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

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ABSTRACT

Background: 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 a noodle food industry, focusing on the environment water quality and its histological impact on the kidney of *Clarias gariepinus*. **Materials and Method:** 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 kidney were assessed under varying concentrations (6.25%, 12.5%, 25%, 50%, and 100%). **Results**: 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 revealed significant kidney damage, including tubular necrosis, glomerular damage, and inflammation.

Conclusion: The severity of kidney damage was directly linked to the concentration of effluent exposure. These findings highlight the need for stricter regulations to protect aquatic ecosystems from the harmful effects of industrial pollution.

Keywords: Histology; Kidney; Effluent; Toxicology; Biomarker; Fish and Water Quality.

INTRODUCTION

Industrial wastewater, if not properly treated, can have severe consequences for aquatic ecosystems. The release of effluents containing high concentrations of organic matter, suspended solids, heavy metals, and other pollutants can lead to a decline in water quality, disruption of aquatic life, and potential human health concerns (Venugopal and Sasidharan, 2021). In the context of the seafood industry, effluents can be particularly problematic due to their high biological oxygen demand (BOD) and chemical oxygen demand (COD) levels, as well as the presence of various compounds such as proteins, oils, and carotenoids (Venugopal and Sasidharan, 2021)

A noodle producing company located at Choba, is a leading manufacturer of instant noodles in Nigeria, producing a wide range of product for domestic and export markets. The company's production facility is located in Choba, a suburb of Port Harcourt, Rivers State Nigeria. The company discharges its effluent directly into nearby water bodies, the Choba axis of New Calabar River (Allison and Paul 2014). Without proper treatment, the discharge of this effluent can have significant consequences on the local aquatic environment, potentially impacting the health and biodiversity of the receiving water bodies (Alimba and Faggio, 2019)

Whole effluent toxicity (WET) testing has become an increasingly important tool for evaluating the environmental impact of industrial wastewater discharges (USEPA, 1984). This approach assesses the combined toxicity of all the constituents in an effluent sample, rather than relying on chemical-specific analyses (USEPA, 1984). In this study, we employed a static renewal whole effluent toxicity testing method using Clarias gariepinus specie to evaluate the toxicity of Choba Indomie's company effluent water. Whole effluent toxicity (WET) testing is a valuable tool for evaluating the overall toxicity of industrial effluents, as it takes into account the combined effects of all the constituents present (Venugopal and Sasidharan, 2021). This is particularly important for complex effluents, like those from the food industry, where the individual chemical components may not fully capture the potential ecological impacts. Traditional chemical-specific analyses can overlook synergistic or antagonistic interactions between the various contaminants in an effluent, leading to an incomplete understanding of its environmental impact (Rubio-Vargas et al., 2021). By using whole effluent toxicity chronic testing with Clarias gariepinus species, we can gain a more comprehensive understanding of the potential effects of the Choba Indomie effluent on the local aquatic ecosystem.

Effluent, the discharged water from industrial or domestic sources, can significantly impact aquatic ecosystems (Forstner, 1995). It often contains a complex mixture of pollutants, including heavy metals, organic compounds, nutrients, and suspended solids (Chapman, 1992). These contaminants can alter water quality parameters such as pH, dissolved oxygen, temperature, and turbidity, creating adverse conditions for aquatic organisms (Bartsch and Schiewer, 2002). The effects of effluent on aquatic life are multifaceted and can range from acute toxicity to chronic impairment. Direct exposure to toxic substances can result in mortality, reduced growth, reproductive failure, and developmental abnormalities (Sprague, 1970). For instance, heavy metals like lead and cadmium can bioaccumulate in aquatic organisms, leading to organ damage and impaired physiological functions (Eisler, 1987). Organic pollutants, such as pesticides and industrial chemicals, can disrupt endocrine systems, causing reproductive abnormalities and population declines (Colborn et al., 1996). In addition to direct toxicity, effluent can indirectly affect aquatic life by altering habitat quality. Nutrient enrichment from domestic and agricultural effluents can lead to eutrophication, resulting in algal blooms and oxygen depletion (Carpenter et al., 1998). Suspended solids can reduce light penetration, affecting primary production and aquatic plant growth (Lewis, 1996). Furthermore, effluent-induced changes in water chemistry can alter species composition and community structure, leading to biodiversity loss (Reynoldson et al., 1997).

The sensitivity of aquatic organisms to effluent varies widely depending on species, life stage, and exposure duration (Chapman, 1992). Fish, as top predators in aquatic food webs, are particularly vulnerable to the bioaccumulation of contaminants (Förlin, 1993). Consequently, they serve as important indicators of ecosystem health and can be used to assess the ecological risks associated with effluent discharges (Bartsch and Schiewer, 2002).

The kidney, as a vital organ responsible for filtration, excretion, and osmoregulation, is particularly susceptible to the toxic effects of environmental contaminants (Hinton et al., 1992). Its strategic role in maintaining homeostasis makes it an ideal target for biomonitoring studies (Klaassen and Amdur, 1991). The kidney, has emerged as a valuable tool for assessing the sublethal effects of environmental contaminants on aquatic organisms like fishes. Histopathological examination of the kidney has been widely employed to assess the impact of various pollutants on aquatic organisms, including fish (Garcí-a-Saavedra et al., 2006). Kidney lesions can serve as early indicators of exposure to toxic substances (Sanders, 1993). Histopathological changes, such as necrosis, inflammation, and cellular degeneration, can provide valuable information on the nature and severity of toxic insult (Hinton et al., 1992). Furthermore, the kidney's ability to accumulate and retain certain contaminants can make it a useful tissue for bioaccumulation studies (Eisler, 1987).

Histological techniques are essential for examining tissue architecture and identifying cellular alterations induced by toxic exposures (Bancroft and Gamble, 2008). In the context of toxicity assessment, these techniques provide invaluable insights into the pathological effects of pollutants on target organs, such as the kidney (Hinton et al., 1992).

MATERIALS AND METHODS

Sampling

Effluent: The effluent used for this study was obtained from the industrial discharge point of a prominent noodle producing company, at the Choba axis of New Calabar River. The choice of this site was informed by its potential impact on the aquatic environment due to the industrial activities.

Effluent samples were collected as a single grab sample from the non-static discharge point of the Indomie Nigeria Limited industry in Choba. The use of a single grab sample was deemed appropriate for this study, considering the focus on the general impact of the effluent rather than temporal variations in its composition.To prevent photo-degradation of potential contaminants within the effluent, samples were collected in black containers, minimizing light exposure.

Fish: Juvenile *Clarias gariepinus* specimens were procured from African Aquaculture Centre (ARAC), a facility owned by Federal Government of Nigerian. ARAC is located at Aluu in Ikwerre Local Government Area (LGA) of Rivers State, Nigeria.

Experimental Design

Stock Solution: Procured *C. gariepinus* specimens were weighed 0.75g and acclimatized to laboratory conditions for a period of two weeks prior to the commencement of the experiment. Following acclimatization, the fish were randomly distributed into six (6) experimental tanks. One tank with untreated freshwater water was used for the control. The other five (5) tanks were treated with various amount effluent concentrations (USEPA, 2002) as follows:

- **Group 1 (Control):** Fish were exposed to underground water, serving as a baseline for comparison.
- **Group 2:** Fish were exposed to 100% effluent concentration.
- **Group 3:** Fish were exposed to 50% effluent concentration (prepared by mixing 5 litres of effluent and 5 litres of underground water).
- **Group 4:** Fish were exposed to 25% effluent concentration (prepared by mixing 2.5 litres of effluent and 7.5 litres of underground).
- **Group 5**: Fish were exposed to 12.5% effluent concentration (prepared by mixing 1.25 litres of effluent and 8.75 litres of underground water).
- **Group 6:** Fish were exposed to 6.25% effluent concentration (prepared by mixing 0.65 litres of effluent and 9.375 litres of underground water).

The effluent concentrations were determined based on the USEPA (2002) Standards for Chronic whole effluent toxicity testing.

Exposure Conditions: Fish were exposed to their respective treatments for a period of 30 days. The experimental setup was maintained under ambient laboratory conditions without any specific control over water quality parameters such as temperature, pH, dissolved oxygen, or light intensity. This approach aimed to simulate natural environmental conditions to which *Clarias gariepinus* would be exposed in its natural habitat.

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 kidney was conducted to assess structural changes caused by effluent exposure. It involved tissue processing of the kidney 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.

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RESULTS

Chemical constituents of Effluent

Table 1; Chemical constituents of Effluent

S/N	Parameter(s) Mg/l	Conc (%)	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.

Kidney Histopathology of Clarias gariepinus.

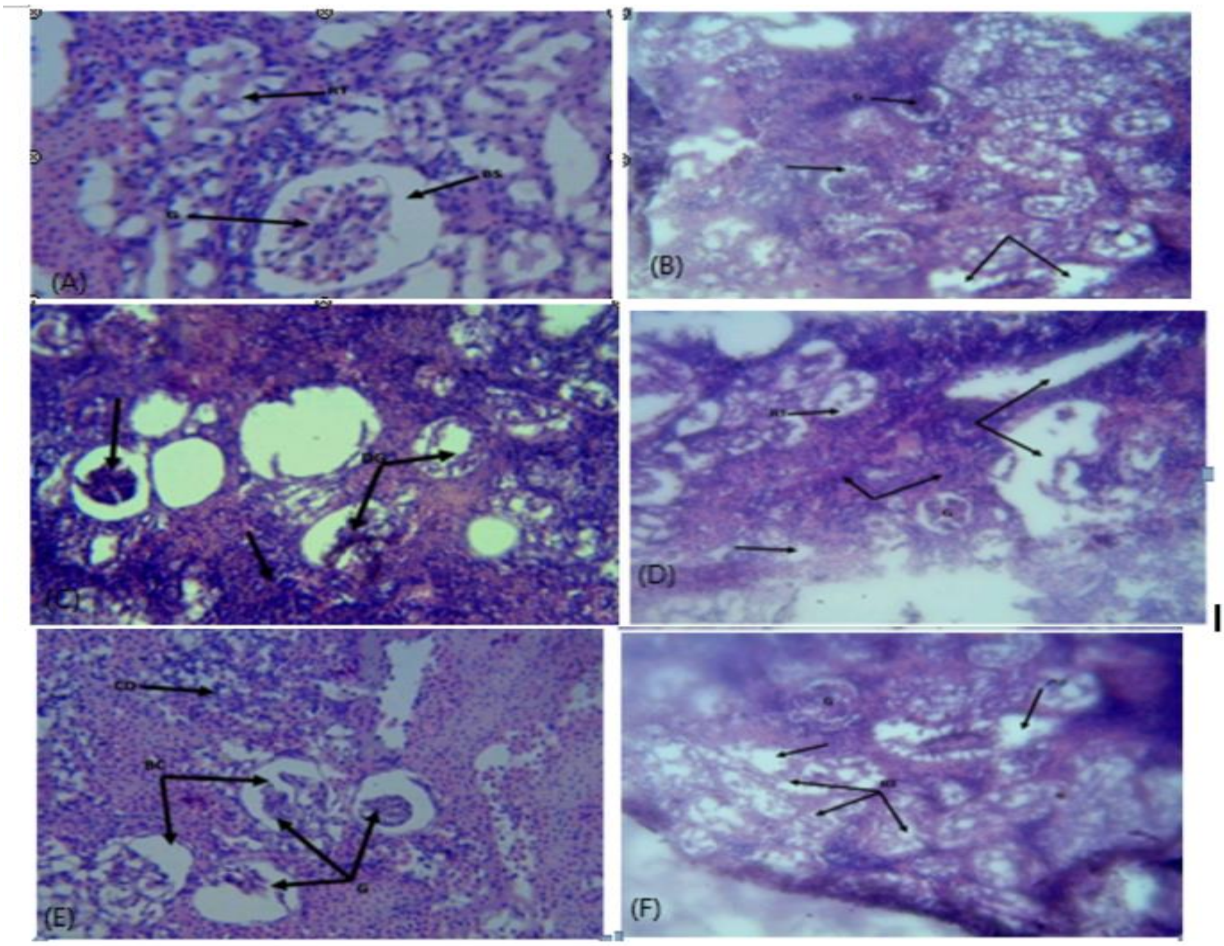

Plate 1 Photomicrograph (H&E x400) of the kidney **A) Control**: showing normal renal corpuscle showing the glomerulus and the Bowman's space well defined (RC) renal tubules (RT), **B) Tank 1 (6.25% effluent)**: mild inflammatory cell infiltration of the glomerulus associated with interstitial and tubular oedema (arrows), **C) Tank 2 (12.5% effluent)**: shrinkage of renal corpuscle (RC) and glomerular deterioration (GD) associated with dilation of Bowman's space (BC); acute cellular degeneration and deformation in the renal tubules architecture (arrows), **D) Tank 3 (25% effluent)**: disruption of renal tubules associated with oedema and diffused inflammatory cell infiltration (arrows), **E) Tank 4 (50% effluent)**: deterioration of glomerulus (G) and dilation of Bowman's space (BC); cellular degeneration (CD) and Shrinkage of renal corpuscle, **F) Tank 5 (100% effluent)**: interstitial oedema; renal tubule disruption associated with multifocal inflammatory cell infiltration.

Table 2: Percentage prevalence of Kidney 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	0	0	0	0	0	0
Progressive Change (PC)						
Hyperplasia	0	0	0	20	20	0
Regressive Change (RC)						
Architectural &Structural alterations	20	20	20	40	20	0
Necrosis	0	0	0	0	0	0
Melano- centres (MMC)	0	0	20	0	20	20
Average % Prevalence	**3.33**	**3.33**	**6.67**	**10**	**10**	**3.33**

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 (Luo et al., 2015; Liu et al., 2021). The elevated Cd levels indicate a high potential for long-term environmental and biological harm, highlighting the need for stricter effluent treatment protocols.Cd exposed to Cd via the consumption of aquatic organisms contaminated with Cd can lead to a range of health problems. Cd accumulates in the kidneys, leading to kidney dysfunction and potential failure.

Histological assessment of kidney samples revealed a clear dose-dependent pattern of renal damage. The control group exhibited normal renal architecture, with well-defined renal corpuscles, glomeruli, Bowman's spaces, and renal tubules. As the concentration of the substance increased, the severity of renal damage also increased. The 6.25% concentration group showed mild inflammatory cell infiltration in the glomerulus, indicating an early stage of immune response to the toxic insult. The 12.5% concentration group displayed more severe damage, including shrinkage of the renal corpuscle, glomerular deterioration, Bowman's space dilation, and tubular degeneration. These changes suggest impaired filtration and reabsorption functions. The 25%, 50%, and 100% concentration groups showed progressively worsening renal damage, characterized by disruption of renal tubules, severe oedema, and widespread inflammatory cell infiltration. These findings indicate significant damage to the kidney's filtration and excretory functions.

CONCLUSION

This study has demonstrated that the noodle producing company`s effluent has a detrimental effect on the kidneys of *Clarias gariepinus*, with the severity of damage increasing with higher concentrations. This information underscores the importance of understanding the potential toxicity of substances in aquatic environments and their impact on aquatic organisms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

All procedures performed in this study are in accordance with the ethical standards of the institutional and/or national research comittee.

Competing Interests

Authors have declared that no competing interests exist.

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