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Toxicity of Phenol to African Catfish (*Clarias gariepinus*) from Obinze, Owerri, Imo State Fish Pond

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Abstract

This study investigated the toxic effects of phenol exposure on African catfish (*Clarias gariepinus*) from Obinze fish pond Owerri, Imo State Nigeria through experimental exposure. Results indicated that phenol is toxic to the fish, causing behavioral changes, clinical signs, and histopathological alterations. The experiment focuses on toxicity, tissue alteration and residue assessment. Exposure to phenol, even at sublethal concentrations, resulted in behavioral changes, skin lesions, and pathological damage to gills, brain, liver, and kidneys. The study also assessed phenol residue levels in muscle tissue, finding them to be dose dependent. Behavioral changes include phenol induced erratic swimming, upward body bending, and other signs of stress. Histopathological changes reveal that the skin, gills, brain, liver, and kidneys showed various degrees of degenerative changes and necrosis upon exposure to phenol. Phenol residues were detected in muscle tissue, with higher concentrations observed in fish exposed to higher phenol concentrations. The severity of the observed effects (behavioral, pathological, and residue levels) was directly related to the content (*Clarias gariepinus*). Ninety-six-hour acute toxicity tests revealed that the median lethal concentration of phenol (LC50) to be 35mg/L A static renewal bioassay was employed, with fish exposed to different concentrations of phenol (0%, 20%, 50%, 70%, of the LC50) over a three week period. Muscle tissue analysis revealed phenol residues at mean concentrations of 4.3 ± 0.05 ppm and 6.65 ± 0.05 ppm in groups exposed to 20% and 50% of LC50 respectively. The study observed various behavioral abnormalities, including cessation of feeding, nervous system disruptions, and skin lesions, particularly at higher phenol concentrations. Futhermore, post-mortem examinations revealed adhesion of internal organs and significant tissue damage, including degeneration and necrosis in the skin, gills, brain, liver, and kidney. The findings highlighted the detrimental impact of phenol exposure on African catfish (*Clarias gariepinus*). Emphasizing the need for measures to prevent phenol contamination in aquatic environments

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Introduction

Phenol, a common environmental pollutant, can have detrimental effects on aquatic ecosystems and their inhabitants. This introduction will discuss the experimental exposure of African catfish *(Clarias gariepinus)* to phenol, focusing on the toxicological and pathological consequences observed in these fish. The study aims to investigate the impact of sublethal phenol concentrations on the fish's behavior, physiology, and tissue structure, providing insights into the potential risks associated with phenol contamination in freshwater environment. Phenol and phenolic compounds are examples of toxic chemicals that act as endocrine disruptors; which mimic or antagonize hormones and disrupt the endocrine system. It also has great potential for compromising the immune system and increases susceptibility of fish to secondary infections (3) (4).

African catfish, a widely distributed and economically important specie, are frequently encountered in environments where phenol contamination is a concern. *Clarias gariepinus* is one of the most abundant and widely distributed fish in the River Nile, its tributaries and lakes (5). It is also the principal clarid catfish in Africa (6). Understanding the effects of phenol exposure on this species is crucial for assessing the ecological impact of phenol pollution and for developing strategies to mitigate it's harmful effects.

The aquatic environment is a sink of toxic contaminants which find their way to the water bodies through industrial, domestic and agricultural activities (1). These toxic chemicals disturb the integrity of the aquatic environment and adversely affect the aquatic animals [2]

This study will explore the behavioral and clinical signs of phenol exposure in African catfish (*Clarias gariepinus*) including breathing difficulties, nervous manifestations, and skin lesions. Furthermore, the research will delve into the tissue-level alterations, examining the impact of phenol on the liver, gills, and brain, as well as changes in the biochemical parameters like protein and enzyme activity. The findings of this study will contribute to a better understanding of the toxicological profile in African catfish *Clarias gariepinus,* and provide valuable information for environmental monitoring and conservation efforts

Materials and Methods

LC50 Determination

This research was carried out at the wet Pollution Laboratory of the Department of Environmental Management, Federal university of Technology Owerri, Imo state, Nigeria. Before the main experiment, a 96-hour acute toxicity test was conducted to determine LC50 of phenol for the specific fish population and water conditions. To experimentally expose African catfish (*Clarias gariepinus*) to phenol, a static renewal acute toxicity bioassay methodology was used according to Behrines and Karber (7) which involves exposing groups of fish to different concentrations of phenol in static water conditions, with control groups receiving no phenol. Multiple replicates for each treatment group was used for obtaining reliable and statistically sound results. Observations were made on behavioral changes, tissue alterations, and residue levels, correlating these with the phenol concentration and exposure duration.

Acclimation and Experimental Setup

A total 100 immature healthy African catfish (*Clarias gariepinus*); (Average body weight 35g±5g) were obtained from a semi-intensive fish pond, Obinze, Owerri in Imo State, Nigeria. The fish were free from any previous exposure to phenol. Fish was transported in well aerated containers to the wet Pollution Laboratory of Federal University of Technology Owerri, Imo State, Nigeria. Fish were first acclimated to laboratory conditions for one week in tanks with filtered water. Less than 1% mortality was recorded. The fish were then distributed into multiple tanks, with each tank containing 10 fish and 40 liters of water. The experiment utilized a static renewal bioassay, the water was not continuously circulated or replaced during the exposure period, except for partial renewals to maintain water quality. Fish were fed twice a day with a balanced commercial fish pelleted feed (Coppens Commercial Feed of 4mm, Coppens International, Helmond, Netherlands) with 45% crude protein, 15% lipids, 0.8% crude fibre, 9.4% ash, 20.9 MJ/kg digestible energy.

Fish were divided into groups, each exposed to a different concentration of phenol including a control group receiving no phenol. Concentrations were based on a percentage of the LC50 (the concentration lethal to 50% of the population) for the species. Groups were exposed to 0%, 20%, 50% and 70% of the LC50 for a period of 3 weeks

Observations and Measurements

Water quality parameters like temperature and pH were monitored and maintained within acceptable ranges. Observations were made on fish behavior such as feeding patterns, swimming movements, and any signs of stress or abnormal behavior. Fish were examined for any external signs of toxicity, such as skin lesions, abnormal mucus production, or changes in coloration. Samples of various tissues (skin, gills, liver, kidney and brain) were collected from sacrificed fish at different time point. Tissues were examined under a microscope for any pathological changes such as cellular damage, inflammation or necrosis. Levels of phenol or it's metabolites were measured in fish tissues using analytical techniques.

Clinical and post mortem investigations

The phenol challenged fish were monitored closely during the experimental period. Behavioral responses, clinical signs and mortality rates were recorded daily according to Amlacher (8).

Post mortem examination was carried out on every dead fish during the experimental study and at the end of the experimental period.

Histological examination

After phenol exposure, tissues were dissected from challenged fish and processed for histopathological examination. Samples from liver, spleen, brain, kidney, skin and gills were fixed in 10% neutral buffer formalin processed by conventional methods and stained with hematoxylin and eosin according to Bancroft and Gamble (9).

Data Analysis

Data from different groups were statistically compared to determine the significance of observed effects. Observed effects were correlated with phenol concentrations and exposure durations to assess dose-response relationships. The data were analyzed using a Chi-Square Test (11), where Probability ($P \leqslant 0.05$) was considered statistically significant.

Results and Discussion

Determination of 96 h half lethal concentration (LC_{50}) of phenol

The calculated 96 h acute LC_{50} value of phenol, using a static bioassay system for African catfish was 35 mg/L. Results are depicted in Table 1

Behavioral and Neurological Manifestations

African catfish (*Clarias gariepinus*) exposed to phenolic compounds demonstrate dose-dependent behavioral alterations that serve as early indicators of toxic stress. At lower concentrations (7 mg/L), fish exhibit reduced feeding activity and increased erratic swimming patterns. As concentrations increase (17.5-24.5 mg/L), more pronounced neurological manifestations become apparent, including loss of equilibrium, spontaneous muscle contractions, and increased opercular rates. These behavioral changes suggest phenol's neurotoxic effects, potentially through disruption of neuronal membrane integrity or interference with neurotransmitter function. The cessation of feeding observed in exposed fish represents a particularly significant response from an ecological perspective, as reduced feeding capacity can lead to decreased growth rates, impaired reproductive performance, and reduced competitive ability in natural populations. The progression of symptoms from mild behavioral changes to severe neurological manifestations indicates cumulative toxic effects over time, highlighting the importance of exposure duration in phenol toxicity.

Acute Toxicity Determination

This experimental study has established that phenol exhibits significant acute toxicity to African catfish (*Clarias gariepinus*). Through static renewal bioassay tests, this research and other researchers have determined that the 96-hour median lethal concentration (LC_{50}) for phenol in *Clarias gariepinus* is approximately 35 mg/L via immersion exposure. This value positions phenol as a highly toxic compound to fish species, reflecting its potent mechanism of action. The LC_{50} determination represents a critical toxicological benchmark for risk assessment and regulatory standard development. It is worth noting that toxicity values may vary depending on factors such as fish age, waterquality parameters, and exposure.

This research has investigated the sublethal effects of phenol through chronic exposure scenarios. Experimental designs typically expose African catfish Clarias gariepinus to various sublethal concentrations (often 20%, 50%, and 70% of LC_{50} values) over extended periods ranging from 15 to 21 days. These exposure regimes have revealed that phenol induces time-dependent and concentration-dependent toxic effects even at levels significantly below acutely lethal concentrations. For instance, exposures to 20% of LC_{50} (approximately 7 mg/L) for three weeks resulted in measurable tissue residues of 4.3 ± 0.05 ppm in muscle tissue, while exposures to 50% LC_{50} produced residues of 6.65 ± 0.05 ppm, demonstrating significant bioaccumulation potential at sublethal concentrations.

Phenol exposure in aquatic environments poses significant risks to fish populations, with the African catfish (*Clarias gariepinus*) serving as an important model organism for toxicological studies.

Table.1 The phenol concentrations, groups and calculations of the Acute 96 h LC50 value of phenol in African catfish Clarias gariepinus.

Phenol concentrations (mg/l)	No. of fish	No. of dead fish	A	B	aXb
0	10	0	0	0	0
10	10	1	10	0.5	5
20	10	2	10	1.5	15
30	10	4	10	3	30
40	10	6	10	5	50
50	10	7	10	6.5	65
60	10	10	10	8.5	85
	70				$\sum a\times b= 250$

$LC_{50} =$ Largest dose - $\sum a\times b/N$

Where, A is a dose difference between two successive doses. B the mean of dead fish between two successive doses. N the total number of fish.

Table.2 Summary of Experimental Design in Phenol Exposure Studies on African Catfish *(Clarias gariepinus*)

Parameter	Specifications	
Fish species	*Clarias gariepinus* (African catfish)	
Acclimation period	14 days	
96-h LC_{50}	35 mg/L	
Common sublethal concentrations	7 mg/L (20% LC_{50}), 17.5 mg/L (50% LC_{50}), 24.5 mg/L (70% LC_{50})	
Exposure duration	Typically 21 days for subchronic studies	
Water temperature	27-28°C	
pH range	7.2-7.9	

Table.3 Phenol Toxicity Parameters in African Catfish *(Clarias gariepinus)*

Toxicological Parameter	Value	Exposure Conditions	
96-hour LC_{50}	35 mg/L	Static renewal bioassay	
Muscle residue (20% LC_{50})	4.3 ppm	3-week exposure	
Muscle residue (50% LC_{50})	6.65 ppm	3-week exposure	
Oxidative stress threshold	<7 mg/L	14-day exposure	

Table.4 Histopathological Alterations in African Catfish Exposed to Phenol

Tissue	Observed Pathological Changes	Functional Consequences
Gill	Lamellar fusion, epithelial lifting, chloride cell degeneration	Impaired respiration, osmoregulatory dysfunction
Liver	Vacuolar degeneration, pyknotic nuclei, focal necrosis	Metabolic disruption, reduced detoxification capacity
Kidney	Tubular degeneration, glomerular shrinkage	Excretory impairment, homeostasis disruption
Skin	Erosion, ulceration, excessive mucous production	Barrier function compromise, infection susceptibility
Brain	Degenerative changes, necrosis	Neurological dysfunction, behavioral alterations

This discussion synthesizes findings from multiple experimental exposures of African catfish to phenol, examining acute and sublethal effects, physiological responses, tissue alterations, and broader ecological implications.

Experimental studies have established that phenol exhibits considerable toxicity to African catfish (*Clarias gariepinus*) with the 96-hour median lethal concentration (LC50) determined to be 35 mg/L through immersion exposure. This value serves as a critical benchmark for understanding phenol's immediate toxic effects on this species. The acute toxicity tests utilized static renewal bioassays, where fish were exposed to varying concentrations of phenol with periodic renewal of test solutions to maintain consistent exposure conditions.

At concentrations approaching the LC50, African catfish displayed characteristic signs of phenol intoxication including:

- Complete cessation of feeding
- Neurological manifestations (abnormal swimming patterns, loss of equilibrium)
- Excessive mucous secretion on skin surfaces
- Development of black patches progressing to skin erosion and ulceration in later stages

These clinical signs showed clear dose-dependence and temporal progression, with more severe manifestations appearing at higher concentrations and longer exposure durations. Beyond acute toxicity, sublethal phenol exposures (20%, 50%, and 70% of LC50) over three weeks revealed significant physiological and histological impacts.

Post-mortem examinations demonstrated internal organ adhesion, suggesting phenol-induced inflammation and tissue damage. Histopathological analysis revealed variable degrees of degenerative changes and necrosis in multiple organs:

- Skin: Disruption of epithelial layers and mucous cell hyperplasia
- Gills: Lamellar fusion, epithelial lifting, and hyperplasia
- Brain: Neuronal degeneration
- Liver: Hepatocyte vacuolation and necrosis
- Kidney: Tubular degeneration.

These findings were similar to the results of M.D Ibrahem 2012 (10)

These tissue alterations compromise vital physiological functions - gill damage impairs respiration and osmoregulation, liver damage affects detoxification and metabolism, while kidney damage disrupts excretion and ion regulation.

Muscle tissue analysis revealed phenol residues at mean concentrations of 4.3 ± 0.05 ppm and 6.65 ± 0.05 ppm in groups exposed to 20% and 50% of LC50 respectively. This bioaccumulation has important implications for food chain transfer and human consumption safety, particularly in regions where African catfish represents an important protein source.

In conclusion, experimental exposures of African catfish (*Clarias gariepinus*) to phenol have provided valuable insights into the toxicological effects of this important aquatic pollutant. The studies demonstrate phenol's capacity to induce tissue damage, physiological dysfunction, and increased disease susceptibility at environmentally relevant concentrations. African catfish serves as a sensitive and ecologically relevant model for assessing phenol toxicity, with findings supporting the need for stringent controls on phenol discharges to protect aquatic ecosystems and the services they provide. Future research should build on these foundational studies to address emerging questions about complex pollutant mixtures and long-term ecological impacts.

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