

Physicochemical Characteristics of Microplastics and Their Effect on Oxidative Stress and Biochemical Responses in Freshwater Fish

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Abstract— Microplastics (MPs) constitute a persistent environmental stressor, exhibiting complex physicochemical behaviors that dictate their bioavailability and ecotoxicity in aquatic ecosystems. While the global proliferation of synthetic polymers is well-documented, the precise mechanistic links between the surface chemistry of microparticles and induced physiological disruptions in freshwater teleosts remain incompletely understood. The present study evaluates the physicochemical characteristics of pristine and environmentally weathered polystyrene (PS) and polyethylene (PE) microplastics, explicitly quantifying their surface functionalization, hydrodynamic diameter, and crystallinity. Utilizing a controlled 21-day continuous flow-through exposure model with *Cyprinus carpio*, this investigation quantifies alterations in hepatic and branchial oxidative stress biomarkers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA). Fourier-transform infrared spectroscopy (FTIR) established a significantly elevated carbonyl index in weathered PS, correlating with augmented reactive oxygen species (ROS) generation in hepatic tissues. The findings indicate that surface oxidation and increased specific surface area—resulting from photo-degradation and mechanical attrition—dramatically amplify the toxicodynamic profile of these polymers. Statistical analysis reveals a biphasic antioxidant response, characterized by initial enzymatic upregulation followed by severe depletion and corresponding lipid peroxidation at higher exposure concentrations (500 µg/L). Ultimately, the data establish a direct relationship between the innate polymer chemistry of weathered microplastics and the severity of oxidative damage in aquatic organisms, highlighting the inadequacy of assessing microplastic toxicity solely based on particle size or nominal concentration.

Keywords— Microplastics, Oxidative stress, Freshwater fish, Polymer chemistry, Biomarkers, Ecotoxicology.

I. INTRODUCTION

The ubiquitous distribution of synthetic polymers across global aquatic environments presents an unprecedented ecotoxicological challenge. Among these contaminants, microplastics (MPs)—defined as polymeric particles with dimensions inferior to 5 millimeters—have emerged as a pervasive threat to freshwater ecosystems. While historical ecotoxicological assessments predominantly concentrated on marine biomes, contemporary research emphasizes that freshwater reservoirs, rivers, and lakes function as primary conduits and terminal sinks for terrestrial plastic debris. The structural integrity and inherent recalcitrance of prevalent polymers, notably polyethylene (PE) and polystyrene (PS), ensure their

environmental persistence, facilitating chronic exposure scenarios for endemic aquatic biota.

Upon entering aquatic systems, macroscopic plastic litter undergoes continuous mechanical attrition and photolytic degradation, progressively fragmenting into micro- and nanoplastics. This environmental weathering dramatically alters the fundamental physicochemical identity of the particles. Pristine polymers, characterized by uniform hydrophobic surfaces and relatively inert chemical profiles, gradually acquire oxygen-rich functional groups—such as carbonyl, hydroxyl, and carboxyl moieties—through extensive oxidative degradation. Consequently, weathered microplastics exhibit augmented specific surface areas, enhanced polarity, and distinct electrostatic profiles compared to their virgin counterparts. These molecular transformations theoretically modulate

the behavior of the particles at the bio-nano interface, dictating their aggregation dynamics, sorption capacities for co-contaminants, and intrinsic biological reactivity.

Freshwater teleosts, representing crucial trophic nodes in aquatic food webs, are particularly susceptible to microplastic ingestion. Species such as the common carp (*Cyprinus carpio*) frequently ingest micro-scale polymers suspended in the water column or deposited within benthic sediments. Following ingestion, the abrasive nature and chemical composition of the particles precipitate severe gastrointestinal mechanical injury. Furthermore, emerging evidence indicates that particles within specific size thresholds can translocate across the intestinal epithelium via paracellular and transcellular pathways, disseminating through the circulatory system to accumulate in secondary detoxification organs, primarily the liver and gills.

The intra-tissue accumulation of microplastics provokes a cascade of cytotoxic responses, predominantly mediated through the excessive generation of reactive oxygen species (ROS). The resulting disruption of redox homeostasis instigates oxidative stress, challenging the organism's endogenous antioxidant defense mechanisms. Key metalloenzymes, including superoxide dismutase (SOD) and catalase (CAT), alongside glutathione peroxidase (GPx), constitute the primary biochemical defense against radical-induced macromolecular damage. When ROS production overwhelms this enzymatic capacity, severe lipid peroxidation occurs, compromising membrane integrity and generating secondary toxic metabolites such as malondialdehyde (MDA).

Despite substantial progress in understanding microplastic-induced oxidative stress, current ecotoxicological paradigms frequently isolate the physical characteristics of the particles—such as size and concentration—from the intrinsic chemical attributes of the polymer matrix. The precise mechanisms by which surface functionalization, polymer crystallinity, and weathering-induced chemical shifts drive specific toxicodynamics remain poorly elucidated. To address this critical knowledge gap, the present study comprehensively evaluates the interplay between the physicochemical properties of pristine and weathered PE and PS microplastics and their specific capacity to induce enzymatic alterations and oxidative damage in *Cyprinus carpio*.

II. MATERIALS AND METHODS

A. Physicochemical Characterization of Microplastics

Pristine polyethylene (PE) and polystyrene (PS) microspheres (nominal diameter 10-50 μm) were obtained

from Sigma-Aldrich. To simulate environmental degradation, a subset of the polymers was subjected to accelerated artificial weathering. The particles were suspended in high-purity deionized water and continuously irradiated using a xenon arc lamp (irradiance 60 W/m²) for 30 days, maintaining a stable temperature of 25°C. Following the weathering protocol, the structural modifications and surface chemistry of both pristine and aged particles were systematically characterized.

Fourier-transform infrared spectroscopy (FTIR) was executed utilizing a Nicolet iS50 spectrometer equipped with an attenuated total reflectance (ATR) module. Spectra were recorded across a wavenumber range of 4000 to 400 cm⁻¹ with a resolution of 4 cm⁻¹. The carbonyl index (CI), functioning as a primary metric of oxidative degradation, was calculated by determining the ratio of the absorbance intensity of the carbonyl band (approximately 1715 cm⁻¹) to the reference methylene band (1465 cm⁻¹).

Morphological and topographic alterations were documented via scanning electron microscopy (SEM) using a Zeiss Auriga instrument. Prior to imaging, samples were sputter-coated with a thin gold-palladium layer to mitigate electron charging effects. Furthermore, the hydrodynamic diameter and effective zeta potential of the microplastics dispersed in the standard exposure medium were quantified through dynamic light scattering (DLS) utilizing a Malvern Zetasizer Nano ZS, ensuring precise measurement of their aggregation behaviors under experimental conditions.

B. Experimental Design and Fish Acclimation

Juvenile common carp (*Cyprinus carpio*), matched for size and weight (average mass 12.5 $\pm$ 1.2 g), were procured from a certified commercial hatchery. Prior to experimental exposure, the specimens were subjected to a 14-day acclimation period in 250 L glass aquaria equipped with continuous bio-filtration. The holding tanks were maintained under a standardized 12:12 hour light/dark photoperiod. Water quality parameters were rigorously controlled: temperature was held at 22 $\pm$ 1°C, pH at 7.4 $\pm$ 0.2, dissolved oxygen (DO) above 7.0 mg/L, and unionized ammonia maintained below 0.05 mg/L.

The *in vivo* exposure bioassay was conducted in a continuous flow-through aquatic system to ensure steady-state toxicant concentrations. The fish were randomly assigned to five distinct experimental cohorts (n = 30 per group, distributed across triplicate tanks): an unexposed control, PE-pristine, PE-weathered, PS-pristine, and PS-weathered. The microplastic concentration for all treatment groups was maintained at 500 $\mu\text{g/L}$, a

concentration reflective of severe contamination scenarios in highly industrialized riverine systems. The exposure duration was strictly maintained for 21 days.

C. Biochemical Assays

1) Tissue Extraction and Processing

Upon completion of the 21-day exposure interval, fish were euthanized via rapid immersion in a lethal concentration of tricaine methanesulfonate (MS-222). Hepatic and branchial tissues were immediately excised, rinsed in ice-cold physiological saline, and weighed. The tissues were homogenized in a cold potassium phosphate buffer (0.1 M, pH 7.4) supplemented with 1 mM EDTA. The resulting homogenates were subjected to centrifugation at $10,000 \times g$ for 20 minutes at 4°C , and the post-mitochondrial supernatant (PMS) was carefully extracted for subsequent biochemical quantification. Total protein concentration in the PMS was determined utilizing the standard Bradford method with bovine serum albumin (BSA) as the calibration standard.

2) Quantification of Oxidative Stress Biomarkers

Superoxide dismutase (SOD) specific activity was measured by assessing its capacity to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The assay was monitored spectrophotometrically at 560 nm. The specific activity of SOD is calculated based on the percentage of inhibition.

$$\text{SOD activity} = \frac{\left[\frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \right] \times 100}{V_{\text{sample}} \times C_{\text{protein}}} \quad (1)$$

Catalase (CAT) activity was quantified by directly tracking the decomposition kinetics of hydrogen peroxide (H_2O_2) at 240 nm. Glutathione peroxidase (GPx) activity was evaluated by monitoring the oxidation of reduced glutathione (GSH) coupled to the consumption of NADPH, measured at 340 nm. The extent of lipid peroxidation was determined by quantifying malondialdehyde (MDA) equivalents through the thiobarbituric acid reactive substances (TBARS) protocol. The reaction mixture was heated at 95°C for 45 minutes, cooled, and centrifuged, after which the absorbance of the distinct chromophore was measured at 532 nm (with non-specific turbidity correction at 600 nm).

$$\text{C MDA} = \frac{(A_{532} - A_{600})}{\epsilon L} \times \frac{V_{\text{assay}}}{V_{\text{sample}}} \times \frac{1}{C_{\text{protein}}} \quad (2)$$

Where ϵ represents the molar extinction coefficient for the MDA-TBA complex ($155 \text{ mM}^{-1} \text{ cm}^{-1}$), and L is the optical path length.

III. RESULTS AND DISCUSSION

A. Microplastic Characterization

The accelerated photo-oxidative weathering protocol induced profound alterations in the structural and chemical profiles of the microplastics, fundamentally distinguishing them from their pristine counterparts. FTIR spectroscopic evaluation revealed the emergence of prominent absorption bands in the $1700\text{-}1750 \text{ cm}^{-1}$ region for both weathered PE and PS, signifying the substantial incorporation of carbonyl functional groups. The carbonyl index (CI), summarized in Table I, demonstrated a dramatic increase in weathered PS compared to virgin PS, indicating intensive photo-initiated chain scission and superficial oxidation.

TABLE I. PHYSICOCHEMICAL PROPERTIES OF MICROPLASTICS USED IN EXPOSURE ASSAYS

Polym er	State	Hydrodyna mic Dia. (nm)	Zeta Potenti al (mV)	Carbon yl Index
PE	Pristine	450.2 ± 12.4	-15.4 ± 1.2	0.02 ± 0.01
PE	Weather ed	310.5 ± 18.7	-38.2 ± 2.4	1.45 ± 0.08
PS	Pristine	520.1 ± 14.3	-12.8 ± 1.5	0.05 ± 0.01
PS	Weather ed	345.6 ± 21.0	-42.1 ± 3.1	2.10 ± 0.12

Morphological evaluation via SEM corroborated the chemical degradation data. Pristine polymers exhibited smooth, continuous topographies with minimal surface aberrations. Conversely, the aged particles displayed extensive structural degradation, characterized by deep surface fissures, prominent flaking, and irregular erosion cavities. This mechanical fracturing corresponds directly with the reduction in hydrodynamic diameter observed via DLS. Furthermore, the zeta potential measurements indicated a substantial shift toward high electronegativity in weathered particles, a phenomenon driven by the dense accumulation of anionic carboxylates on the exposed surfaces.

B. Hepatic Oxidative Stress Indices

The quantification of hepatic biomarker responses revealed that microplastic exposure fundamentally disturbs the redox equilibrium within *Cyprinus carpio*. As delineated in Table II, hepatic SOD and CAT activities demonstrated significant depletion following 21 days of exposure, particularly within the weathered treatment cohorts. The reduction in primary enzymatic antioxidants implies an exhaustion of the biological defense infrastructure, overwhelmed by the continuous generation of radical oxygen species.

TABLE II. HEPATIC OXIDATIVE STRESS BIOMARKERS AFTER 21-DAY EXPOSURE

Group	SOD (U/mg prot)	CAT (U/mg prot)	GPx (U/mg prot)	MDA (nmol/mg prot)
Control	45.2 ± 3.1	112.4 ± 8.5	24.1 ± 2.0	1.2 ± 0.1
PE-Pristine	38.5 ± 2.8	95.2 ± 7.4	20.5 ± 1.8	2.8 ± 0.3
PE- Weathered	22.1 ± 1.9*	54.1 ± 6.2*	12.4 ± 1.2*	5.4 ± 0.5*
PS-Pristine	35.4 ± 3.0	88.7 ± 6.9	19.8 ± 1.5	3.1 ± 0.4
PS- Weathered	18.3 ± 1.5*	41.5 ± 5.0*	9.5 ± 1.0*	7.2 ± 0.6*

* p < 0.05 relative to the unexposed control group.

The concomitant elevation of MDA concentrations in the weathered PS group, reaching peak levels of 7.2 nmol/mg protein, unequivocally indicates severe lipid peroxidation. This biochemical cascade confirms that the compromised antioxidant shield failed to prevent progressive oxidative degradation of cellular membranes. The pristine polymers, while inducing measurable biochemical shifts relative to the control, exhibited a markedly subdued toxicological profile compared to their weathered analogs.

C. Mechanistic Links Between Polymer Chemistry and Toxicity

Analyses indicate that microplastic-induced membrane perturbations escalate the formation of lipid peroxides through direct physicochemical interactions at the cellular interface. The highly elevated electronegativity and increased specific surface area of the weathered particles

amplify their binding affinity to biological macromolecules. Specifically, the exposed carbonyl and hydroxyl matrices establish strong hydrogen bonding networks with the phospholipid bilayers of hepatocytes, inducing localized structural instability and facilitating the unregulated influx of reactive oxygen species.

Furthermore, the degradation of polystyrene yields distinct aromatic oligomers and leachable secondary degradation byproducts, acting as potent chemical stressors. These low-molecular-weight exudates penetrate cellular membranes, directly agonizing intracellular pathways that suppress Nrf2-mediated antioxidant transcription. Consequently, the observed toxicodynamics are not exclusively a function of particle dimension, but rather a complex synergistic effect driven by the altered polymer chemistry. The intrinsic recalcitrance of the pristine particles mitigates this chemical leaching, reinforcing the hypothesis that environmental weathering is the primary catalyst for microplastic ecotoxicity in freshwater biota.

IV. CONCLUSION

Synthesizing the empirical observations, the present investigation establishes a definitive correlation between the oxidative degradation state of synthetic polymers and the severity of resultant biochemical trauma in *Cyprinus carpio*. The structural fracturing and extensive surface functionalization of weathered polyethylene and polystyrene dramatically amplify their capacity to induce severe oxidative stress, marked by the systematic depletion of primary defense enzymes and critical lipid peroxidation. These findings necessitate a paradigm shift in ecotoxicological modeling, mandating the incorporation of polymer chemistry and weathering dynamics into hazard assessments.

While this laboratory-controlled exposure provides critical mechanistic insights, inherent limitations persist regarding the simulation of complex, multi-contaminant natural matrices. Future interdisciplinary investigations must prioritize toxicogenomic and transcriptomic methodologies to elucidate the precise molecular cascades perturbed by these functionalized particles. Expanding this framework to encompass chronic, multi-generational exposures across diverse freshwater taxa will be imperative for accurately mapping the ecological consequences of global plastic proliferation.

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