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Single and combined effects of microplastics and wastewater on kidney of *Cyprinus carpio*

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Abstract

The rapid pace of urbanisation and industrialisation has led to the accumulation of different pollutants such as domestic sewage, agricultural runoff and industrial waste in water bodies. Additionally, microplastics (MPs) are problematic due to their toxicity, environmental persistence, abundance and ability to adsorb different contaminants. Among different aquatic organisms, fishes are considered as an exceptional indicator to study water quality and the well-being of the aquatic ecosystem due to their sensitivity to alterations in environmental conditions. In addition, they are an important protein source; however, toxicants in them could affect the health of human beings consuming them. Therefore, the current study was designed to study the combined effects of low-density polyethylene (LDPE) MPs and wastewater on histomorphology of kidney of minor carp *Cyprinus carpio*. The results showed that significant alterations such as paracellular spaces, damaged hematopoietic tissue, detachment of renal tubule from basal lamina, damaged renal tubules, paracellular spaces, vacuolization, necrosis and exudate were found in kidneys of fingerlings belonging to treated groups. Moreover, organ index of kidney also indicated a significant ($p \leq 0.05$) rise in different treatment groups in comparison to the control group. Overall, the results indicated that the combination of LDPE MPs and wastewater affects kidneys of *C. carpio* fingerlings in a duration- and dose-dependent manner with limited reversibility.

Keywords: Freshwater fish, kidney, microplastics, wastewater, water pollution

1. Introduction

Water is an essential natural resource that sustains all life forms on Earth; however, the rapid pace of urbanisation and industrialisation has led to the daily discharge of about 2×10^6 tons of different pollutants including domestic sewage, agricultural runoff and industrial waste (Mahmoud *et al.*, 2026) ^[14]. Wastewater contains a plethora of anthropogenic contaminants such as pharmaceutically active compounds, heavy metals, bisphenol-A, nanomaterials, organic pollutants and endocrine-disrupting chemicals (Klecka *et al.*, 2010; Mehdi *et al.*, 2021; Vaid and Vashishat, 2024) ^[12, 15, 23]. Among these, plastic pollution has become a pervasive environmental concern due to its persistence, mismanagement and abundance. Plastic refers to synthetic and organic polymers that are widely used in different industries due to their low production cost, durability, strength and versatility (Kumar *et al.*, 2018) ^[13]. Plastic particles are categorised from macroplastics (≥ 25 mm) and mesoplastics (5-25 mm) to microplastics (MPs) (20 μ m-5 mm) (Gigault *et al.*, 2018) ^[5]. However, their resistance to biodegradation has led to a significant accumulation in terrestrial and aquatic environments, posing severe ecological and health risks (Rakib *et al.*, 2022) ^[19]. Among plastic particles of different size groups, MPs are of increasing concern for aquatic organisms due to their toxicity, environmental persistence, abundance and ability to adsorb different contaminants (Vaid and Vashishat, 2025) ^[24].

Among different aquatic biota, fishes are considered as an exceptional bioindicator to study water pollution due to their sensitivity to environmental fluctuations (Cvetkovic *et al.*, 2010; Vaid and Hundal, 2019) ^[4, 25]. Various studies reported that MPs may induce behavioural alterations, genotoxicity, oxidative stress, histopathology and mortality (Motivarash *et al.*, 2024) ^[16]. In addition, they are an important protein source and act as a sensitive indicator of overall well-being of the aquatic ecosystem (Vaid *et al.*, 2024; Bakhasha *et al.*, 2025) ^[26, 2]. Among various organs, kidneys are essential excretory organs responsible for eliminating metabolic wastes and xenobiotics; however, prolonged accumulation of various toxic

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substances may pose significant health issues in fishes (Ortiz *et al.*, 2003) ^[18]. Moreover, toxicant-induced histological and functional alterations in kidneys can disrupt homeostasis (Iqbal *et al.*, 2004) ^[9]. Despite escalating concerns over water contamination, research addressing the combined effects of MPs and other contaminants on fish remains limited. Therefore, the present study was designed to study the combined effects of low-density polyethylene (LDPE) MPs and wastewater on histomorphology of kidney of minor carp *Cyprinus carpio*.

2. Materials and Methods

2.1 Collection of wastewater and fingerlings

For conducting experiments, wastewater was collected from the sewage water treatment plant, Bhattian, India. The sampling site was situated within the municipal boundaries about 11 km from Punjab Agricultural University, India. *C. carpio* fingerlings were collected from Aujla fish company fish farm, India and acclimatized for 14 days in 100 l glass tanks. Fingerlings were fed with commercial feed/day at 2% of body weight. Fingerlings were not fed for 24 h prior to the commencement of the experiment. The experimental protocol adhered to the standard guidelines of the Organisation for Economic Co-operation and Development (OECD, 1992) ^[17].

2.2 Preparation of MPs

Polyethylene plastic pellets (Sigma-Aldrich) were used for the preparation of MPs following the methodology described by Wong *et al.* (2014) ^[27]. Briefly, 30 pellets were pre-heated with toluene (6 ml) and Tween-20 (0.2 ml) and subjected to ultrasonication for 30 min, followed by spreading onto a Petri plate and air-drying to completion. The resulting MPs were subsequently utilised for toxicity bioassays.

2.3 Toxicity bioassays- Acute and Chronic

The acute bioassay was conducted according to the standard guidelines of the Organisation for Economic Co-operation and Development (OECD, 1992) ^[17]. For lethal concentration (LC₅₀) calculation, 2 batches ($n = 7$) of fingerlings each were treated with various concentrations of wastewater (20, 40, 60, 80 and 100%) and LDPE MPs (350-750 mg/l) separately for a period of 96 hours. The results showed no mortality in fingerlings exposed to MPs; thus, sub-lethal concentrations were selected based on environmentally relevant levels, *i.e.*, 1 and 2 mg/l MPs (Rochman *et al.*, 2017) ^[21]. The LC₅₀ of wastewater, determined using POLO software (Robertson *et al.*, 2007) ^[20], was 64.47%. After that, the LC₅₀ value of the combination of wastewater (20, 40, 60, 80 and 100%) and MPs (2 mg/l MPs) was found to be 53.72%. Chronic bioassay concentrations were selected as 1/10th and 1/20th of the LC₅₀ for wastewater alone and for the combined treatment [2 mg/l MPs + wastewater (1/10th and 1/20th of the LC₅₀)], respectively. The experiment was performed in triplicate ($n = 3$) with 15 fingerlings in each replicate for 60 days (Table 1). Thereafter, the surviving fingerlings were transferred to tanks containing dechlorinated water for another 60-day recovery period. In parallel, a control ($n = 3$) with 15 fingerlings in each replicate was also maintained having de-chlorinated water only.

Table 1: Experimental design for chronic toxicity bioassay

Groups	Details of treatments
T ₀ (Control)	Fish feed
T ₁	1 mg/l MPs + Fish feed
T ₂	2 mg/l MPs + Fish feed
T ₃	1/10 th LC ₅₀ of wastewater + Fish feed
T ₄	1/20 th LC ₅₀ of wastewater + Fish feed
T ₅ (COMB1)	1/10 th LC ₅₀ of wastewater + 2 mg/l MPs + Fish feed
T ₆ (COMB2)	1/20 th LC ₅₀ of wastewater + 2 mg/l MPs + Fish feed

$n = 3$ per replicate per treatment

2.4 Histomorphology of kidney

Histomorphology of the kidney was done using the procedure of Humason (1975) ^[7]. Briefly, kidneys were collected and fixed in 10% neutral buffered formalin for 24-48 h, processed with a gradient of ethanol, cleared in xylene and embedded in paraffin. Serial sections (3–5 μ m) were obtained using a rotary microtome, mounted on glass slides and stained with haematoxylin and eosin. Microscopic evaluation and image acquisition were performed using a LEICA DM5000 B light microscope equipped with a digital imaging system.

2.5 Organ index of kidney

Organ index of kidney for different groups was determined following the classification system described by Bernet *et al.* (1999) ^[3]. For each experimental group, kidneys from 3 fingerlings were analysed and 3 randomly selected histological sections per fingerling were evaluated. Histological lesions were categorised into reaction patterns. Each lesion was assigned a severity score ranging from 0 (Absent) to 6 (Severe) based on the degree of tissue damage, and an importance factor (1–3) was applied according to its pathological relevance. The total histopathological index and the organ-specific index were subsequently calculated using the formula 1, where r_p , a , r_p , a , o and w indicate reaction pattern, alteration, score value, organ and importance factor respectively.

$$\text{Organ index} = \sum_{r_p} \sum_{a} (a_{o r_p a} \times w_{o r_p a})$$

2.6 Statistical analysis

Data were expressed as mean $\pm$ standard error. Significant differences among treatment groups were evaluated using two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test in Statistix 10.00 software, with significance set at $p \leq 0.05$.

3. Results

3.1 Histomorphology of kidney

Normal morphological structure of kidneys of *C. carpio* consists of renal tubules, glomerulus and hematopoietic tissue. After 15 days exposure, fingerlings belonging to control group showed renal tubules and hematopoietic tissue (Figure 1A). In case of T₁ group, the morphological examination of kidneys depicted detachment of renal tubule from basal lamina, paracellular spaces and damaged hematopoietic tissue (Figure 1B). Similarly, fingerlings exposed to T₂ group showed detachment of renal tubule from basal lamina, paracellular spaces, vacuolization and damaged hematopoietic tissue (Figure 1C). Likewise, fingerlings reared in T₃-T₆ groups for 15 days revealed detachment of renal tubule from basal lamina, paracellular spaces, enlarged spaces in lumen, vacuolization and

damaged hematopoietic tissue (Figures 1D-G). The results showed increase in severity of damage with increase in the concentration of test chemicals.

Similarly, fingerlings belonging to control group after 30 days showed normal renal tubules (Figure 2A). However, fingerlings reared in 1 and 2 mg/l MPs for 30 days revealed vacuolization, detachment of renal tubule from basal lamina, damaged renal tubule and damaged hematopoietic tissue in kidneys of *C. carpio* fingerlings (Figures 2B-C). Similar alterations were observed in kidney of fingerlings exposed to T₃ and T₄ groups (Figures 2D-E). Kidney of fingerlings exposed to T₅ and T₆ groups also indicated alterations such as detachment of renal tubule from basal lamina, paracellular spaces, vacuolization and damaged hematopoietic tissue (Figures 2F-G). Apart from these, severe renal alterations such as tubular necrosis and irregular arrangement of tubular cells, enlargement of Bowman space tissue, collapsed and degenerated glomeruli and enlarged space in lumen were also observed in T₅ and T₆ groups.

After 45 days, normal renal tubules were found in kidney of *C. carpio* belonging to control group (Figures 3A). Morphological examination of kidney of fingerlings exposed to different treatment groups i.e. T₁-T₆ for 45 days exhibited aberrations such as detachment of renal tubule from basal lamina, paracellular spaces, vacuolization, damaged hematopoietic tissue, necrosis, tubular necrosis,

irregular arrangement of tubular cells, collapsed and degenerated glomeruli; and enlarged space in lumen (Figures 3B-G). Similarly, normal renal tubules were found in kidney of *C. carpio* belonging to control group (Figures 4A). Kidneys of fingerlings exposed to different groups i.e. T₁-T₆ for 60 days showed similar aberrations such as detachment of renal tubule from basal lamina, paracellular spaces, vacuolization, damaged hematopoietic tissue, necrosis, exudate and damaged renal tubules (Figures 4B-G). The results clearly indicated dose and duration-dependent effect of LDPE MPs and wastewater on fingerlings. Additionally, the results indicated that the effect of exposure of COMB1 and COMB2 was more severe in kidneys than MPs and wastewater alone.

After recovery period, less severe alterations were observed in the kidney of treatment groups indicating incomplete recovery. After recovery period, fingerlings exposed to control, T₁ and T₂ showed normal structures of kidneys (Figures 5A-C). Similarly, fingerlings exposed to T₃ and T₄ groups revealed normal histological structure with slight aberrations such as exudate formation, detachment of renal tubule from basal lamina and vacuolization in kidney (Figures 5D-E). Alterations such as damaged hematopoietic tissue, paracellular spaces, exudate formation, vacuolization and damaged renal tubules were observed in T₅ and T₆ groups after recovery period (Figures 5F-G).

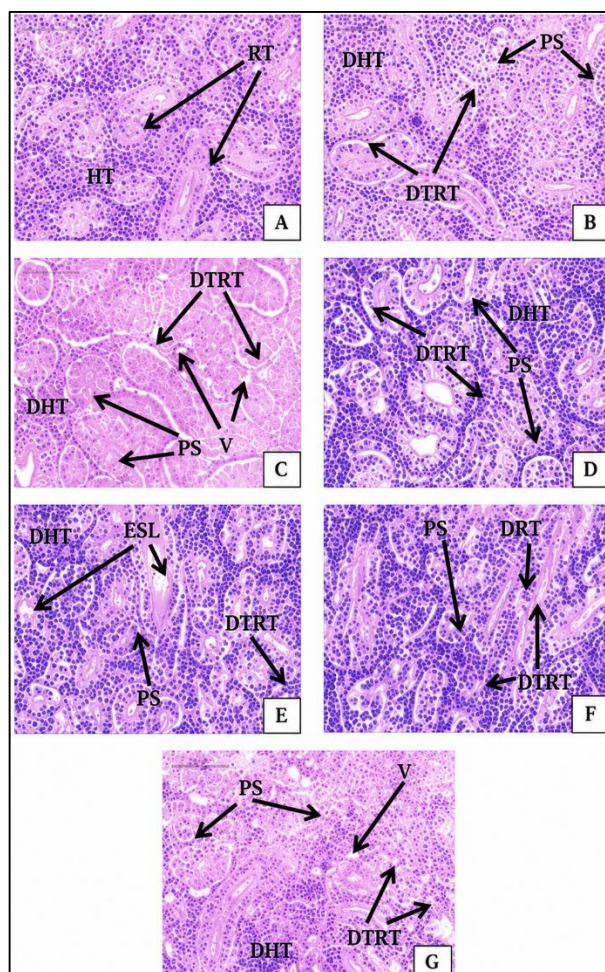


Fig 1: T.S. of kidney of *Cyprinus carpio* fingerlings exposed to different treatment groups for 15 days. A) Control showed renal tubules (RT) and haematopoietic tissue (HT), B) T₁, C) T₂, D) T₃, E) T₄, F) T₅ and G) T₆ showed detachment of renal tubule from basal lamina (DTRT), damaged haematopoietic tissue (DHT), paracellular spaces (PS), damaged renal tubule (DRT), vacuolization (V) and enlarged spaces in lumen (ESL). T₁: 1 mg/l LDPE MPs, T₂: 2 mg/l LDPE MPs, T₃: 1/10th LC₅₀ of wastewater, T₄: 1/20th LC₅₀ of wastewater, T₅: 1/10th LC₅₀ of wastewater + 2 mg/l LDPE MPs, T₆: 1/20th LC₅₀ of wastewater + 2 mg/l LDPE MPs

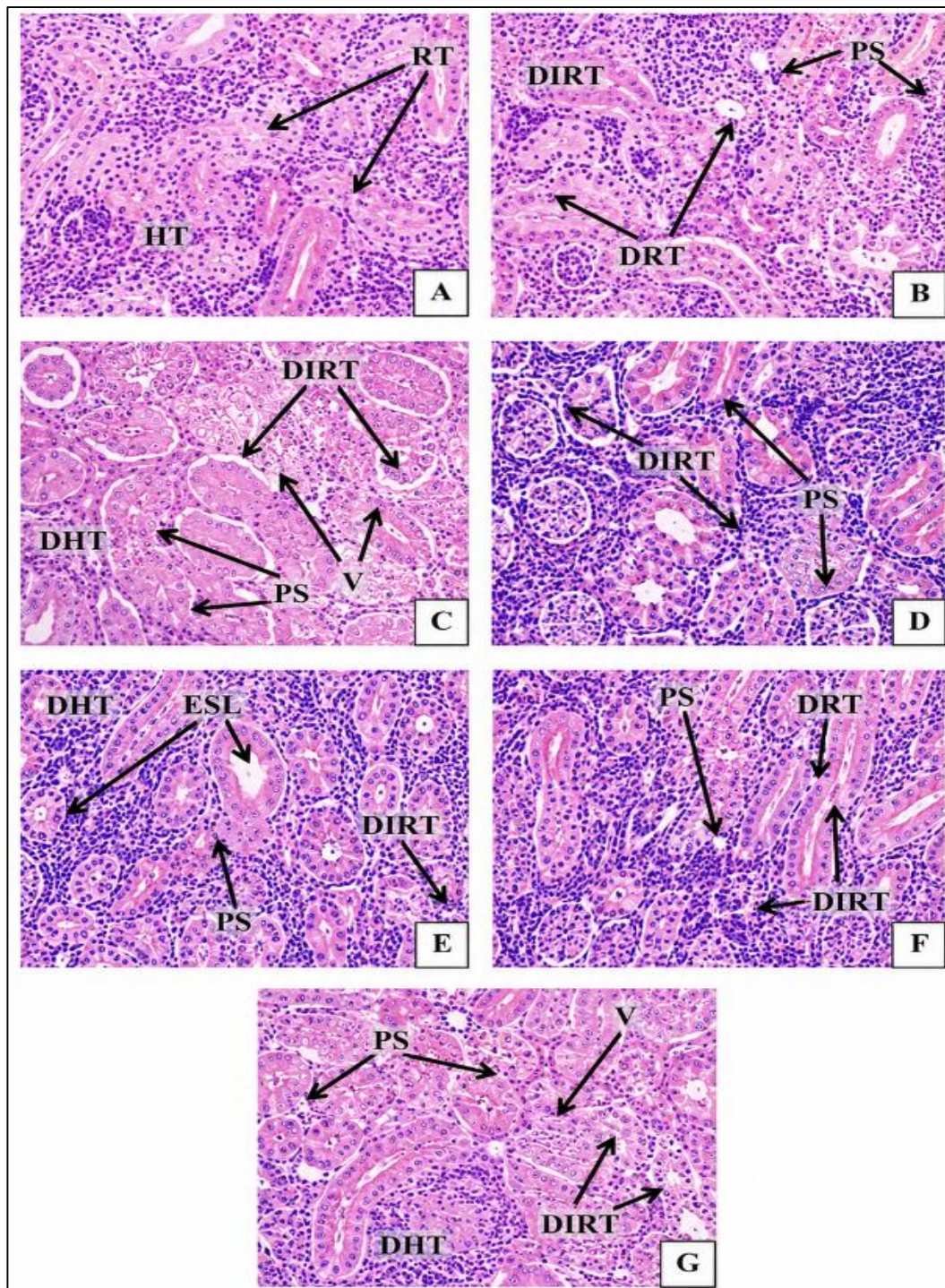


Fig 2: T.S. of kidney of *Cyprinus carpio* fingerlings exposed to different treatment groups for 30 days. A) Control showed renal tubules (RT), B) T₁, C) T₂, D) T₃, E) T₄, F) T₅ and G) T₆ showed detachment of renal tubule from basal lamina (DRT), damaged haematopoietic tissue (DHT), paracellular spaces (PS), damaged renal tubule (DRT), vacuolization (V), damaged glomerulus (DG), tubular necrosis (TN), enlargement of Bowman space tissue (EBS) and enlarged spaces in lumen (ESL). T₁: 1 mg/l LDPE MPs, T₂: 2 mg/l LDPE MPs, T₃: 1/10th LC₅₀ of wastewater, T₄: 1/20th LC₅₀ of wastewater, T₅: 1/10th LC₅₀ of wastewater + 2 mg/l LDPE MPs, T₆: 1/20th LC₅₀ of wastewater + 2 mg/l LDPE MPs

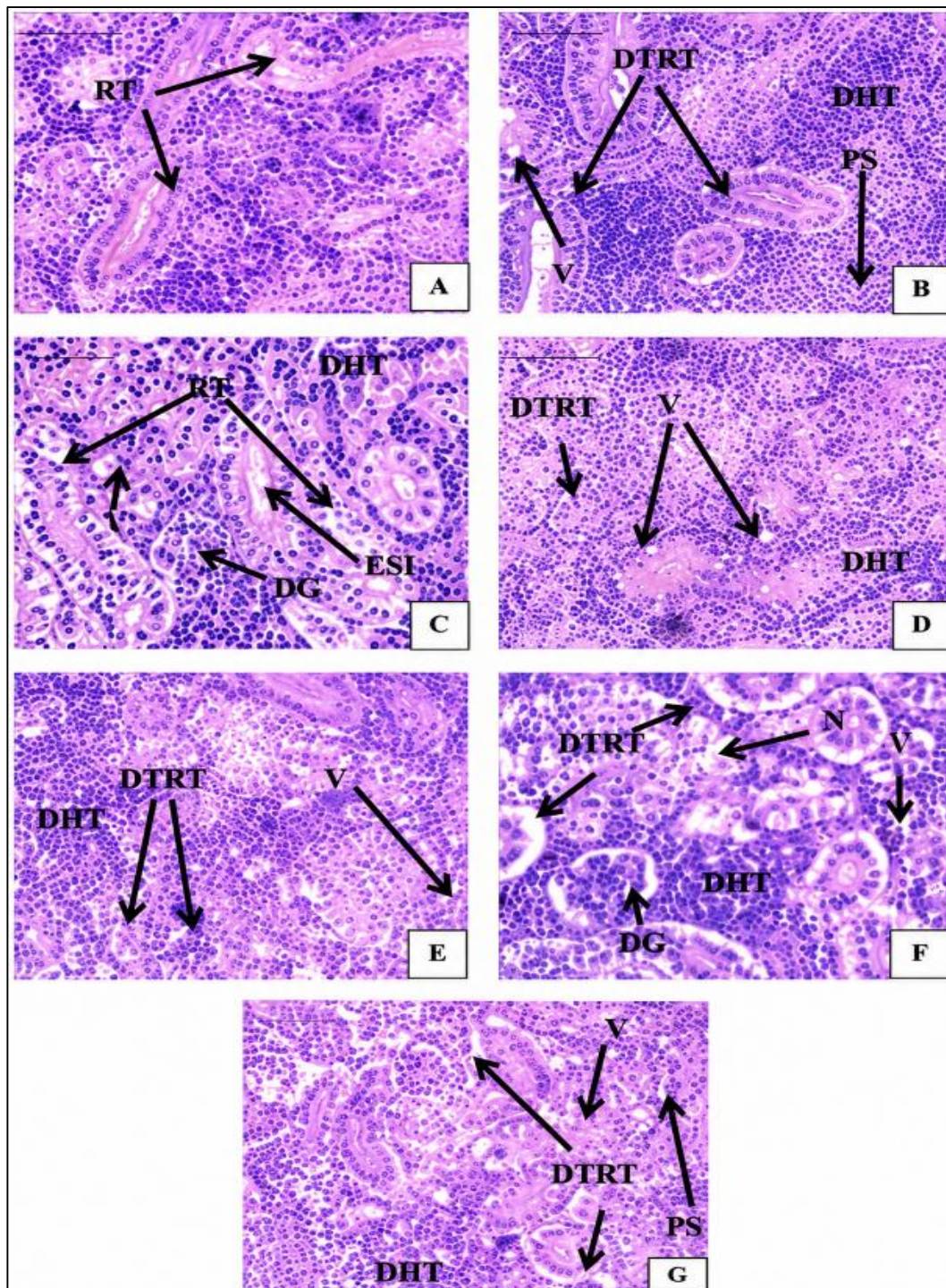


Fig 3: T.S. of kidney of *Cyprinus carpio* fingerlings exposed to different treatment groups for 45 days. A) Control showed renal tubules (RT), B) T₁, C) T₂, D) T₃, E) T₄, F) T₅ and G) T₆ showed detachment of renal tubule from basal lamina (DTRT), damaged haematopoietic tissue (DHT), paracellular spaces (PS), vacuolization (V), damaged glomerulus (DG), tubular necrosis (TN) and enlarged spaces in lumen (ESL). T₁: 1 mg/l LDPE MPs, T₂: 2 mg/l LDPE MPs, T₃: 1/10th LC₅₀ of wastewater, T₄: 1/20th LC₅₀ of wastewater, T₅: 1/10th LC₅₀ of wastewater + 2 mg/l LDPE MPs, T₆: 1/20th LC₅₀ of wastewater + 2 mg/l LDPE MPs

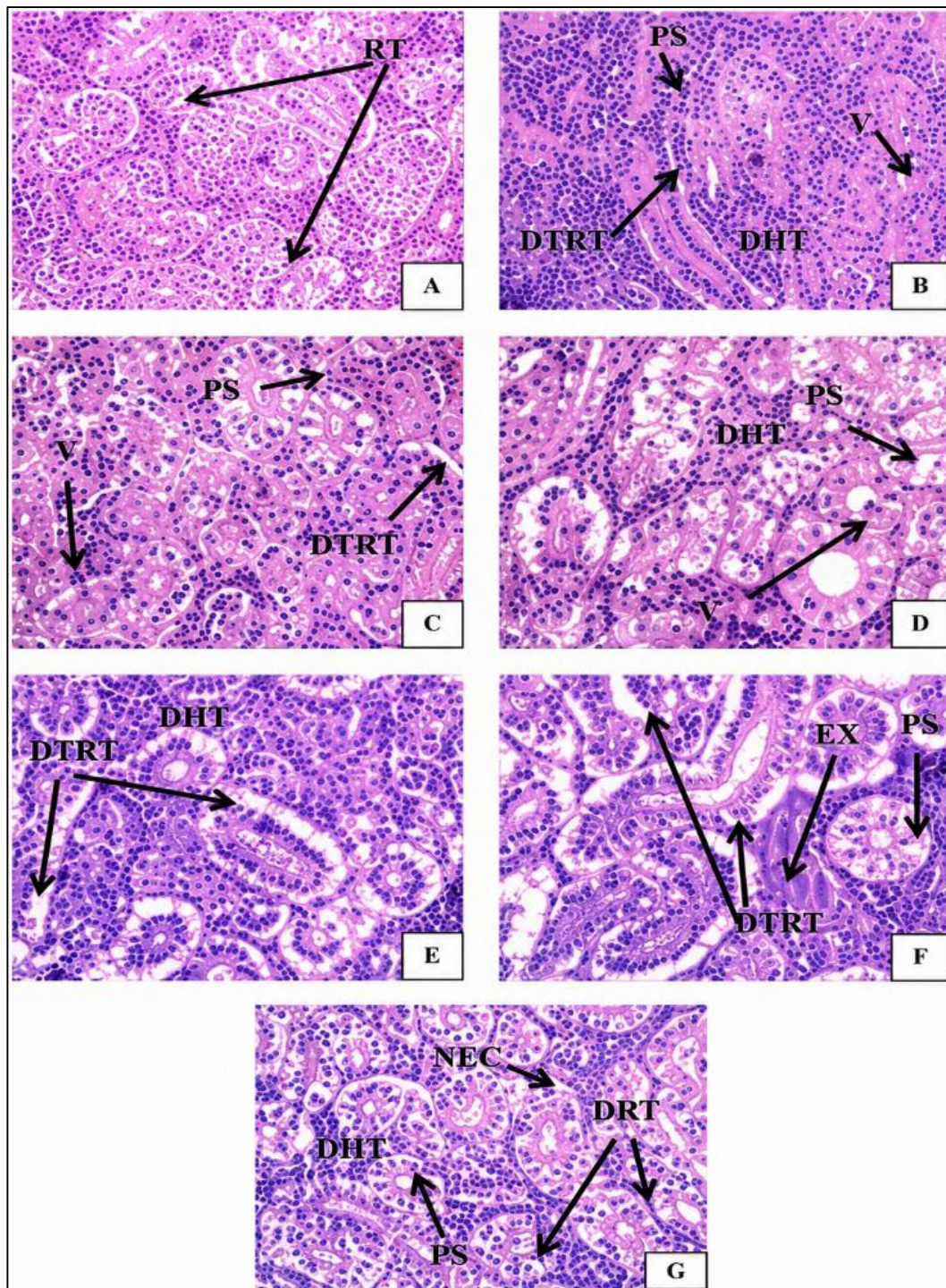


Fig 4: T.S. of kidney of *Cyprinus carpio* fingerlings exposed to different treatment groups for 60 days. A) Control showed renal tubules (RT), B) T₁, C) T₂, D) T₃, E) T₄, F) T₅ and G) T₆ showed detachment of renal tubule from basal lamina (DTRT), damaged haematopoietic tissue (DHT), paracellular spaces (PS), damaged renal tubule (DRT), vacuolization (V), exudate (EX) and necrosis (NEC). T₁: 1 mg/l LDPE MPs, T₂: 2 mg/l LDPE MPs, T₃: 1/10th LC₅₀ of wastewater, T₄: 1/20th LC₅₀ of wastewater, T₅: 1/10th LC₅₀ of wastewater + 2 mg/l LDPE MPs, T₆: 1/20th LC₅₀ of wastewater + 2 mg/l LDPE MPs

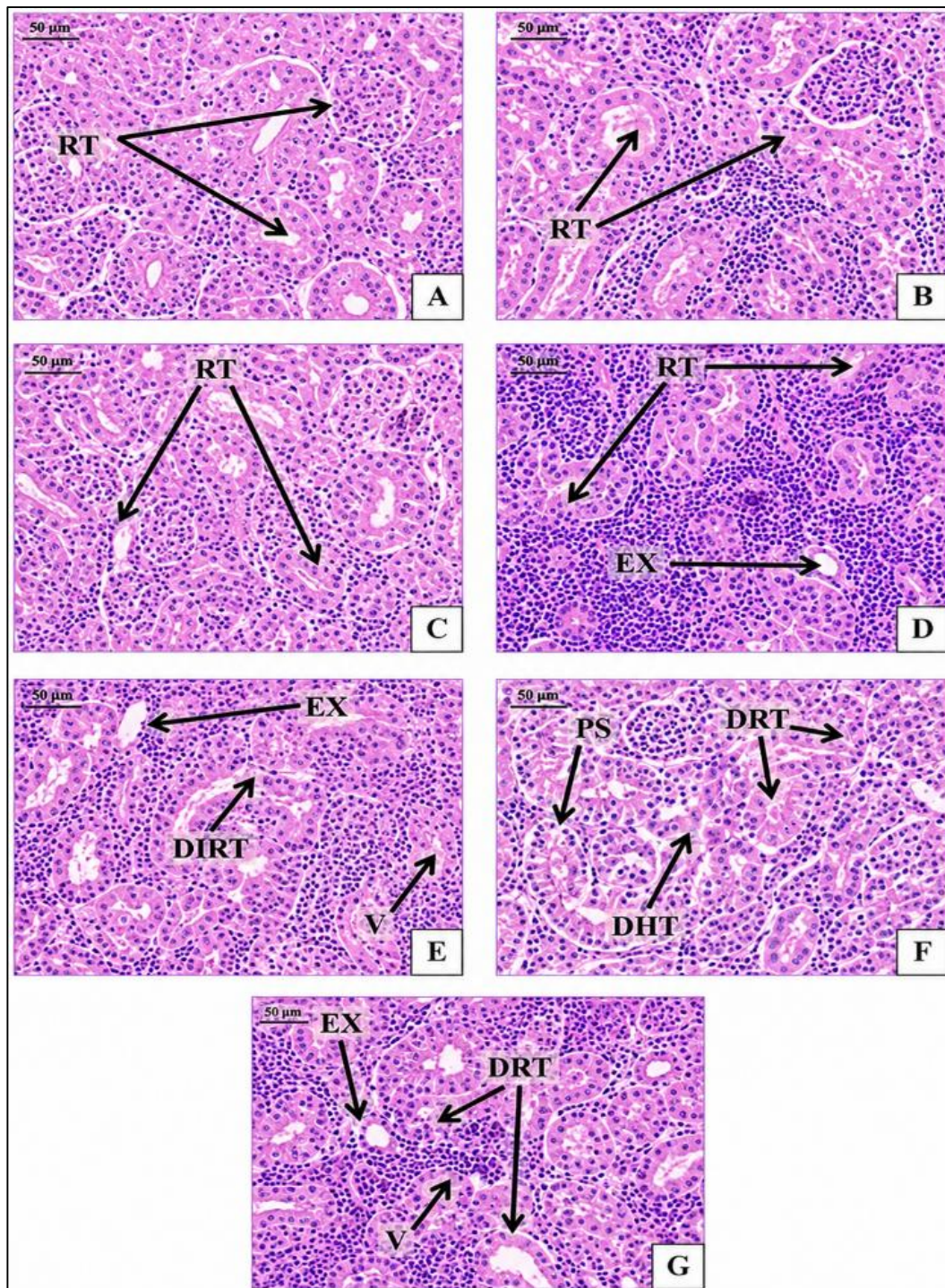
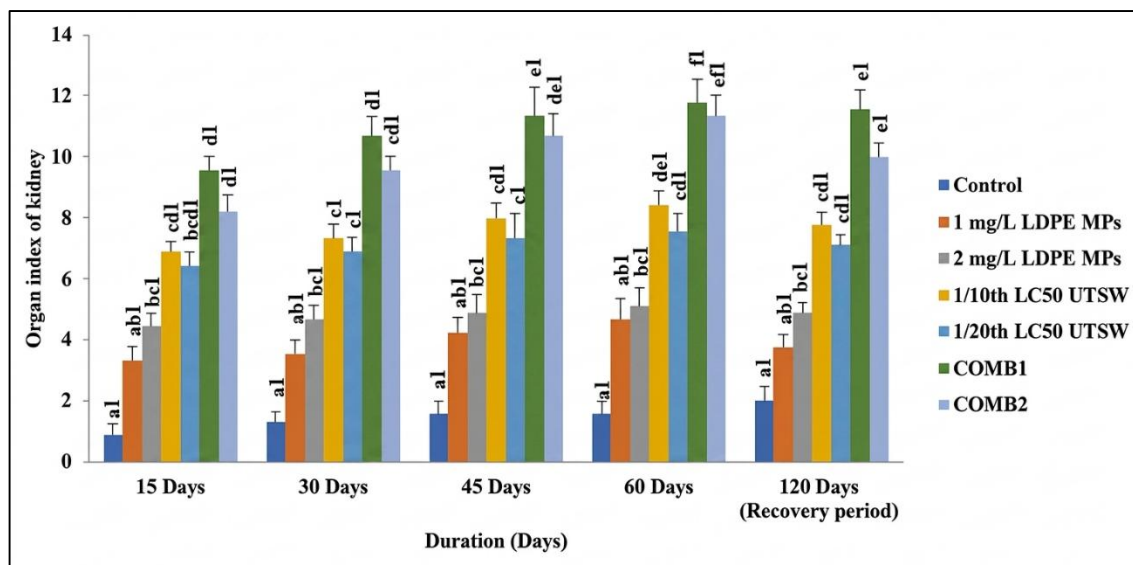


Fig 5: T.S. of kidney of *Cyprinus carpio* fingerlings exposed to recovery period. A) Control, B) T₁, C) T₂, D) T₃, E) T₄, F) T₅ and G) T₆ showed renal tubules (RT), detachment of renal tubule from basal lamina (DRT), damaged haematopoietic tissue (DHT), damaged renal tubule (DRT), vacuolization (V) and exudate (EX). T₁: 1 mg/l LDPE MPs, T₂: 2 mg/l LDPE MPs, T₃: 1/10th LC₅₀ of wastewater, T₄: 1/20th LC₅₀ of wastewater, T₅: 1/10th LC₅₀ of wastewater + 2 mg/l LDPE MPs, T₆: 1/20th LC₅₀ of wastewater + 2 mg/l LDPE MPs

3.2 Organ index of kidney: In the present study, organ index of kidneys of fingerlings belonging to different treatment groups i.e. T₁-T₆ increased non-significantly ($p > 0.05$) after 60 days in comparison to 15 days (Figure 6). After 60 days of exposure, organ index of kidneys of

fingerlings belonging to different treatment groups increased significantly ($p \leq 0.05$) in comparison to control group except fingerlings exposed to 1 mg/l MPs. Along with this, incomplete recovery was observed in different groups compared to control group except 1 mg/l LDPE MPs.



Different alphabetic superscripts (a-f) indicate significant ($p \leq 0.05$) difference among groups
 Same numeric superscripts (1) indicate significant ($p \leq 0.05$) difference w.r.t duration of exposure
 COMB1 and COMB2 refer to 1/10th LC₅₀ of wastewater + 2 mg/l LDPE MPs and 1/20th LC₅₀ of wastewater + 2 mg/l LDPE MPs, respectively

Fig 6: Organ index of kidney of *Cyprinus carpio* fingerlings reared in different treatments after regular time intervals

4. Discussion

Kidney is an essential excretory organ and it plays a pivotal role in maintaining homeostasis (Iqbal *et al.*, 2004) [9]. Kidneys of fish are sensitive to chemical alterations in the aquatic bodies because they are constantly exposed to chemicals dissolved in water (Hussain *et al.*, 2019) [19]. Their roles in excretion and detoxification of toxicants from blood expose them to contaminants (Kim and Kang, 2015) [11]. Kidney plays role in excretion of metabolic waste and selective reabsorption which helps in maintaining volume and pH of various body fluids. Therefore, any alterations in histomorphology of kidney could lead to corresponding alterations in behavioural and physiological parameters (Ortiz *et al.*, 2003) [18].

Histopathological alterations such as destructive renal tubules, necrosis and shrinkage of renal cortex were observed in the kidney of fish. Histological effects of 1, 3 and 5% of LDPE and polyethylene terephthalate MPs on kidneys of *Oreochromis niloticus* were studied by Ali *et al.* (2023) [1]. In a study conducted by Hamed *et al.* (2021) [6], effects of different concentrations of MPs (1, 10 and 100 mg/l) were examined on *O. niloticus* juveniles for 15 days and 15 days post-exposure. Concentration dependent histopathological alterations such as vacuolated tubules, glomerular atrophy, increase in inter-tubular space, inflammatory cells, congestion of blood capillaries, loss of basophilic cytoplasm, shrinkage of convoluted tubules, deformation, vacuolization and fatty tubules were observed in kidneys of experimental groups. The results also showed alterations in kidney even after post-exposure. The results suggested that MPs cause range of alterations from biochemical alterations in single cells to lesions in entire tissue affecting vitality of fish.

The degree of histological alterations in kidneys of fish *O. niloticus* obtained from a polluted site (downstream of Chakbandi drain) was observed by Shahid *et al.* (2022) [22]. Histopathological alterations such as degenerated renal tubules, necrosis, delineated lymphocytic infiltration and obliteration in Bowman's space were observed in kidneys of MPs-exposed groups. Similarly, the effects of water

pollution on kidneys of fishes (*Wallago attu* and *Cirrhinus mrigala*) collected from Chenab River were studied by Hussain *et al.* (2019) [8]. Alterations such as dilation of glomerular capillaries, hyperaemia, degeneration of renal tubule cells, glomerulonephritis, absence of Bowman's space, vacuolization, necrosis, degeneration, hypertrophy and shrinkage of glomeruli were observed in sections of kidneys. However, fish belonging to control group showed normal histoarchitecture of kidneys.

In support of the findings of the current study, histopathological alterations such as necrosis, vacuolization, hypertrophy, degeneration, dilation of glomerular capillaries and contraction of glomeruli were observed in kidneys of *Channa punctatus* exposed to different concentrations of wastewater (60-100%) from Tung Dhab Drain for 15 and 30 days by Kaur and Dua (2014) [10]. Zhu *et al.* (2019) [28] conducted the experiment to study the effects of different concentrations of polystyrene MPs (500, 1000 and 2000 µg/g) on kidneys of *Oreochromis melastigma* for 10 weeks. The alterations such as glomerulopathy and nephrogenesis were observed in kidneys of *O. melastigma* exposed to PS MPs. However, the alterations might have occurred due to leaching of additives from MPs.

5. Conclusion

The present study showed significant alterations such as detachment of renal tubule from basal lamina, paracellular spaces, vacuolization, damaged hematopoietic tissue, necrosis and exudate in kidneys of fingerlings belonging to treated groups. Likewise, organ index of kidney of different groups also showed a significant ($p \leq 0.05$) increase in comparison to the control group except fingerlings treated with 1 mg/l MPs. Moreover, the results showed incomplete recovery in different groups compared to control group except 1 mg/l LDPE MPs. The results indicated that the combination of LDPE MPs and wastewater may impact kidneys of *C. carpio* fingerlings in a duration- and dose-dependent manner with limited reversibility following exposure, highlighting potential long-term risks to aquatic health.

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7. Declarations

The authors declare no competing interests.

Author contribution: The paper was conceived and designed by Vibhu Vaid and Nisha Vashishat. Both the authors have read and approved the final manuscript.

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