Original Article
DOI: https://doi.org/10.70749/ijbr.v4i1.2702

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Scruitinizing the Effects of Microplastic (Polyethylene) on Minerals and Liver Enzyme Profile Of Common Carp (*Cyprinus carpio*)

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ARTICLE INFO

Keywords: Microplastics, Mineral profile, Liver Enzymes, Polyethylene, Toxicology, *Cyprinus carpio*

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Declaration
Authors' Contribution
Conceptualization and supervision of the experiment: Ahsan Khan. Conduction of the experiment: Sameena Zahid and Mahnoor Amin. Writing a draft: Hafsa Iqbal. Reviewing the draft. Asma and Ahsan Khan.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 17-11-2025 Revised: 04-01-2026
Accepted: 19-01-2026 Published: 30-01-2026

ABSTRACT

Purpose: This study investigates the effects of polyethylene microplastics (PE-MPs) on the mineral (electrolyte) profile specifically sodium (Na^+), potassium (K^+), chloride (Cl^-) and on the liver enzymes including creatine phosphokinase (CPK), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH of *Cyprinus carpio* (common carp). **Method:** Using controlled laboratory conditions, carp were exposed to three different concentrations of PE-MPs (5, 25, and 50 mg/L) over two-time intervals (7 and 14 days), simulating acute and sub-chronic exposure. **Results:** The data revealed dose- and time-dependent alterations in both key serum enzymes (ALT and LDH), indicative of hepatocellular damage and oxidative stress, and in electrolyte levels. The data showed significant ionic imbalance, notably a consistent drop in potassium, alongside variable changes in sodium and chloride associated with enzyme patterns suggesting muscle necrosis (from declining CPK) and hepatic exhaustion (from a complex ALP response). These findings support the hypothesis that microplastics, especially those chemically modified, possess strong bio-interactive capabilities that can disrupt cellular and physiological homeostasis in aquatic organisms. Also demonstrated that even osmoregulatory short-term exposure to PE-MPs can disturb ionic homeostasis in fish, potentially impairing function and overall metabolic health. **Conclusion:** This research contributes vital insight to freshwater ecotoxicology by demonstrating the compounded toxicity of functionalized plastic particles, thereby extending previous findings. Consequently, the study underscores the urgent need for mitigation measures and calls for further exploration into the sub-lethal effects on ecosystems, aquaculture, and human food safety.

INTRODUCTION

Microplastics (MPs) are widely distributed in aquatic ecosystems and occur not only in marine environments but also in freshwater systems (1,2). The world is becoming increasingly threatened by plastic pollution (3). Upon being in the natural habitat, plastics exist in processes of physical degradation, and biodegradation towards the formation of microplastics (MP). It is not only caused by the fact that such fragmentation complicates their detection and elimination, but it also enhances their bioavailability (4). The small size of MPs makes them highly available to aquatic biota, and they have been found in fish, causing multiple toxicological effects, such as intestinal damage, neurotoxicity, oxidative stress, inflammation, and energy metabolism disruption (5-9). Recently conducted studies suggest that the liver is a part of the organism that is affected to the greatest extent in the fish, exposed to microplastics (10).

The most important transit routes to the transfer of plastic debris to the water ecosystem are rivers, runoff, urban and industrial sewage (11). The effect of physical and chemical factors in the seas and oceans other than the UV rays can make the compounds to be reduced in size (12). This reduction can increase the bioavailability of the plastics towards aquatic life especially the filter feeder animals to a very large extent. The major faction of micro-plastics that detects its presence in the aquatic ecosystem comprises polyvinyl chloride (PVC), polyethylene (PE), polyvinyl alcohol (PA) along with their derivatives (13). The estimate provided by Carbery*et al.*(14) is that more than 8 million tons of plastic will end up being a part of the seas and even the oceans on a yearly basis, which implies that above 690 species of marine life will be affected.

MPs have the potential to impose direct damage on the sensitive organs of fish such as gills, digestive system, kidneys and other tissues. Moreover, they serve as

vectors and releasing absorbed chemicals such as heavy metals, organic compounds or pharmaceutics when released to the environment and as a consequence, make these substances more bioavailable to enter aquatic organisms and impair their health and immune functions (15). The accumulation of MPs in marine systems have been well reported before (16,17), while previous studies have extensively focused on quantifying the presence of marine debris in aquatic organisms, fewer studies have delved into the physiological effects of ingested microplastics in cultured fish (18). In addition, no report is available on the endocrine and immunological consequences of MPs in marine caged fish.

As MPs are of minute sizes, they are highly accessible to the organisms living in water, and were found in fishes, and their rate of toxicological effects is high, among which are intestinal tissue damage, neurotoxicity, oxidative stress, inflammation, and modification of the energy metabolism (5-7). Because they are smaller and of higher penetration capacity, they can easily find their passages through the skin, mouth and gills and gather up in the gills by binding on the gill filament that causes distortion and functionality of the gills such as ionic balance, gaseous exchange and osmoregulation (19). The surplus number of MPs in intestine will cause intestinal obstruction, intestinal locomotor disorders and the development of intestinal inflammatory response, thereby, loss of appetite, energy metabolism and nutrient absorption (20). Besides, accreted MPs in these organs can enter through the endothelium, or lymphatic circulation of the intestine, enter the circulation, and translocate to other body organs, among which liver is also included (21).

Upon exposure and ingestion, MPs can introduce high levels of xenobiotics toxins to internal organs, potentially resulting in reduced fitness (22) through disruption of liver functions as the primary organ for detoxification and triggering enzymes involved in detoxification (23). Besides being xenobiotics, ingested plastics transfer hazardous chemicals into fish organs and induce toxicity in liver (24). Furthermore, MP related additives can also adversely affect the immune system of fish, cause neurotoxicity and oxidative stress in aquatic animals by affecting acetylcholinesterase (AChE) levels, and lipid peroxidation (LPO) respectively (25). The results of previous studies said that the hematological and biochemical parameters might change based on the exposure of MPs to aquatic animals (25).

Currently, the presence and the toxic effects of MPs have been well documented in marine ecosystem (26). However, even though all harmful consequences of plastic documented for marine biota may be relevant for freshwater organisms as well, limited information is available to estimate ecological effects in freshwater ecosystem (27). Therefore, freshwater biota may be at a greater risk of MP exposure compared to the marine organisms (27).

Common carp (*Cyprinus carpio*) is the species of the order Cypriniformes and the family Cyprinidae, which is regarded as the biggest family of the freshwater species widely distributed across the globe, serves as a valuable model organism for studying aquatic toxicology due to its ecological and economic importance (28). It is being considered as a potential organism for commercial aquaculture in Asia and some European countries as it has a very high adaptive capability to both environment and food (29). Understanding how polyethylene microplastics affect the mineral profile and liver enzyme profile of *Cyprinus carpio* is crucial for assessing the potential ecological risks posed by microplastics pollution.

MATERIALS AND METHODS

Experimental Setup

Carboxylate-modified polyethylene microplastics (PE-MPs) with a particle size of 1 to 5 mm were procured from a certified commercial supplier. The study was conducted in the Limnology and Marine Laboratory, Department of Zoology, University of Swabi, Pakistan. The experimental organism selected for the study was the Common carp (*Cyprinus carpio*) specimens were obtained from a local hatchery in Peshawar.

After a 14-day acclimation period in 80L aerated aquaria, fish were randomly distributed into groups. The experimental design included a control group (without exposure to microplastics) and treatment groups exposed to three different concentrations of polyethylene microplastics: 5, 25, and 50 mg/L. Exposure durations were set for 7 and 14 days, respectively, to evaluate both short-term and prolonged effects of microplasticsexposure.Themicroplastics were dispersed evenly in the water column of treated aquaria. Aeration was provided continuously, and water was partially renewed every 48 hours. Fish behavior, feeding activity, and mortality were recorded daily throughout the experimental period.

Blood Collection and Analysis

At the end of each exposure period (7 and 14 days), fish were carefully removed from the aquaria for sample collection. To minimize stress, the fish were first anesthetized using a standard dose of clove oil solution, following ethical guidelines for handling live specimens. Once anesthetized, blood samples were collected either from the caudal peduncle or via direct cardiac puncture using a sterile 5 cc syringe. The harvesting was conducted in human ways of treatment to cause harm to the fish to minimal levels.

The collected blood was immediately transferred into gel tubes, which were pre-labeled and kept under cold conditions. Proper transfer of the blood into the gel tubes was done and centrifuged. These tubes were then subjected to centrifugation at 4000 revolutions per minute (rpm) for 1 minute to separate the serum from whole blood. A centrifugation of 4000 RPM of 1 minute was used to separate serum and the blood cells. This action was necessary to get clear serum so that biochemical tests would be performed. The resulting serum was carefully extracted using micropipettes and stored at −20°C until further analysis.The mineral and enzymatic profiles of the serum samples were analyzed using a biochemical analyzer (Mindray). The minerals tested included essential elements such as potassium,

sodium, and chloride. These elements are crucial indicators of physiological health and can reflect the impact of environmental stressors such as microplastics. The step played a vital role to determine the possible biochemistry changes in the carp that could have been caused by the exposure to PE-MPs.

Serum mineral analysis helped to evaluate the extent of physiological disruption caused by polyethylene microplastics exposure, allowing comparisons between control and treatment groups. Enzymatic profile also gave helpful information on whether the activities involved in the metabolic process of the fish have been perturbed or not, which may be the hint of the effect of polyethylene microplastics particles on the fish at the molecular level. The changes in mineral concentrations were further correlated with exposure duration and microplastics concentration to determine dose-dependent and time-dependent effects. The collected data served as an essential component in assessing the sub-lethal impacts of microplastics on fish health, especially in relation to ionic regulation, osmoregulation, and overall metabolic stability.

RESULTS

Investigation shows that *Cyprinus carpio* is highly susceptible to carboxylate-modified polyethylene micro plastic (PE-MP), which induces concurrent and dose- and time-dependent disruption of key biochemical markers and ionic homeostasis.

7 Days Exposure

At 5 mg/L after 7 days, mild but significant increases in CPK, ALT, ALP, and LDH indicate muscular and hepatic stress, coinciding with significant increases in sodium and chloride levels and notable potassium depletion. The mid-range dose of 25 mg/L initially had no impact on electrolytes but exacerbated biochemical toxicity at higher concentrations.

At 50 mg/L, the biochemical alterations were more severe, with significant rises in ALT and LDH indicating serious liver injury and cellular disturbance, alongside marked electrolyte imbalance.

14 Days Exposure

Prolonged exposure to 14 days intensified these pathophysiological effects. The 5 mg/L exposure significantly reduced potassium and slightly raised chloride, while 25 mg/L specifically lowered potassium. The highest dose of 50 mg/L markedly diminished all three electrolytes sodium, potassium, and chloride while biochemical signs of chronic poisoning emerged, including inhibited ALP and variable LDH, suggesting cell fatigue and organ malfunction. Interestingly, CPK showed a non-linear response, rising at low levels but decreasing upon higher exposure, possibly due to enzyme leakage from advanced muscle damage.

The integrated findings demonstrate that microplastics exposure causes acute and chronic multiorgan stress, critically determined by concentration and duration. The collective disruption of enzyme profiles and severe electrolyte imbalance likely reflects impaired Na^+/K^+ ATPase function and osmoregulatory failure, posing a serious threat to fish health and aquatic ecosystem stability.

Comparative Analysis

The integrated analysis of enzyme and electrolyte profiles, supported by statistical comparison (t-test, $p < 0.05$ and $p < 0.01$), reveals that polyethylene micro plastic (PE-MP) exposure induces significant, dose- and duration-dependent physiological stress in *Cyprinus carpio*, marked by cellular damage and severe ion regulatory disruption. Table 1 (7 days exposure), primarily indicates significant elevations in key enzymes (ALT, ALP, and LDH) and a distinct pattern of electrolyte imbalance (elevated Na^+ and Cl^-, decreased K^+) were observed at 5 and 50 mg/L, indicating initial metabolic disturbance and osmoregulatory cost.

Notably, electrolyte levels remained statistically unchanged after 7 days at 25 mg/L, the enzyme profiles showed significant elevations. This indicates that cellular stress and damage are detectable earlier in the toxicological progression than systemic osmoregulatory failure. Extending exposure to 14 days (table 2) intensified this toxicity, 5 mg/L and 25 mg/L doses, the data reveal a distinct and significant pattern of chronic metabolic and osmoregulatory stress that differs from both the acute 7-day response, shifting the response toward more severe cellular injury, as evidenced by a massive increase in creatine phosphokinase (CPK) at 50 mg/L, and a profound, dose-independent depletion of potassium coupled with declining sodium and chloride at the highest dose. This progression from initial stress to over cellular damage, alongside the consistent vulnerability of potassium homeostasis, underscores a critical toxicological threshold where prolonged exposure leads to compromised energy metabolism, cellular integrity, and osmotic balance.

Table No. 1

Enzyme Profile of Cyprinus carpio after 7-Days Polyethylene Microplastics (PE-MP) Exposure

Dose	Creatine phosphokinase (U/L)		Alanine aminotransferase (U/L)		Alkaline phosphatase (IU/L)		Lactate dehydrogenase (U/L)	
	Control Group	Treated Group	Control Group	Treated Group	Control Group	Treated Group	Control Group	Treated Group
5mg/L	5044.00±2.65	5322.00±2.00**	45.00± 2.00	144.00± 1.00**	55.00± 4.00	297.00± 3.00**	1014.00±2.00	1202.00±2.65**
25mg/L	5044.00± 2.00	1755.00±3.00**	45.00± 2.00	770.00± 1.00**	55.00± 4.00	281.33± 2.52**	1014.00±2.00	2817.00±3.00**
50mg/L	5044.00± 2.00	3320.00±.00	45.00± 2.00	560.00± 3.00**	55.00± 4.00	150.00± 2.00**	1014.00±2.00	2504.00±3.00**

P Value	Asterisks denote a significant difference from the control group within the same dose row, as determined by t-test: *p < 0.05, **p < 0.01.

Values are expressed as a mean± standard deviation (SD) for three replicates (n=3)

Table 2

Mineral Level in Cyprinus carpio after 14-Days Polyethylene Microplastics (PE-MP) Exposure

Dose	Sodium (mmol/L)		Potassium (mmol/L)		Chloride (mm0l/L)	
	Control Group	Treated Group	Control Group	Treated Group	Control Group	Treated Group
5mg/L	133.00±2.00	135.00±3.00**	5.2000±0.2000	1.7500±0.0300	97.00±2.00	101.00±1.00**
25mg/L	133.00±2.00	131.00±3.00**	5.200±0.200	1.400±0.300*	97.00±2.00	95.00±3.00**
50mg/L	133.00±2.00	118.00±3.00**	5.2000±0.2000	1.1700±0.0200	97.00±2.00	89.00±4.00**
P Value	Asterisks denote a significant difference from the control group within the same dose row, as determined by t-test: *p < 0.05, **p<0.01.					

Values are expressed as a mean± standard deviation (SD) for three replicates (n=3)

DISCUSSION

Exposure of *Cyprinus carpio* to carboxylate-modified polyethylene microplastics (PE-MPs) caused dose- and time-dependent biochemical and ionic changes. Over 7 days, the lowest concentration (5 mg/L) induced early stress, marked by slight increases in CPK, ALT, ALP, and LDH, alongside elevated sodium and chloride and reduced potassium, indicating disrupted electrolyte regulation. At 25 mg/L for 7 days, CPK decreased while ALT, ALP, and LDH increased, suggesting severe hepatic and cellular damage with minimal ionic disturbance. The highest concentration (50 mg/L) led to sustained tissue damage, suppressed CPK, elevated ALT and LDH, and significant sodium and chloride increases with potassium reduction, confirming a clear dose-response effect.

Over 14 days, these effects intensified: 5 mg/L caused cumulative stress with high ALT and LDH, slight CPK decline, and significant potassium and chloride changes. At 25 mg/L, a spike in CPK indicated necrotic processes, while ALT and LDH declined, potentially due to enzyme exhaustion, and potassium remained significantly reduced. At 50 mg/L, all biomarkers indicated severe chronic toxicity, with depressed enzyme levels and significant reductions in sodium, potassium, and chloride, reflecting systemic ionic and metabolic collapse.

The findings align with Darabi*et al.* (30), which examined PVC-MPs in juvenile common carp. While Darabi et al. focused on ingestion and histopathological damage, both studies show that higher concentrations and longer exposures induce significant physiological disruptions, highlighting the importance of biochemical monitoring alongside tissue analysis.

Similarly, Banaei*et al.* (31) reported significant alterations in ALT and LDH in *Cyprinuscarpio* exposed to polyethylene microplastics, confirming tissue damage and physiological stress. Jahan *et al.* (32) observed systemic toxicity, including growth reduction and tissue damage in *Oreochromis niloticus*, which complements our findings of specific ionic imbalances, suggesting that early electrolyte disruption may precede histopathological lesions.

The results also converge with Jeyavani*et al.* (33), demonstrating clear toxic effects of carboxylate-modified PE-MPs in freshwater fish. Likewise, Rashid *et al.* (34) highlighted chronic dietary toxicity, with metabolic and intestinal disturbances and hepatic toxicity. The present study identifies early-warning biochemical events, notably severe hypokalemia and elevated ALT and LDH, which precede histological and nutritional impairments, expanding the understanding of microplastic-induced toxicity.

Finally, these biochemical disruptions support a common mechanistic pathway described by Gheorghe *et al.* (35) for polystyrene microplastics, where oxidative stress underlies hepatic and systemic tissue damage, linking electrolyte imbalance to cellular energy failure and systemic exhaustion.

CONCLUSION

The study demonstrates that carboxylate-modified PE-MPs induce dose- and time-dependent biochemical and ionic disturbances in *Cyprinus carpio*. Early electrolyte imbalances, particularly hypokalemia, combined with elevated ALT and LDH, serve as sensitive indicators of hepatic and systemic stress. Collectively, these findings confirm that microplastic exposure disrupts ionic homeostasis, triggers oxidative stress, and can progress to severe tissue and metabolic damage in freshwater fish.

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