INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 5, p. 184-191, 2022

RESEARCH PAPER OPEN ACCESS

Effects on liver somatic index, erythrocyte nuclear abnormalities and biliary metabolites of PAH in *Oreochromis niloticus* exposed to water-borne crude oil

GDSR. Piumali[1], SHNP. Gunawickrama[2], KB. Suneetha Gunawickrama[*1]

[1]*Department of Zoology, Faculty of Science, University of Ruhuna, Wellamadama, Matara, Sri Lanka*
[2]*Institute for Combinatorial Advance Research and Education, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka*

Key words: Bile metabolites, Biomarkers, Crude oil, ENA, LSI, Polyaromatic hydrocarbons

http://dx.doi.org/10.12692/ijb/21.5.184-191 Article published on November 15, 2022

Abstract

Exposure to crude oil has detrimental effects on both marine and freshwater biota, yet the focus on the latter is relatively low, especially in lentic environments with minimal dispersion. The present study investigated the short-term effects of sub-lethal crude oil on *Oreochromis niloticus* with reference to liver somatic Index (LSI), erythrocyte nuclear abnormalities (ENA), and bile PAH metabolite types. Two groups of sub-adult fish of the same brood cohort were maintained in a static renewal system, i.e., the treatment group with crude oil dispersed in water (nominal v/v approximation of 50 ppm simulating a slight oil slick) in three replicates, and one control group with no crude oil (n=15/ tank). Sampling was done initially, and on the 4th, 8th, 12th and 16th day post-exposure. Three bile PAH metabolite types were estimated by fixed wavelength fluorescence (FF). LSI and standardized bile florescence values at each sampling point were expressed as % difference from the control. Relative LSI calculated as deviation from the control increased by water-borne crude oil in the fish over 16-day period. Higher ENA counts ($p<0.05$) were found in the exposed group (nuclear buds, notched nuclei, and lobbed nuclei) on 16th day compared to the pre-exposure fish. Relative values of FF-detected, protein-standardized naphthalene and phenanthrene metabolites showed more than 30% and 130% increase respectively on day 12 compared to control fish. The results showed that crude oil can induce changes in LSI, ENA and bile metabolite levels in freshwater fish *O. niloticus*.

* **Corresponding Author:** Suneetha Gunawickrama ✉ suneetha@zoo.ruh.ac.lk

Introduction

Among many organic contaminants, crude oil is a major source of pollution affecting the structure and function of aquatic environments (Islam and Tanaka, 2004). Crude oil is a complex mixture of organic compounds, mainly consisting of hydrocarbons, non-hydrocarbon compounds such as sulphur, Nitrogen and Oxygen compounds, and some heavy metals (Onwurah *et al.*, 2007; Kponee *et al.*, 2015; Adipah, 2019). Due to accidental spill events, crude oil can reach both marine and fresh waters, but higher attention is received on marine oil spills perhaps due to their larger impact range and higher frequency of occurrence. Freshwater spills tend to be less frequent than marine spills, however, an oil spill reaching freshwater bodies can have greater impact on the biota due to lower dispersion and dilution potential. Therefore, studies about the evaluation and damage caused by petroleum products on the freshwater ecosystem have become important, but relatively less numerous (Van der Oost *et al.*, 1998; Nikanorov and Stradomskaya, 2009; Ibemenuga, 2013).

Effects on biota from pollutant exposure can vary from acute toxic lethal effects to chronic sub-lethal effects which can be detected from the molecular level to ecosystem levels (Van der Oost *et al.*, 2003). Fishes are commonly used as indicators of pollution in aquatic habitats because they cannot escape from the detrimental effect of various toxic contaminants added to aquatic habitats (Sanchez *et al.*, 2008; Ibemenuga, 2013). The integrative biomarker approach has been recommended by many studies to reveal a multitude of toxicant effects on fish (Marigómez *et al.*, 2004; Ghisi *et al.*, 2017; Santos *et al.*, 2017). Oil slicks may remain in lentic environments unaffected by dispersion thus the short-term effects can be expected to accumulate. Laboratory experiments can reveal any short-term effects of exposure to a sublethal quantity of crude oil slick in water that is continuous and unaffected by dilution, and such data will be important in long-term risk assessment attempts. This study focused on biomarkers of exposure at three levels; PAH metabolites at the molecular level, erythrocyte nuclear abnormalities at the cellular level, and change in Liver Somatic Index (LSI) at the organ level of fish. The present study investigated the short-term effects of sub-lethal water-borne crude oil exposure that simulate a lentic slight oil slick on freshwater fish *Oreochromis niloticus* with reference to liver somatic Index (LSI), erythrocyte nuclear abnormalities (ENA), and excretory levels of bile PAH metabolites within a 16-day period in a controlled laboratory experiment.

Materials and methods

Experimental setup

The experimental study was done using sub-adults of *Oreochromis niloticus* (total length 10-12cm) as a test organism and using Murban crude oil (from Ceylon Petroleum Corporation), at the wet laboratory of the Department of Zoology, University of Ruhuna. Fish from Muruthawela fish breeding Centre, National Aquaculture Development Authority (NAQDA), Sri Lanka was used, and the experiment was conducted in January 2020. Two experimental groups of fish, a treatment group with crude oil dispersed in water (with a nominal level of 50 ppm) in three replicates, and a control group with no crude oil in water (15 fish per tank) preceded by 10-day acclimatization were maintained in fiberglass tanks (dia. 0.7m x 0.5m). All fish originated from the same brood cohort were used to remove any parental effects on the results. Fish were fed with a commercial fish diet at 2% of average body weight once a day, and moderate aeration was maintained in a static renewal system. Exposure was renewed once in four days. Sampling was done pre-exposure and subsequently on the 4th day, 8th day, 12th day, and 16th day post-exposure, to collect data on liver and body weight, to prepare blood smears for erythrocyte counting, and to collect bile for PAH metabolite analysis.

Data collection and analysis

The experiment utilized the fish from the same brood cohort to exclude parental effects, thus facing a limitation on sample size. On the pre-exposure day, 2 fish from each tank were taken to be used as a reference point in the relative value calculations for treated fish. At post-exposure sampling points, 3 fish from each treatment tank (total n=9 per sampling day) and 2 fish from the control tank were randomly sampled. After the fish were anesthetized, the total

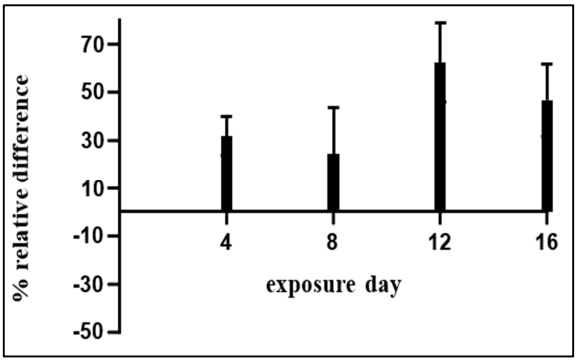
wet weight and the total length of the fish were measured. A drop of cardiac blood was drawn and thin blood smears were prepared followed by Giemsa staining. Liver weight (Wt) was measured to calculate the liver somatic index LSI (Liver Wt/body Wt x 100). A bile sample was collected to measure the bile fluorescence for three types of metabolites i.e. naphthalene type, phenanthrene type, and pyrene type. Each bile sample was diluted at 1:1000 in 50/50 (v/v, ethanol to water) before measuring fluorescence. Fluorescence intensity was measured using the fixed wavelength fluorescence (FF) method, using the LUMINA Florescence spectrometer (Thermo Scientific) with the help of Luminous Software-Wave Scan. FF was determined at the following excitation/emission wavelengths, with slit size of 20 nm: excitation/emission Naphthalene type 290/335, pyrene type 380/430, phenanthrene type 256/380 (Miller *et al.*, 1999; Fuentes Rios *et al.*, 2005). The values were normalized to the total protein absorbance levels of the relevant bile sample at 280 nm measured by a UV/VIS spectrophotometer (UH5300 HITACHI). Erythrocytes (total 5000) were counted while enumerating the cells with selected abnormalities (ENA) under the microscope (mag. 10x100). PH value, temperature, dissolved oxygen concentration (DO) and total Ammonia concentration (Color Assay kit) in each tank were measured for water quality monitoring.

Due to limited sample size in the control group, LSI values and standardized bile fluorescence values at each sampling point were expressed as a deviation from reference (% difference from the control), and any comparisons where required among different time points within the treatment group were done by non-parametric tests. Relative frequencies of ENA types were non-parametrically compared among sampling days in the treatment group using the Kruskal-Wallis test and Conover post hoc analysis.

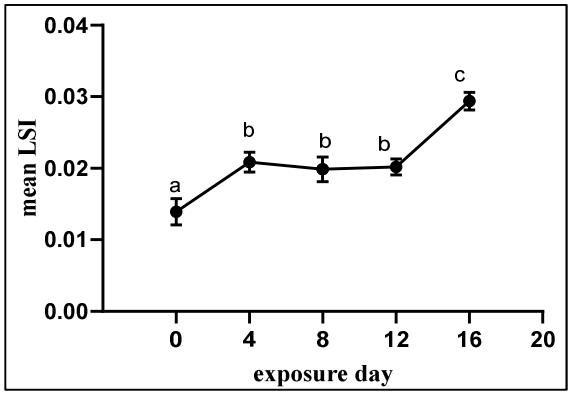
Results

Liver somatic index (LSI)

After exposure to crude oil, the percentage relative difference of LSI of fish in the treatment group was high relative to the fish in the control group at all sampling points and it was significantly high on day 12 and day 16 (Fig. 1a). It is apparent that the liver weight has increased due to crude oil exposure. The LSI of fish in the treatment group showed significant variation among sampling times within the 16 days of the exposure period. After exposure to crude oil, LSI in treatment groups was significantly higher than the pre-exposure, even after 4 days of exposure to crude oil (Fig. 1b), while no significant change occurred thereafter until the 12th day. The overall change from pre-exposure to day 16 was significant.



(a)



(b)

Fig. 1.(a). Percentage relative differences of Liver Somatic Index of *Oreochromis niloticus* exposed to crude oil slick (50 ppm crude oil in water), and (b) change of mean (± SD) LSI in the treatment group over 16-day period of exposure (non-shared superscripts indicate differences among exposure days at $p<0.05$, Kruskal-Wallis test).

Erythrocyte nuclear abnormalities (ENA)

According to the Kruskal-Wallis test, relative frequency (per 1000 RBC's) of erythrocyte nuclear abnormalities (ENA) varied among sampling points in the treatment group showing significant induction with time (Fig. 2).

Three types of erythrocyte nuclear abnormalities (nuclear buds, notched nuclei, and lobed nuclei) were reliably enumerated (Fig. 3). Four days after exposure to crude oil, the relative frequency of nuclear buds and lobed nuclei significantly increased while the relative frequency of notched nuclei increased after 8 days of exposure. No micronuclei were observed in any group.

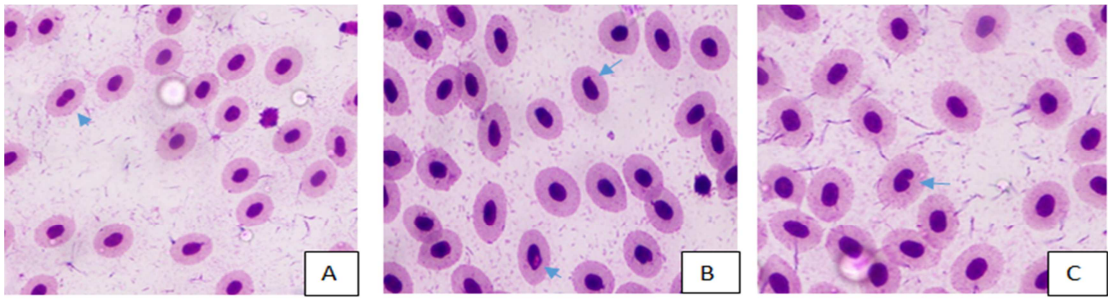


Fig. 2. Photomicrography (×100) of different erythrocyte nuclear abnormalities of *Oreochromis niloticus,* (A) lobbed nuclei, (B) nuclear buds, and (C) notched nuclei (indicated by arrows).

PAH biliary metabolite types

Fluorescence intensity at designated excitation/ emission points standardized by the total protein levels revealed that naphthalene, pyrene and phenanthrene types of PAH metabolites were either high or low relative to the fluorescence value of the control group (Fig. 4). Fluorescence intensity of phenanthrene and naphthalene clearly increased with time upon crude oil exposure, significantly on days 12 and 16. Relative values of FF-detected, protein-standardized naphthalene, and phenanthrene metabolite types showed more than 30% and 130% increase respectively from day 12 compared to control fish but not pyrene type. Pyrene type did not show any increase or discernible trend in response to exposure time and requires further attention as negative readings were obtained.

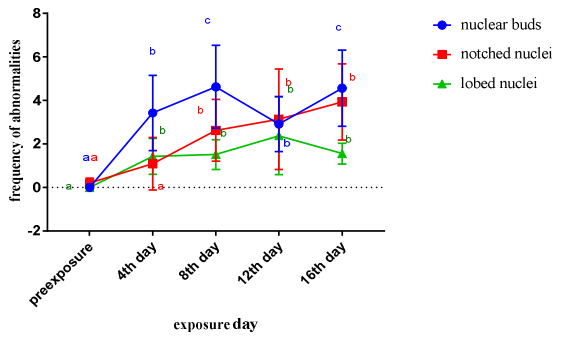


Fig. 3. Frequencies of ENA types in *Oreochromis niloticus* exposed to 50 ppm crude oil slick over 16-day period (non-shared superscripts indicate differences among exposure days at $p<0.05$, Kruskal-Wallis test).

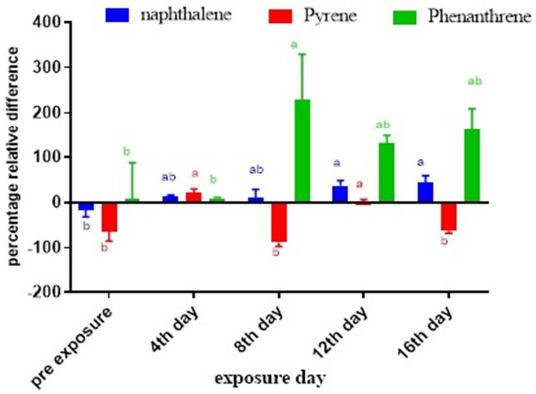


Fig. 4. Percentage relative differences of fluorescence values of Naphthalene, Pyrene, and Phenanthrene type bile metabolites over 16-day exposure to 50 ppm crude oil (non-shared superscripts indicate differences among exposure days at $p<0.05$, Kruskal-Wallis test).

Discussion

LSI has been used as an organ-level indicator of fish wellbeing. A previous study by Papoulias *et al.* (2014) reported that there was an increase in LSI of fish collected from the Kalamazoo River, Michigan following discharges of diluted Bitumen crude oil, and it was attributed to the poor health status of fish after exposure to crude oil. Enhanced LSI has been reported in fish exposed to oil industrial waste effluents (Corredor-Santamaría *et al.*, 2021). When toxic and carcinogenic compounds accumulate in the liver, liver tissues may undergo hypertrophy and may begin to swell (Fletcher *et al.*, 1982). But if the exposure to a toxic substance is getting worse or fish

were exposed for longer periods of time, then the liver starts to suffer from necrosis and cirrhosis (Marina *et al.* 2007). Cirrhosis may lead to the shrinking of the liver subsequently leading to a lower LSI (Pantung *et al.*, 2008). If exposure to toxic compounds no longer occurs in the long run, the liver can be returned to its normal condition, because the swelling of liver cells is a reversible process. In the present study within the 16 days of the exposure period, liver weight increased disproportionate to the normal increase in body weight, so the LSI value was increased. A possible explanation can be liver swelling due to the accumulation of toxic polycyclic aromatic hydrocarbons and disruption of liver metabolism and subsequent swelling. If the exposure period is much longer, there may be a possibility of a decrease in the LSI value due to further liver damage. Results of the control tank revealed that there is no significant increase in LSI of fish which overweighs the normal weight increase due to any growth that occurred through the experiment period. That means the only reason for the relative increase in LSI is linked to the exposure to crude oil slick. Elevated LSI in fish exposed to PAH contaminated water has been reported in fish linking the effects to liver hypertrophy but other pollutants may affect the LSI differently as shown by Kusuma Devi and Prabawo (2017) who reported that LSI values of both Nile Tilapia and *Cyprinus carpio* treatment group had a lower LSI value than the control group when exposed to heavy metals for 1.5 months.

Having a significant induction of ENA in the crude oil exposed group indicates that exposure to the oil slick exerts a genotoxic effect on RBCs within a short time. Nuclear abnormalities may occur due to the damages that happen inside the cell such as chromosome breaks, chromatid breaks, and chromatid deletions (Yadhav and Trivedi, 2006). Fernandes *et al.* (2007) reported that notched nuclei were formed due to the aneuploidy. In the present study, only the types that could be reliably enumerated were considered, and micronuclei were not found. In published research, micronuclei test has been used as an effective marker of contaminant exposure (Barseine *et al.*, 2006; Gomes *et al.*, 2015). Gunawickrama *et al.* (2016) reported that frequency of erythrocyte nuclear abnormalities such as micronuclei, nuclear buds, fragmented nuclei, notched, lobed and other types of erythrocyte nuclear abnormalities were increased by the long term continuous and renewed exposure of *Oreochromis* juveniles to 25ppm crude oil over 90 days of exposure period. Significant induction of micronuclei may not have occurred due to shorter exposure period although the nominal oil concentration was two-fold in the present study. Absence of common ENA types in the control group was somewhat anticipatory as revealed in other studies as either absent or present in very low frequencies (Barsiene *et al.*, 2006).

In the fish liver, PAHs are rapidly transformed into more hydrophilic metabolites, and they are excreted into the bile and then stored in the gallbladder prior to the subsequent excretion (Aas *et al.*, 1998; Van der Oost *et al.*, 2003). Most of the PAH and their metabolites have characteristic florescence properties, and the exposure effects of PAH in fish can be assessed by the presence of PAH metabolites in fish bile (Pathiratne *et al.*, 2010). Increased naphthalene and phenanthrene type bile metabolites detected by the present study indicate short term exposure responses in fish to a minor crude oil slick. According to a laboratory study done by Pathiratne *et al.* (2010), fluorescence values of Nile tilapia which exposed to the naphthalene, phenanthrene and pyrene were higher than the unexposed group. In that study, naphthalene values were increased nine-fold compared to the control group one day after exposure and three fold after 3 days of exposure but phenenthrene was increased in a dose dependent manner, and similar trends were reported for pyrene fluorescence values as well.

Pulster *et al.* (2017) reported that naphthalene and phenanthrene concentrations in Florida pompano increased by up to 200 times from 24 hours after injection to 72 hours of injection of Deepwater Horizon crude oil. Similar increases in concentrations over time were observed in red drum (Pulster *et al.*, 2017) which suggested that phenanthrene may be the principal biotransformation product regardless of the

species, exposure route or whether phenanthrene is introduced as a single compound or as a complex compound like crude oil. In this research too, both naphthalene and phenanthrene types always showed a positive deviation from the control value suggesting an obvious increase linked to the exposure. Pyrene type did not show such a clear trend, however. It can be suggested that similar responses may occur in fish living in any type of environment if they were exposed to PAH-contaminated waters. Ranasinghe and Pathiratna (2015) analyzed the bioavailability of PAHs in fish residing in the koggala lagoon, Sri Lanka, and revealed that *Mugil cephalus* and *Lutjanus rusellii* from the seaward side of the lagoon and *Etroplus suratensis* collected from the landward site of the lagoon have significantly higher bile fluorescence levels of naphthalene and phenanthrene type compared to the *Oreochromis mossambicus* captured from the innermost landward site of the lagoon. The same study however reported significantly high fluorescence values of pyrene in fish captured from the seaward side of the lagoon than in fish from the innermost site of the lagoon. Analysis of feral fish exposed to a similar kind of minor oil slick may shed further light on the bile metabolite levels in exposed fish. Fluorescence values of different PAH metabolites can vary with the dietary pattern of the fish (Insausti *et al.*, 2009). The concentration of PAH metabolites may be influenced by the species (Pulster *et al.*, 2017), age, sex, and maturity, but the size of the fish does not influence the concentration of phenanthrene and pyrene (Baali *et al.,* 2016). The observed changes cannot be attributable to such confounding factors, as the experiment was conducted using the fish of the same brood cohort and sexually immature stage. Throughout the experiment period, observed water quality parameters (pH 6.4-7.4, temperature 27.9-28.7 ^{0}C, DO: 0.008-0.012mg/L, ammonia concentration: <1mg/L) did not show any significant difference among tanks (results not shown), so there was no any effect from water quality to the observed results.

Conclusion

The present study reports short-term biomarker responses in freshwater fish *Oreochromis niloticus* exposed experimentally to crude oil in water (50 ppm v/v). Liver somatic index (LSI) calculated as a percentage difference from the control increased over a 16-day exposure period. Higher ENA counts ($p<0.05$) were found in the exposed group (nuclear buds, notched nuclei, and lobbed nuclei) on the 16th day compared to the pre-exposure fish. Relative values of FF-detected, protein-standardized naphthalene, and phenanthrene metabolite types showed more than 30% and 130% increase respectively from day 12 compared to control fish but not pyrene type.

The results showed that water-borne crude oil can induce changes in LSI, ENA, and PAH bile metabolite levels in freshwater fish *O. niloticus*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Department of Chemistry, University of Ruhuna is thanked for the use of UV/Visible spectrophotometer. Dr. Janitha Walpita at the Instrument Center, Department of Chemistry, University of Sri Jayewardenepura is acknowledged for fluorometry. The authors are grateful for the wet laboratory and other laboratory facilities at the Department of Zoology, and the microscopic imaging facility at the Faculty of Fisheries and Marine Science & Technology, University of Ruhuna.

References

Aas E, Beyer J. 1998. PAH in fish bile detected by fixed wavelength fluorescence. Marine Environmental Research **46(1-5),** 225-228.

Adipah S. 2019. Introduction of Petroleum Hydrocarbons contaminants and its human effects. Journal of Environmental Science and Public Health **3(1),** 1-9.

Agah H, Leermakers M, Elskens M, Fatemi SMR, Baeyens W. 2009. Accumulation of trace metals in the muscles and liver tissues of five fish species from the Persian Gulf. Environmental Monitoring and Assessment **157,** 499-514.

Baali A, Kammann U, Qoraychy I, Yahyaoui A. 2016. Bile metabolites of polycyclic aromatic hydrocarbons (PAHs) in three species of fish from Morocco. Environmental Science **28(1),** 25. DOI: 10.1186/s12302-016-0093-6.

Barseine J, Dedonyte V, Rybakovas A, Andreikenaite L, Andersen OK. 2006. Investigation of micronuclei and other nuclear abnormalities in peripheral blood and kidney of marine fish treated with crude oil. Aquatic Toxicology **78,** 99-104.

Corredor-Santamaría W, Mora-Solarte DA, Arbeli Z, Navas JM, Velasco-Santamaría YM. 2021. Liver biomarkers response of the neotropical fish *Aequidens metae* to environmental stressors associated with the oil industry. Heliyon **7(7),** https://doi.org/10.1016/j.heliyon.2021.e07458.

Fernandes TCC, Mazzeo DEC, MarinMolares MA. 2007. Mechanism of micronuclei formation in polyploidizated cells of *Allium Cepa* exposed to trifluralin herbicide. Pest Biochemistry Physiology **88,** 252-259.

Fletcher GL, King MJ, Kiceniuk JW, Addison RF. 1982. Liver hypertrophy in winter flounder following exposure to experimentally oiled sediments. Comparative Biochemistry and Physiology C: Comparative Pharmacology **73(2),** 457-62. DOI: 10.1016/0306-4492(82)90153-8.

Fuentes-Rios D, Orrego R., Rudolph A, Mendoza G, Gavila'n JF, Barra R. 2005. EROD activity and biliary fluorescence in *Schroederichthys chilensis* (Guichenot 1848): Biomarkers of PAH exposure in coastal environments of the South Pacific Ocean. Chemosphere **61,** 192-199.

Ghisi NC, Oliveira EC, Guiloski IC, de Lima SB, de Assis HCS, Longhi SJ, Prioli AJ. 2017. Multivariate and integrative approach to analyze multiple biomarkers in ecotoxicology: A field study in Neotropical region. Science of the Total Environment **609,** 1208-1218.

Gomes JMM, Ribeiro HJ, Procopio MS, Alvarenga BM, Castro ACS, Dutra WO, Silva JBBD, Correa JD. 2015. What the erythrocyte nuclear alterations frequencies could tell us about genotoxicity and macrophage iron storage. PLOS One **10(11),** 1-22.

Gunawickrama SHNP, Panawala PDSM, Gunawickrama KBS. 2016. Impaired growth and erythrocyte nuclear lesions of immature *Oreochromis niloticus* exposed to waterborne crude oil: persistent responses. Sri Lanka Journal of Aquatic Science **21(2),** 113-124.

Ibemenuga KN. 2013. Impacts of crude oil on freshwater fish fauna, its control and management measures. Animal Research International **10(3),** 1799-1804.

Insausti D, Maite C, Francesco M, Joan EC, Montserrat S. 2009. Biliary florescent aromatic compounds (FAcs) measured by fixed wavelength florescence (FF) in several marine fish species from NW Mediterranean. Marine Pollution Bulletin **58(11),** 1635-1642.

Islam MS, Tanaka M. 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Marine Pollution Bulletin **48(7-8),** 624-649.

Kaponee KZ, Chiger A, Kakulu II, Vorhees D, Heigher-Bernays W. 2015. Petroleum contaminated water and health symptoms: A cross sectional pilot study in a rural Nigerian community. Environmental Health **14,** 86.

Kusuma Dewi N, Prabowo R. 2017. Determination of Liver Somatic Index (LSI) and Gonadosomatic Index (GSI) value of Carp (*Cyprinus carpio*) and Nile Tilapia (*Perca fluviatilis*). International Journal of Scientific and Research Publications **7(6),** 220-223.

Marigómez I, Soto M, Orbea A, Cancio I, Cajaraville MP. 2004. Biomonitoring of environmental pollution along the Basque coast, using molecular, cellular and tissue-level biomarkers: an integrative approach. Oceanography and Marine Environment of the Basque Country, Elsevier Oceanography Series, Elsevier, Amsterdam **70,** 335-364.

Marina M, Carmago and Claudia Martinez BR. 2007. Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. Neotropical Ichthyology **5(3),** 327-336.

Miller HC, Mills GN, Bembo DG, Macdonald JA, Evans CW. 1999. Induction of cytochrome P4501A (CYPIA) in *Trematomus bernachi* as an indicator of environmental pollution in Antarctica: assessment by quantitative RT-PCR. Aquatic Toxicology **44,** 183-193.

Nikanorov AM, Stradomskaya AG. 2009. The role of biogenic hydrocarbons in the assessment of oil pollution of freshwater bodies. Water Resources **36,** 57-63. https://doi.org/10.1134/S0097807809010059.

Onwura INE, Ogugua VN, Onyike NB, Ochonogor AE, Otitoju OF. 2007. Crude oil spills in environment, effects and some innovative clean-up biotechnologies. International Journal of Environmental Research **1(4),** 307-320.

Pantung N, Helanderkg, Helander HF, Cheevaporna V. 2008. Histopathological alterations of hybrid walking catfish (*Clarias macrocephalus x Clarias gariepinus*) in acute and sub-acute Cadmium exposure. Environment Asia **1,** 22-27.

Papoulias DM, Veléz V, Nicks DK, Tillitt DE. 2014. Health Assessment and Histopathologic Analyses of Fish Collected from the Kalamazoo River, Michigan, Following Discharges of Diluted Bitumen Crude Oil from the Enbridge Line 6B. US. Department of the Interior, US. Geological Survey.

Pathiratne A, Hemachandra CK, Pathiratne KAS. 2010. Assessment of bile florescence patterns in a tropical fish, Nile tilapia (*Oreochromis niloticus*) exposed to naphthalene, phenanthrene, pyrene and chrysene using fixed wavelength florescence and synchronous florescence spectrometry. Bulletin of Environmental Contamination & Toxicology **84,** 554-558.

Pulster EL, Main K, Wetzel D, Murawski S. 2017. Species-specific metabolism of naphthalene and phenanthrene in 3 species of marine teleosts exposed to Deepwater Horizon crude oil. Environmental Toxicology and Chemistry **36(11),** 3168-3176. https://doi.org/10.1002/etc.3898.

Ranasingha RATCS, Pathiratne A. 2015. Histological alterations and polycyclic aromatic hydrocarbon exposure indicative bile florescence patterns in fishes from Koggala lagoon, Sri Lanka. Journal of National Science Foundation Sri Lanka **43(1),** 65-73.

Sanchez W, Katsiadaki I, Piccini B, Ditche JM, Porcher JM. 2008. Biomarker responses in wild three-spined stickleback (*Gasterosteus aculeatus* L.) as a useful tool for freshwater biomonitoring: A multiparametric approach. Environment International **34(4),** 490-498.

Santos R, Joyeux A, Besnard A, Blanchard C, Halkett C, Bony S, Devaux A. 2017. An integrative approach to assess ecological risks of surface water contamination for fish populations. Environmental Pollution **220,** 588-596.

Van der Oost R, Beyer Jonny, Vermeulen NPE. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology **13(2),** 57-149.

Van der Oost R, Lopes SCC, Komen H, Satumalay K, Van den Bos R, Heida H, Vermeulen NPE. 1998. Assessment of environmental quality and inland water pollution using biomarker responses in caged carp (*Cyprinus carpio*); use of a bioactivation: Detoxification ratio as Biotransformation index (BTI). Marine Environmental Pollution **46,** 315-319.

Yadav KK, Trivedi SP. 2006. Evaluation of Genotoxic potential of chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations. Asian Pacific Journal of Cancer Prevention **7,** 472-476.