

Some Physiological, Histopathological, and Molecular Alterations in Common Carp (*Cyprinus carpio* L.) Exposed to Polystyrene Microplastics

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Abstract

Background :Polystyrene microparticles (PS-MPs) are becoming more accepted as deleterious to freshwater ecosystems. However, there are not many ecotoxicological studies that have correlated pollutant concentration in the field with the fish species in the river systems of the Middle East. The aim of the study was to evaluate the physiological as well as the histopathological and molecular aspects of the impact of chronic exposure to ecologically relevant concentrations of PS-MPs on juvenile common carp of the Tigris River.

Materials and Methods : Juvenile common carp (*Cyprinus carpio*; average weight 14.8 1.9 g) were used for studying the chronic effects of PS-MPs via laboratory studies for 60 days using commercially available standardized spherical PS-MPs (Sigma Aldrich No. 43302) with dimensions ranging between 1 and 10 μ m. Four concentrations were used that were 0, 0.01, 1, and 1 mg/L which were developed based on the field concentrations provided for the Tigris River at the Samarra site (median values of 0.14 ± 0.09 mg/L and 95th percentile values of 0.78 mg/L). Effects were evaluated through measurement of blood count and serum biochemistry (biomarkers of oxidative stress including malondialdehyde [MDA], superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]), histopathological evaluations of gill and liver with semi-quantitative method of HAI (based on Bernet method) and expression analysis of genes of interest (*il-1 β* , *tnf- α* , *sod*, and *cat*) through RT-qPCR technique.

Results One hundred percent survival was recorded by each group and important effects were only noted in the 1 mg/L group [MF13.1]: there was a reduction in specific growth rate (14.3%, $P < 0.01$), there was a decline in RBC count and haemoglobin levels ($P < 0.05$), serum ALT level was increased (from 18.3 ± 1.4 to 31.6 ± 2.3 U/L, $P < 0.001$), and hepatic MDA was at elevated levels (1.74 times higher at $P < 0.01$). There were no important changes recorded for the control group and groups exposed to low and medium concentrations. HAI scores were higher in both liver and gill tissues as concentration increased (Kruskal-Wallis test $P < 0.001$). At a transcriptional level, *il-1 β* and *tnf- α* were up-regulated by 2.27 and 1.84 times respectively, while *sod* and *cat* genes were down-regulated, by 0.71 and 0.74 times (but only with reference to the high concentration).

Conclusions: The findings demonstrate that PS-MPs at chronic environmentally relevant concentrations have damaging effects on the physiology of juvenile common carp. Thus, testing juvenile common carp, a species that is both economically and ecologically important in the Middle East, provides an appropriate model.

Keywords: *C. carpio*; polystyrene microplastics; Tigris River, Samarra, Iraq[MF15.1]; oxidative stress; histopathology; RT-qPCR; ecotoxicology

1. Introduction

Microplastic particles (≤ 5 mm) are widely recognized as reliable indicators of anthropogenic pollution in freshwaters globally, detected throughout aquatic environments (e.g. river bed sediments, pelagic water, biota across all trophic levels) (19)(8). The polymer type most widely studied is polystyrene (PS) because of its extensive use in packaging and the foaming process, and its capacity to sorb hydrophobic organic pollutants and thus transport them into the guts of fish, the mechanism referred to as the Trojan Horse effect (18)(5). Laboratory studies on teleosts have demonstrated growth suppression, immune dysfunction and tissue damage following consumption of PS micro-particles (12), (7) however most studies used

exposure concentrations that were one to three orders of magnitude higher than environmentally realistic concentrations, severely limiting the applicability of these data for ecological risk assessments (11) The Tigris River flows through major urban areas in northern and central Iraq, and regularly receives direct inputs of domestic wastewater and solid waste. Concentrations of PS-MPs in surface waters at our field site in Samarra (0.003 mg/L - 0.87 mg/L; median 0.14 mg/L) are comparable to those reported from highly polluted urban rivers in Asia. In Iraq, common carp (*Cyprinus carpio*) are the major commercially exploited freshwater fish species and a primary source of animal protein in the region (1)

2. Materials and Methods

2.1 Environmental Monitoring and Justification of Experimental Concentrations

Surface water samples were obtained from five monitoring stations located along the 12km stretch of the Tigris River as it passes through Samarra city (34°12'N, 43°52'E) from March to October 2024 (36 sampling events per station; 3 replicates of 1 L per event). The samples underwent a sequential filtration process: through stainless steel sieves (500 then 50 μ m), followed by filtration with a 0.45 μ m membrane filter. The particles were identified using micro-Raman

spectroscopy (Thermo Scientific DXR2xi) and μ FT-IR (Bruker LUMOS II). PS-MP accounted for $23.4 \pm 4.1\%$ of total particles found; concentrations ranged from 0.003 to 0.87 mg L⁻¹ (median 0.14 ± 0.09 mg L⁻¹; 95th percentile 0.78 mg L⁻¹). The experimental concentrations (0.01, 0.1, and 1 mg L⁻¹) were selected to encompass this environmental range, in accordance with best practice recommendations(11).

2.2 Experimental Design and Ethical Compliance

This study was conducted in accordance with the guidelines for the care and use of laboratory

(4) animals issued by the Iraqi Ministry of Higher Education and Scientific Research. Since there was no formal animal care and use committee at Samarra University at the time of the study, the procedures were reviewed and approved by the

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Institutional Research Committee and implemented in accordance with the principles of EU Directive EU/2010/63 and followed the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020) and OECD Test Guideline 215 (2022). After a 14-day acclimation period, 120 juvenile carp (14.8 ± 1.9 g) were randomly distributed into

four treatment groups ($n = 30$ per group; 3 tanks $\times$ 10 fish/tank in 150-L glass aquaria) that were maintained at $24 \pm 1^\circ\text{C}$ with a 12:12-h photoperiod.

Table 1. Experimental groups, nominal polystyrene microplastic (PS-MP) concentrations, and their relationship to Tigris River monitoring data.

Exposure Period	Replicates	No. of fish (n)	Environmental Relevance	Concentration (mg L^{-1})	Treatment Group
60 days	3	30	Particle-free medium	0	Negative control
60 days	3	30	Below field median	0.01	Low
60 days	3	30	Near field median (0.14 mg L^{-1})	0.1	Medium
60 days	3	30	Above 95th percentile (0.78 mg L^{-1})	1.0	High

2.3 Preparation and Physicochemical Characterisation of PS-MPs

Commercially standardized spherical PS particles (Sigma-Aldrich No. 43302; nominal diameter $1 \mu\text{m}$ - $10 \mu\text{m}$; dynamic light scattering (DLS) hydrodynamic diameter = $5.6 \pm 1.31 \mu\text{m}$; polydispersity index (PDI) = 0.093 ± 0.020 ; zeta potential = -18.4 ± 2.19) were used instead of particles collected from field sites. The strongly negative zeta potential indicates stable electrostatic colloidal stability at aquaristic salinities, with a PDI value < 0.1 reflecting near-monodisperse size distribution, and thus establishing both of these factors are required for a reliable, quantitatively measurable dose-response. Three experimental imperatives governed the choice of these particles: i. particle consistency between replicates and inter-laboratory reproducibility; ii. the exclusion of co-

contaminants (organic pollutants, trace metals, and biofilms) which would be associated with heterogeneous field collected particles and introduce confounding attributions in the observation of effects to PS-MPs; and iii. the establishment of biologically interpretable dose-response metrics permanently affixed to Tigris River field sampling. The physical-chemical characterization methodology used in this work were DLS, determination of PDI, zeta potential, and monitored colloidal stability over the 48-hour inter-exchange time; are the standard methods mirrored by the current peer-reviewed ecotoxicological literature dealing with commercial reference particles (4)(6)(11) and completely meet all physicochemical characterization requirements as per OECD. All

stock suspensions were made by using probe sonication (Qsonica Q55; 10 minutes on ice) and then diluted prior to each water exchange to minimize aggregation; zeta potential was

remeasured at 48 hours ($p=0.43$) and confirmed that there was no agglomeration during the inter-exchange period.

2.4: Sampling at End of Experimentation

On day 60 (experimental completion), ten fish from each group will be euthanized via cervical transection after anesthetic induction with MS-222. Blood will be collected via caudal vein into

EDTA-coated vacutainers. Gill and liver tissue will be excised and transferred to: a) histopathological processing using 10% neutral buffered formalin and b) frozen in liquid nitrogen for analysis of oxidative stress (OS) and extraction of RNA, subsequently stored at -80°C .

2.5: Physiological and Biochemical Endpoints

Subjects' blood samples will be analyzed for complete blood counts (CBCs) using a Mindray BC-5000 Vet, within 2 hours of collection. Samples for serum biochemistry will also be analyzed on a Mindray BS-430, using Randox reagents, including: ALT, AST, ALP, total protein, albumin, creatinine, urea, glucose, and triglycerides. Cortisol will be quantified using a competitive ELISA (Cayman Chemical; detection limit: 6.6 pg/mL).

Samples of hepatic tissues will be coagulated with 10:1 (v:v) ice-cold phosphate buffer, then homogenized, and centrifuged at 10,000 X g for 15 minutes at 4°C . Malondialdehyde (MDA) measurement will be obtained using the TBARS method (Ohkawa et al., 1979); superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) will be measured using Randox kits, normalizing values to tissue protein via the Bradford (1976) assay.

2.6: Histopathological Assessments

Tissues will be prepared for histopathological analysis: gill and liver specimens will be processed through graded ethanol, cleared with xylene, and embedded in paraffin. Specimens will be cut into 5 μm sections using a Leica RM2235 microtome, and then, H&E stained, periodic acid-Schiff (PAS), and Masson's trichrome stained. Two board-certified veterinary pathologists, blinded to treatment allocation, will independently score each histological section using a semi-quantitative scoring method, (2)to determine a

Histopathological Alteration Index (HAI) for each organ.

Table 2. Semi-quantitative scoring criteria for histopathological assessment (adapted from Bernet et al., 1999).

Weighting Factor	Descriptive Criterion	Category	Score
—	No detectable histological change	Normal	0
1	Focal, reversible lesion; <25% of section area affected	Mild	1
1 or 10*	Multifocal lesion; 25–50% of section area affected	Moderate	2
10	Diffuse, irreversible lesion; >50% of section area affected	Severe	3

2.7 Gene expression was assessed by RT-qPCR

to evaluate the RNA obtained using TRIzol reagent and an optional DNase treatment of the isolated total RNA for purification of the isolated RNA. Samples with an RNA Integrity Number (RIN) less than 7.5 were not included in this analysis. A cDNA library from the total RNA was generated using the PrimeScript RT Kit (Takara Bio) and prepared for RT-qPCR with LightCycler 96 RT-qPCR assay instruments with TB Green Premix, with 3 biological replicates of each assay. The following 4 target genes were chosen to examine the 2 mechanistic pathways of interest: antioxidant capacity (superoxide dismutase [sod] and catalase [cat]) and inflammatory signaling

(interleukin 1 beta [il-1 β] and tumor necrosis factor [tnf- α]). This panel of genes was specifically limited to the four listed genes, to increase mechanistic specificity, reproducibility and reduce the impact of the multiple testing correction typically required for all analyses involving multiple genes. The geNorm analysis ($M < 0.5$; $V_{\square/\square} = 0.11$) confirmed the stability of the following two reference genes, β -actin and elongation factor 1 alpha (effective brings the mean value of the two groups to one); for quantification of relative expression was conducted using the efficiency-corrected (16)

Table 3. Primer sequences, GenBank accession numbers, PCR efficiencies, and amplicon sizes for *Cyprinus carpio*.

Efficiency (%)	Amplicon (bp)	Sequence (5'→3')	Direction	GenBank Accession	Gene
98.4	185	TGCAGCCAGATGAAGACAATG	Forward	NM_001308527.1	il-1 β
		CAGCAGCACATCAAGCCATC	Reverse		

96.2	197	ATGGCCTCCGTCAGAGAGGG	Forward	AY836796.1	tnf- α
		AGGGCTTGGAGCAGAAGAAC	Reverse		
97.6	192	GCAGCATGGTTTCCACAATG	Forward	NM_001327447.1	sod
		CCTGTCTCCAGCACCATCTC	Reverse		
98.1	188	GCAGACAGAGGCAACCAAAG	Forward	NM_001308626.1	cat
		GGACCAGCAGGTCCTTCATC	Reverse		
99.5	207	GAGAAGATGACCCAGATCATGTT	Forward	NM_001123525.1	β -actin †
		TCGTCCCAGTTGGTGACAAT	Reverse		
98.9	183	CCTGGACAAGCTGAAGGCTG	Forward	AB056104.1	efl α †
		GCCTGGAGTTGGTACCAGGA	Reverse		

2.8 Statistical Analysis

Continuous variables were analyzed statistically using one-way ANOVA and Tukey's post hoc multiple comparison test for pairwise comparisons of the four treatment groups. Histopathology scores were measured non-parametrically and analyzed with the Kruskal-Wallis dataframe; Dunn and Bonferroni correction was applied to all the nonparametric ordinal histogram comparisons. Gene expression results were adjusted using the efficiency of the (16)model for calculating gene

expression. The Pearson product moment correlation coefficient was used to correlate pairs of the hepatic oxidative stress markers, fold changes in gene expression, and the Hay Infection Index (HAI) scores; a correlation heatmap was developed to visualize the mechanism of action for the oxidative stress markers. A two-tail α level of 0.05 was used to define statistical significance for all analyses. All statistical analyses were conducted using SPSS version 26.

3. Results

3.1 Survival, Growth, and Behavior

All experimental groups showed 100% survival within each of the 60 days, which demonstrated that none of the exposure concentrations produced acute toxicity. Starting on day 28, fish in the 1 mg L⁻¹ treatment group exhibited a decrease in their feed-strike activity and an increase in the average

rate of opercular beating compared with the control group (82 ± 4 vs. 74 ± 3 beats per minute; $P = 0.06$) and these findings suggest that fish in this group were exhibiting signs of sub-lethal respiratory stress. The specific growth rate of the fish in the 1 mg L⁻¹ treatment group was

significantly less than that of the fish in the control group at the end of the study (1.08 ± 0.09 vs. $1.26 \pm 0.07\%$ per day; $P = 0.009$; Cohen's $d =$

0.89). There were no significant reductions in the specific growth rate of fish in either the low or medium concentrations.

3.2 Hematological Parameters

A significant main effect of treatment concentration was found for the RBC count ($F_{3,12} = 9.4$; $P = 0.006$), hemoglobin ($F_{3,12} = 7.8$; $P = 0.011$), and hematocrit ($F_{3,12} = 6.3$; $P = 0.018$) when analyzed with one-way ANOVA. Post-hoc analysis of the data provided for each

variable showed that only the RBCs of the 1 mg L^{-1} group were significantly different from the controls (Tukey; Table 4). Total WBC count and mean corpuscular volume did not significantly differ between any of the treatment groups.

Table 4. Haematological parameters in *C. carpio* after 60-day PS-MP exposure (mean $\pm$ SEM; $n = 10$).

High (1.0)	Medium (0.1)	Low (0.01)	Control (0)	Parameter
1.97 ± 0.06 b	2.17 ± 0.09 a	2.25 ± 0.07 a	2.31 ± 0.08 a	RBC ($\times 10^6 \mu L^{-1}$)
7.2 ± 0.3 b	7.9 ± 0.3 a	8.2 ± 0.3 a	8.4 ± 0.3 a	Haemoglobin (g dL^{-1})
25.3 ± 0.8 b	27.1 ± 1.0 a	27.9 ± 0.8 a	28.4 ± 0.9 a	Haematocrit (%)
128.4 ± 3.9	124.8 ± 3.6	123.6 ± 2.9	122.8 ± 3.2	MCV (fL)
37.6 ± 2.6	33.4 ± 2.3	30.1 ± 2.0	28.3 ± 1.8	WBC ($\times 10^3 \mu L^{-1}$)
64.8 ± 2.7	68.5 ± 2.4	70.2 ± 1.9	71.4 ± 2.1	Lymphocytes (%)
24.1 ± 2.1	21.2 ± 1.8	19.4 ± 1.6	18.3 ± 1.4	Neutrophils (%)

3.3 Serum Biochemistry

There were significant differences observed within ALT, AST and creatinine concentrations (ALT, $F_{3,12} = 14.2$; $P = 0.002$) (AST, $F_{3,12} = 9.7$; $P = 0.005$) (creatinine, $F_{3,12} = 6.8$; $P = 0.014$) with each significant

pairwise comparison occurring exclusively between 1 mg L^{-1} and control groups). Concentrations of total protein, albumin, urea, glucose and triglycerides were not statistically different across any of the groups

3.4 Hepatic Oxidative Stress

There was a statistically significant increase in MDA concentrations at both 0.1 mg L^{-1} and 1 mg L^{-1} ($F_{3,12} = 18.3$; P Less than 0.001) and SOD, CAT, and GPx activities did not show significant reductions until the highest exposure level (Table 6). The data collectively supports the

concept of a gradual accumulation of redox imbalance as lipid peroxides accumulate differently across concentrations while antioxidant enzyme stores do not decline until after the greatest level of exposure which indicates that the amount of up-regulation

produced is inadequate to account for additional

increases at that exposure level .

Table 6. Hepatic oxidative stress markers in *C. carpio* after 60-day PS-MP exposure (mean $\pm$ SEM; n = 10).

High (1.0)	Medium (0.1)	Low (0.01)	Control (0)	Biomarker
5.54 $\pm$ 0.38 c***	4.12 $\pm$ 0.31 b*	3.44 $\pm$ 0.25 a	3.18 $\pm$ 0.22 a	MDA (nmol mg ⁻¹ protein)
37.4 $\pm$ 2.6 b**	48.6 $\pm$ 3.4 ab	53.2 $\pm$ 2.9 a	56.8 $\pm$ 3.1 a	SOD (U mg ⁻¹ protein)
15.3 $\pm$ 1.4 b*	18.9 $\pm$ 1.7 ab	21.1 $\pm$ 1.5 a	22.4 $\pm$ 1.6 a	CAT (U mg ⁻¹ protein)
33.7 $\pm$ 3.0 b*	41.3 $\pm$ 3.6 ab	46.1 $\pm$ 3.1 a	48.6 $\pm$ 3.3 a	GPx (mU mg ⁻¹ protein)

3.5 Results

of Histopathological Analysis The inter-rater reliability for histopathological evaluations was found to be very high (ICC = 0.83 (95% CI = 0.76-0.89), well exceeding the predetermined value of ≥ 0.75 . The results of the statistical analyses (Kruskal-Wallis) provide further evidence that the concentration effects obtained for each group (Gill = HAI; Gill = HWI) were significant (Gill = H = 28.7; $P < 0.001$); Liver = H = 24.3; $P < 0.001$). All of the significant statistical pairwise comparisons were localized to the 1 mg/L group.

Gill: The fish exposed to the 0.01 mg/L group had histopathologically indistinguishable gills when compared to the controls (HAI=0); fish exposed to 0.1 mg/L demonstrated focal lamellar fusion (< 25% of total secondary lamellae) and mild hyperplasia of chloride cells (HAI = 4.2 $\pm$

0.9); and fish exposed to 1.0 mg/L had multifocal, moderate lamellar fusion (30-45% of total secondary lamellae) along with epithelial lifting and goblet cell proliferation (HAI = 16.8 $\pm$ 2.3; $P < 0.01$ v control). Fibrovascular and squamous metaplasia, typically associated with > 10 mg/L, were not observed in any groups. Liver: Fish exposed to 0.1 mg/L had focal periportal glycogen-rich vacuolation (Grade 1), and individualized nuclear pyknosis (HAI = 8.4 $\pm$ 1.4; $P = 0.07$, not significant); whereas fish exposed at 1.0 mg/L had multifocal vacuolar degeneration (25-40% of hepatic parenchyma), periportal macrophage infiltration, and rare bile duct proliferation within the liver (HAI = 22.6 $\pm$ 3.1; $P < 0.01$). The lack of any fibrosis observed in the Masson stained tissue indicates that the liver injury is of a non-fibrotic, subacute classification.

3.6 Gene Expression (RT-qPCR)

At 1 mg/L of the test substance, both il-1 β and tnf- α were significantly elevated; 2.27 fold ($P < 0.01$) and 1.84 fold ($P < 0.05$), respectively; whereas, sod transcripts were significantly decreased to 0.71 fold ($P < 0.05$) and cat decreased to 0.74 fold ($P < 0.05$). No gene

reached statistical significance at 0.01 mg/L. The relationship of the Molecular results to the Biochemical results establishes the complete mechanistic relationship between Molecular (Transcriptional) and Biochemical endpoints: as the relative downregulation of both sod and cat

occurred on the transcription level, that finding corroborates the loss of bioactivity of the enzymes associated with their biochemical depletion in

contrast to the use of $\text{Il-1}\beta$ and $\text{tnf-}\alpha$, which demonstrate upregulation at the transcriptional level.

Table 7. Relative hepatic gene expression in *C. carpio* after 60-day PS-MP exposure (mean $\pm$ 95% CI; n = 10).

High (1.0)	Medium (0.1)	Low (0.01)	Control (0)	Gene
$2.27 \pm 0.32^{**}$	$1.61 \pm 0.24^*$	1.14 ± 0.19	1.00 (ref.)	$\text{il-1}\beta$
$1.84 \pm 0.28^*$	1.38 ± 0.22	1.11 ± 0.18	1.00 (ref.)	$\text{tnf-}\alpha$
$0.71 \pm 0.12^*$	0.88 ± 0.16	0.96 ± 0.14	1.00 (ref.)	sod
$0.74 \pm 0.11^*$	0.85 ± 0.14	0.98 ± 0.15	1.00 (ref.)	cat

4. Discussion

Across measurement levels, a threshold dose-effect relationship was observed across all endpoints whereby only an exposure concentration of 1 mg L^{-1} (95th percentile of the Tigris field distribution) produced statistically significant findings at this concentration and there were no statistically significant findings for the 0.01 mg L^{-1} group compared to controls. This pattern was also found by (4) and (6) in their respective meta-analyses on PS-MPs and redox pressure in aquatic systems. Most relevantly, the significant elevation of oxidative stress (MDA) at the lowest exposure concentration of 0.1 mg L^{-1} indicates that aquatic species residing in close proximity to the Tigris field median (0.14 mg L^{-1}) may have already experienced a subclinical but detectable level of redox stress. The blood profile exhibited by fish exposed to the Tigris field was characterized by decreased RBC counts, decreased haemoglobin levels and decreased haematocrit without evidence of increased WBC counts, indicating that erythropoiesis rather than acute inflammatory leucocytosis was the primary target of low-level PS-MP exposure. This finding is corroborated by (10) who found similar results

for erythropoiesis in *Labeo rohita* exposed to a PS-MP concentration of 1 mg L^{-1} . The elevation of hepatic MDA supports the conclusion that PS particle surfaces catalyse lipid peroxidation, leading to decreased integrity of the erythrocyte membrane and reduced lifespan of the erythrocyte. The elevation of hepatic transaminases related to hepatic vacuolar degeneration and periportal inflammation, but without evidence of fibrosis, suggests that the liver injury produced in this study is sub-acute and partially reversible. This is in stark contrast to the hepatocyte necrosis and bridging fibrosis that (12) documented in zebrafish exposed to a PS-MP concentration of $50\text{--}100 \text{ mg L}^{-1}$, clearly demonstrating that environmentally-calibrated concentrations of PS-MPs produce an entirely different— and significantly less severe— injury profile compared to the higher concentrations. The small creatinine increase, in addition to the histological changes related to this increase in the kidney, indicates that kidney injury is also minimal at the Tigris environmental concentration (0.14 mg L^{-1}).

5. Conclusions

This study provides an ecotoxicology assessment of chronic environmentally relevant polystyrene microplastic (PS-MP) exposure in *Cyprinus carpio* from an Iraqi riverine system utilizing multiple endpoints and field calibration. Statistically significant sublethal impairments were documented only in the 1mgL⁻¹ treatment group and were mechanistically consistent; antioxidant depletion (i.e. MDA increase, SOD/CAT/GPx decreases) preceded, or were coincident with, increased expression of pro-inflammatory genes (i.e. IL-1 β and TNF- α) and these biological changes were reflected in histopathological changes (i.e. HAI increase) in

both gill and liver tissues. The estimated threshold concentration for biological effects occurred in the range of 0.1 to 1 mgL⁻¹, which spanned the concentration range observed in Tigris River water samples; this indicates a risk of ecologically relevant harm to fishes inhabiting this portion of the basin. Therefore, this work has established a scientific basis for developing microplastic specific water quality criteria for river systems within the Mesopotamia Region and illustrates the necessity for conducting systematic seasonal monitoring of microplastic concentrations and polymer type within these river systems.

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