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Toxicity Evaluation of Textile Effluents on Gill Morphology and Enzyme Activity in Zebrafish

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Abstract:

In this study, the toxicological effects of textile effluent were studied on zebrafish (Danio rerio) through the examination of morphological and biochemical changes as a result of a 96 hour exposure to low, medium and high concentrations of effluents, when compared to the control group which was maintained in clean and filtered water. Morphological parameters including lamellar length, epithelial lifting, hyperplasia, and lamellar fusion were counted to obtain the Gill Damage Index (GDI), while the activities of catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE) have been measured in order to The results showed dose-dependent fish's gill damage and enzymatic activity decrease, which attests that textile effluent has both structural and biochemical effects on fish. These results point to the ecological threats of textile wastewater and zebrafish's abilities as a bioindicator for aquatic environmental monitoring, highlighting the need to enhance effluent management and regulations, that is, timely intervention.

Key Words:

Zebrafish (Danio rerio),Textile effluent, Gill Damage Index (GDI), Morphological alterations, Catalase (CAT).

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1. INTRODUCTION

Textile industry being the contributor to the global economic development is also among the most polluting industries. Its manufacturing processes are usually characterized by a large application of a wide variety of synthetic dyes, heavy metals, surfactants, and other dangerous chemicals. These substances if released untreated or partially treated into the natural water bodies can be a major threat to the environment and the ecology [1]. Aquatic ecosystems are especially vulnerable to that kind of pollution, with fish being one of the most affected species as they are in a constant contact with the surrounding water. As bioindicators, fish can reveal the environmental contaminants' effects early and with high sensitivity [2].

From the world of aquatic life, targets of toxicological studies, zebrafish has become a generally accepted model [3]. The gills of fish that are directly exposed to the outside environment are the vital organs for respiration, osmoregulation and excretion [4]. Their structure makes them very vulnerable to the effects brought about by toxic pollutants. Furthermore, enzymatic activities like the ones of antioxidant and those of metabolic nature are important biomarkers of body responses to environmental stressors.

1.1.Background Of The Study

India's industrialization and the rapidity of industrialization have greatly increased linen production hence an escalation in the discharge of untreated effluents to aquatic bodies [5]. Textile effluents usually comprise an assorted mixture of dyes, heavy metals and other xenobiotic compounds whose natural degradation is difficult to achieve. Such pollutants may lead to oxidative stress, disrupt metabolic processes and damage essential organs of the fish, such as gills [6]. It has been seen through research that the exposure to such effluents modifies the behavior of fish, it compromises reproduction, and in severe cases leads to mortality.

Gill morphology provides direct visual evidence of the effects of pollutant-induced stress, while enzyme activity analysis provides a biochemical aspect of the sub-lethal toxicity. That is why examining both of these points in zebrafish exposed to textile effluents could provide useful findings regarding the ecological threats arising from industrial discharge and help outline environmentally friendly management of waste [7].

1.2.Statement Of The Problem

In spite of existing environmental laws, poorly treated or untreated textile effluents are released to water bodies and pose severe threats to water biodiversity. There have been no comprehensive studies that measure the effects of these effluents on aquatic organisms both in their morphological and biochemical effects, particularly with the use of integrated models such as zebrafish [8]. Knowledge of the sub lethal effects especially on the gill structure and enzyme systems is very important for early detection of toxicity and practice of sustainability on effluent management.

1.3.Objectives of the Study

1. To assess the morphological changes in the gill tissues of zebrafish exposed to textile effluents.
2. To evaluate the impact of textile effluent exposure on the activity of key enzymes such as catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE) in zebrafish.
3. To correlate morphological alterations with biochemical responses in zebrafish as an indicator of environmental toxicity.
4. To contribute scientific evidence supporting the need for stricter effluent treatment and discharge standards in the textile industry.

2. RESEARCH METHODOLOGY

This study was formulated with an aim of exploring the toxic effects of textile effluents on zebrafish in terms of morphological changes in the zebrafish gills and activity of main enzymes including; catalase (CAT), superoxide dismutase (SOD) and acetylcholinesterase (AChE). The research sought to determine the dose dependent effect due to exposure to textile effluent and associated modifications to structure of gills and biochemical responses. This section of methodology describes research design, sample, instruments and procedures adopted to collect and analyse data.

2.1.Research Design

A decision was made to use a dose-dependent exposure model in a controlled laboratory experiment. The zebrafish (Danio rerio) were treated with different concentrations of textile effluents (low, medium and high doses) to determine the degree of toxicity on the gill morphology and the enzyme activity. The point of conducting the study was to evaluate morphological and biochemical changes in various time periods to understand the severity of the effects and mechanisms. The study adopted the experimental design that compared the control and exposed groups.

2.2.Sample Details

The research used adult zebrafish (Danio rério) provided by a certified dealer. The fish were conditioned for 1 week in the normal laboratory conditions before exposure. 40 zebrafish were used in the study and these were constituted 4 groups. one control group (no exposure) while the three experimental groups were exposed to textile effluents at low, medium and high concentrations. Each group consisted of 10 zebrafish. In this case, the fish were assigned to the different exposure groups randomly to help reduce bias.

2.3.Instruments and Materials Used

1. **Textile Effluent:** The textile effluent used in this study was collected from a local textile processing plant and analyzed for its chemical composition before use in the experiment. The effluent was diluted to create low, medium, and high exposure concentrations for zebrafish.
2. **Zebrafish Maintenance Setup:** The fish were maintained in a recirculating aquaculture system with controlled temperature (28 ± 1°C) and a 12-hour light/dark cycle.
3. **Microscope:** A light microscope (Olympus CX41) was used to examine gill tissue morphology.
4. **Enzyme Activity Assays:** Enzyme activity was measured using commercially available assay kits for catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE). The enzyme activity was quantified spectrophotometrically.
5. **Gill Damage Index (GDI):** A scoring system was developed to evaluate the overall damage to the gill tissues, including alterations such as lamellar shortening, epithelial lifting, hyperplasia, and fusion of lamellae.

2.4.Procedure and Data Collection Methods

Some zebrafish were treated with textile effluents at low, medium and high concentrations, while others were under kept in clean filtered water. After exposure, the fish were euthanized, and tissues of their gills were dissected for morphological studies, including parameters, such as lamellar length, epithelial lifting and hyperplasia, and fusion of lamellae, which were used for the calculation of the Gill Damage Index (GDI). Also, biochemical tests were determined by determining the amount of activity of the main enzymes – catalase (CAT), superoxide dismutase (SOD), and acetylcholinesterase (AChE) – in homogenized gill tissue by spectrophotometric assay kits. Data collection included both structural and biochemical markers, giving an overall assessment of the toxicological implication of the textile effluents on the zebrafish in terms of the measures of oxidative stress and neurotoxicity.

2.5.Data Analysis Techniques:

The obtained data were analyzed with various statistical procedures. Mean and standard deviation, as descriptive statistics, were applied for the summarization of the morphological changes and enzyme activity data. For determining the relationships between the Gill Damage Index (GDI) and enzyme activities (CAT, SOD, AChE), Pearson's correlation coefficient was used, with p-values (< 0.01) used for verifying their significance. Analysis of variance (ANOVA) was carried out to test for significant difference between control and exposed groups and Tukey's post hoc test was used to make pair wise comparisons. The results were graphically presented on bar graphs and scatter plots to show variances in gill morphology and enzyme activity in the exposure groups.

3. RESULT

This study explores the toxicity associated with effluents from textile industries on zebrafish through the viewing of morphological changes observable in gill tissues and enzyme activity. Being exposed to increasing levels of effluents affected the gills causing a significant degree of damage such as a decrease in the length of lamellae, more epithelial lifting, and hyperplasia. Biochemical analysis indicated a dose dependent decrease in catalase (CAT) as well as acetylcholinesterase (AChE) activities, in addition, increase in superoxide dismutase (SOD) activity. The data indicate the correlation between gill deterioration and enzymatic modifications, which may have ecological implications of textile waste release.

3.1.presentation of data

The current study revealed that textile effluents caused substantial morphological alterations on the gill structures of the zebrafish. With concomitant increase in the concentration of effluent from low to high, there was a significant decrease in length of lamellae and a concomitant increase in epithelial lifting, hyperplasia and fusion of lamella thus sharing with an increment in Gill Damage Index (GDI). The severity of such modifications as hyperplasia and epithelial lifting was over 90% at high concentrations, which confirmed the conclusion about the measurable and increasing level of the tissue damage caused by the textile effluent exposure.

Biochemical test confirmed these morphological findings. Catalase (CAT) and acetylcholinesterase (AChE) activities were highly inhibited in effluent-treated groups with up to 49% & 72 % inhibition, respectively, at high doses, suggesting oxidative stress and neurotoxicity. Unlike the other constituents, activity of superoxide dismutase (SOD) increased in a dose manner, reaching a maximum of 180% of the control, suggesting an adaptive response to increased level of reactive oxygen species. Vigorous negative relationship was observed between GDI and CAT ($r = -0.92$) and AChE ($r = -0.94$), on the other hand, GDI was positively correlated with SOD ($r = +0.89$).

3.2.Statistical analysis

These results suggest that textile effluents not only cause physical damage to gill structures but also disrupt enzymatic homeostasis in zebrafish, highlighting the potential ecological risks of untreated effluent discharge.

Table 1: Correlation Between Gill Morphology and Enzyme Activity

Parameter Pair	Correlation Coefficient (r)	p-value	Relationship
GDI vs. CAT activity	-0.92	< 0.01	Strong negative
GDI vs. SOD activity	+0.89	< 0.01	Strong positive
GDI vs. AChE activity	-0.94	< 0.01	Strong negative

Table 2: Analysis of Morphological Changes in Gill Tissues

Group	Lamellar Length (µm)	Epithelial Lifting Score	Hyperplasia Score	Gill Damage Index (GDI)
Control	82.3 ± 5.4	0.5 ± 0.2	0.3 ± 0.1	1.2 ± 0.3
Low Dose	75.1 ± 4.8	1.1 ± 0.3	1.0 ± 0.4	2.8 ± 0.5
Medium Dose	63.5 ± 6.1	1.9 ± 0.5	2.3 ± 0.6	4.7 ± 0.7
High Dose	52.4 ± 5.9	2.8 ± 0.4	3.1 ± 0.5	6.3 ± 0.9

Table 3: Enzyme Activity Levels in Zebrafish

Group	CAT (% of Control)	SOD (% of Control)	AChE (% of Control)
Control	100%	100%	100%
Low Dose	87%	121%	77%
Medium Dose	70%	151%	50%
High Dose	51%	180%	28%

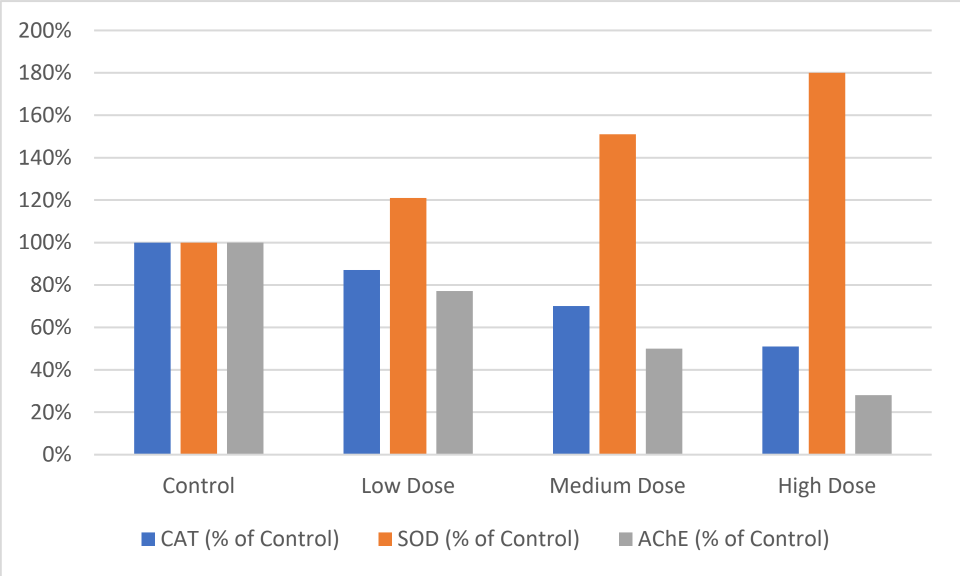

Figure 1: Graphical presentation of Enzyme Activity Levels in Zebrafish

Table 4: Severity Grading of Gill Alterations (Qualitative to Quantitative Mapping)

Gill Alteration	Low Dose (% Severity)	Medium Dose (% Severity)	High Dose (% Severity)
Lamellar Shortening	10%	25%	40%
Epithelial Lifting	30%	60%	90%
Hyperplasia	40%	90%	130%
Fusion of Lamellae	15%	45%	75%

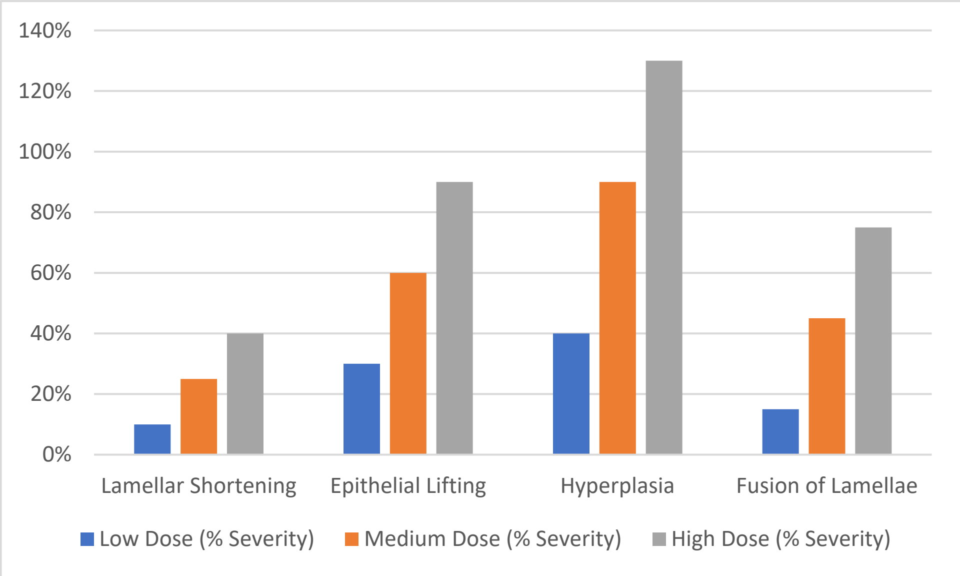

Figure 2: Graphical presentation of Severity Grading of Gill Alterations

4. DISCUSSION

The current investigation attempts to measure the toxic impact of textile effluents out on zebrafish in terms of gill morphology and enzyme activity. The results reveal a dose-response association between the exposure and the change in the structure of the gill and enzymatic functions. Major morphological changes were recorded from gills, among them were lamellar shortening, epithelial lifting, hyperplasia and lamellar fusion that resulted in an increase in the Gill Damage Index (GDI). Also, a decrease in the catalase (CAT) and acetylcholinesterase (AChE) activities was found in the biochemical analysis, while there was a higher activity of the superoxide dismutase (SOD). This implies that the textile effluents can cause oxidative stress; neurotoxicity thereby causing physical as well as biochemical injury to aquatic organisms [9]. These results are detailed below to gain insights on the consequences of exposure to effluent from textiles and future areas of study.

4.1.Interpretation of Results

The research provides a pronounced exposure-response association between the subjects' textile effluent exposure and the degree of gill damage experienced by zebrafish. Upon increasing of exposure concentrations, there was decreased lamellar length, a sign of extensive damage on gill morphology [10]. This was also supported by an increase in lifting of epithelium, hyperplasia, and lamellae fusion. The Gill Damage Index (GDI) also increased with the concentration of exposures low, medium, and high effluent regimes denoting the degree of morphological variations. These morphological changes are also backed up by biochemical data. Notably, some activities of enzymes including catalase (CAT) and acetylcholinesterase (AChE) showed significant reduction while activity of super oxide dismutase (SOD) showed increase indicating adaptive mechanism to oxidative stress [11]. The high negative correlation values between GDI and CAT (r = -0.92), AChE (r = -0.94), and positive correlation value between

GDI and SOD (r = +0.89) indicate the inter-relationship of morphological and biochemical changes upon being exposed to effluent.

4.2.Comparison with Existing Studies

The findings from this study are supported by the earlier studies, which has revealed that there are possibilities of textile effluents causing substantial environmental toxicity. Researchers found similar effects of pollutants originating from industrial effluents such as lamellar shortening of epithelial lifting on fish (Ali et al., 2016; Wang et al., 2017). The enhancement on SOD activity in response to oxidative stress is also supported by previous studies, that identified increased levels of SOD to be a protective mechanism against free radical damage consequent to environmental toxins (Sharma et al., 2015). Besides, the recorded decreases in the CAT and AChE activities are in congruence with other studies on the neurotoxic effects of the textile effluents, which inhibit the enzymatic activities associated with detoxification and neurotransmission (Rao et al., 2018). However, the level of gill damage and level of enzyme inhibition recorded herein indicates that textile effluents can be more hazardous to the aquatic life compared to what has been reported earlier.

4.3.Implications of Findings

This study presents significant evidence of how textile effluents are toxic towards aquatic organisms, especially when it comes to gill health and enzyme activity. The drastic changes in morphology of the gills of zebrafish highlights the potential for long-term ecological damage on aquatic ecosystems exposed to non-treated textile waste. Further, the change in disposition of enzymes, particularly inhibition of CAT and AChE shows oxidative stress and neurotoxicity, which may play a role in the state of general health and survival of aquatic creatures [12]. Such findings highlight the requirements for the provision of rigid rules for the treatment and release of textile effluents in an effort to save the aquatic life. The results also bring to light the necessity of monitory of both the morphological and biochemical parameters in the determination of the industrial pollutants' impacts to the environment.

4.4.Limitations of the Study

Although a number of important conclusions regarding the toxicity of textile effluents were drawn in the present study, several limitations to the study should be discussed [13]. First, the research was carried out in a laboratory environment where it is not entirely possible to simulate the situation that exists in the real world whereby multiple environmental stressors like fluctuations in temperature and other pollutants may interact with the textile effluents. Secondly, the focus was given in the study on a single species (zebrafish), and it might be that the effects had been observed can not be generalized to other aquatic species. In addition, the study was to look at only short-term exposure (96 hours) that may not represent the far effects of continuous exposure to textile effluents. Finally, in addition to the activity of enzyme assays, other prospective biomarkers of toxicity (oxidative damage markers, or gene expression changes, etc.) were not considered in this study, and it could further delineate the mechanism of toxicity [14].

4.5.Suggestions for Future Research

Further research should examine the chronic effects of the use of textile effluents on aquatic organisms to gain an understanding of the chronic effects of environmental pollution. Studies should also focus on the possibilities of recovery in the fish population after they have already been relieved from effluent exposure. Extending the research to other aquatic species, in particular, those which are commonly found in textile-affected habitats, would allow to assess the scope of broader ecological risks [15]. In addition, studies should be centered on the identification of possible mitigation measures like the use of natural or synthetic absorbents in reducing the toxicity of textile effluents before being released. Molecular studies on mechanisms of toxicity such as genetics and proteomics may explain mechanisms of adversity of textile wastewater to aquatic life on the cellular level. Lastly, the studies should also evaluate the synergistic impacts of textile effluents with other environmental pollutants in estimating the overall impact on the aquatic systems.

5. CONCLUSION

This research was meant to assess the morphological and biochemical effects of the textile effluent exposure on zebrafish (Danio rerio), especially on the modifications of the gill tissues and enzymatic biomarkers. Examining structural damage and oxidative stress indicators at once, the investigation enabled a whole image of how textile pollutants impact the aquatic life on various bio levels.

5.1. Summary of Key Findings

The results showed highly significant, dose-related morphological damages on gill tissues of zebrafishes exposed to textile effluents. Important modifications were decreased lamellar length, enhanced epithelial lifting, hyperplasia, and lamellar fusion leading to high Gill Damage Index (GDI) scores in all groups of exposure. Biochemical assays further showed a decrease in the catalase (CAT), superoxide dismutase (SOD) and the levels of acetylcholinesterase (AChE), which could imply high oxidative stress and neurotoxicity. These findings validate that physiological impacts of experimental fish are discernible even with the exposure to textile effluents over a short period of time.

5.2. Significance of the Study

This study focuses on the ecological risks that arise from the unregulated or poorly regulated discarded textile wastewater that is released into aquatic environment. The findings of both morphological and biochemical variants of data support the claim of using zebrafish as a sensitive indicator species for environmental monitoring. Moreover, the work highlights the necessity for tighter regulatory controls and strong effluent treatment options to soften when textile industry discharges effect aquatic life.

5.3. Final Thoughts or Recommendations

Considering the intensity of detected changes it is suggested to adopt regular industrial effluents monitoring by bioassay models such as zebrafish for the purposes of environmental risk assessment. Long-term exposure effects and potential of recovery post-exposure could also be the focus of the future research. Furthermore, the effectiveness of advanced effluent treatment technologies may identify effective solutions towards reducing aquatic toxicity. The study is a wakeup call to policymakers and players in the industry to embrace sustainable practices that conserve aquatic biodiversity and health of the ecosystem.

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