bioindication and biomarker responses of earthworms: A tool for soil pollution assessment. DOI: 10.1007/978-3-030-89984-4_23

Ebenezer, A. and Eremasi, Y. (2012). Determination of heavy metals in water sediments and *Tilapia zilli* from Kolo Creek, Ogbia Local Government Area, Bayelsa State, Nigeria. *Scientia Africana*., 11(1): 44 – 52.

Ellman, G.L., Courtney, K.D., Andres, V.J. and Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 7: 88- 95.

Ellman, G.L. (1959). Tissue sulfhydryl groups. *Arch Biochem Biophys*. 82: 70- 77.

Emoyan, O.O., Tesi, G.O., Ohwo, E. and Odali, E.W. (2021). Quantification, sources, and associated risks of 16-priority polycyclic aromatic hydrocarbons from selected land-use impacted soils. *Ovidius University Annals of Chemistry*, 32(1): 53:62

Fragoso, N.M., Hodson, P.V. and Zambon, S. (2006). Evaluation of an exposed assay to measure uptake of sediment PAH by fish. *Environ Monit Assess* 116: 481 – 511.

Habig, W.R., Pabst, M.J. and Jakoby, W.B. (1974). Glutathione transferase: a first enzymatic step in mercaturic acid formation. *J. Biol. Chem.* 249: 7130-7139.

Inengite, A.K., Oforka, N.C. and Osuji, L.C. (2010). Evaluation of polycyclic hydrocarbons in sediment of Kolo Creek in the Niger Delta. *Int. J Appl Environ Sci*, 5(1): 127 - 143.

Iniaghe, P.O.,Tesi, G.O. and Iniaghe, O.P. (2013). Environmental Degradation and Sustainable Development in Nigeria's Niger Delta Region: An overview. *Journal of Sustainable Development in Africa*, 15(3): 61-78

Iwegbue, C.M.A., Ehigbor, M.J., Tesi, G.O., Eguavoen, O.I. and Martincigh, B.S. (2020). Occurrence, sources and exposure risk of polycyclic aromatic hydrocarbons (PAHs) in street dusts from the Nigerian megacity, Lagos. *Polycyclic Aromatic Compounds* http://doi.org/10.1080/10406638.2020.1716027

Jacob, J. and Seidel, A. (2020). Review: Biomonitoring of polycyclic aromatic hydrocarbons in human urine. *J. Chromatogr B*, 778: 31 – 47.

Jovanovic, A.M., Durst, S. and Nick, P. (2010). Plant cell division is specifically affected by nitrotyrosine. *Journal of Experimental Botany*, 61(3): 901 -909.

Kaplan,J.H. and Groves, J. (1972) Liver and blood cell catalase activity in tumor-bearing mice. *Cancer Res*. 32: 1190-1194.

Kolakowska, A. and Bartosz, G. (2010). Antioxidatants. In: Sikorski Z. and Kolakowska, A. (Eds.), Chemical, Biological and Functional Aspects of Food Lipid. CRC Press Boca Raton FL USA. Pp: 163 -184.

Maity,S., Banerjee, R., Goswami, P., Chakrabarti, M. and Mukherjee, A.(2018). Oxidative stress responses of two different ecophysiological species of earthworms (*Eutyphoeus Walton* and *Eisenia fetida*) exposed to Cd – contaminated soil. *Chemosphere*, 203: 307 -317.

Misra, H.P. and Fridovich, I. (1972) The role of superoxide ion in the auto-oxidation of epinephrine and a simple essay for superoxide dismintase. *J Biol Chem*.247: 3170 -3175

Moin, V.M. (1986). Simple and specific method of measurement of glutathione peroxidase activity in the erythrocytes. *Laboratornoe delo* 12: 724-727.

Nasr, L.N., Arief, M.H., Abdel –Aleem, A.H. and Malhat, F.M. (2010). Polycyclic aromatic hydrocarbons (PAHs) in aquatic environment at El Menofiya Governorate. *Egypt J. Applied Sci Res*, 6(1): 13 -21.

Olowu, R.A., Ayejuyo, O.O., Adewuyi, G.O., Adejoro, I.A., Denloye, A.A.B., Babatude, A.O. and Ogundayo, A.L. (2010). Determination of heavy metals in fish tissues, water

and sediment from Epe and Badagry Lagoons, Lagos, Nigeria. *Electronic J. Chem*., 7(1): 215 – 221.

Onokare, E.P., Tesi, G.O., Odokuma, L.O. and Sikoki, F.D. (2022). Effectiveness and toxicity of chemical dispersant in oil spill aquatic environment. *Asian Journal of Water, Environment and Pollution* 19(2): 41-46

Osioma, E. and Ejoh, A.S. (2021). Acitivities of acetylcholinesterase, oxidative and nitrosative stress markers in *Clarias gariepinus* exposed to 'Uproot', a glyphosate herbicide. *Inter J Zoological Invest*. 7(1): 51 -66.

Silva, C., Oliveira, C., Gravato, C. and Almeida, J.R. (2013). Behaviour and biomarkers as tools to assess the acute toxicity of benzo(a)pyrene in the common prawn *Palaemon serratus*. Mar Environ Res, 90: 39 -46.

Tesi, G.O., Iwegbue, C.M.A., Emuh, F.N. and Nwajei, G.E. (2016). Ladgo Dam floods disaster of 2012: An assessment of the concentrations, sources and risks of polycyclic aromatic hydrocarbons in floodplain soils of the lower reaches of River Niger, Nigeria. *Journal of Environmental Quality*, 45: 305-314

Varanasi, U., Stein, J.E., Nishimoto, M. (1989). Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAHs) in fish. In: Varanasi, U. (Ed.), Metabolism of polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press, Boca Raton, FL, pp: 9 - 149.

Yaguo, E.B., Egbo, M.W. and Goldie, J. (2021). Total petroleum hydrocarbon accumulation in gills and muscles tissue of *Tilapia Spp* in Kolo Creek Imiringi Bayelsa State. *Res J Environ Sci Toxicol* 2(2): 010 – 014.

Zeb, A., Li, S., Wu, J., Lian, J., Liu, W. and Sun, Y. (2020). Insight into the mechanisms underlying the remediation potential of earthworms in contaminated soil: A critical review of research progress and prospects. *Sci Total Environ*, 740: 104 -145.