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

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A B S T R A C T

Industrial effluents discharged into aquatic environments pose significant ecological and biological threats, particularly to aquatic species like Clarias gariepinus. This study investigated the chronic toxicity of effluents from the noodles food industry on the histology of the male gonads 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 samples were analyzed for TC constituents, and their effects on fish were assessed under varying concentrations (6.25%, 12.5%, 25%, 50%, and 100%). Results revealed that most effluent parameters, such as copper, chromium, and nickel, were within NESREA and USEPA limits, while cadmium levels (0.037 mg/L) exceeded permissible thresholds, highlighting significant pollution concerns. Histological analysis of the male gonads showed progressive damage with increasing effluent concentration. Control groups displayed healthy testicular structures and active spermatogenesis, whereas effluent-treated groups exhibited testicular lobule atrophy, basement membrane degeneration, and severe necrosis at 100% concentration. The findings highlight the reproductive toxicity and ecological risks posed by cadmium-contaminated effluents, emphasizing the need for improved effluent treatment and stricter regulatory enforcement. This study underscores the critical need for sustainable industrial practices to protect aquatic ecosystems and ensure biodiversity conservation.

Keywords: Histology; Male Gonad, Effluent; Toxicology, Biomarker; Fish and Water Quality.

1. Introduction

Industrial effluents are liquid wastes generated from various industrial processes and are typically discharged into nearby water bodies, often without adequate treatment. These effluents contain a wide range of contaminants, including heavy metals, organic compounds, oils, suspended solids, and various chemicals used in industrial operations. The composition and concentration of pollutants in industrial effluents vary depending on the nature of the industry and its production processes. When improperly managed, these effluents contribute significantly to environmental pollution, posing a threat to aquatic ecosystems, public health, and biodiversity (Ekhaise & Anyasi, 2021).

The rapid industrialization of urban areas in developing countries has led to an increase in environmental pollution, particularly in water bodies. Rivers and streams, which often serve as natural recipients of industrial effluents, are increasingly subjected to the discharge of untreated or inadequately treated waste waters. The Choba River in Rivers State, Nigeria, is no exception, as it receives significant amounts of effluents from various industries, including the food industry.

One of the key industries contributing to this contamination is the popular noodles production company, which is located near the Choba River. The effluent discharged by this company contains various contaminants that can potentially harm aquatic life. This effluent, rich in organic and inorganic substances, can lead to the deterioration of water quality, thereby affecting the health of aquatic organisms, particularly fish species that inhabit these waters (Odoemelam et al., 2014).

Aquatic toxicology is a branch of environmental science that focuses on understanding the effects of chemical pollutants on aquatic organisms and ecosystems. It encompasses the study of how toxic substances affect the health, behavior, and survival of aquatic life, as well as the mechanisms through which these substances cause harm. The principles of aquatic toxicology are fundamental to assessing and managing the risks posed by pollutants in aquatic environments. Central to aquatic toxicology is the concept of toxicity, which refers to the degree to which a substance can cause harm to living organisms. Toxicity is often measured through dose-response relationships, which describe how the severity of the effect changes with varying concentrations of the toxicant. These relationships are typically represented by dose-response curves, which illustrate the range of doses that cause different levels of effect, from no observable effect to lethal outcomes (Gauthier & Vijayan., 2020).

Indicators of toxicity in aquatic systems are key to assessing the health of aquatic environments and understanding the impact of pollutants on aquatic organisms. These indicators encompass a range of bio-morphological, biochemical, and physiological responses that provide insights into the extent of toxic exposure and the potential harm being inflicted on aquatic species.

Bio-morphological indicators are often the first line of evidence in toxicity assessments, as they reflect the responses of organisms to toxicants. Behavioral changes are a common indicator, with exposed organisms displaying altered feeding patterns, swimming behavior, or social interactions. These behavioral changes can be the result of nervous system damage or stress induced by exposure to toxicants. For example, fish exposed to heavy metals may exhibit erratic swimming or reduced activity due to neurotoxic effects (Adams et al., 2019). Physiological changes, such as altered respiration rates, are also significant indicators of toxicity. They are often critical to acute responses of the exposed organism (Allison and Ogoun, 2023). For example, respiratory distress is a critical sign of waterborne pollution and often serves as a reliable marker of acute exposure. (Kramer et al., 2020). At the biochemical level, toxicity can manifest as oxidative stress, enzyme inhibition, and membrane damage. Oxidative stress, caused by the generation of reactive oxygen species (ROS), can lead to bio-morphological cellular damage and impaired metabolic processes. Aquatic organisms exposed to toxicants often display elevated levels of antioxidants or lipid peroxidation, which can be used as biochemical markers of stress (Barton & Iwama, 1991; Georgieva, 2021; Farag et al., 2023; Saha, 2022). Additionally, the inhibition of key enzymes involved in detoxification, such as catalase and glutathione peroxidase, is indicative of exposure to bio-chemical pollution.

Histological changes, such as tissue damage or abnormal cell growth, are also used to assess toxicity. These changes are often observed in critical organs like the liver, kidneys, or gonads of aquatic species. For instance, effluent exposure has been shown to cause liver necrosis, gill damage, and reproductive dysfunction in fish, which are signs of chronic toxicity (Rehman et al., 2018). Histopathological analysis allows for the detection of sub-lethal effects that may not be immediately apparent through behavioral or physiological observations.

The African catfish, Clarias gariepinus, is a prominent species in the Choba River and is widely used in toxicological studies due to its hardiness and high resistance to pollutants. However, prolonged exposure to sub-lethal concentrations of contaminants, such as those found in industrial effluents, can lead to chronic toxicity, which may not be immediately lethal but can cause significant physiological and histopathological alterations over time (Kori-Siakpere et al., 2009; Doherty et al. (2011).

The gonads of fish, which include the testes in males and ovaries in females, are essential organs responsible for reproduction. Histopathological studies on fish gonads provide insight into the effects of toxicants, particularly from environmental pollution, on reproductive health. Exposure to industrial effluents, agricultural runoff, and other contaminants can result in significant alterations in the structure and function of these organs, affecting reproductive success and, ultimately, population dynamics. One of the most critical effects of toxicants on fish gonads is impaired gametogenesis, the process by which sperm and eggs are produced. In male fish, this manifests as disrupted spermatogenesis, which includes the formation, maturation, and release of spermatozoa. Toxicant exposure can lead to a reduction in the number of spermatocytes (immature sperm cells) or their degeneration. For example, industrial effluents containing heavy metals or endocrine-disrupting chemicals (EDCs) can cause necrosis of spermatogenic cells, leading to reduced sperm count and motility (Jobling & Tyler, 2003; Marchand et al., 2010; Soffker & Tyler, 2012; Mínguez-Alarcón, 2012). This impairment can severely affect the reproductive potential of the fish. In addition to spermatogenesis, the structural integrity of the testicular tissue can also be compromised.

Histopathological analysis often reveals signs of testicular atrophy, which involves the shrinkage or degeneration of the testes. This is characterized by a reduction in the size and number of seminiferous tubules, which are the structures where spermatogenesis occurs. Toxicants such as pesticides, polycyclic aromatic hydrocarbons (PAHs), and industrial chemicals can cause the collapse of these tubules, preventing proper sperm development (Wu et al., 2025).

Histological studies, which involve the microscopic examination of tissues, are particularly useful in assessing the impact of pollutants on aquatic organisms. In this study, the male gonads of Clarias gariepinus were selected for histological examination to assess the chronic toxicity of the effluent discharged into the Choba River by the noodles food company. The male gonads are sensitive indicators of reproductive health and can reveal sub-lethal effects of contaminants that may not be detected through other means, such as behavioral or mortality studies (Agbabiaka et al., 2020).

Studies have identified general histopathological effects of industrial effluents on fish, there is limited research detailing the specific histopathological changes in the male gonads of Clarias gariepinus exposed to varying concentrations of effluents. Detailed histological studies are needed to elucidate the specific types of cellular damage and reproductive impairment caused by these effluents. This knowledge is essential for understanding the long-term consequences of effluent exposure on fish populations and their reproductive health (Akaninwor et al., 2007).

This study was conducted to provide insight into the potential long-term effects of industrial effluents on the reproductive health of fish, with a focus on understanding how chronic exposure to these pollutants affects the histological structure of the male gonads of Clarias gariepinus. This research is crucial in developing strategies for monitoring and mitigating the impact of industrial activities on aquatic ecosystems, ensuring the sustainability of fish populations, and maintaining the ecological balance of rivers like the Choba.

2. Materials and Method

2.1 Study Type

The study type is a short-term chronic whole effluent toxicity laboratory testing in receiving waters of freshwater organism for a duration of 30 days (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 the following:

1. ***Static renewal*** - The test organisms are exposed to a fresh solution of the same concentration of sample every 24 h 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.
2. ***Key Outcome:*** The key outcome measured in this study were the histopathological changes observed in the gonads of C. gariepinus.
3. ***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%

2.2. Study Design

This study employed an experimental design involving the exposure of Clarias gariepinus to different concentrations of industrial effluents. Fish were divided into experimental groups subjected to varying effluent concentrations (6.25%, 12.5%, 25%, 50%, and 100%) and a control group maintained in clean water (USEPA, 2002). The experiment was carried out for a 30-day duration, ensuring consistent environmental conditions such as temperature, pH, and aeration.

2.3. Sampling

Effluent Sampling: 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.

2.4. Evaluation

Chemical Analysis: To understand the chemical composition of the effluents used in this study, samples were analyzed for target parameters: Cd, Cr, Cu, Pb, Ni and PAH. These parameters were compared against the standards set by NESREA (National Environmental Standards and Regulations Enforcement Agency) and USEPA (United States Environmental Protection Agency) effluent guidelines to assess compliance.

Histological Assessment: The histological examination of the male gonads was conducted to assess structural changes caused by effluent exposure. It involved tissue processing of the male gonads and qualitative analysis of prepared tissue slides (Drury and Wallington, 1980; Allison and Paul, 2014). This was done at the Environmental Histology laboratory of the Department of Anatomy, school of Basic Medical Science, University of Port Harcourt. The percentage prevalence of observed histological abnormalities was calculated for each group, providing a measure of effluent toxicity

3. Result

3.1. Effluent Quality

Table 1 Chemical constituents of effluent used for the study

S/N	**Parameter(s) Mg/l**	**Concentration (mg/l)**	**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.

The table provides a comparative analysis of key chemical parameters in the effluent, measured against the NESREA and USEPA standards:

- PAH (<0.001%): Well below the NESREA (0.10) and USEPA (0.30) standards, indicating safety.
- Copper (0.079 mg/L): Within permissible limits of both NESREA (1.2 mg/L) and USEPA (1.3 mg/L), making it safe.
- Lead (<0.001 mg/L): Safe under NESREA (0.1 mg/L) but exceeding the stricter USEPA standard (0.015 mg/L).
- Cadmium (0.037 mg/L): Exceeds both NESREA (0.003 mg/L) and USEPA (0.015 mg/L) thresholds, indicating toxicity.
- Chromium and Nickel (<0.001 mg/L each): Well within the limits for both NESREA (0.1 mg/L for Chromium, 0.5 mg/L for Nickel) and USEPA (0.1 mg/L for both), making them safe.

The results show cadmium levels as the primary concern for aquatic toxicity, necessitating stricter effluent management strategies.

3.2. Histological Assessment

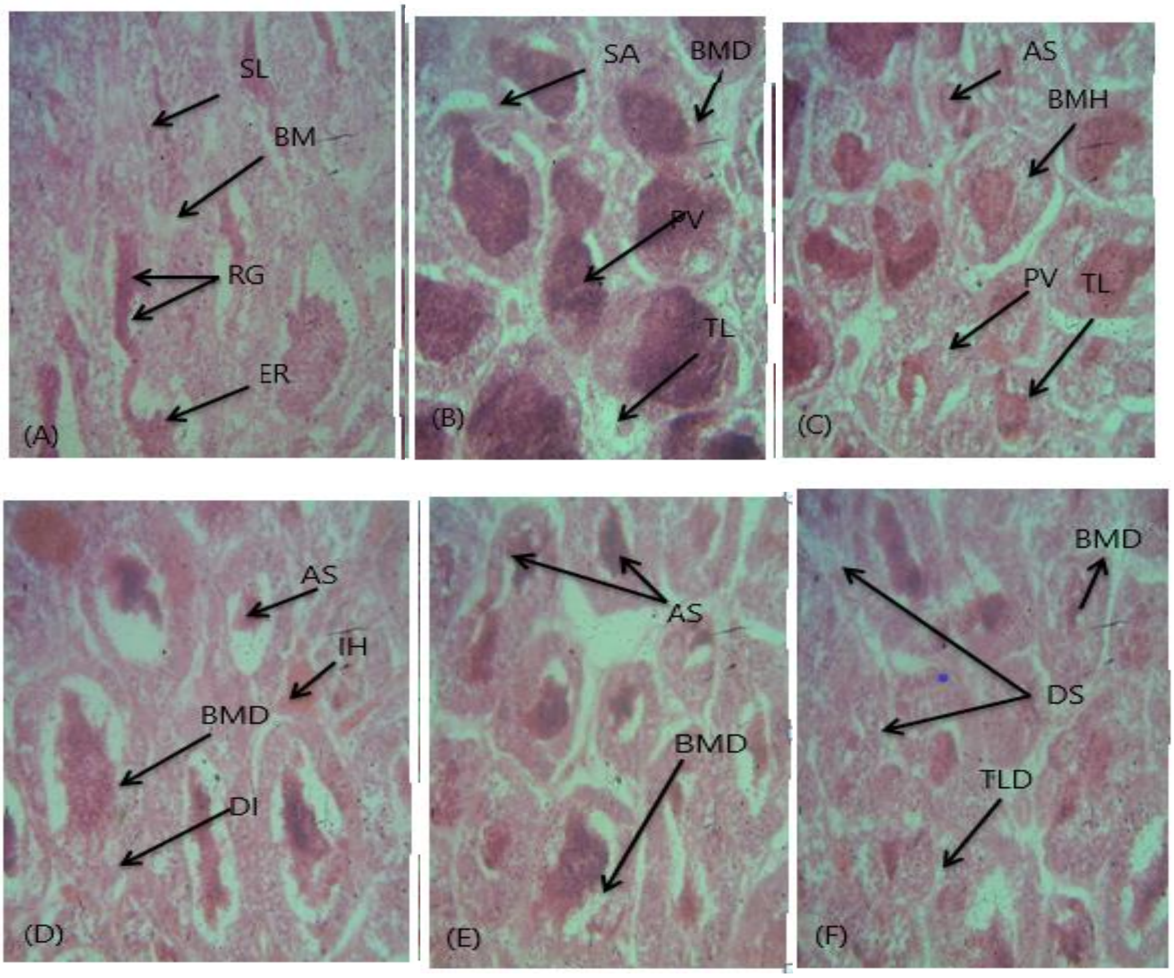

Figure 4. 2: Photomicrographs of the Testes

Photomicrographs (H&E:400X) showing: A.) – Control Testes – Seminiferous Lobules (SL) with Seminiferous tubules (ST) in different stages of spermatogenesis (RG-Regressive Gonad, ER – Early Recrudesce, TR - Terminal Recrudesce); B.)Tank 1 (6.25% effluent) – Showing previtelogenic stages (PV) and testicular lobule atrophy (TLA) C.)Tank 2 (12.5% effluent) – Showing previtelogenic stages (PV), testicular lobule atrophy (TLA) Atrophy of spermatozoa (AS), basement membrane degeneration (BMD)..D.)Tank 3 (25% effluent) – Showing spermatogenic inhibition, lobular structural alteration (SA), basement membrane degeneration (BMD), interstitial haemorrhage (IH). diffuse basement detachment (DMD).diffuse basement detachment (DMD).E.)Tank 4 (50% effluent) – Showing basement membrane degeneration (BMD), and Atrophy of spermatozoa (AS).E.)Tank5 (100% effluent)– Showing generalised degeneration of basement membrane (BMD),spermatozoa (DS) and testicular lobule (TLD).

Photomicrographs highlight progressive testicular damage with increasing effluent concentration:

- Control Group: Healthy seminiferous tubules in various spermatogenesis stages.
- 6.25% Effluent: Minor atrophy.
- 12.5% to 50% Effluent: Degenerative changes, including basement membrane degeneration (BMD), testicular lobule atrophy, and atrophy of spermatozoa.
- 100% Effluent: Severe degeneration across all testicular structures, signifying profound reproductive toxicity.

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

ALTERATIONS	**% PREVALENCE**					
	tank 1 (n=5)	**tank 2 (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**

Histopathological changes align with effluent concentration:

Interstitial Oedema: Present across all tanks (20% prevalence), including control.

- Architectural Alterations and Necrosis: Increase significantly at higher concentrations (e.g., 40% necrosis in 100% effluent).
- Melano-Macrophage Centers (MMC): Highlight the immune response to toxicity, with higher prevalence at 25% and 100% effluent concentrations.

4. Discussion

The results of this study provide critical insights into the chronic toxicity of industrial effluents from the noodles food industry on the male gonads of Clarias gariepinus. The findings demonstrate significant histological impacts, correlating with effluent concentration. The effluent chemical profile revealed that while parameters like PAH, Cu, Cr, and Ni were within NESREA and USEPA permissible limits for effluent, Cd concentrations exceeded both standards. This is concerning as Cd is a potent bioaccumulative toxicant that disrupts cellular functions and has been linked to reproductive toxicity in aquatic organisms (Emmanuel & Aluko, 2021). The elevated Cd levels indicate a high potential for long-term environmental and biological harm, highlighting the need for stricter effluent treatment protocols.

Histological observations showed a progressive testicular damage with increasing effluent concentration. The control group exhibited normal seminiferous tubules with active spermatogenesis, while the 6.25% effluent group showed minor atrophy. At higher concentrations, structural integrity was severely compromised, including testicular lobule atrophy, basement membrane degeneration, and spermatozoa depletion. By 100% effluent concentration, diffuse necrosis and severe degeneration were apparent. These findings align with reports that heavy metals like cadmium disrupt endocrine functions and spermatogenesis, leading to reduced fertility and reproductive failure (Jobling & Tyler, 2003; Marchand, 2010 Hassan et al. 2015). The prevalence of melano-macrophage centers (MMCs) at higher concentrations further indicates a heightened immune response to toxic stress. The significant histopathological damage observed highlights the inadequacy of current effluent management practices by the noodles producing company. While some parameters meet NESREA standards, the exceedance of Cd levels underscores the need for stricter regulatory enforcement. The USEPA's more stringent Cd threshold provides a better benchmark for mitigating ecological risks.The adverse effects observed in Clarias gariepinus raise broader ecological concerns, as this species is a vital component of aquatic food webs. Disruption to its reproductive health could cascade through the ecosystem, affecting predator-prey dynamics and fish populations. Furthermore, the contamination of water bodies poses risks to communities relying on these resources for drinking water and fishing

5. Conclusion

The study demonstrates that industrial effluents, particularly cadmium contamination, pose severe risks to the reproductive health and survival of Clarias gariepinus. Histological changes emphasized the urgent need for improved effluent treatment and compliance with stricter environmental standards. These findings underscore the critical role of ongoing monitoring and regulatory enforcement to safeguard aquatic ecosystems and public health.

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