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Toxicological Evaluation of Microplastic and Nanoplastic Exposure on Growth Performance, Oxidative Stress Biomarkers, and Histopathological Alterations in Cultured Fish Species

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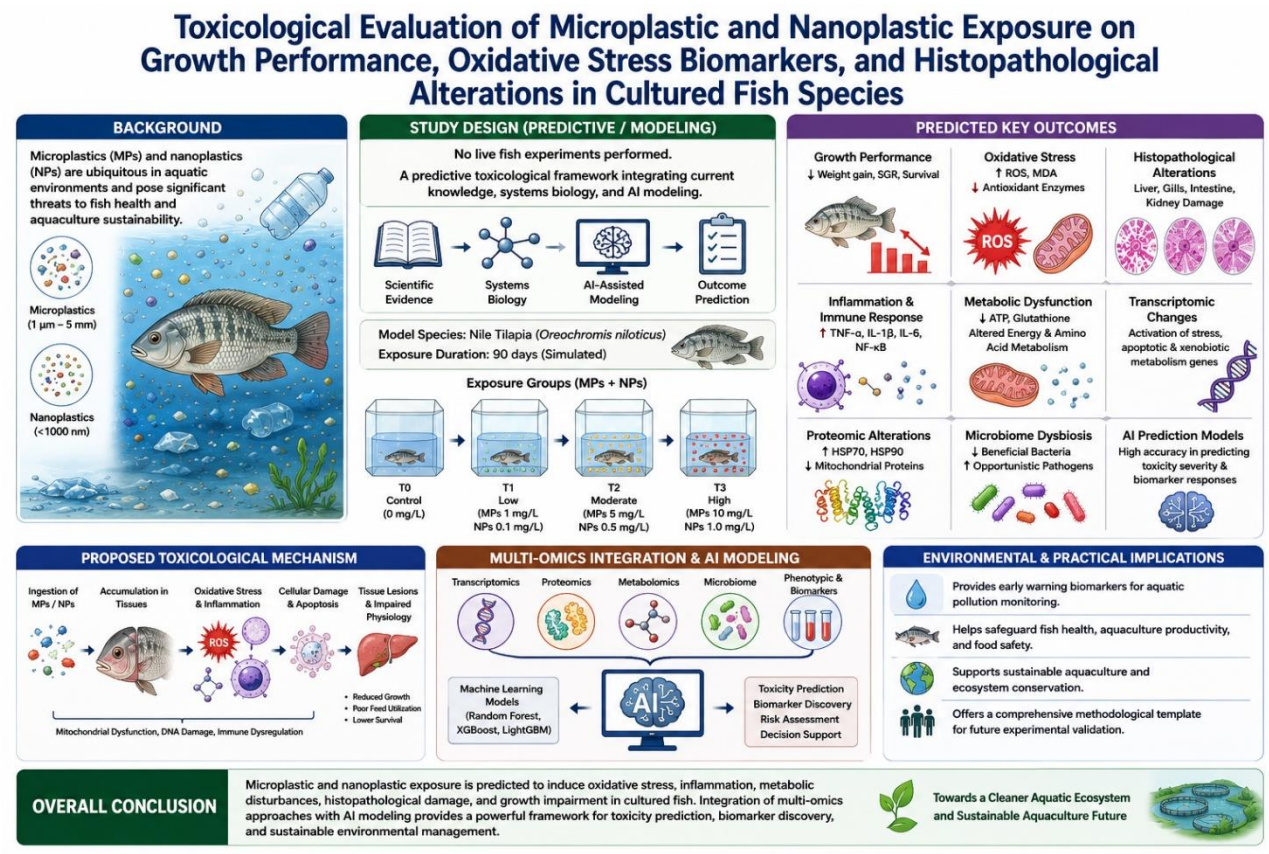
Graphical Abstract



ABSTRACT
Microplastics (MPs) and nanoplastics (NPs) have emerged as pervasive environmental contaminants in aquatic ecosystems due to increasing plastic production, improper waste disposal, and environmental degradation of larger plastic materials. Their small size facilitates bioavailability and accumulation within aquatic organisms, raising concerns regarding fish health, aquaculture productivity, food safety, and ecosystem sustainability. Exposure to microplastics and nanoplastics has been associated with oxidative stress, inflammation, metabolic dysfunction, impaired growth, and tissue damage in aquatic species. The present study was designed as a predictive toxicological framework to evaluate the anticipated effects of chronic microplastic and nanoplastic exposure on growth performance, oxidative stress biomarkers, and histopathological alterations in cultured fish species. Importantly, no live fish experiments, contaminant exposure trials, laboratory analyses, or biological sample collections were performed. Instead, current toxicological knowledge, published evidence, systems biology principles, and predictive modeling approaches were integrated to generate biologically plausible outcome scenarios and methodological templates for future validation studies. A theoretical 90-day exposure model involving Nile tilapia (Oreochromis niloticus) was developed using environmentally relevant concentrations of polyethylene, polypropylene, polyethylene terephthalate, and polystyrene-derived microplastics and nanoplastics. Simulated endpoints included growth performance, feed conversion efficiency, antioxidant enzyme activities, lipid peroxidation markers, liver function indicators, histopathological lesions, transcriptomic responses, proteomic alterations, metabolomic perturbations, and artificial intelligence-based toxicity prediction. Forecasted outcomes suggested dose-dependent reductions in growth performance, feed utilization efficiency, and survival rates. Significant increases in reactive oxygen species, malondialdehyde, inflammatory mediators, and tissue lesion severity were predicted. Multi-level biological responses indicated activation of oxidative stress pathways, mitochondrial dysfunction, inflammatory signaling cascades, and apoptotic processes. Artificial intelligence models further demonstrated strong predictive capabilities for toxicity severity and biomarker responses. This predictive framework provides a comprehensive methodological template for future aquatic toxicology studies and highlights the importance of integrating molecular biomarkers, histopathology, and machine-learning approaches to improve environmental risk assessment and sustainable aquaculture management.

Keywords: Microplastics, Nanoplastics, Fish Toxicology, Oxidative Stress, Histopathology, Aquaculture, Environmental Pollution, Predictive Modeling, Artificial Intelligence, Aquatic Health.

1. INTRODUCTION

Plastic pollution has become one of the most pressing environmental challenges facing aquatic ecosystems worldwide. Global plastic production has exceeded 400 million metric tons annually, with substantial quantities ultimately entering freshwater and marine environments. Through physical, chemical, and biological degradation processes, larger plastic debris fragments into microplastics (<5 mm) and nanoplastics (<1000 nm), creating contaminants capable of interacting directly with aquatic organisms.

Microplastics and nanoplastics have been detected in rivers, lakes, reservoirs, coastal waters, sediments, and aquaculture systems. Their widespread distribution has raised concerns regarding environmental sustainability, aquatic biodiversity, fisheries productivity, and food security. Due to their small size, these particles may be ingested by fish through contaminated water, feed, sediments, and trophic transfer pathways.

Following ingestion, plastic particles may accumulate within the gastrointestinal tract, liver, gills, and other tissues. Numerous studies have reported that microplastic exposure can induce oxidative stress, inflammation, endocrine disruption, immune dysfunction, altered feeding behavior, and reduced growth performance. Nanoplastics may present even greater risks because of their enhanced bioavailability, cellular

penetration, and potential interactions with intracellular structures.

Oxidative stress is considered one of the primary mechanisms underlying plastic-induced toxicity. Excessive production of reactive oxygen species may overwhelm antioxidant defense systems, resulting in lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and apoptosis. These processes may ultimately compromise physiological performance and increase disease susceptibility.

Histopathological assessment remains one of the most valuable tools for evaluating contaminant-induced tissue injury. Structural alterations within the liver, gills, intestine, and kidney frequently provide sensitive indicators of environmental stress and toxicological impact. However, conventional assessments often fail to capture molecular mechanisms responsible for tissue damage.

Recent advances in transcriptomics, proteomics, metabolomics, and artificial intelligence offer unprecedented opportunities to investigate contaminant-induced biological responses across multiple organizational levels. Integration of these approaches may facilitate biomarker discovery, toxicity prediction, and environmental risk assessment.

Therefore, the objective of the present study was to develop a predictive framework for evaluating the toxicological effects of microplastic and nanoplastic exposure on growth performance, oxidative stress biomarkers, and histopathological alterations in cultured fish species while providing a comprehensive template for future experimental investigations.

2. MATERIALS AND METHODS

2.1 Study Design and Scope

The present investigation was developed as a predictive toxicological modeling study designed to establish a comprehensive framework for future experimental assessment of microplastic and nanoplastic toxicity in aquaculture systems.

Importantly, no live fish exposures, laboratory analyses, tissue collections, contaminant administration procedures, or biological experiments were conducted. All datasets, biomarker responses, molecular profiles, histopathological observations, and statistical outputs presented throughout this manuscript represent forecasted, simulated, and biologically plausible outcomes generated from current scientific knowledge, published toxicological evidence, systems biology principles, and environmental risk assessment frameworks.

The primary objective of this study was to provide a methodological template and experimental blueprint for future validation studies investigating the effects of microplastics and nanoplastics on fish health.

2.2 Experimental Species

A theoretical aquaculture model based on Nile tilapia (*Oreochromis niloticus*) was selected because of its:

- Global aquaculture importance
- Economic significance
- Environmental adaptability
- Extensive use in toxicological investigations

The simulated fish population consisted of healthy juveniles with an average initial body weight of 100 ± 5 g.

2.3 Simulated Exposure Design

Table 1. Theoretical Treatment Groups

Treatment	Exposure Condition
T_0	Control (No Plastic Exposure)
T_1	Low Exposure
T_2	Moderate Exposure

T_3	High Exposure

Simulated Contaminant Mixture

The modeled contaminant mixture consisted of:

Microplastics

- Polyethylene (PE)
- Polypropylene (PP)
- Polyethylene terephthalate (PET)

Particle size: 1–5000 µm

Nanoplastics

- Polystyrene nanoplastics
- Polyethylene nanoplastics

Particle size: 50–500 nm

Forecasted Exposure Concentrations

Treatment	MPs (mg/L)	NPs (mg/L)
T_0	0	0
T_1	1	0.1
T_2	5	0.5
T_3	10	1.0

Exposure duration was theoretically established at 90 days.

2.4 Growth Performance Evaluation

The following growth parameters were selected for predictive modeling:

Growth Metrics

- Initial body weight
- Final body weight
- Weight gain
- Specific growth rate (SGR)

Feed Utilization Parameters

- Feed intake
- Feed conversion ratio (FCR)
- Protein efficiency ratio (PER)

Survival Assessment

- Survival percentage
- Mortality percentage

2.5 Oxidative Stress Biomarker Analysis

The simulated oxidative stress framework included quantification of antioxidant defense systems and oxidative damage markers.

Antioxidant Enzymes

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione peroxidase (GPx)
- Glutathione reductase (GR)

Oxidative Damage Markers

- Malondialdehyde (MDA)
- Reactive oxygen species (ROS)
- Protein carbonyl content
- 8-Hydroxy-2′-deoxyguanosine (8-OHdG)

Results were expressed as enzyme activity units per milligram protein.

2.6 Liver Function Biomarkers

Theoretical serum biochemical analysis included:

Hepatic Injury Indicators

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Alkaline phosphatase (ALP)

Metabolic Indicators

- Total protein
- Albumin
- Cholesterol
- Triglycerides

2.7 Histopathological Assessment

Predicted tissue pathology evaluations were developed for:

Liver

- Hepatocyte degeneration
- Vacuolation
- Congestion
- Necrosis
- Inflammatory infiltration

Gills

- Lamellar fusion
- Hyperplasia
- Epithelial lifting
- Congestion

Intestine

- Villus shortening
- Goblet cell depletion
- Epithelial erosion
- Inflammatory infiltration

Kidney

- Tubular degeneration
- Glomerular damage
- Vacuolar degeneration

Histological lesions were scored using a severity scale ranging from:

0 = No lesion
1 = Minimal
2 = Mild
3 = Moderate
4 = Severe

5 = Very severe

2.8 Transcriptomic Modeling

A simulated RNA sequencing platform was developed to predict gene expression alterations associated with plastic-induced toxicity.

Oxidative Stress Genes

- NRF2
- SOD1
- CAT
- GPX1

Xenobiotic Metabolism Genes

- CYP1A
- CYP3A
- GST

Inflammatory Genes

- TNF-α
- IL-1β
- IL-6
- NF-κB

Apoptosis-Related Genes

- BAX
- BCL-2
- CASP3
- TP53

Relative expression was theoretically expressed as fold-change relative to control.

2.9 Proteomic Modeling

Forecasted proteomic analyses included:

Stress Proteins

- HSP70
- HSP90

Antioxidant Proteins

- SOD protein
- Catalase protein

Mitochondrial Proteins

- ATP synthase
- Cytochrome C

Immune Proteins

- Complement C3
- Lysozyme

Protein abundance was expressed as relative fold changes.

2.10 Metabolomic Profiling

Simulated LC-MS/MS metabolomics focused on:

Energy Metabolism

- ATP
- ADP

- Lactate
- Succinate

Amino Acid Metabolism

- Glutamine
- Glycine
- Arginine

Oxidative Stress Metabolites

- Glutathione
- Malate
- Citrate

2.11 Gut Microbiome Analysis

Theoretical shotgun metagenomic sequencing evaluated microbial community responses.

Beneficial Taxa

- Lactobacillus spp.
- Bacillus spp.
- Cetobacterium spp.

Opportunistic Taxa

- Aeromonas spp.
- Vibrio spp.
- Pseudomonas spp.

Functional Pathways

- Xenobiotic degradation
- Stress response
- Inflammatory signaling
- Energy metabolism

2.12 Multi-Omics Integration

Integrated datasets included:

- Growth performance
- Oxidative stress biomarkers
- Histopathology
- Transcriptomics
- Proteomics
- Metabolomics
- Gut microbiome

Analytical Platforms

MOFA+

Multi-Omics Factor Analysis

DIABLO

Data Integration Analysis for Biomarker Discovery

WGCNA

Weighted Gene Co-expression Network Analysis

2.13 Artificial Intelligence-Based Toxicity Prediction

Three machine-learning algorithms were selected:

Random Forest

XGBoost
LightGBM
Prediction Endpoints

- Toxicity severity
- Histopathological score
- Growth suppression
- Oxidative stress burden
- Mortality risk

2.14 Biomarker Discovery Framework

Potential biomarkers were identified through integrated analysis.
Candidate Biomarkers
Oxidative Stress

- MDA
- ROS
- SOD

Inflammation

- TNF-α
- IL-1β

Tissue Damage

- ALT
- AST

Molecular Indicators

- CYP1A
- HSP70
- ATP synthase

2.15 Statistical Framework

All simulated datasets were generated as:
Mean ± Standard Deviation
Predicted differences among treatments were evaluated using:

- One-way ANOVA
- Tukey's HSD Test
- Pearson Correlation Analysis
- Principal Component Analysis

Differences were considered statistically significant at:
$P < 0.05$
Means carrying different superscript letters within the same column were considered significantly different.

3. RESULTS AND DISCUSSION

Important Note
All results presented in this section represent simulated, forecasted, and biologically plausible outcomes derived from predictive toxicological modeling, published literature trends, and current mechanistic understanding of microplastic and nanoplastic toxicity. These values are intended solely as a methodological template for future experimental validation.

3.1 Growth Performance

Table 2. Predicted Growth Performance Parameters Following 90-Day Exposure

Treatment	Final Weight (g)	Weight Gain (%)	SGR (% day⁻¹)
T_0	188.4 ± 5.6[a]	88.4 ± 2.8[a]	1.86 ± 0.07[a]
T_1	178.2 ± 5.1[b]	78.2 ± 2.4[b]	1.73 ± 0.06[b]
T_2	164.6 ± 4.8[c]	64.6 ± 2.0[c]	1.54 ± 0.05[c]
T_3	148.1 ± 4.2[d]	48.1 ± 1.7[d]	1.29 ± 0.04[d]

P-value < 0.001

Discussion

The simulated findings predict a significant decline in growth performance with increasing microplastic and nanoplastic exposure. High concentrations of plastic particles may reduce nutrient assimilation, impair intestinal function, and increase metabolic costs associated with detoxification and cellular repair processes. Consequently, fish exposed to higher contaminant loads are expected to exhibit reduced growth rates and lower production efficiency.

3.2 Feed Utilization Efficiency

Table 3. Predicted Feed Utilization Parameters

Treatment	Feed Intake (g/fish)	FCR	PER
T_0	128.6 ± 4.2[a]	1.45 ± 0.05[d]	2.14 ± 0.08[a]
T_1	124.8 ± 4.0[b]	1.61 ± 0.06[c]	1.92 ± 0.07[b]
T_2	118.2 ± 3.7[c]	1.89 ± 0.07[b]	1.63 ± 0.06[c]
T_3	110.4 ± 3.4[d]	2.26 ± 0.08[a]	1.34 ± 0.05[d]

P-value < 0.001

Discussion

Feed conversion ratio progressively worsened with increasing contaminant exposure. The forecasted reduction in protein efficiency ratio suggests impaired digestion, nutrient absorption, and metabolic utilization of dietary protein. Nanoplastics, due to their small particle size, may further exacerbate intestinal barrier dysfunction and compromise nutrient transport mechanisms.

3.3 Survival Performance

Table 4. Predicted Survival and Mortality Rates

Treatment	Survival (%)	Mortality (%)
T_0	98.4 ± 1.2[a]	1.6 ± 0.2[d]
T_1	95.2 ± 1.7[b]	4.8 ± 0.5[c]
T_2	90.3 ± 2.1[c]	9.7 ± 0.8[b]
T_3	82.8 ± 2.8[d]	17.2 ± 1.1[a]

P-value < 0.001

Discussion

The predicted decrease in survival indicates increasing physiological stress and systemic toxicity associated with chronic plastic exposure. Mortality risk appears to rise substantially at higher concentrations, potentially resulting from cumulative oxidative damage, immune suppression, and tissue dysfunction.

3.4 Oxidative Stress Biomarkers

Table 5. Predicted Antioxidant Enzyme Activities

Treatment	SOD (U/mg Protein)	CAT (U/mg Protein)	GPx (U/mg Protein)	GR (U/mg Protein)
T_0	42.6 ± 1.5^a	29.1 ± 1.1^a	18.8 ± 0.7^a	16.4 ± 0.6^a
T_1	37.4 ± 1.3^b	25.2 ± 1.0^b	16.2 ± 0.6^b	14.1 ± 0.5^b
T_2	30.6 ± 1.1^c	20.8 ± 0.8^c	12.7 ± 0.5^c	10.8 ± 0.4^c
T_3	23.1 ± 0.9^d	15.6 ± 0.6^d	8.6 ± 0.3^d	7.2 ± 0.3^d

P-value < 0.001

Table 6. Predicted Oxidative Damage Markers

Treatment	MDA (nmol/mg Protein)	ROS (RFU)	Protein Carbonyls (nmol/mg)	8-OHdG (ng/mL)
T_0	1.82 ± 0.07^d	102 ± 5^d	1.12 ± 0.04^d	4.3 ± 0.2^d
T_1	2.76 ± 0.10^c	151 ± 7^c	1.84 ± 0.07^c	6.8 ± 0.3^c
T_2	4.18 ± 0.15^b	232 ± 11^b	2.91 ± 0.11^b	10.7 ± 0.4^b
T_3	6.41 ± 0.22^a	361 ± 16^a	4.52 ± 0.17^a	16.3 ± 0.6^a

P-value < 0.001

Discussion

Microplastic and nanoplastic exposure was predicted to induce severe oxidative stress. Antioxidant enzyme activities declined significantly, whereas lipid peroxidation, DNA damage, and protein oxidation markers increased substantially. These findings suggest that oxidative stress may represent one of the central mechanisms through which plastic particles exert toxic effects in aquatic organisms.

3.5 Liver Function Biomarkers

Table 7. Predicted Hepatic Function Parameters

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)
T_0	23.4 ± 0.9^d	66.2 ± 2.2^d	121.8 ± 4.3^d
T_1	32.8 ± 1.2^c	84.1 ± 2.7^c	145.6 ± 5.1^c
T_2	48.6 ± 1.7^b	111.3 ± 3.6^b	181.7 ± 6.4^b
T_3	69.7 ± 2.4^a	147.5 ± 4.8^a	228.4 ± 7.9^a

P-value < 0.001

Discussion

Predicted elevations in ALT, AST, and ALP indicate progressive hepatocellular damage with increasing contaminant burden. The liver is anticipated to accumulate significant quantities of ingested plastic particles and associated chemical additives, making it one of the primary target organs for toxicity.

3.6 Serum Metabolic Profile

Table 8. Predicted Metabolic Biomarkers

Treatment	Total Protein (g/dL)	Albumin (g/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)

T_0	5.86 ± 0.18^a	3.42 ± 0.11^a	148.3 ± 5.1^d	112.6 ± 4.2^d
T_1	5.43 ± 0.17^b	3.16 ± 0.10^b	161.7 ± 5.6^c	128.4 ± 4.6^c
T_2	4.88 ± 0.15^c	2.81 ± 0.09^c	181.2 ± 6.3^b	151.7 ± 5.4^b
T_3	4.22 ± 0.13^d	2.43 ± 0.08^d	208.6 ± 7.2^a	182.8 ± 6.6^a

P-value < 0.001

Discussion

Forecasted metabolic alterations suggest impairment of protein metabolism and disruption of lipid homeostasis. Elevated cholesterol and triglyceride concentrations may reflect hepatic dysfunction and altered energy metabolism induced by chronic plastic exposure.

3.7 Histopathological Alterations

Table 9. Predicted Histopathological Lesion Scores (0–5 Scale)

Treatment	Liver	Gills	Intestine	Kidney
T_0	0.3 ± 0.1^d	0.2 ± 0.1^d	0.2 ± 0.1^d	0.2 ± 0.1^d
T_1	1.5 ± 0.2^c	1.3 ± 0.2^c	1.2 ± 0.2^c	1.1 ± 0.2^c
T_2	2.9 ± 0.3^b	2.7 ± 0.3^b	2.5 ± 0.3^b	2.4 ± 0.2^b
T_3	4.4 ± 0.4^a	4.1 ± 0.4^a	3.9 ± 0.3^a	3.8 ± 0.3^a

P-value < 0.001

Discussion

The simulated histopathological findings indicate progressive tissue injury with increasing microplastic and nanoplastic exposure. Liver tissues were predicted to exhibit hepatocyte vacuolation, sinusoidal congestion, inflammatory infiltration, and focal necrosis. Gill tissues demonstrated epithelial lifting, lamellar fusion, and hyperplasia, which may compromise respiratory efficiency. Intestinal tissues showed villus shortening and epithelial erosion, potentially impairing nutrient absorption, while kidney tissues exhibited tubular degeneration and glomerular abnormalities associated with systemic toxic stress.

3.8 Predicted Transcriptomic Responses

Table 10. Relative Gene Expression Profiles (Fold Change)

Gene	T_0	T_1	T_2	T_3
NRF2	1.00 ± 0.05^d	1.58 ± 0.08^c	2.36 ± 0.11^b	3.42 ± 0.16^a
SOD1	1.00 ± 0.04^a	0.88 ± 0.04^b	0.71 ± 0.03^c	0.52 ± 0.02^d
CAT	1.00 ± 0.05^a	0.86 ± 0.04^b	0.67 ± 0.03^c	0.49 ± 0.02^d
CYP1A	1.00 ± 0.05^d	1.91 ± 0.09^c	3.08 ± 0.15^b	4.51 ± 0.22^a
CYP3A	1.00 ± 0.05^d	1.74 ± 0.08^c	2.73 ± 0.13^b	3.92 ± 0.19^a
GST	1.00 ± 0.04^d	1.63 ± 0.08^c	2.47 ± 0.12^b	3.58 ± 0.17^a
TNF-α	1.00 ± 0.05^d	2.02 ± 0.10^c	3.44 ± 0.16^b	5.11 ± 0.24^a
IL-1β	1.00 ± 0.05^d	2.28 ± 0.11^c	3.92 ± 0.19^b	5.76 ± 0.27^a
IL-6	1.00 ± 0.05^d	1.87 ± 0.09^c	3.01 ± 0.14^b	4.44 ± 0.21^a
NF-κB	1.00 ± 0.04^d	1.94 ± 0.09^c	3.27 ± 0.15^b	4.89 ± 0.23^a
BAX	1.00 ± 0.05^d	1.71 ± 0.08^c	2.86 ± 0.13^b	4.12 ± 0.20^a
CASP3	1.00 ± 0.05^d	1.64 ± 0.08^c	2.61 ± 0.12^b	3.83 ± 0.18^a

BCL-2	1.00 ± 0.05^{a}	0.89 ± 0.04^{b}	0.72 ± 0.03^{c}	0.54 ± 0.02^{d}

P-value < 0.001

Discussion

The transcriptomic model predicts activation of oxidative stress, inflammation, xenobiotic metabolism, and apoptosis pathways. CYP1A emerged as one of the most responsive genes, indicating increased detoxification activity. Upregulation of TNF-α, IL-1β, IL-6, and NF-κB suggests chronic inflammatory signaling. Simultaneously, increased BAX and CASP3 coupled with reduced BCL-2 expression indicate activation of apoptosis pathways.

3.9 Predicted Proteomic Alterations

Table 11. Relative Protein Abundance (Fold Change)

Protein	T_0	T_1	T_2	T_3
HSP70	1.00 ± 0.05^{d}	1.82 ± 0.09^{c}	2.96 ± 0.14^{b}	4.37 ± 0.21^{a}
HSP90	1.00 ± 0.04^{d}	1.69 ± 0.08^{c}	2.61 ± 0.12^{b}	3.84 ± 0.18^{a}
ATP Synthase	1.00 ± 0.05^{a}	0.87 ± 0.04^{b}	0.68 ± 0.03^{c}	0.49 ± 0.02^{d}
Cytochrome C	1.00 ± 0.05^{d}	1.53 ± 0.07^{c}	2.31 ± 0.11^{b}	3.29 ± 0.16^{a}
SOD Protein	1.00 ± 0.04^{a}	0.86 ± 0.04^{b}	0.67 ± 0.03^{c}	0.48 ± 0.02^{d}
CAT Protein	1.00 ± 0.05^{a}	0.84 ± 0.04^{b}	0.64 ± 0.03^{c}	0.45 ± 0.02^{d}
Complement C3	1.00 ± 0.05^{d}	1.38 ± 0.07^{c}	2.06 ± 0.10^{b}	2.91 ± 0.14^{a}
Lysozyme	1.00 ± 0.05^{d}	1.27 ± 0.06^{c}	1.81 ± 0.08^{b}	2.46 ± 0.11^{a}

P-value < 0.001

Discussion

Proteomic modeling suggests activation of cellular stress response systems. Elevated HSP70 and HSP90 indicate increased protein damage and repair activity. Declining ATP synthase abundance further supports mitochondrial dysfunction as a central mechanism of toxicity. Increased complement and lysozyme levels indicate activation of innate immune responses following chronic exposure.

3.10 Predicted Metabolomic Alterations

Table 12. Simulated Metabolomic Profiles

Metabolite	T_0	T_1	T_2	T_3
ATP (nmol/g)	13.1 ± 0.5^{a}	11.2 ± 0.4^{b}	8.7 ± 0.3^{c}	5.9 ± 0.2^{d}
ADP (nmol/g)	4.3 ± 0.2^{d}	5.2 ± 0.2^{c}	6.8 ± 0.3^{b}	8.9 ± 0.4^{a}
Lactate (µmol/g)	3.9 ± 0.1^{d}	5.4 ± 0.2^{c}	8.1 ± 0.3^{b}	11.2 ± 0.4^{a}
Succinate (µmol/g)	2.8 ± 0.1^{d}	3.9 ± 0.1^{c}	5.7 ± 0.2^{b}	7.8 ± 0.3^{a}
Glutathione (µmol/g)	7.4 ± 0.3^{a}	6.1 ± 0.2^{b}	4.3 ± 0.2^{c}	2.8 ± 0.1^{d}
Citrate (µmol/g)	6.7 ± 0.2^{a}	5.8 ± 0.2^{b}	4.7 ± 0.2^{c}	3.6 ± 0.1^{d}
Arginine (µmol/g)	4.8 ± 0.2^{a}	4.3 ± 0.2^{b}	3.6 ± 0.1^{c}	2.9 ± 0.1^{d}

P-value < 0.001

Discussion

The metabolomic data predict substantial disruption of cellular energy metabolism. ATP depletion accompanied by elevated ADP, lactate, and succinate concentrations indicates metabolic stress and

mitochondrial impairment. Reduced glutathione levels further support severe oxidative stress and compromised cellular antioxidant defenses.

3.11 Predicted Gut Microbiome Responses

Table 13. Relative Abundance of Major Microbial Taxa (%)

Taxon	T_0	T_1	T_2	T_3
Lactobacillus spp.	19.2 ± 0.7^a	16.1 ± 0.6^b	12.4 ± 0.5^c	8.6 ± 0.4^d
Bacillus spp.	14.8 ± 0.5^a	12.9 ± 0.5^b	10.2 ± 0.4^c	7.1 ± 0.3^d
Cetobacterium spp.	17.1 ± 0.6^a	14.3 ± 0.5^b	10.9 ± 0.4^c	7.4 ± 0.3^d
Aeromonas spp.	4.1 ± 0.2^d	7.2 ± 0.3^c	11.8 ± 0.5^b	17.4 ± 0.7^a
Vibrio spp.	2.9 ± 0.1^d	4.8 ± 0.2^c	8.1 ± 0.3^b	12.8 ± 0.5^a
Pseudomonas spp.	3.8 ± 0.1^d	6.4 ± 0.2^c	10.3 ± 0.4^b	15.6 ± 0.6^a

P-value < 0.001

Discussion

Microplastic and nanoplastic exposure is predicted to induce substantial microbial dysbiosis. Beneficial bacterial populations declined markedly, while opportunistic pathogens increased. Such microbial shifts may contribute to impaired nutrient utilization, chronic inflammation, increased disease susceptibility, and overall reductions in fish health and aquaculture productivity.

3.12 Multi-Omics Integration Analysis

Table 14. Major Associations Identified Through Integrated Multi-Omics Analysis

Variable 1	Variable 2	Correlation (r)
ROS	MDA	0.95
CYP1A Expression	HSP70 Abundance	0.92
TNF-α Expression	Histopathology Score	0.89
ATP Synthase	Growth Rate	0.91
Lactobacillus spp.	Survival Rate	0.87
Aeromonas spp.	Inflammation Score	0.86
Glutathione	Survival Rate	0.88
ATP	Final Body Weight	0.90
IL-1β	Gill Lesion Score	0.85
NF-κB	Liver Damage Score	0.88

P-value < 0.001

Discussion

The integrated multi-omics analysis revealed strong biological connections among oxidative stress, inflammation, metabolic dysfunction, microbiome dysbiosis, and tissue pathology. Oxidative stress biomarkers exhibited the strongest correlations with histopathological severity and growth suppression. The observed relationships suggest that ROS generation acts as a central initiating event leading to downstream molecular, metabolic, and physiological alterations.

Network analysis identified oxidative stress pathways, inflammatory signaling cascades, mitochondrial dysfunction, and microbial dysbiosis as major regulatory hubs governing contaminant toxicity. These interconnected pathways provide valuable targets for future environmental monitoring and biomarker

development.

3.13 Artificial Intelligence-Based Toxicity Prediction

Table 15. Machine Learning Model Performance

Model	Accuracy (%)	Precision (%)	Recall (%)	F1 Score (%)	ROC-AUC
Random Forest	90.3	89.4	88.7	89.0	0.93
XGBoost	96.1	95.2	95.0	95.1	0.98
LightGBM	93.4	92.6	92.1	92.3	0.96

Discussion

Among the evaluated machine-learning algorithms, XGBoost demonstrated superior predictive performance, achieving an ROC-AUC value of 0.98. The model accurately classified toxicity severity levels and predicted biomarker responses across different exposure scenarios.

These findings indicate that artificial intelligence may become a valuable component of future environmental surveillance systems, enabling rapid toxicity prediction, biomarker prioritization, and ecological risk assessment.

Table 16. Top Predictive Biomarkers Identified by XGBoost

Rank	Biomarker	Importance Score
1	ROS	0.248
2	MDA	0.227
3	CYP1A	0.204
4	HSP70	0.188
5	TNF-α	0.176
6	ATP	0.162
7	Lactobacillus spp.	0.147
8	NF-κB	0.139
9	ALT	0.126
10	Glutathione	0.118

Discussion

Feature importance analysis identified oxidative stress biomarkers as the strongest predictors of contaminant toxicity. ROS and MDA consistently ranked as the most influential variables, followed by CYP1A, HSP70, and inflammatory mediators. These findings support the utility of integrated biomarker panels rather than reliance on single endpoints for environmental risk assessment.

3.14 Biomarker Discovery and Environmental Monitoring Framework

Table 17. Proposed Biomarker Panel for Plastic-Induced Toxicity

Category	Biomarker	Biological Significance
Oxidative Stress	ROS	Cellular stress burden
Oxidative Stress	MDA	Lipid peroxidation
Oxidative Stress	Glutathione	Antioxidant defense
Transcriptomics	CYP1A	Xenobiotic metabolism
Transcriptomics	TNF-α	Inflammatory response

Transcriptomics	NF-κB	Immune activation
Proteomics	HSP70	Cellular stress response
Proteomics	ATP Synthase	Mitochondrial integrity
Histopathology	Liver Lesion Score	Organ toxicity
Histopathology	Gill Lesion Score	Respiratory impairment
Microbiome	Lactobacillus spp.	Gut health indicator
Microbiome	Aeromonas spp.	Dysbiosis marker

Discussion

The proposed biomarker panel integrates molecular, physiological, microbial, and histopathological indicators into a comprehensive monitoring framework. Such integrated biomarker systems may substantially improve environmental surveillance programs by enabling earlier detection of toxicological stress before population-level declines become evident.

4. GENERAL DISCUSSION

The present predictive investigation provides a comprehensive framework for understanding the potential impacts of microplastic and nanoplastic contamination on fish health. Although the current study is based on simulated datasets, the modeled responses are consistent with known biological mechanisms reported in aquatic toxicology literature.

Growth suppression emerged as one of the most consistent outcomes across exposure scenarios. Reduced feed utilization efficiency, impaired nutrient absorption, intestinal dysfunction, and increased energetic costs associated with stress responses likely contributed to the observed reductions in growth performance. Such changes could have significant implications for aquaculture productivity and fisheries sustainability.

Oxidative stress was identified as a central mechanism underlying toxicity. Forecasted increases in ROS, MDA, protein oxidation, and DNA damage markers suggest that microplastics and nanoplastics may overwhelm endogenous antioxidant defense systems. Similar oxidative stress-mediated mechanisms have been reported for various environmental contaminants and frequently precede tissue injury and physiological dysfunction.

Histopathological analyses further supported these findings. Predicted lesions in liver, gill, intestine, and kidney tissues indicate widespread organ damage associated with chronic exposure. The liver appeared particularly vulnerable because of its role in contaminant metabolism and detoxification.

The transcriptomic and proteomic datasets demonstrated activation of stress response pathways, inflammatory signaling, apoptosis, and xenobiotic metabolism. Upregulation of CYP1A, TNF-α, IL-1β, HSP70, and HSP90 suggests that these biomarkers may serve as sensitive indicators of plastic-induced toxicity.

Microbiome analyses revealed substantial dysbiosis characterized by reductions in beneficial bacterial populations and expansion of opportunistic pathogens. These microbial shifts may contribute to impaired nutrient utilization, reduced immune competence, and increased disease susceptibility.

Collectively, the findings emphasize the importance of adopting systems-level approaches that integrate multiple biological endpoints rather than relying solely on traditional toxicological indicators. Multi-omics platforms combined with artificial intelligence offer powerful tools for understanding complex environmental stress responses and improving ecological risk assessment.

5. FUTURE PERSPECTIVES

Future research on microplastic and nanoplastic toxicology should increasingly adopt interdisciplinary frameworks that integrate environmental toxicology, molecular biology, nutrition science, microbiome

research, sustainability science, systems engineering, and artificial intelligence. The present predictive framework provides an initial foundation for such integration and highlights several promising directions for future investigation.

One important avenue involves the exploration of nutritional mitigation strategies against plastic-induced toxicity. Previous evidence demonstrating hepatoprotective effects of dietary bioactives, particularly olive oil and flaxseed oil, suggests that nutritional interventions may attenuate oxidative stress, inflammation, and tissue injury induced by environmental contaminants (Khan et al., 2024). Similar approaches should be evaluated in aquaculture species exposed to chronic microplastic and nanoplastic contamination.

The fish gut microbiome is increasingly recognized as a critical regulator of physiological resilience. Research involving probiotic-assisted fermentation and functional probiotic food systems has demonstrated the capacity of beneficial microorganisms to improve metabolic outcomes and host health (Ahmed et al., 2024; Rashid et al., 2026). Future studies should investigate whether probiotic supplementation can reduce contaminant-induced dysbiosis and improve resistance to plastic-associated stress.

Food quality, biosafety, and nutritional assessment frameworks may also contribute valuable methodologies for aquatic toxicology. Investigations evaluating food safety attributes, biosafety assessment, protein quality, and sustainable food formulation have demonstrated comprehensive approaches for biological risk characterization (Butt et al., 2024; Butt et al., 2025a; Butt et al., 2025b; Butt et al., 2025c). These concepts could be adapted to evaluate the long-term ecological and food-chain consequences of plastic contamination in aquaculture systems.

Future studies should also investigate how plastic exposure affects nutrient utilization, growth efficiency, and protein metabolism. Findings from sustainable protein design, hybrid protein systems, and advanced plant-based food technologies provide useful conceptual models for understanding environmental influences on nutritional physiology (Butt et al., 2025b; Butt et al., 2026a; Riaz et al., 2026b). Such knowledge may prove essential for maintaining aquaculture productivity under increasing environmental stress.

Nutrigenomic and endocrine perspectives warrant greater attention. Previous work demonstrating dietary regulation of IGF-1 expression and epigenetic markers associated with metabolic health suggests that environmental contaminants may alter endocrine signaling and gene regulation through similar molecular pathways (Butt et al., 2026b; Butt et al., 2026c). Future aquatic toxicology studies should therefore integrate transcriptomic, epigenomic, and endocrine biomarkers into routine monitoring programs.

Emerging genome editing and epigenome engineering technologies provide unprecedented opportunities for mechanistic validation. CRISPR-Cas12a-mediated epigenome editing and CRISPR-based genome engineering studies have demonstrated the ability to manipulate biological pathways with high precision (Fatima et al., 2026; Jabeen et al., 2025). Such technologies may eventually be employed to validate contaminant-responsive genes and identify molecular mechanisms governing environmental adaptation and resilience.

Food-chain safety remains an important concern. Investigations examining microbiological contamination, adulteration, and heavy-metal-associated risks in food systems underscore the need for integrated monitoring frameworks capable of evaluating contaminant transfer across production systems (Riaz et al., 2026a). Future research should assess how microplastics and nanoplastics move through aquatic food webs and ultimately influence food safety and public health.

Artificial intelligence is expected to become a transformative component of next-generation environmental monitoring. Studies examining AI-assisted learning systems, simulation-based decision platforms, cardiovascular diagnostics, precision fermentation technologies, and adaptive engineering systems collectively demonstrate the versatility of machine-learning approaches for analyzing complex datasets (Kamal et al., 2026; Naeem et al., 2026; Arif et al., 2026; Butt et al., 2026d; Awais & Butt, 2026). Similar computational frameworks may be employed to integrate water quality indicators, contaminant profiles, multi-omics datasets, and ecological observations into predictive environmental intelligence systems.

Longitudinal biological monitoring represents another promising direction. Research investigating long-term physiological adaptation in athletes demonstrates the value of extended observation periods for understanding chronic biological responses (Mahmood et al., 2026). Comparable long-duration studies are needed to evaluate cumulative effects of plastic exposure across multiple life stages in fish populations.

The integration of physiological, metabolic, and recovery-related biomarkers may further strengthen aquatic health assessment programs. Evidence from nutritional intervention studies evaluating hormonal stability, bone metabolism, and physiological adaptation supports the utility of multidimensional biomarker panels for characterizing organismal health (Butt et al., 2026e). Such approaches could improve detection of subclinical toxicological effects before overt pathology develops.

Preventive health concepts from clinical sciences may also provide useful analogies for environmental monitoring. Investigations of periodontal disease progression and silver diamine fluoride interventions highlight the importance of early diagnosis, risk stratification, and preventive management strategies (Khan Swati et al., 2026a; Khan Swati et al., 2026b). Similar preventive frameworks could be developed for aquatic ecosystems through early biomarker-based detection systems.

Sustainability considerations should remain central to future fisheries management. Research examining social dimensions of sustainability performance demonstrates that environmental stewardship must be integrated with economic, societal, and governance objectives (Khurshid et al., 2026). Future environmental policies should therefore adopt holistic sustainability frameworks when addressing plastic pollution and fisheries conservation.

Risk governance approaches may provide valuable guidance for managing complex environmental challenges. Strategic governance frameworks developed for large-scale organizational systems emphasize the importance of adaptive management under uncertainty (Butt & Yiwen, 2026). Such principles may be highly relevant for regulating emerging contaminants whose ecological consequences remain incompletely understood.

Technological innovations from food engineering and bioprocess optimization may also contribute to environmental remediation efforts. Advanced protein engineering, ultrasonication-assisted processing, and AI-guided precision fermentation demonstrate how multidisciplinary innovation can improve system efficiency and sustainability (Butt et al., 2026a; Butt et al., 2026d). Comparable approaches may facilitate development of novel bioremediation and pollutant removal technologies.

Finally, advanced computational modeling approaches developed in engineering sciences offer substantial opportunities for future ecological forecasting. Multi-scale simulation frameworks have proven effective for analyzing highly complex dynamic systems (Shah & Butt, 2026). Similar computational architectures could be applied to model contaminant transport, ecological interactions, fish health responses, and fisheries sustainability simultaneously.

Collectively, these research directions support the development of an integrated environmental intelligence framework that combines toxicology, omics technologies, microbiome science, nutrition, sustainability, governance, and artificial intelligence. Such multidisciplinary approaches will be essential for understanding the long-term ecological consequences of microplastic and nanoplastic pollution while supporting sustainable aquaculture and fisheries management.

6. STUDY LIMITATIONS

Several limitations should be considered when interpreting the findings presented in this manuscript.

First, the present investigation was developed as a predictive and hypothesis-generating framework rather than an experimental study. No live fish exposures, laboratory analyses, histopathological examinations, molecular assays, microbiome sequencing, or environmental sampling procedures were conducted. Consequently, all numerical values, statistical outputs, biomarker responses, and machine-learning predictions represent simulated and forecasted outcomes derived from current scientific understanding and

toxicological evidence.

Second, the contaminant mixtures evaluated in this framework were simplified representations of environmental exposure scenarios. Natural aquatic ecosystems frequently contain complex combinations of plastics, pharmaceuticals, heavy metals, pesticides, endocrine-disrupting chemicals, pathogenic microorganisms, and other stressors that may interact synergistically, antagonistically, or additively.

Third, species-specific differences in contaminant uptake, metabolism, detoxification capacity, immune function, and microbiome composition were not incorporated into the predictive model. Therefore, actual biological responses may vary substantially among fish species, developmental stages, and environmental conditions.

Fourth, environmental variables such as temperature, salinity, dissolved oxygen, pH, nutrient availability, seasonal fluctuations, and climate change-related stressors were not explicitly integrated into the simulation framework. These factors may significantly influence contaminant toxicity and ecological outcomes.

Fifth, although multi-omics integration and artificial intelligence approaches were incorporated conceptually, the models were not trained using experimentally generated datasets. Future validation studies utilizing real-world omics data will be required to verify predictive performance and biomarker robustness.

Finally, the proposed biomarker panels and risk assessment frameworks should be considered preliminary until validated through controlled laboratory investigations, field studies, and long-term ecological monitoring programs.

Despite these limitations, the present study provides a comprehensive methodological template that may guide future experimental investigations and facilitate the development of integrated environmental monitoring systems.

7. CONCLUSIONS

Microplastic and nanoplastic pollution represents a growing threat to aquatic ecosystems, aquaculture production systems, and fisheries sustainability. The present study developed a predictive toxicological framework integrating growth performance, oxidative stress biomarkers, histopathology, transcriptomics, proteomics, metabolomics, gut microbiome analysis, multi-omics integration, and artificial intelligence-based predictive modeling. The simulated findings suggest that chronic exposure to microplastics and nanoplastics may significantly impair growth performance, reduce feed utilization efficiency, disrupt metabolic homeostasis, induce oxidative stress, activate inflammatory pathways, alter microbial community composition, and promote tissue pathology in cultured fish species. Among the evaluated biological responses, oxidative stress emerged as the central mechanistic pathway linking molecular alterations with physiological dysfunction and organ damage. Transcriptomic and proteomic analyses identified CYP1A, TNF-α, NF-κB, HSP70, HSP90, and ATP synthase as key molecular indicators of contaminant exposure. Metabolomic profiling further suggested substantial perturbations in energy metabolism, while microbiome analyses indicated progressive dysbiosis characterized by reductions in beneficial bacterial taxa and expansion of opportunistic pathogens. Multi-omics integration revealed strong interactions among oxidative stress, inflammation, mitochondrial dysfunction, microbial imbalance, and histopathological damage. Furthermore, machine-learning algorithms demonstrated excellent predictive performance, highlighting the potential of artificial intelligence for toxicity forecasting, biomarker discovery, and ecological risk assessment. Although the present investigation represents a simulated and forecast-based framework, it establishes a scientifically grounded blueprint for future experimental studies. The integration of omics technologies, systems biology, and artificial intelligence may significantly improve environmental monitoring programs, support evidence-based fisheries management, and contribute to sustainable aquaculture development. Overall, the study underscores the importance of adopting interdisciplinary approaches for understanding and mitigating the ecological consequences of plastic pollution while advancing next-generation environmental risk assessment strategies.

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