Histology Based Whole Effluent Chronic Toxicity Testing of Noodles Processing Company Waste, using the Liver of *Clarias gariepinus* as a Biomarker.

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Abstract
This study investigated the chronic toxicity of effluents from a noodle food industry, focusing on the environment water quality and its histological impact on the liver of Clarias gariepinus. The following known aquatic contaminants from food industries were selected as the effluent target chemical (TC) of concern: cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni) and polyaromatic hydrocarbons (PAH). Effluent concentrations of 6.25%, 12.5%, 25%, 50%, and 100% were tested, with a control group maintained in clean water. Histopathological analysis of the liver was conducted to assess structural alterations linked to effluent toxicity. Effluent analysis result revealed Results revealed that Cu levels (0.037 mg/L) exceeded permissible regulatiro thresholds, highlighting significant contaminantion concerns. Histological analysis revealed graded liver damage, highlighting the significant impact of the studied industrial effluents on aquatic life.

Keywords: *Histology; Liver, Effluent; Toxicology, Biomarker; Fish and Water Quality.*

Introduction

Effluent toxicity refers to the adverse effects of industrial and municipal discharges on aquatic ecosystems (Ahmed et al., 2021). These discharges often contain a variety of contaminants, including heavy metals, organic compounds, and nutrients, which can be detrimental to aquatic life (Ebol et al., 2020). Chronic exposure to these pollutants can lead to significant ecological imbalances, affecting not only individual species but entire aquatic communities (Scholz & McIntyre, 2015).

Whole Effluent Toxicity (WET) refers to the aggregate toxic effect to aquatic organisms from all pollutants contained in a facility's wastewater (effluent). It is one of the ways to implement the Clean Water Act's prohibition of the discharge of toxic pollutants in toxic amounts (USEPA, 2002). The objective of aquatic toxicity tests with whole effluents or pure compounds is to estimate the "safe" or "no effect" concentration of these substances, which is defined as the concentration which will permit normal propagation of fish and other aquatic life in the receiving waters. The endpoints that have been considered in tests to determine the adverse effects of toxicants include death and survival, decreased reproduction and growth, locomotor activity, gill ventilation rate, heart rate, blood chemistry, histopathology, enzyme activity, olfactory function, and terata (USEPA, 1999; USEPA, 2002).These data are used for environmental permits development and to determine compliance with permit toxicity limits.

Partial life-cycle toxicity tests with embryo-larval and early life-stages (ELS) of fish was proposed as the most sensitive in establishing water quality criteria (McKim, 1977; USEPA, 2002). This was due to the high cost of full life-cycle fish toxicity tests and the emerging

consensus that the ELS test data usually would be adequate for estimating chronically safe concentrations. Hence, there was a rapid shift by aquatic toxicologists to 30 - 90-day ELS toxicity tests for estimating chronically safe concentrations (USEPA, 2002)

Whole effluent chronic toxicity tests are performed as a part of self-monitoring permit requirements, compliance biomonitoring inspections, toxics sampling inspections, and special investigations. Modifications of these tests are also used in toxicity reduction evaluations and toxicity identification evaluations to identify the toxic components of an effluent, to aid in the development and implementation of toxicity reduction plans, and to compare and control the effectiveness of various treatment technologies for a given type of industry, irrespective of the receiving water (USEPA, 1992; USEPA, 2002). The validity of the freshwater chronic methods in predicting adverse ecological impacts of toxic discharges was demonstrated in field studies (USEPA, 1984; Birge et al., 1989; Eagleson et al., 1990; USEPA, 20002).

A noodle producing company, located at Choba, Port Harcourt, Rivers State, Nigeria, discharges substantial amounts of effluents into nearby New Calabar River water body at Choba. A chemical environmental study have shown that these effluents contain residual chemicals from production processes, which they surmise might lead to pollution of aquatic environments (Chindah et al., 2011). The impact of such pollution is profound, as it can cause biochemical, physiological, and structural changes in aquatic organisms. These changes may impair growth, reproduction, and survival, ultimately leading to population declines and loss of biodiversity (Mustafa et al., 2024).

Noodles are a type of food made from unleavened dough which is either rolled flat and cut, stretched, or extruded, into long strips or strings. Noodles are a staple food in many cultures and made into a variety of shapes. The most common noodles are those derived from either Chinese cuisine or Italian cuisine. Effluents from food processing industries, like the noodle companies, are typically discharged into nearby water bodies, often with insufficient treatment. This can result in significant contamination, affecting both the quality of the water and the health of aquatic organisms (Woke et al., 2013). The effluents may contain high levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, and other pollutants that can deplete dissolved oxygen in the water, leading to hypoxic conditions that are detrimental to aquatic life (Bashir et al., 2020).

Effluent discharge from the the noodle producing company at Choba has been reported to have caused noticeable changes in the physicochemical properties of the New Calabar River, including alterations in pH, temperature, turbidity, and nutrient level (Nwankwoala & Angaya, 2017). These changes can have cascading effects on the river's ecosystem, impacting the diversity and abundance of aquatic organisms. Fish species, such as *Clarias gariepinus*, which are common in the river, are particularly vulnerable to these pollutants due to their habitat and feeding habits (Ajima et al., 2015).

Understanding effluent toxicity and its environmental impact is essential for developing effective management strategies to mitigate pollution and protect aquatic life. Regulations and monitoring programs must be enforced to ensure that industrial discharges meet acceptable environmental standards, thereby safeguarding aquatic ecosystems and their biodiversity (Cao et al., 2017).

Histopathological changes in fish are key indicators of toxicant exposure and serve as critical biomarkers in aquatic toxicology. These changes are the result of complex mechanisms triggered by the interaction between toxicants and cellular structures, leading to various forms of cellular and tissue damage. Understanding these mechanisms is essential for interpreting the histopathological effects observed in toxicological studies and assessing the potential risks posed by environmental pollutants. The change are as follows (Allison et al., 2023):

1. Oxidative Stress and Cellular Damage: It involves the generation of reactive oxygen species (ROS) within cells. These ROS radicals can cause significant damage to cellular components such as lipids, proteins, and DNA. Lipid peroxidation, a common outcome of oxidative stress, leads to the disruption of cell membranes, resulting in cellular necrosis and apoptosis (Tang et al., 2019).
2. Inflammatory Responses**:** Toxicant-induced oxidative stress and direct chemical irritation can also trigger inflammatory responses in fish tissues. In response to toxicant exposure, immune cells such as macrophages are recruited to the site of damage, releasing cytokines and other signaling molecules that exacerbate tissue injury.
3. Metabolic Disruption: Cellular role in metabolizing toxicants can lead to the accumulation of intermediate metabolites that are themselves toxic, further exacerbating cellular damage. For example, the biotransformation of certain organic pollutants through cytochrome P450 enzymes can produce reactive metabolites that bind to cellular macromolecules, leading to protein denaturation and enzyme inhibition (Guengerich, 2003) .
4. Genotoxicity and Carcinogenesis: Toxicants can also induce histopathological changes through genotoxic mechanisms, where damage to DNA leads to mutations, chromosomal aberrations, and altered gene expression. Persistent genotoxic stress can result in the formation of pre-neoplastic lesions and, eventually, tumorigenesis in liver tissues(Karin & Dhar, 2016).
5. Mitochondrial Dysfunction: Mitochondria, the energy-producing organelles in cells, are also key targets of toxicant-induced damage. Toxicants such as heavy metals can disrupt mitochondrial function by inhibiting key enzymes involved in the electron transport chain, leading to a decrease in adenosine triphosphate (ATP) production and an increase in ROS generation. Mitochondrial dysfunction can result in energy depletion, triggering apoptosis or necrosis depending on the severity of the damage (Chan et al., 2013).
6. Cell Death Pathways: The ultimate consequence of severe toxicant exposure is cell death, which can occur via apoptosis (programmed cell death) or necrosis (uncontrolled cell death). Apoptosis is a regulated process that involves cell shrinkage, chromatin condensation, and DNA fragmentation, often as a response to irreparable damage. Necrosis, on the other hand, is typically associated with acute toxicant exposure and is characterized by cell swelling, membrane rupture, and the release of cellular contents that can trigger inflammation (Doonan & Cotter, 2008).

Histological studies are a fundamental aspect of toxicology, providing crucial insights into the cellular and tissue-level effects of toxic substances on organisms. By examining tissue samples under a microscope, researchers can identify specific morphological changes that occur as a result of exposure to various contaminants. These changes can include cellular degeneration, necrosis, inflammation, and hyperplasia, among others (Liao et al., 2010; Allison and Paul, Allison, 2014: Allison and Ogoun, 2024). The liver is often the focal point of histological studies in toxicology due to its primary role in detoxification and metabolism. As the organ responsible for processing and neutralizing harmful substances, the liver is particularly susceptible to damage from toxicants. Histopathological analysis of liver tissue can reveal early signs of toxicity, such as hepatocellular vacuolation, bile duct proliferation, and fibrosis, which may precede more overt clinical symptoms (Cullen, 2005; Allison and Ogoun, 2024).

Histological methods provide a detailed view of the impact of toxicants at the cellular level, which is essential for understanding the mechanisms of toxicity. These studies are particularly valuable in assessing chronic toxicity, where long-term exposure to low levels of pollutants may cause subtle yet significant changes in tissue structure and function (Lehman-McKeeman,

2013). Chronic toxicity studies often involve the use of biomarkers, which are specific histological or biochemical changes that can be linked to exposure to particular toxicants (Nikinmaa, 2014).

In addition to their diagnostic value, histological studies are also used to evaluate the efficacy of remediation efforts and regulatory policies aimed at reducing environmental pollution. By comparing histological data from organisms in contaminated and reference sites, researchers can assess the success of interventions designed to mitigate the effects of toxic substances (Stentiford et al., 2003).

The liver of fish is particularly vulnerable to contaminants due to its central role in detoxification processes. Histological changes in the liver can serve as early indicators of environmental stress, making it a critical organ for toxicity studies. By examining the histological changes in the liver of *Clarias gariepinus*, researchers can assess the chronic effects of effluent exposure and gain insights into the potential long-term impacts on fish health and aquatic ecosystems (Marchand et al., 2009)

Materials and Method

Source of Effluent

The source of effluent is from noodles producing company effluent discharge point at the Choba axis of the New Calabar River (NCR) in Obio-Akpo Local Government Area (LGA) of Rivers State, Nigeria. The New Calabar River is a major water body in Port Harcourt. This river is a critical resource for the local community, providing water for domestic use, fishing, and agriculture. The contamination of the New Calabar River with industrial effluents would poses a severe threat to the health and livelihoods of the people who depend on it.

Test site

Whole effluent chronic toxicity study was done in the Histochemistry Laboratory of the Department of Anatomy, University of Port Harcourt. China, Rivers State, Nigeria.

Study Specie

The African catfish, *Clarias gariepinus*, is widely recognized as a significant species for ecotoxicological studies due to its unique biological and ecological characteristics. Native to Africa, *C. gariepinus* is a resilient and adaptable species, capable of thriving in various aquatic environments, including those subject to anthropogenic pollution Marchand et al., 2009; Allison and Paul, 2014).

These species are vital component of both commercial and subsistence fisheries across Africa, contributing significantly to food security and local economies (Weyl et al., 2016). Understanding the impact of pollutants on *C. gariepinus* is therefore crucial not only for environmental protection but also for safeguarding the livelihoods of communities that rely on this species.

Clarias gariepinus is also an excellent bioindicator species. Due to its benthic nature and feeding habits, *C. gariepinus* is directly exposed to pollutants present in sediments and water, making it a sensitive indicator of environmental quality (Marchand et al., 2009). Changes in the health and behaviour of *C. gariepinus* can provide early warnings of ecological disturbances, helping to identify and address pollution sources before they cause widespread damage (Quintaneiro et al., 2006). Moreover, *C. gariepinus* has been extensively studied in various toxicological research, providing a wealth of baseline data that can be used for comparative analyses. The availability of such data enhances the reliability and relevance of new studies, facilitating a deeper understanding of pollutant effects and contributing to the

development of more effective environmental regulations and management strategies (EEA, 2018)

Study Design

Study Guideline: United State Environmental Protection Agency (USEPA) "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater organisms" was used for the laboratory study (USEPA, 2002). This guideline describes chronic toxicity tests for use in the National Pollutant Discharge Elimination System(NPDES) Permits Program to identify effluents and receiving waters containing toxic materials in chronically toxic concentrations.This Standard allows for the use of a semi-static method. The endpoint response was a measure of the morphological changes of test fish exposed to a test substance for a period of 30 days. The standard permits to adapt this method for use in freshwater with appropriate modifications in test conditions (temperature, food, fish marking technique).

Study Duration: The study is a 30 days duration whole effluent chronic testing of the effects of noodles processing company effluent on the histology of liver of *Clarias gariepinus*. This approach allows for a controlled investigation of the toxicological impact of varying concentrations of effluent on the test organisms over a the specified period.

Key Outcome: The key outcome measures in this study were the survival rate of the fish and the histopathological changes observed in the liver tissues. The survival rate was monitored throughout the exposure period to assess the acute and sublethal effects of the effluent at different concentrations. Liver histology was examined at the end of the exposure period to identify any structural alterations indicative of toxic effects, such as necrosis, inflammation, and other pathological changes.

Sample Collection

Effluent Sample Collection: The industrial effluent used in this study was collected directly from the discharge point of the industry, ensuring that it represented the actual waste being released into the surrounding environment. Collection was done using clean, non-reactive containers to prevent contamination. The samples were then transported to the laboratory under controlled conditions, where they were stored at low temperatures to preserve their chemical integrity until further analysis and use in the experimental setup.

Fish Sample Collection: The fish used in this study were *Clarias gariepinus* fingerlings, obtained from African Regional Aquaculture Centre (ARAC). ARAC is a reputable government owned fish hatchery, located in Aluu, Port Harcourt, Rivers State. Early life stage (fingerlings) were used for this study. They had an average weight of 0.75g, making them suitable for the toxicological experiment. Upon collection, the fingerlings were acclimatized in clean, aerated water for a period of two weeks before being introduced to the experimental conditions. This acclimatization period allowed the fish to stabilize physiologically, reducing the impact of transport and handling stress on the study's outcomes. The fingerlings were then randomly assigned to the different experimental groups, each exposed to varying concentrations of the effluent as described in the research design.

Experimental Design

Experiment Type: Static renewal tests - The test organisms are exposed to a fresh solution of the same concentration of sample every 24 hrs or other prescribed interval, either by transferring the test organisms from one test chamber to another, or by replacing all or a portion of solution in the test chambers.

Test Solution : The test solution is fresh water, which was used to simulated fresh surface water.

Stock Solution: The experiment was structured to include multiple treatment groups, each exposed to different concentrations of the industrial effluent, specifically 6.25%, 12.5%, 25%, 50%, and 100%. A control group, which was not exposed to any effluent, was also maintained to provide a baseline for comparison (USEPA, 2002). The effluent was diluted using clean underground water, which served as the dilution medium to create the different exposure concentrations. This design enables the determination of dose-response relationships and the identification of the concentration levels at which significant toxic effects manifest.q

Test Chamber: Twenty (20) liters of chemically inert vessels (plastic Tanks) were used for this study. Each tank was stocked with 5 fish, a stock capacity that was enough to allow for proper growth and maintenance of dissolved oxygen concentration. This is in compliance with the guideline loading rate criteria (OECD, 2013). The test chambers was randomly positioned in the test area and shielded from unwanted disturbance. The test was carried out without adjustment of pH. Nevertheless, where there is evidence of marked changed in the pH of the Tank water after addition of the test substance, the test would be repeated, adjusting the pH of the stock solution to the tank water before addition of the test substance. The PH adjustment will be made (preferably with HC1 or Na0H) in such a way that the stock solution concentration is not changed to any significant extent and that no chemical reaction or physical precipitation of the test substance is caused (ISO, 1994; USEPA, 2002; CEPA, 2004).

Test Conditions: Water temperature was maintained within standard limits ambient air temperature. The temperature of test solutions was measured by placing the thermometer directly into the test solutions. Temperature was recorded continuously in at least one test vessel for the duration of each test. Dissolved oxygen (DO) concentration and pH was checked at the beginning of each test and daily throughout the test period (USEPA, 2002).Light quality was set at laboratory illumination. Photo ambient period was set at a minimum of ration of 12 hours light to 12hours dark, with a light intensity maintained at 10 to 20 µE/m2/s. Feeding was at least once daily, the quantity of food being kept constant and related to the initial fish weight, at least 2% body weight (ISO, 1994; CEPA, 2004).

Validity of Test: For the conditions of validity, ISO (1994) and USEPA (2002) conditions for the validity of test were adopted for this study:

- The mortality in the controls should not exceed 10% at the end of test.
- The dissolved oxygen concentration should be at least 60% of the air saturation value throughout the test
- In semi-static procedures, aeration can be used, provided it does not lead to a significant loss of test substance
- There should be evidence that the concentration of the substance being tested has been satisfactorily maintained (it should be at least 80% of the nominal concentration) over the test period. The results should be based on measured concentration if the deviation from the nominal concentration is greater than 20%

Chemical Analysis of Effluent

The physicochemical analysis of the industrial effluent focused on determining the concentration of specific heavy metals known to pose significant environmental and health risks. The parameters analyzed included Nickel (Ni), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Copper (Cu). Additionally, the effluent was tested for the presence of Polycyclic Aromatic Hydrocarbons (PAHs), a group of organic pollutants often associated with industrial activities. Heavy metals and PAHs were selected for analysis due to their potential to cause toxic effects in aquatic organisms, including fish.

Histological Evaluation:

At the end of the experiment, 5 fish from each tank was sacrificed and the target organ of gills were excised and preserved in 10% buffered formalin solution and sent to the laboratory for tissue preparation. Tissue preparation was done in accordance to Drury and Wallington (1980) and (Allison and Paul, 2014). A Light microscopy (Olympus BH2) was used to identify and interpret (histological description) tissue slides and micrograph specimens at 40X, X100 and X400 magnification. The percentage prevalence of tissue histopathology was noted.

Result

Determine the Chemical components of the Effluent used for the study.

Table 1: Chemical quality of Effluent.

S/N	Parameter(s) Mg/l	Concentration	National standard (NESREA)	International standard (USEPA)	Remarks
1	PAH	<0.001	0.10	0.03	Below the standard, hence it is safe.
2	COPPER, Cu	0.079	1.2	1.3	Below the standard, hence it is safe.
3	LEAD, Pb	<0.001	0.1	0.015	Below the standard, hence it is safe.
4	CADMIUM, Cd	**0.037**	0.003	0.015	Above the standard, hence it is toxic.
5	CHROMIUM, Cr	<0.001	0.1	0.1	Below the standard, hence it is safe.
6	NICKEL, Ni	<0.001	0.5	0.1	Below the standard, hence it is safe.

Determine the effect of effluent on the histology of the liver of Clarias gariepinus.

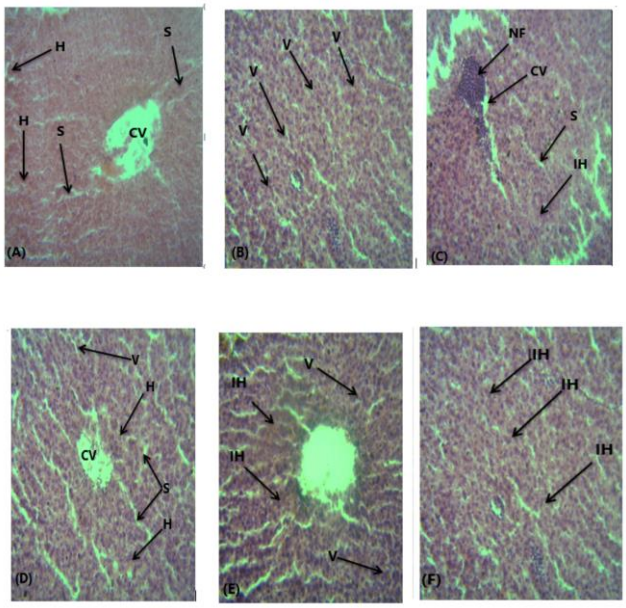

Plate 1; Photomicrographs (H&E:400X) showing: A.) – Control Liver showing normal architecture central vein,intercellular heamorrhage, with normal sinusoid spacing B.) **Tank 1**

(6.25% effluent): –showing Diffuse Macrosteatosis with structural alteration; C.) **Tank 2 (12.5% effluent)**: – Showing Necrotic Foci (NF), Diffuse intercellular heamorrhage (IH), Central vein (CN) and sinusoid space; D.) **Tank 3 (25% effluent)**: – Showing diffuse cellular vacuolation (V), intercellular heamorrhage (IH), Sinusoid space (S) and central vein; E.) **Tank 4 (50% effluent)**: - Showing Showing diffuse cellular vacuolation (V), intercellular heamorrhage (IH) and central vein; F.) **Tank 5 (100% effluent)**: - Showing diffuse intercellular heamorrhage (IH).

Table 2: Percentage prevalence of Liver histopathology of fishes exposed to effluent in different tanks.

ALTERATIONS	**PREVAILANCE**					
	Tank 1 (n-5)	**Tank2 (n-5)**	**Tank 3 (n-5)**	**Tank 4 (n-5)**	**Tank 5 (n-5)**	**Control (n-5)**
Circulatory Disturbance (CD)						
Intercellular haemorrhage	0	0	0	1	0	0
Interstitial Oedema	20	20	20	20	20	20
Progressive Change (PC)						
Hyperplasia	0	0	0	20	20	0
Regressive Change (RC)						
Architectural &Structural alterations	20	20	20	20	20	0
Necrosis	0	0	20	20	40	0
Melano-macrophage centres (MMC)	0	0	20	0	20	20
Average % Prevalence	6.33	6.33	13.33	13.33	20	6.67

Discussion

The chemical analysis result of this study showed that the effluent had a high level of cadmium (Cd) present in the effluent. Cd is a naturally occurring element in the Earth's crust, typically found in combination with other elements such as oxygen, chlorine, or sulfur. It is a transition metal and is known for its toxic properties to both humans and the environment. Cd is non-essential biologically, meaning it has no beneficial role in living organisms and is toxic even at low concentrations. Generally, cadmium binds strongly to organic matter where it will be immobile in soil and be taken up by plant life, eventually, entering the food supply. Soluble forms migrate in water. Insoluble forms of Cd are immobile and will deposit and absorb to sediments (ATSDR, 2012b). Most of the Cd that enters the body goes to the kidney and liver and can remain there for many years. A small portion of the cadmium that enters the body is excreted slowly in urine and feces. The body can change most Cd to a form that is not harmful, but too much Cd can overload the ability of the liver and kidney to change the Cd to a harmless form, which might result in kidney and liver diseases respectively.

Histopathological evaluation showed that all concentration of the whole effluent caused multiple liver lesions. The ommonly observed lesions were structural alterations, diffuse macrosteatosis, necrotic foci, diffuse intercellular heamorrhage, diffuse cellular vacuolation and intercellular heamorrhage. Cd is defined by USEPA as potentially hazardous to most forms

of life, and is considered to be toxic and relatively accessible to aquatic organism (DWAF, 1996). In one study that uses comparative acute toxicity testing of 63 heavy metals, cadmium was the most toxic (Borgmann et al., 1998). In another study that uses comparative acute toxicity testing of 63 heavy metals, Cd was the most toxic (Borgmann et al., 1998). The histological alteration in this study is consistent with lesions observed in *O.mossabicus* liver (structural alterations; congestion of blood vessels and sinusoids; granular degeneration of hepatocytes; fat accumulation; intercellular deposit; necrosis; hypertrophy of hepatocytes; mild inflammation) (Ackermann, 2008).

CONCLUSION

This study has been able to show that noodle processing industrial effluent discharge into the Choba axis of the New Calabar River contains hazardous chemical, Cadmium. The histological study has been able to prove that the effluent at all experimented concentration are causing deleterious health effect on aquatic organism. Hence, the noodle processing company industrial effluent can be scientifically surmised to be polluting the New Calabar River.

References

Ackermann, C. (2008). A quantitative and qualitative histological assessment of selected organs of *Oreochromis mossambicus* after acute exposure to cadmium, chromium and nickel. MSc dissertation. University of Johannesburg: Johannesburg.

Ahmed, T., Noman, M., Ijaz, M., Ali, S., Rizwan, M., Ijaz, S., Hameed, A., Ahmad, S., Wang, Y., Sun, G., & Li, B. (2021). Current trends and future prospective in nanoremediation of heavy metals contaminated soils: A way forward towards sustainable agriculture. *Ecotoxicology and Environmental Safety, Volume 227,112888.*

Ajima, M. N. O., Ogo, A. O., Audu, B. S., & Ugwoegbu, K. C. (2015) Chronic diclofenac (DCF) exposure alters both enzymatic and haematological profile of African catfish, Clarias gariepinus. Drug Chem. Toxicol. 38(4), 383-390.

Allison, T. A., & Paul, C. W. (2014). Histological based biomonitoring: a baseline ecotoxicological evaluation of New-Calabar River using Chrysichthys nigrodigitatus. *Int J Environ Poll Res*, *2*(3), 17-41.

Allison, T. A., & Ogoun, T. R., (2024). Histological Based Environmental Monitoring of the Lower Nun River: Using the Histo-morphometry of Kidney, Gills, Liver, and Testes of Coptodon zillii. Journal of Advances *in Medical and Pharmaceutical Sciences Volume 26, Issue 10,* Page 20-39, 2024; Article no.JAMPS.117884 ISSN: 2394-1111

Allison, T. A., Isirima, J. C., Osunwoke, E. A., & Bob-manuel, I. F. (2023). Allison's Toxicology Histopathology: Volume 1- Lesion Pathomechanism and Diagnosis. Igiri press and libraries (IPL). ISBN 9789781022951

ATSDR (Agency for Toxic Substances and Disease Registry), (2012a). Public Health Statement on Chromium. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry

Bashir, I., F. A., Lone, F. A., & Bhat, R. A. (2020). Concerns and Threats of Contamination on Aquatic Ecosystems. In book: Bioremediation and Biotechnology, Sustainable Approaches to Pollution Degradation (pp.1-26). DOI:10.1007/978-3-030-35691-0_1

Birge, W. J., Blac, J. A., Short, T. M., & Wester- man, A. G. (1989). A Comparative Ecological and Toxicological Inves- tigation of a Secondary Wastewater Treatment Plant Efflux- ent and Its Receiving Stream. *Environmental Toxicology and Chemistry, Vol. 8, No. 5, 1989*, pp. 437- 450.

Borgmann, U., Cheam, V., Norwood, W. P., & Lechner, J. (1998). Toxicity and bioaccumulation of thallium in Hyalella azteca, with comparison to other metals and prediction of environmental impact. *Environmental Pollution, Volume 99, Issue 1,* Pages 105-114.

Cao, P., Lu, C., & Yu, Z. (2017). Agricultural nitrogen fertilizer uses in the continental U.S. during 1850-2015: a set of gridded time-series data [dataset]. PANGAEA, https://doi.org/10.1594/PANGAEA.883585,

CEPA (California Environmental Protection Agency), (2004). Overview of Freshwater and Marine Toxicity Tests: A Technical Tool for Ecological Risk Assessment. Ecotoxicology Unit Reproductive and Cancer Hazard Assessment Section Office of Environmental Health Hazard Assessment California Environmental Protection Agency.

Chan, F. K. M., Moriwaki, K., & De Rosa, M. J. (2013). Detection of necrosis by release of lactate dehydrogenase activity. *Immune Homeost. Methods Protoc. 979*, 65–70.

Chang, X., Kobayashi, T., Senthilkumaran, B., Kobayashi-Kajura, H., Sudhakumari, C. C., & Nagahama, Y. (2005). Two types of aromatase with different encoding genes, tissue distribution and developmental expression in Nile Tilapia (*Oreochromis mossambicus*). *Gen. Comp. Endocrinol., 141*: 101-115.

Chindah, A. C., Braids, S. A., & Obunwo, C. (2011). Water Quality of Streams Receiving Municipal Waste Water in Port Harcourt, Niger Delta, Nigeria. In book: Waste Water - Evaluation and Management. DOI:10.5772/16186

Cullen J. M. (2005). Mechanistic classification of liver injury. *Toxicol Pathol* ;33:6–8.

Doonan, F., & Cotter T. G. (2008). Morphological assessment of apoptosis. *Methods ;44(3)*:200-4.

DWAF (Department of Water Affairs and Forestry), (1996). South African Water Quality Guidelines Volume 7. Aquaticecosystem Pretoria: DWAF Ecotoxicology and Environmental Safety. 1(2): 151

Ebol, E. L., Donoso, C. H., Saura, R. B. D., Ferol, R. J. C., Mozar, J. R. D., Bermon, A. N., Manongas, J., Libot, J. C. H., Cara, J., Matabilas, C. J., Jumawan, J. C., & Capangpangan, R. Y. (2020). Heavy Metals Accumulation in Surface Waters, Bottom Sediments and Aquatic Organisms in Lake Mainit, *Philippines. Inter. Letters Nat. Sci. 79*: 40-49 Edokpayi, JN; Odiyo, JO; Popoola, OE; Msa

Eagleson K. W. et al. 1990. Comparison of measured instream biological responses with responses predicted using the Ceriodaphnia dubia chronic toxicity test. *Environ. Toxicol. Chem. 9*: 1019-1028.

EEA (European Environmental Agency) (2018). Air quality in Europe - 2018 Report. EN PDF: TH-AL-18-013-EN-N - ISBN: 978-92-9213-990-2

Drury and Wallington (1980).Drury, R.A. and Wallington, E.A., (1980). Carleton' s Histology Technique. 5th Edition, Oxford University Press, Newyork.

Guengerich, F. P. (2003). Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. *Arch Biochem Biophys: 409(1)*:59-71.

ISO (International Organization for Standardization) (1994). Methods of determining the long-term toxicity of substances to freshwater fish. ISO-10390: Geneve, Switzerland

Karin, M. & Dhar, D. (2016). Liver carcinogenesis: from naughty chemicals to soothing fat and the surprising role of NRF2, *Carcinogenesis, Volume 37, Issue 6*: Pages 541–546.

Lehman-McKeeman, L. D., (2013). Systems and computational biology as foundations for toxicology research. *Toxicologist 132*: 821

Liao, M., Zhang, H., Yu, S., Chen, C., Huang, C., Xu, J., & Huang, P. (2010). Effects of cadmium and mercury alone and in combination on the soil microbial community structural diversity. J. Xu, P. Huang (Eds.), Molecular Environmental Soil Science at the Interfaces in the Earth's Critical Zone, Springer, Berlin, Heidelberg, pp. 337-341

Marchand, M. J, van Dyk, J..C, Pieterse, G. M., Barnhoorn, E. J., & Bornman, M. S. . (2009). Histopathological alterations in the liver of the sharptooth catfish Clarias gariepinus from polluted aquatic systems in South Africa. *Environmental Toxicology 24*: 133–147.

McKim, J. M. (1977)..Evaluation of Tests with Early Life Stages of Fish for Predicting Long-Term Toxicity. *Journal of the Fisheries Research Board of Canada, 34*, 1148-1154.

McIntyre, J. K, Davis, K. W., Hinman, C., Macneale, K. H., Anulacion, N. F. Scholz, N. L., & Stark, J. D. (2015). Soil bioretention protects juvenile salmon and their prey from the toxic impacts of urban stormwater runoff. *Chemosphere, Volume 132:* Pages 213-219.

Mustafa, A.D., Juahir, H., Yunus, K., Amran, M. A., Hasnam, C. N. C., Azaman, F., Abidin, I. Z., Azmee, S. H. & Sulaiman, N.H. (2015). Oil spill related heavy metal: a review. Malaysian. *Journal of Analytical Sciences. 19(6)*: 1348 1360.

Nikinmaa, M. (2014). An introduction to aquatic toxicology. Elsevier Inc. Academic Press.

Nwankwoala H. O. & Angaya Y. B., (2017). An Evaluation of Heavy Metals Concentration in the Choba Section of the New Calabar River, Eastern Niger Delta. *Scientific Review, Academic Research Publishing Group, vol. 3(6):* pages 54-61.

OECD (The Organization for Economic Cooperation and Development), (2013). OECD Guidelines for the Testing of Chemicals: Fish, Early-life Stage Toxicity. Test. 210. Adopted: 26 July 2013

Quintaneiro, C, Monteiro, M, Pastorinho, R, Soares, A. M., Nogueira, A. J., Morgado, F., & Guilhermino, L. (2006). Environmental pollution and natural populations: a biomarkers case study from the Iberian Atlantic coast. *Pollut Bull. ;52(11)*:1406-13..

Stentiford, G. D., Evans, M., Bateman, K., & Feist, S. W. (2003). Co-infection by a yeast like organism in Hematodinium-infected European edible crabs (Cancerpagurus) and velvet swimming crabs (*Necorapuber*) from the English Channel. *Dis. Aquat. Org. 54*,195– 202.

Tang, D., Kang, R., Berghe, T. V., Vandenabeele, P., & Kroemer, G. (2019). The molecular machinery of regulated cell death. *Cell Res. 29*, 347–364.

USEPA, (United State Environmental Protection Agency), (1984). Effluent and Ambient Toxicity Testing and Instream Community Response on the Ottawa River, Lima, Ohio. Environmental Research Laboratory Agency, Duluth MN 55804

USEPA (US Environmental Protection Agency) (1999). A Risk Assessment Multiway Exposure Spreadsheet Calculation Tool. Washington DC: United States Environmental Protection Agency.

USEPA (United State Environmental Protection Agency), (2002). Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. EEPA-821-R-02-014.

Weyl, O. L. F., Daga, V. S., Ellender, B. R. & Virulent, J. R. S. (2016). A review of Clarias gariepinus invasions in Brazil and S outh Africa. *Journal of Fish Biology.* doi:10.1111/jfb.12958, available online at wileyonlinelibrary.com

Woke, G. N., Babatunde, B. B., & Wokoma A. (2013). Effects of Indomie Industrial effluent discharge on fish fauna of New Calabar River, Port Harcourt. *Glob. J. Environ. Sci. Vol. 12* 41-47