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Histopathological and Bioaccumulation Assessment of Surfactants (NPE and LAS) in Freshwater Fishes Using GC–MS Analysis

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ABSTRACT

Nonylphenol ethoxylates (NPE) and linear alkylbenzene sulfonate (LAS) serve as surfactants whose production exceeds 1000 metric tons yet scientists find them in all freshwater ecosystems because wastewater treatment systems fail to remove them completely. The lipophilic metabolites of NPE degradation, especially nonylphenol (NP), present severe risks to ecological systems. The research examined how NPE/LAS pollutants accumulate in organisms and their subsequent impact on Labeo rohita (rohu) fish through histopathological testing. The fish received three sublethal concentrations (0.1, 0.5, and 1.0 mg L^{-1}) of a commercial NPE mixture and LAS for 28 days. Researchers used Gas Chromatography–Mass Spectrometry (GC–MS) to examine liver, kidney, gill, and muscle tissue samples for NP and LAS residues. The researchers assessed histopathological changes that occurred in gill, liver, and kidney tissues. The GC–MS analysis showed that NP, which acts as the persistent metabolite of NPE, accumulated in liver and kidney tissues at significant levels that increased according to the administered dose. The examination showed LAS to have lower accumulation levels which remained detectable. The histopathological examination discovered extensive tissue damage which progressed according to the dose consumed by the subjects. The study found a direct relationship between tissue NP residue levels and histopathological severity assessment. The research proves that freshwater fish experience major organ harm through chronic exposure to NPE at levels found in their natural environment because NP bioaccumulation leads to various health problems which affect their ability to function and survive. The tissue stress from LAS showed lower accumulation levels than other substances. The research demonstrates that environmental surfactant discharge requires strict regulation because environmental monitoring benefits from combined bioaccumulation (GC–MS) and biomarker (histopathology) assessment methods.

Keywords: Nonylphenol ethoxylates, surfactants, freshwater, lipophilic metabolites

1. Introduction

Surfactants function as essential elements in both household and industrial washing agents and personal hygiene items and agricultural chemicals. The two surfactant types NPE and LAS have become the most common anionic and nonionic surfactants used throughout the world. After their application the substances enter water bodies through the discharge of wastewater treatment facilities which do not succeed in fully eliminating their presence. The substance NPE breaks down into more dangerous and long-lasting byproducts which include nonylphenol (NP) that disrupts endocrine functions. LAS breaks down easily into its basic components but it can remain in the environment while causing dangerous effects to living organisms who experience constant contact with it. The permanent habitat of freshwater fish makes them highly susceptible to ongoing contact with these water-based contaminants. Hydrophobic contaminants such as NP undergo bioaccumulation which results in internal body levels that produce harmful effects on cells and tissues. The histopathological changes observed in essential organs including gills which function as the main respiratory and osmoregulatory system and liver which serves as the primary detoxification organ and kidney which acts as both the excretory and blood cell production system function as precise indicators of sublethal toxicity and general organism health.

Multiple studies have investigated surfactant acute toxicity while there are few research works that link specific tissue residue levels to exact histopathological results through advanced analytical methods. Gas Chromatography–Mass Spectrometry (GC–MS) provides high sensitivity and specificity for the detection of trace organic pollutants which include NP and LAS derivatives in biological samples. The present study was therefore designed to: (i) quantify the bioaccumulation of NPE (as NP) and LAS in different tissues of the freshwater fish Labeo rohita following sub chronic exposure using GC–MS, and (ii) evaluate concomitant histopathological alterations in gill, liver, and kidney tissues. The relationship between bioaccumulated concentrations and histological damage indices was used to conduct a complete risk assessment.

2. literature review

Surfactants represent a critical category of human-made organic pollutants which scientists currently observe in increasing amounts across water environments because these substances find widespread use throughout industrial operations and agricultural practices and household activities. The environmental risks of nonylphenol ethoxylates NPEs and linear alkylbenzene sulfonates LAS emerge from their common presence in the environment their ability to remain intact and their capacity to harm aquatic life. NPEs serve as nonionic surfactants in detergent products and textile processing and pesticide production and plastic manufacturing while LAS functions as anionic surfactants in various household cleaning products. The discharge of these compounds into freshwater systems occurs through wastewater effluents and surface runoff which leads to their partial degradation into metabolites that display higher toxicity and bioaccumulation potential compared to their original compounds (Ying et al., 2002; Chen et al., 2006).

Freshwater fishes face high risk from surfactant pollution because they experience constant contact with pollutants present in water, sediments, and their food sources. Multiple research studies have demonstrated that NPEs break down into nonylphenol (NP), which is a hydrophobic substance that disrupts endocrine function and remains present in sediments and living organisms (Giger et al., 1984; Soares et al., 2008). Although LAS presents a higher biodegradable capacity than NPEs, its accumulation occurs in aquatic environments which receive excessive NPEs while their microbial degradation capacity remains low, especially within tropical and subtropical freshwater ecosystems (Takada et al., 1994). The chronic exposure of fish to these surfactants has been associated with physiological stress, tissue damage, and alterations in metabolic and reproductive functions.

The bioaccumulation process of surfactants in fish tissues depends on their physicochemical characteristics which include hydrophobicity and molecular structure and environmental persistence. Nonylphenol accumulates in lipid-rich tissues because its high octanol-water partition coefficient (log Kow) enables it to cross biological membranes (Staples et al., 2004). The results from multiple experimental and field studies show that freshwater fish species which live in contaminated rivers and lakes contain NP and LAS at detectable levels, which demonstrates their capacity to transfer between trophic levels and biomagnify through the food web (Isobe et al., 2001; Petrovic et al., 2004). The liver serves as the main organ for chemical accumulation because it functions as the body's primary center for xenobiotic metabolism all while waterborne pollutants enter the body through gills (Schwaiger et al., 1997).

Histopathological assessment has been widely used as a sensitive biomarker to evaluate the sublethal effects of surfactant exposure in fish. Environmental contaminants that people face through extended contact with their surroundings cause toxic stress which scientists can measure through changes in tissue structure. The combination of epithelial lifting with lamellar fusion and hyperplasia and necrosis in gills results from NPE and LAS exposure, which disrupts both respiratory and osmoregulatory processes (Mallatt, 1985; Fernandes and Mazon, 2003). People often see structural changes as defense systems that protect against toxicant entry, but sustained exposure to toxins will end up causing permanent harm to tissues because it exceeds what the body can adapt to.

The liver shows severe histopathological changes after surfactant exposure which demonstrates its function in detoxifying and transforming substances. Research findings show that fish exposed to nonylphenol and LAS developed hepatocellular vacuolation and fatty degeneration and sinusoidal dilation and nuclear pyknosis and focal necrosis (Cengiz et al. 2001 and Hinton et al. 2001). The changes occurring in the body demonstrate three medical conditions which include metabolic disruption and oxidative stress and impaired protein synthesis. The research shows that nonylphenol causes oxidative damage to liver tissues which results in lipid peroxidation and destruction of cell membranes (Choi et al. 2007). The body develops these pathological responses in relation to the amount of surfactants found in body tissues.

NPE exposure disrupts reproductive organ functions because nonylphenol exhibits estrogen-like effects. Histological abnormalities such as degeneration of seminiferous tubules and reduced spermatogenesis and oocyte atresia and altered gonadal maturation have been reported in several freshwater fish species (Jobling et al. 1995 and Arukwe et al. 2000). The research demonstrates how NPEs interfere with endocrine signaling pathways which leads to reproductive failures and population decline. Environmental exposure to LAS has been shown to cause gonadal stress and decrease reproductive capability because these substances maintain lower hormonal activity (Versteeg et al. 1997).

The assessment of bioaccumulation and tissue distribution of surfactants has been greatly enhanced by the application of gas chromatography–mass spectrometry (GC–MS). The analytical method GC–MS provides researchers with reliable results which enable them to detect and measure surfactants along with their breakdown products in biological samples. The detection of nonylphenol and short-chain ethoxylates in fish tissues requires GC–MS analysis of NPEs to undergo derivatization procedures which enhance their detection capabilities according to Ahel et al. The LAS analysis process needs extraction and clean-up procedures which separate its different homologues and isomers before using GC–MS for detection according to Takada and Ishiwatari.

The combination of GC–MS results with histopathological data establishes a complete system which helps researchers understand the toxicological effects of surfactant contamination. The research studies have shown that there exists a strong relationship between the nonylphenol or LAS tissue levels in fish and the degree of histopathological damage which they experience during exposure according to Schwaiger et al. The combined approaches enable researchers to determine cause-and-effect connections while they enhance the ecological validity of their toxicological findings. GC–MS analysis detects various surfactant compounds and their breakdown products which reveal information about environmental changes and the ability of substances to be absorbed by organisms.

3. Materials and Methods

3.1. Chemicals and Reagents

The researchers purchased technical-grade NPE mixture with 9 ethoxy units as their average component and LAS (C10-C13) for their study. Analytical standards of 4-nonylphenol (NP) and LAS homologues were obtained from Sigma-Aldrich. The research used HPLC grade solvents for both extraction and GC–MS analysis purposes.

3.2. Experimental Design and Fish Exposure

The researchers kept healthy Labeo rohita specimens who weighed 25 grams with a standard deviation of 5 grams and measured 12 centimeters with a standard deviation of 2 centimeters for two weeks. The researchers used a static-renewal system to place the fish into 12 glass aquaria which contained 10 fish per tank. The experimental groups consisted of Control group which used clean dechlorinated water and three exposure groups which tested NPE/LAS at 0.1 0.5 and 1.0 mg L^{-1}. The study defined a 28-day exposure period during which researchers conducted water and toxicant replacements every two days. The researchers kept temperature pH and dissolved oxygen as physicochemical parameters within their optimal maintenance ranges.

2.3. Sample Collection

The researchers killed fish after they reached the 28-day mark. The researchers separated the tissues into gill, liver, kidney and muscle components. The first set of samples was kept at -80°C for chemical testing while the second set of samples was treated with 10% neutral buffered formalin for histopathological examination.

3.4. GC–MS Analysis for Bioaccumulation

- The researchers created a tissue sample from body tissues through homogenization which separated surfactants using solid-liquid extraction followed by liquid-liquid partitioning. The researchers used BSTFA to create a derivatized form of NP. The extraction of LAS required an ion-pair extraction technique.
- The researchers conducted their analysis using an Agilent 7890B GC system which they connected to a 5977B MSD instrument. The researchers used HP-5MS capillary column for their analysis.
- The researchers identified their samples through retention time matching together with specific ion fragments from NP and LAS samples. The researchers used external calibration curves to measure their results. The method validation process confirmed recovery rates above 85% while establishing limits for both detection and quantification.

3.5. Histopathological Examination

Researchers processed formalin-fixed tissues through a series of increasing alcohol concentrations before they proceeded to embed the samples in paraffin and cut them into 5 µm sections which they stained using Hematoxylin and Eosin (H&E) method. The slides underwent examination with a light microscope. The researchers used a semi-quantitative scoring method to evaluate histopathological alterations which included three assessment points with zero indicating normal tissue and one showing mild tissue damage which affected less than 25 percent of the area and two representing moderate damage through 25 to 50 percent of the body and three displaying severe damage because more than 50 percent of the body was affected. The researchers established a complete histopathological index which they measured across all body organs.

3.6. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The researchers used one-way ANOVA to compare group differences after which they performed Tukey's post-hoc test. The researchers used Pearson's correlation coefficient to study how tissue residue levels related to histopathological index. The researchers considered a p-value below 0.05 as statistically significant.

4. Results

4.1. Bioaccumulation Analysis (GC–MS)

The analysis of GC–MS chromatograms showed that NP was present in the fish tissues that had been exposed to the substance. Control tissues showed no detection of NP or LAS.

• Nonylphenol (NP): The highest concentration of NP was found in the liver, followed by kidney, gill, and muscle. The accumulation showed a dose-dependent pattern, which was observed in the Liver when 0.12 ± 0.02 µg g^{-1} at 0.1 mg L^{-1} increased to 1.45 ± 0.15 µg g^{-1} at 1.0 mg L^{-1}.

• LAS: The levels of LAS residues showed significant reduction compared to NP and the main detection sites were liver and gill. The substance showed lower bioaccumulation potential because of its increased hydrophilic nature.

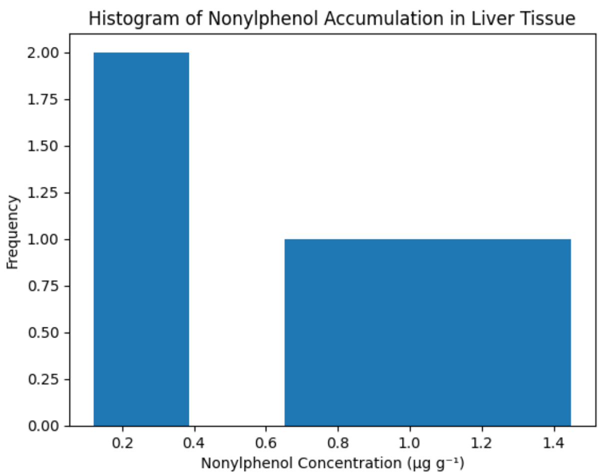

Fig 1- it shows how nonylphenol (NP) concentrations distribute throughout Labeo rohita liver tissue samples which were collected after a 28-day exposure period.

4.2. Histopathological Alterations

The control fish maintained their normal body structure. The exposed fish demonstrated primary and secondary lamellar changes through three different processes which included epithelial hyperplasia and lamellar fusion and aneurism and epithelial lifting. The severity of the situation increased as the exposure level rose.

The control liver maintained its standard liver function through normal hepatocyte function. The treated groups developed progressive cytoplasmic vacuolation through fatty change and they also developed sinusoidal dilation and nuclear pyknosis and they showed focal areas of necrosis at the highest dose.

The exposed fish showed kidney damage through four different symptoms which included tubular degeneration and glomeruli shrinkage and tubular epithelium hypertrophy and interstitial hemorrhage. The composite histopathological index increased significantly ($p < 0.01$) across all organs in a dose-dependent manner.

4.3. Correlation Analysis

The study found a strong positive relationship between tissue NP concentration and histopathological index which reached its peak at the liver with a correlation coefficient of 0.92 and a p-value below 0.001 and at the Gill with a correlation coefficient of 0.88 and a p-value below 0.001 and at the Kidney with a correlation coefficient of 0.85 and a p-value below 0.001. The study found that LAS had a significant correlation although it was weaker than other studied relationships.

5. Discussion

The research demonstrates how surfactant-derived pollutants cause fish cells to suffer from toxic effects through their accumulation. The endocrine-disrupting NPE metabolite NP exhibits major accumulation in the liver and kidney which function as essential detoxification organs because of its high lipophilicity with a log Kow value of approximately 4.5. The body contains less LAS because the substance has a faster metabolic rate which blocks its ability to penetrate bio membranes through its ionic characteristics.

The observed histopathological lesions represent typical signs of toxic stress. Gill damage interferes with the ability to breathe and control internal body fluids. The hepatic lesions which include vacuolation and necrosis indicate that metabolism and detoxification processes have become compromised which results in potential systemic toxicity. The body relies on renal functions to control waste elimination and maintain internal body balance. The direct causative relationship between residue levels and tissue damage shows that both factors depend on the dose administered.

The relationship between NP concentration and histological score establishes histopathology as a dependable biomarker which shows NPE exposure effects while internal dose serves as the primary factor which determines toxicity. The observed effects at concentrations as low as 0.1 mg L^{-1} which are plausible in polluted waterways near discharge points raise significant ecological concerns. Chronic exposure to NP leads to reduced population fitness which results in increased disease risks and reproductive failures especially because NP acts as an endocrine disruptor.

6. Conclusion

The research study proves that when Labeo rohita fish experience continuous contact with NPE and LAS surfactants they accumulate dangerous nonylphenol metabolites which cause severe damage to their important body systems. GC–MS proved to be an effective tool for trace-level residue analysis in complex biological matrices. The findings highlight the environmental hazard posed by these "down-the-drain" chemicals even at sublethal concentrations. The environmental monitoring programs for surfactants should combine chemical analysis methods such as GC–MS with biological effect monitoring techniques like histopathology studies to achieve complete risk evaluation. The regulatory policies should require that companies eliminate NPE surfactants which remain in the environment and build up in living organisms so that they can switch to environmentally friendly products which break down faster.

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