Oxidative Stress Induced by Exposure of Microplastics in Labeo Rohita

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Research Article

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Abstract

The present study revealed the oxidative stress induced by the exposure of low density polyethylene microplastics (LDPE MPs) in *Labeo rohita*. Fingerlings were divided into four (control, T1, T2 and T3) groups. Fingerlings of T1, T2 and T3 groups were exposed respectively to 2, 20 and 200 mg/L of LDPE MPs for 45 days. A control was also maintained during experiment to which no MPs were added. Oxidative stress studies revealed a decrease in the activity of hepatic antioxidative enzymes i.e. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) in all treated groups in dose and time dependent manner. In gills, an increase in the activities of SOD, CAT and GPx but decrease in the activities of GR and GST was found in T1 group after 30 days of exposure. However, decrease in activities of these enzymes was observed in all treated groups in dose dependent manner after 45 days of exposure. Moreover, the activities of antioxidative enzymes were found to be decreased in time dependent manner. In kidneys, increase in the activities of antioxidative enzymes was observed in T1 group followed by their decrease in T2 and T3 groups in dose and time dependent manner. However, Lipid peroxidation was found to be increased in vital organs of all treated groups in dose and time dependent manner indicating MPs induced oxidative stress in these organs due to increased production of reactive oxygen species.

1. Introduction

Plastics are synthetic polymers with high molecular weights, made up of linked long chain of similar repeating units called monomers which are derived from petroleum or fossil fuels. These are cheap, strong, durable, water resistant materials thus making it a material of choice for manufacturers of several items of daily use including packaging, clothing and electrical appliances (Horton 2019).

For many years, extreme use and disposal, ineffective material recycling and dumping of plastics on land and sea lead to accumulation of mismanaged plastic litter in the environment. The properties of plastic materials such as durability, longevity and resistance to degradation due to presence of hydrocarbons allow their persistence in the natural environment. Thus, deposition and accumulation of plastics in the natural environment has become an emerging subject of environmental urgency (Asmonaite 2019).

Both marine and freshwater environments are contaminated with plastics all over the world. This huge presence of plastics in the water bodies have adverse effects on the aquatic ecosystems and related economic activities (Boucher and Friot 2017). Plastic waste has two forms: large plastic wastes and small plastic particles less than 5 mm in size termed as microplastics (Thevenon et al. 2014). Large sized plastic wastes are easily visible and studies of these large plastic wastes have revealed a number of undesirable social, ecological and economic impacts. These impacts vary from reduced food intake, intestinal injury, entanglement, intestinal blockage and death of animals. As MPs are not visible due to their small size so their harmful impacts on the animals are not easily identified (Boucher and Friot 2017). These ingested MPs may have many negative effects on marine organisms ranging from physical to physiological effects. These effects include internal abrasions and blockage of digestive tract which

may cause starvation (Gall and Thompson 2015), histopathological changes in the intestine, behavioural changes and change in lipid metabolism (Jovanovic 2017) in fish. There is increasing concern about study of harmful effects of MPs on human health as they may move independently or may adsorb other toxic chemicals while moving in the food chain (Eriksen et al. 2014). Ingestion of these MPs is not only harmful to humans alone but also to ecological health due to accumulation of pathogens on surfaces of plastics.

On the basis of their origin, MPs can be classified into primary and secondary MPs. Primary MPs, manufactured in the form of microbeads, capsules, pellets or fibers are directly released into the environment in the form of small particles. Examples include use of microbeads in personal care products such as in cosmetics, soaps, toothpaste and use of microfibers in textiles. Secondary MPs are produced by the degradation of larger plastic products into smaller plastic pieces through photodegradation and other weathering processes of improper management of wastes such as discarded plastic bags (Boucher and Friot 2017).

To date, several studies have been carried out on the contamination of marine environment by MPs and their effects on various marine organisms including marine fishes. Although studies on MPs in freshwater environments are scarce than marine water studies, plastics have been reported in freshwater fish, insects and invertebrates also (Anderson 2019). Among aquatic organisms, fish are highly susceptible to MPs ingestion due to their buoyancy, attractive colouration and great resemblance to food (Jovanović et al. 2018). Thus, it becomes important to protect and preserve populations of freshwater fish from pollutants of appearing concern like MPs (Pinheiro et al. 2017).

2. Materials And Methods

2.1. Preparation of test chemical

Low Density Polyethylene (LDPE) in pellet form (CAS: 9002-88-4; melting point: 116ºC; density: 0.925g/ml at 25ºC) was purchased from SIGMA-ALDRICH Company, USA. 30 LDPE pellets were taken in each glass test tube. To it, 6 ml of toluene (used as solvent) was added. Then 200 µl of Tween-20 as surfactant was added by using a micropipette and mouth of each glass test tube was covered with aluminium foil/ parafilm to prevent the evaporation of toluene on heating. The test tubes were kept in water bath shaker pre-heated at 95ºC until LDPE pellets were completely dissolved in toluene. After this process, 6 ml of double distilled water (DDW) pre-heated at 95ºC was added in each glass test tube containing the dissolved LDPE at 95ºC. The hot mixture was immediately homogenized at 10,000 rpm for about 30 seconds. The resultant mixture was rapidly spread on wide glass petriplate with the help of spatula and left undisturbed for few days or till the mixture was completely air dried. The obtained LDPE powder was sieved using siever to obtain fine particles of LDPE. The left over large particles were then crushed with the help of pestle and mortar. These crushed particles were then sieved to obtain fine LDPE powder. This formulation of LDPE powder was used as LDPE MPs in the present study.

2.2. Procurement and acclimatization of fingerlings

Healthy fingerlings of *Labeo rohita* (9 ± 2 cm in length) were procured from private fish farm, Ludhiana, Punjab, India. These were acclimatized to laboratory conditions for fortnight in plastic tanks of 100 litres capacity filled with 80 litres of de-chlorinated tap water with adequate values of temperature, pH, alkalinity, conductivity, hardness, dissolved oxygen and biochemical oxygen demand. A natural photoperiod of 12 hour light:dark cycle was maintained in the laboratory. Each tank was fitted with electrically operated two water aerators (to fulfil the oxygen demand) and two water filters (to avoid accumulation of excreta) during the acclimatization period. Fingerlings were fed with commercial fish food (Trade name: Tokyu floating type fish feed) once a day in afternoon during acclimatization period. No feed was given to the fingerlings one day before the start of an experiment. The experimental protocol met the standard guidelines given by Organisation for Economic Co-operation and Development (OECD) (1992).

2.3. Exposure of fingerlings to LDPE MPs

In the present study, fingerlings were divided into four groups- 1st group served as control and 2nd, 3rd and 4th served as MPs treated groups with three replicates per group. A total of nine fingerlings (n = 9) were kept in each tank (35 litres capacity) containing 25 litres of de-chlorinated tap water thus resulting in the use of total 108 fingerlings and 12 tanks. For exposure concentration selection, existing literature was thoroughly studied to find an environmental relevant concentration of PE MPs in freshwater environments. Rochman et al (2017) stated the presence of maximum of 2.8 mg/L of PE MPs in the freshwater environments so accordingly, the present study denoted the concentration of 2 mg/L in water as environmental relevant concentration of PE MPs and this concentration is used in lowest treatment group (T1) in our study. Moreover, the other two concentrations used in the present study were taken as 20 and 200 mg/L in water (T2 and T3 respectively), which are 10 and 100 times higher than this environmental relevant concentration respectively and represented the worst-case scenario of contamination of MPs in the aquatic environment.

The experimental design used in the present study during chronic toxicity test is shown in the following table:

GROUPS	TREATMENTS
Control	Commercial fish feed
T1	Commercial fish feed + **2** mg/L LDPE MPs (environmental relevant concentration)
T2	Commercial fish feed + **20** mg/L LDPE MPs (10 times the environmental relevant concentration)
T3	Commercial fish feed + **200** mg/L LDPE MPs (100 times the environmental relevant concentration)

During the whole experiment, water was completely changed after every 5th day to avoid the accumulation of excreta and the MPs were added to restore its concentration in each tank.

2.4. Assessment of oxidative stress

Fingerlings were sacrificed by putting them for 10–15 minutes in the beaker containing 2–3 drops of clove oil mixed with 1 litre of tap water. After sacrificing, fingerlings were carefully cut opened from the ventral side i.e. the anal fin along the belly up to the operculum using small sharp surgical scissors (taking care not to cut into the fingerling's internal organs and prevent leakage of bile from gall bladder). Various organs i.e. gills, liver, kidneys were dissected out with the help of scalpel, forceps and a pair of sharp surgical scissors for oxidative stress studies.

After sacrificing, 3 fingerlings (n = 3) from each tank were dissected at an interval of 30 days and 45 days to collect the required tissues. For preparing tissue (gills, liver, intestine, muscle and kidney) homogenate, 0.5 g of each tissue was taken in centrifuge tube containing 2 ml of freshly prepared PBS solution and homogenised for 2 minutes following centrifugation at 3,000 r.p.m. for 10 minutes. After centrifugation, the supernatant was collected in 2 ml eppendorf microtubes and stored in deep freezer at -20^{o}C for oxidative stress analysis.

The activities of antioxidative enzymes i.e. superoxide dismutase (SOD) (Marklund and Marklund 1974), catalase (CAT) (Aebi 1983), glutathione peroxidase (GPx) (Hafeman et al. 1984), glutathione-S-transferase (GST) (Habig et al. 1974) and glutathione reductase (GR) (Carlberg and Mannervik 1985) and lipid peroxidation (LPO) (Stocks and Dormandy 1971) was assessed in vital organs of fingerlings of all groups.

2.5. Statistical analysis

All values are expressed as mean ± S.E. Two way analysis of variance (ANOVA) and Tukey's post hoc test was used for determining significant differences among treatments i.e. control, T1, T2 and T3 groups using Statistical Package for Social Sciences (SPSS) version 16 for windows. Values with different alphabetical superscript(s) are considered significant at $p < 0.05$.

3. Results And Discussion

Results revealed a decrease in the activity of hepatic antioxidative enzymes i.e. SOD, CAT, GST, GR and GPx was observed in all treated groups in dose and time dependent manner (shown in tables). In gills, an increase in the activities of SOD, CAT and GPx but decrease in the activities of GR and GST was found in T1 group after 30 days of exposure. However, a significant decrease in activities of these enzymes was observed in all treated groups in dose dependent manner after 45 days of exposure. Moreover, the activities of antioxidative enzymes were found to be decreased in time dependent manner (shown in tables). In kidneys, increase in the activities of antioxidative enzymes was observed in T1 group followed by their decrease in T2 and T3 groups in dose and time dependent manner (shown in tables). However,

LPO was found to be increased in vital organs of all treated groups in dose and time dependent manner (shown in table VI).

Oxidative stress can be defined as a disturbed balance between the formation of damaging reactive species i.e. reactive oxygen species (ROS) and reactive nitrogen species (RNS) and a living organism's ability to deal with them. Increase in the production of ROS and RNS or decrease in the antioxidant defense against ROS and RNS can cause oxidative damage of molecules (Halliwell and Gutteridge 2015). Thus, the control of oxidative stress is crucial for the normal functioning of any organism (Prokic et al. 2018). To provide protection against the attack of potentially destructive reactive species, organisms which utilize oxygen have developed a bio-chemical cellular antioxidative system. This system involves numerous enzymatic, non-enzymatic components and biochemical pathways designed to prevent cellular damage ((Halliwell and Gutteridge 2015, Prokic et al. 2019).

Exposure to xenobiotics results in the elevation in the concentrations of reactive species and/or subsequent decline in antioxidant defences against the produced reactive species. Approach of assessing the levels of oxidative stress is widely used in studies which deals with the mechanisms of environmental toxicity and ecotoxicity in living organisms exposed to contaminants (Bartoskova et al. 2013, Regoli and Giuliani 2014, Faggio et al. 2016). Oxidative stress is measured directly through the production of free radicals, indirectly via antioxidant defences against the reactive species, and by assessing the end-results of oxidative stress and oxidative damage. Most of the studies which examine the oxidative stress induced by MPs have involved two components of oxidative stress i.e. antioxidant defences and oxidative damage (Prokic et al. 2019).

The anti-oxidative system plays an important role in the process of detoxification and removal of harmful toxicants from the organism's body. Thus, knowledge about the response of the anti-oxidative system can provide crucial information of the possible underlying expenses of xenobiotic poisoning and an organism's ability to respond to it. A stressful condition can either stimulate or inhibit the working of anti-oxidative system, depending upon the duration of exposure and severity of the stressor (Lushchak 2011). From the set of components of anti-oxidative system, the enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) have been studied in assessing the effects of MPs (Prokic et al. 2019).

SOD, CAT and GPx are regarded as three major enzymes of first line defense mechanism that directly helps to remove harmful ROS. The enzyme SOD works by catalyzing the dismutation of toxic superoxide anion (O_2-) to molecular oxygen (O_2) and less toxic hydrogen peroxide (H_2O_2). This H_2O_2 is further eliminated by the combined action of CAT and GPx. These enzymes works by catalyzing the reduction of H_2O_2 to harmless products. GPx can also act on other peroxides (Halliwell and Gutteridge 2015). Glutathione (GSH), being non-enzymatic component of the anti-oxidative system, is directly involved in scavenging a broad variety of free radicals or indirectly through the working of GSH-dependent system which includes three enzymes i.e. GPx, GST and GR. GSH acts as a co-factor in GPx reactions. GST conjugates the GSH to the breakdown products of lipid peroxides thus playing its role in preventing

oxidative damage. Glutathione is regarded as one of the principal components of the antioxidative system as it prevents oxidation of proteins and lipids caused in organisms by exposure to different environmental xenobiotics. GR helps in maintaining the normal functioning of GSH by reducing the oxidized form of glutathione (GSSG) to its reduced form (GSH) (Oost et al. 1998, Prokic et al. 2019).

The antioxidative system of organisms exposed to MPs revealed a variety of responses, ranging from induction and reduction to changes which are non-significant, depending on the size, type and concentrations of MPs and the studied tissues and organisms.

Lipid peroxidation (LPO) is a self-reliant chain reaction of molecular events that causes oxidative damage to cell membranes, lipo-proteins and other structures containing lipids (Nam 2011). Alterations in the structure and function of lipid bilayers by peroxidation of membrane lipids changes membrane permeability and encourages penetration of toxicants in the cell (Ayala et al. 2014). Organisms exposed to xenobiotics usually show increase in LPO (Ferreira et al. 2005) hence, measuring the levels of malondialdehyde (MDA) as the product of LPO is a extensively used biomarker of exposure to environmental pollutants (Prokic et al. 2019).

The liver is the main organ which plays a vital role in the detoxification of pollutants as it is the site of storage of many substances, hence, exposure and accumulation of pollutants may affect its vital functions. Gills are paired organs in fish which possess osmoregulatory, respiratory and excretory functions. Their direct contact with the surrounding aquatic environment makes them more prone to exposure of various pollutants. Kidneys are the paired organs that are supposed to be exposed to various pollutants due to its excretory role.

Decrease in the activity of anti-oxidative enzymes in the liver with the increasing concentration of MPs and exposure period may be attributed to the fact that stress induced by the overproduction of ROS in the cells exceeds the self-clearing approach of the antioxidative defence system thereby inhibiting the anti-oxidative enzymes activity. The time dependent decrease in the GPx activity in the liver of all treated groups might be due to an increased production levels of hydroperoxides (Ajima et al. 2017), supposed tobe caused by the adverse effect of LDPE MPs (Iheanacho and Odo 2020).

However, at low concentrations of MPs, the activity of antioxidative enzymes is not always inhibited and consequently the ROS levels are not significantly increased. This suggests that the antioxidative system of fish is resistant to oxidative stress induced by low concentration of MPs, but once the concentration of MPs exposure is increased, the antioxidative capacity of fish is destroyed and fish could not resist the oxidative stress induced by the MPs (Yang et al. 2020). Moreover, increase in the exposure period of MPs to fish is thought to play an important role in decrease in the antioxidative capacity of fish due to continous contact of vital organs (like gills) with MPs. These facts could possibly explain the trends of antioxidative enzymes activities observed in gills and kidneys after exposure to MPs in the present study.

In the present study, LPO levels were found to be increased in all vital organs of fingerlings of all treated groups in a dose and time dependent manner in comparison to control group. This might be due to the

increased levels of MDA, produced as a result of increased ROS, which causes damage to structure of cell membranes and cells hence causing oxidative stress in fingerlings (Umamaheswari et al. 2020).

There are enough studies reporting the decrease in the activities of antioxidative enzymes in the liver of MPs exposed fish but studies involving the changes in the activities of these enzymes in gills and kidneys after exposure to MPs are lacking.

Decrease in the SOD and CAT activity in the liver of European sea bass (*Dicentrarchus labrax* L.) was also reported by Espinosa et al. (2019) on exposure of fish to100 or 500 mg of virgin PE-MPs/ kg diet for 3 weeks. However, GR activity was not affected by MPs exposure. Moreover, no significant effects on the activities of these anti-oxidative enzymes were observed when fish were exposed to PVC MPs diets for 3 weeks.

Marine medaka were exposed to 2, 20 and 200 µg/L of PS MPs in a semistatic system for 60 days. After exposure period, the activities of various antioxidative enzymes and MDA content in gills and liver were recorded. In liver, the activity of GPx in liver was found to be significantly decreased in all exposure groups. But, fish groups exposed to high concentrations of MPs (i.e. 20 and 200 µg/L MPs) showed significant decrease in the activities of CAT and GST in liver and increase in the activity of SOD and GST in gills. Regarding SOD activity, it showed a significant decline only in highest (200 µg/L MPs) treated group. Moreover, the MDA content was found to be significantly declined in both gills and liver of all treated groups which indicated that MPs caused excess production of ROS resulting in increased LPO in these organs, thus suggesting the MPs induced oxidative stress in these organs of fish (Wang et al. 2019).

Similar to the findings of the present study, adult male zebrafish exposed to 10 µg/L and 100 µg/L of polystyrene MPs in water for a period of 35 days resulted a dose and time dependent significant decrease in the activities of antioxidant enzymes (SOD, CAT and GPx) and significant increase in the LPO levels in the liver of fish. However, the activity of GST was found to be increased in the liver of MPs exposed groups in a dose and time dependent manner which may be due to increased efficiency of GST to detoxify the ROS produced as a result of MPs exposure (Umamaheswari et al. 2020).

Exposure of *Clarias gariepinus* juveniles to 0.5, 1.5 and 3.0% PVC MPs in their diet for 45 days resulted in a time dependent decrease in the activities of CAT and GPx in the liver of juveniles. However, the activity of SOD was found to be increased in all treated groups after 30 days followed by its decrease after 45 days of exposure. Moreover, the levels of LPO were found to be significantly increased progressively in all treated groups in a time dependent manner (Iheanacho and Odo 2020).

In contrary, there are several studies reporting the increase in the activities of antioxidative enzymes and consequently decline in the LPO levels in various organs of fish exposed to MPs. This could be due to the use of different species of fish and their life stages; different types, shapes, sizes of MPs; different concentrations and exposure.

4. Conclusion

Chronic exposure of *Labeo rohita* fingerlings to low density polyethylene microplastics caused oxidative stress in their vital organs (gills, liver and kidneys) as depicted by alterations in the activities of antioxidative enzymes and increase in the lipid peroxidation levels in these organs in dose and time dependent manner.

5. Declarations

Animal Research (Ethics): Not applicable
Consent to Participate (Ethics): Not applicable
Consent to Publish (Ethics): Not applicable
Plant Reproducibility: Not applicable
Clinical Trials Registration: Not applicable
Author Contribution: The Corresponding author conducted and pen down this research work under the guidance of second author. Both authors read and approved the final manuscript.
Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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7. Tables

Table I: Effect of microplastics exposure on the specific activity of superoxide dismutase (SOD) (units/mg protein) in vital organs of *Labeo rohita* fingerlings after 30 and 45 days of treatment period

Vital organ	Treatment period (days)	Treatments			
		Control	T1(2mg/L)	T2(20mg/L)	T3(200mg/L)
Liver	30	147.05±0.58^{a}	123.18±0.62^{b}	92.63±0.39^{d}	66.09±0.58^{f}
	45	147.16±0.66^{a}	118.85±0.52^{c}	86.09±0.67^{e}	53.38±0.10^{g}
Gills	30	139.92±0.51^{b}	174.55±0.27^{a}	121.27±0.22^{d}	66.25±0.64^{f}
	45	141.41±0.80^{b}	126.44±0.50^{c}	102.31±0.18^{e}	34.13±0.59^{g}
Kidneys	30	161.07±0.60^{b}	189.75±0.90^{a}	106.49±0.57^{d}	94.00±0.45^{e}
	45	159.01±0.58^{b}	111.65±0.75^{c}	72.38±0.48^{f}	60.48±0.81^{g}

All values are expressed as mean±S.E. (n=3)

Values with different alphabetical superscripts differ significantly at p<0.05.

Table II: Effect of microplastics exposure on the the specific activity of catalase (CAT) (μmoles of H$_2$O$_2$ decomposed/min./mg protein) in vital organs of *Labeo rohita* fingerlings after 30 and 45 days of treatment period

Vital organ	Treatment period(days)	Treatments			
		Control	T1(2mg/L)	T2(20mg/L)	T3(200mg/L)
Liver	30	69.42±0.75^{a}	63.27±0.73^{b}	44.10±0.58^{d}	22.31±0.38^{f}
	45	68.83±0.30^{a}	55.69±0.40^{c}	40.46±0.43^{e}	18.65±0.53^{g}
Gills	30	26.71±0.36^{bc}	33.06±0.62^{a}	21.92±0.48^{d}	19.63±0.35^{e}
	45	27.65±0.46^{b}	24.81±0.51^{c}	19.16±0.60^{e}	16.84±0.10^{f}
Kidney	30	36.57±0.29^{c}	40.56±0.24^{a}	34.86±0.44^{d}	32.35±0.28^{e}
	45	36.56±0.29^{c}	38.22±0.13^{b}	33.38±0.23^{e}	30.46±0.24^{f}

All values are expressed as mean±S.E. (n=3)

Values with different alphabetical superscripts differ significantly at p<0.05.

Table III: Effect of microplastics exposure on the specific activity of glutathione reductase (GR) (µmoles of NADPH oxidized/min./mg protein) in vital organs of *Labeo rohita* fingerlings after 30 and 45 days of treatment period

Vital organ	Treatment period(days)	Treatments			
		Control	T1(2mg/L)	T2(20mg/L)	T3(200mg/L)
Liver	30	19.00±0.29^{a}	17.24±0.30^{b}	13.70±0.35^{c}	8.60±0.35^{d}
	45	19.30±0.18^{a}	16.26±0.37^{b}	9.94±0.39^{d}	4.09±0.13^{e}
Gills	30	20.45±0.29^{ab}	18.95±0.16^{bc}	16.32±0.67^{de}	13.14±0.26^{f}
	45	20.42±0.21^{a}	17.18±0.60^{cd}	15.04±0.13^{e}	10.57±0.30^{g}
Kidney	30	12.36±0.02^{b}	14.65±0.07^{a}	11.47±0.26^{c}	9.53±0.24^{d}
	45	12.42±0.03^{b}	14.50±0.10^{a}	10.14±0.08^{d}	8.18±0.15^{e}

All values are expressed as mean±S.E.(n=3)

Values with different alphabetical superscripts differ significantly at $p<0.05$.

Table IV: Effect of exposure of different concentrations of microplastics on the specific activity of glutathione-S-transferase (GST) (µmoles of GSH-CDNB conjugate formed/min./mg protein) in vital organs of *Labeo rohita* after 30 and 45 days of treatment period

Vital organ	Treatment period(days)	Treatments			
		Control	T1(2mg/L)	T2(20mg/L)	T3(200mg/L)
Liver	30	4.74±0.13^{a}	4.03±0.15^{bc}	3.47±0.22^{cd}	2.42±0.04^{e}
	45	4.66±0.21^{ab}	3.18±0.09^{d}	2.15±0.08^{e}	1.45±0.05^{f}
Gills	30	14.33±0.20^{ab}	13.54±0.05^{b}	11.56±0.22^{c}	8.34±0.22^{e}
	45	14.67±0.19^{a}	10.37±0.20^{d}	9.42±0.25^{d}	4.77±0.29^{f}
Kidney	30	9.49±0.20^{a}	10.41±0.31^{a}	7.12±0.23^{b}	5.11±0.69^{c}
	45	9.72±0.04^{a}	9.90±0.50^{a}	5.33±0.17^{c}	3.30±0.15^{d}

All values are expressed as mean±S.E. (n=3)

Values with different alphabetical superscripts differ significantly at p<0.05.

Table V: Effect of exposure of different concentrations of microplastics on the specific activity of glutathione peroxidise (GPx) (µmoles of NADPH oxidized/min./mg protein) in vital organs of *Labeo rohita* fingerlings after 30 and 45 days of treatment period

Vital organ	Treatment period (days)	Treatments			
		Control	T1(2mg/L)	T2(20mg/L)	T3(200mg/L)
Liver	30	33.67±0.46^{a}	28.00±0.50^{b}	22.16±0.72^{c}	17.09±0.59^{d}
	45	32.56±0.31^{a}	25.75±0.33^{b}	18.51±0.40^{d}	11.25±0.44^{e}
Gills	30	28.75±0.48^{ab}	30.09±0.65^{a}	26.18±0.29^{c}	18.53±0.38^{d}
	45	30.06±0.07^{a}	27.45±0.35^{bc}	20.36±0.71^{d}	12.55±0.40^{e}
Kidney	30	17.71±0.17^{c}	22.03±0.21^{a}	16.19±0.17^{d}	14.72±0.37^{e}
	45	17.90±0.38^{c}	20.02±0.25^{b}	14.57±0.35^{e}	12.66±0.06^{f}

All values are expressed as mean±S.E. (n=3)

Values with different alphabetical superscripts differ significantly at p<0.05.

Table VI: Effect of exposure of different concentrations of microplastics on the lipid peroxidation (LPO) (nmoles of MDA produced/min./mg protein) in vital organs of *Labeo rohita* fingerlings after 30 and 45 days of treatment period

Vital organ	Treatment period (days)	Treatments			
		Control	T1(2mg/L)	T2(20mg/L)	T3(200mg/L)
Liver	30	2.26±0.14^{f}	3.98±0.06^{e}	6.05±0.12^{d}	10.43±0.25^{b}
	45	2.51±0.17^{f}	5.27±0.27^{d}	8.49±0.26^{c}	13.18±0.37^{a}
Gills	30	1.52±0.04^{g}	2.49±0.08^{f}	3.17±0.05^{e}	5.39±0.07^{b}
	45	1.58±0.05^{g}	3.71±0.05^{d}	4.68±0.09^{c}	7.13±0.06^{a}
Kidney	30	4.63±0.02^{d}	4.93±0.15^{d}	5.86±0.08^{c}	6.61±0.14^{b}
	45	4.65±0.03^{d}	5.02±0.08^{d}	6.76±0.11^{b}	7.64±0.17^{a}

All values are expressed as mean±S.E.(n=3)

Values with different alphabetical superscripts differ significantly at $p<0.05$.