Consumption and Impacts of Water-Borne Polypropylene Microplastics on *Daphnia Similis*

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Abstract

Polypropylene microplastics are the primary pollutant in aquatic ecosystems and their toxicity assessment study remains limited. The proposed study focuses on acute exposure of polypropylene microplastic in *Daphnia similis,* which alters the biochemical parameter due to the accumulation of microplastics in the digestive tract. Moreover, the commercially available polypropylene bags have been converted into microplastic using xylene. FTIR results showed the absence of xylene residue in prepared microplastics particles and it was spherical shaped, size range (FE-SEM) from 11.86μm – 44.62μm. The outcome of the impact reveals that acute exposure to polypropylene microplastic in *D. similis* leads to immobilization. Further, the biochemical results showed that ingestion of microplastics increases reactive oxygen species (ROS) production. This leads to an increase in the antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and a non-antioxidant enzyme of reduced glutathione (GSH) and also oxidative stress effects in lipid (lipid peroxidation - LPO), protein (Carbonyl protein - CP) gradually increases due to production of free radicals in *D. similis*. In addition, the neurotransmitter enzyme acetylcholinesterase (AChE) level diminished. These results reflect that accumulation of polypropylene microplastic should exacerbate harmful pollutants on filter-feeding organisms.

Introduction

In the middle of the 19th century, plastic production and consumption rate increased every year due to its durability, versatility, strength and insulating capacity. For example, in 2018, the global plastic production rate increased to 359 million tonnes (Plastic Europe 2019). This accumulated microplastic under the action of physical (photo radiation, weathering conditions), chemical and natural abrasion were converted into fragmented particles, including micro (< 5mm) and nano-sized (< 100nm) reported by Bilal and Iqbal, 2020. Ultimately, 80% of microplastics were originated from the terrestrial ecosystem (Jambeck et al. 2015; Dusi et al. 2016) and 20% of microplastics were originated from ocean-based sources (Wright et al. 2013; Karbalaei et al. 2018). However, their poor disposal management, such as landfilling, incineration, recycling, and pyrolysis (Panda et al. 2010), remains in the environment.

Generally, freshwater is the primary intermediator for transporting microplastic debris from land to the ocean, which causes threads and harm to biodiversity. For example, Miller et al. (2017) reported that the Hudson river in the USA transport 300 million microfibers into the Atlantic Ocean approximately every day. Likewise, microplastic particles were noted in the surface, sediments, and even in organisms of the freshwater ecosystem (Xiong et al., 2018; Tan et al. 2019; Yuan et al. 2019; Yang et al. 2020). Moreover, microplastics were easily consumed by aquatic organisms via contact, entanglement, ingestion & inhalation. These microplastic particles translocate into the various organ of aquatic organisms lead to accumulation (Lu et al. 2016; Paul-Pont et al. 2016; Ding et al. 2018; Yu et al. 2018; Zhang et al. 2019; Elizalde-Velázquez et al. 2020) and severe adverse effects in aquatic fauna such as inflammation, alter the biochemical and physiological parameter, changes in behaviour, decrease feed intake, damage in gastrointestinal tract, growth & reproduction retardation leads to genotoxicity and finally attain death (Lu

et al. 2016; Ding et al. 2018; Yu et al. 2018; Xu et al. 2020b). This problem promotes the crucial worries about the ecological and human health effects of microplastic via tropic transfer.

It is a thermoplastic polymer and derived from a monomer of propylene petrochemical products. It was having a wide range of applications in the field of the food industry (packaging container, plastic bags), pharmaceutical industry (disposable syringe, specimen bottle), electronic products, personal products (hairdryers, feeding bottles, litter trays, toys, plastic boxes etc.), textile industry (clothing, diapers) & automobile sector and some other industrial applications (Grigore et al. 2017; Hwang et al. 2019). Thus, constant polypropylene material-based products and unusual disposal methods create a significant pollutant in the environment. Some researchers should be investigating the microplastic impacts in freshwater zooplankton *Daphnia* sp under laboratory conditions. Several studies reported that bioaccumulation, acute and chronic toxicity, toxic effects in morphological level, reproduction, embryonic development in *Daphnia* (Jemec et al. 2016; Ogonowski et al. 2016; Rehse et al. 2016, Frydkjær et al. 2017; Rist et al. 2017; Aljaibachi and Callaghan 2018; Kelpsiene et al. 2020; Xu et al. 2020a). Moreover, previous study results revealed that the accumulation of microplastic particles depends on the based upon types, species, particle size, exposure duration and food availability (Jemec et al. 2016; Cui et al. 2017; Aljaibachi et al. 2018; Lin et al. 2019; Espinosa et al. 2019; Kelpsiene et al. 2020; Xu et al. 2020a). Nevertheless, there is a lack of knowledge on biochemical effects on *Daphnia* sp when exposed to microplastic particles. Especially there is no research available on polypropylene microplastic toxicity and its impacts in *Daphnia*.

This study aimed to assess acute exposure (24 hours) of polypropylene microplastic in *D. similis* and their accumulation was investigated. Additionally, to evaluate the impacts of biochemical parameters such as enzymatic antioxidant and non-enzymatic antioxidant enzyme [Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), reduced glutathione (GSH), Glutathione -S- transferase (GST)]; oxidative stress level [Carbonyl protein (CP), Lipid peroxidation (LPO)] and neurotransmitter enzyme [Acetylcholine esterase (ACHE)] were also assessed.

Materials And Methods

Preparation of microplastic particles

The polypropylene bags were purchased from the local market (51-micron thickness; density-0.85 – 0.92 g/cm^3) Karaikudi, Tamil Nadu. Polypropylene bags were cut into small pieces and they were sterilized using Tween 20 solution followed by 70% ethanol. Then it was boiled with xylene at 60 °C and obtained solute was air-dried, washed with distilled water and kept in a hot air oven at 60 °C up to evaporation of water (Das and Kumar 2015). Finally, this powdered polypropylene microplastic was filtered through a 50µm pore size filter.

Preparation of microplastic particles for toxicity assessment

Microplastic particles were immersed in 4% crystal violet and vortexed well (Supplementary figure 1) and elimination of extra stain by frequently washing with sterile water and dried in a hot air oven. Then 1000mg /L concentration stock suspension was prepared and this stock suspension was sonicated for 20 minutes and further, this solution was stirred at 600rpm for 20 minutes.

Physiochemical characterization of Polypropylene microplastic samples

Polypropylene plastic was identified by Attenuated Total Reflection-Fourier transform infrared spectroscopy (ATR- FTIR) for commercially available polypropylene film and Fourier transforms infrared spectroscopy (FTIR) for powdered polypropylene microplastic.10 milligrams of microplastic particles were mixed with potassium bromide and make into a pellet. The pellet was kept in a sample holder and scanned with 4500cm^{-1} – 400 cm^{-1}. Field Emission - Scanning Electron Microscope (FE-SEM) was used to determine the polypropylene microplastic's surface morphology and their particle size. FTIR and SEM analysis characterized powdered polypropylene microplastic samples.

Culture conditions of *Daphnia similis*

D. similis was used as test organisms for evaluating the polypropylene microplastic toxic effects. In the present study, *D. similis* stock culture was maintained in our laboratory for approximately three months before the study. Culture organisms alternative feed with blue-green algae and yeast for maintained at 22 ±2°C and pH – 7.2 – 7.8 in a photoperiod of 16:8 (light: dark). Water renewed twice for a week. The *D. similis* culture was maintained by following the previous method of Vijayakumar et al. (2018).

Acute toxicity experiment

Acute toxicity test was conducted according to OECD test guidelines 202 (2004). Newborn neonates of *D. similis* less than 24 hours old were selected and was transferred to a glass test container with microplastic particles. Controls contained only test medium without polypropylene microplastics. Three repetitions per treatment were done. In each container, add 20 animals and were exposed to different concentrations (0, 12.5, 25, 50, 100 mg/L) of microplastic added to the test medium. Acute toxicity study was conducted for up to 24 hours. (Previously, we experimented on polypropylene microplastic exposure for up to 48 hours. As results show that (Data not shown) animals were destroyed. Ingestion, accumulation and adherence of polypropylene microplastic particles can be visualized under the stereomicroscope.

Biochemical parameter analysis

After 24 hours of exposure, experimental water was filtered and collect the animals. Collected animals were rinsed with deionized water and ice-cold 0.1M phosphate buffer saline (PBS - pH7.4) to obliterate adherent microplastic particles. Then, these animals were homogenized using 1.5 mL of ice-cold PBS (pH 7.4) for 10 minutes and centrifuged at 10,000 rpm for 15 minutes (Galhano et al. 2020). The supernatant was collected and stored at -20°C used for further enzyme analysis.

The biochemical markers associated with antioxidant enzyme activity (Catalase, CAT; Glutathione peroxidase, GPx; Superoxide dismutase, SOD), biotransformation (Glutathione S- transferase, GST), non-enzyme antioxidant activity (Reduced glutathione, GSH) and neurotransmission (acetylcholinesterase, AChE), oxidative stress (Lipid peroxidation, LPO; Carbonyl protein, CP), were determined. The protein content of the *D. similis* was determined by the dye-binding method of Bradford (1976) using bovine serum albumin as standard.

Antioxidant and Non- antioxidant enzyme activity

SOD activity was done by following the method of Suzuki (2000). Absorbance was measured at 560nm and expressed as U/mg of protein. Enzyme CAT activity was determined by the following method of Cohen et al. (1970). Catalase enzyme can decompose substrate hydrogen peroxide into hydrogen and water and monitor at 240nm in ELISA reader and expressed in U/mg of protein. GPx activity determines the NADPH oxidation presence of H_2O_2 and reduced glutathione (GSH) following the procedure Habig et al. (1974) and measured at 340nm. This activity was represented as U/mg of protein. GST activity was determined by Habig et al. (1974) method using the substrate as 1-chloro 2,4- dinitrobenzene (CDNB) for 96 well microtiter plate and measured at 340nm. Unit of GST activity denoted in U/mg of protein. Reduced glutathione (GSH) was measured by adding DTNB formed yellow colour and absorbed at 412nm. This procedure followed Jollow et al. (1974) and their concentration expressed at µmol GSH mg^{-1}.

Oxidative stress effects on protein and lipid

LPO was determined by measure the amount of thiobarbituric acid and malondialdehyde complex, from which thiobarbituric acid was used as a substance, followed by Buege and Aust (1978). The spectrophotometrically was measured at 532nm and expressed at µmol MDA mg^{-1} protein. Carbonyl protein (CP) quantification was measured at 360nm in ELISA reader following by the method of Reznick and Packer (1994) and oxidation of CP expressed by µmol carbonyl mg^{-L} protein.

Acetylcholine esterase activity

AChE activity was measured by the following procedure of Ellman et al. (1961) and acetylcholine iodide used as the substrate, spectrophotometrically measured at 412nm and their unit expressed as U/mg of protein.

Statistical analysis

All assays were done in Triplicates. Data were processed with Microsoft excel and one way of variance (ANOVA) followed by Tukey's HSD test. Results were expressed standard error, and the adapted level of significance is $p < 0.05$.

Results

Physiochemical characterization of polypropylene microplastic

Determination of the chemical composition of polypropylene microplastic by FTIR

Fourier-transform infrared spectroscopy analysis was carried out to identify the chemical bond present in polypropylene plastic material. ATR- FTIR spectra of virgin polypropylene commercial film (Figure 1a) has shown dominant absorptions peak at 2950-2840 cm^{-1} (symmetrical and asymmetrical stretching of CH_3); 1456 $cm^{-1}$and 1376 cm^{-1} (Symmetrical bending CH_3); 1168 cm^{-1} (C-H Rocking), 974 cm^{-1} (Rocking and stretching of C-C and CH_3); 842 cm^{-1} (C-H and CH_3 rocking, C= C bending), 810 cm^{-1} (asymmetric and symmetric stretching –H- C-H bending). Similar results have shown that xylene treated polypropylene microplastic particles and these microplastic particles do not have any xylene residue. FTIR spectra of polypropylene microplastic particles results (Figure 1b) showed that 2962-2839 cm^{-1}, 1462 cm^{-1}, 1377 cm^{-1}, 1168 $cm^{-1,}$ 972 $cm^{-1,}$ 840 cm^{-1}, 810 due to symmetrical and asymmetrical stretching of CH_3, Symmetrical bending CH_3 (1462 $cm^{-1}$and 1377 cm^{-1}), C-H Rocking, Rocking and stretching of C-C and CH_3, C-H, CH_3 rocking and C= C bending, 809 cm^{-1} (asymmetric and symmetric stretching –H- C-H bending).

FE-SEM and TEM

FE-SEM analysis results show our polypropylene microplastic was spherical; the size of the polypropylene microplastic particles ranged from 11.86µm – 44.62µm. (Figure 2a & 2b).

Biodistribution and adherence of polypropylene microplastic in *D. similis*

24 hours exposure of polypropylene microplastic particles in *D. similis* at different concentration as follow as 0, 12.5mg/L, 25mg/L, 50mg/L and 100mg/L. Microplastic ingestion in *D. similis* was proved by accumulation in the digestive track within 24 hours of exposure (Figure 3). Accumulation of microplastic in *D. similis* directly proportional to exposure concentration and also adherence in their external appendages such as head, shell spine, post abdominal claw, antennae, thoracic leg, abdominal setae (Figure 4). The animal attains immobilization within 24 hours. Further biochemical parameter impacts were analyzed by the collection of animals from treated and control groups.

Biochemical parameters in *D. similis* exposed to polypropylene microplastic

Antioxidant and Non- antioxidant enzyme activity

Antioxidant and non-antioxidant enzyme results were reported in Figure 5 (i). *D. similis* exposed to polypropylene microplastic for 24 hours showed a significant increase ($P< 0.05$) in SOD activity at high concentration (100mg/L) compared to the control group. Similarly, this activity also exhibited significant effects in 25 mg/ L and 50mg/L (Figure 5-i-a). Enormous increases ($P< 0.05$) in CAT enzyme (Figure 5-i-b) activity was observed on each polypropylene microplastic exposed concentration (12.5, 25, 50 mg/L) at 24 hours in *D. similis* compared to the microplastic unexposed group. Compared to the control group *D.*

similis exposed to polypropylene microplastic for 24 hours, the treated group (12.5, 25, 50, 100 mg/L) shows significantly increased (P< 0.05) GPx activity (Figure 5-i-c). Bio- transferase GST enzyme (Figure 5-i-d) activity moderately increased in *D. similis* exposed to polypropylene microplastic at 24 hours compared to the control group. Nonantioxidant GSH enzyme level (Figure 5-i-e) remarkably increases (P< 0.05) during exposure to polypropylene microplastic at high concentrations (25,50,100mg/L) compared to the control group of *D. similis.* Since there is no variance in control and 12.5mg/L microplastic exposure group

Oxidative stress effects on protein and lipid

D. similis, treated (24hours) with polypropylene microplastic, expressed increased (P< 0.05) carbonyl protein level (50mg/L and 100mg/L) as compared to the control group. Moreover, there is no significant difference between 12.5mg/L and 25mg/L exposed group (Figure 5-ii-f). Similarly, the lipid peroxidation activity in *D. similis* exposed to polypropylene microplastic for 24 hours increased remarkably at each concentration compared to control group animals. Especially in 100mg/L, LPO activity significantly elevated (P< 0.05) while remaining concentrations (12.5, 25, 50 mg/L) have increased the lipid peroxidation activity related to control (Figure 5-ii-g)

Acetylcholine esterase activity

Acetylcholine esterase activity in *D. similis* significantly decreased at different (12.5, 25, 50, 100mg/L) concentrations exposed to polypropylene microplastic for 24 hours compared to control groups (Figure 5-iii-h).

Discussion

Several studies have been highlighted on commercially available microplastic (Ding et al. 2018) and primary (personal care product) microplastic and also assess the toxic effects in freshwater organisms (Jemec et al. 2016; Kokalj et al. 2018). Only a few studies stated that preparation of microplastic particles using organic solvent and determine their toxic effects in *D. magna* (Schür et al. 2020). In this study, we prepared polypropylene microplastic particles using xylene. Moreover, our FTIR results revealed no persistence of xylene residues in our prepared polypropylene microplastic (Figure 1a & b).

Furthermore, several studies focus on polystyrene and other microplastics such as Nylon, PET polymer. Nevertheless, only a few reports are available of polypropylene microplastic impacts. For example, exposure to polypropylene microplastic in human-derived cells (Hwang et al. 2019), leads to cytokine production from immune cells causes cytotoxicity in freshwater plankton *Hyalella Azteca* (Au et al. 2015) leads to retarded growth. In addition, Li et al., 2020 reported that polypropylene and polyethylene (24%) microplastic particles are distributed equally in freshwater ecosystems. So, we have chosen the polypropylene microplastic due to its major concern pollutant and its toxicity-based study was still limited in freshwater organisms. In several studies, microplastic particles are tagged with fluorescent dye to

determine their accumulation in organisms. However, this study is innovatively used to prepare crystal violet dye as a staining agent for microplastic particles. (Supplementary Figure 1).

In FTIR, analysis Polypropylene main peak were observed, and this study our prepared polypropylene microplastic results (Figure 1a & 1b) similarly denoted by Tiwari et al., 2019 (2961-2842cm^{-1}, 972cm^{-1} and 841cm^{-1}), Putra et al., 2018 (2866 cm^{-1},2837 cm^{-1},1460 cm^{-1} and 1378 cm^{-1}), Sathish et al., 2020 (2915 cm-1,2945 cm^{-1}, 1455 cm^{-1}- 800cm^{-1}) There is no peak variation between the virgin polypropylene materials and xylene treated polypropylene microplastics in FTIR analysis. This report revealed the absence of xylene residue in our microplastic particles. Usually, microplastic particles are different in shapes, such as granular/pellet, spherical or bead, fiber/line, foam and flakes/fragmented particles in a natural environment reported by Zhu et al. 2019 and Campanale et al. 2019. This study prepared spherical (Figure 2a) shaped with irregular (rough) surface polypropylene microplastic particles. Generally, *Daphnia* sp feeds the particle size from 1µm - 50 µm (Ebert 2005). We prepared polypropylene microplastic particles in the range of about 11.86µm – 44.62µm (Figure 2a & 2b). So, we prepared our zooplanktonic organisms to ingest microplastic particles easily.

Planktonic organisms have more sensitive to exposure to different polymeric substances. So, it causes harmful effects and alters the physiological, biochemical and molecular levels.In the freshwater ecosystem, zooplanktons are the primary food source of most fishes and play a vital role in the carbon and nutrient cycle. *D. similis* was filter-feeding organisms that cannot distinguish their food particles from microplastic. Moreover, this study shows that polypropylene microplastic particles are easily ingested and accumulated (Figure 3) in the gastrointestinal tract of Cladocera. Moreover, it was adherence in their appendages (Figure 4). similar results revealed by Elizalde-Velázquez et al. (2020) described polystyrene microplastic exposure in *D. magna* shown to accumulate in their gastrointestinal tract (Elizalde-Velázquez et al. 2020).This accumulated microplastic in organisms alters the physiological and biochemical parameters, diminishes the energy status of organisms and collapses entire metabolisms (Eltemsah and Bøhn 2019).

In this study, acute exposure to polypropylene microplastic in *D. similis* causes immobilization within 24 hours. Accumulation of polypropylene microplastic in the digestive tract of *D. similis,* which causes oxidative stress and alters the biochemical parameter (Figure 5) by producing an uneven number of electrons in oxygen molecules, leads to the formation of free radicals in their body. These free radical molecules easily react with other biomolecules causes oxidative stress or damage. This leads to an increased reactive oxygen species (ROS) (Piddington et al. 2001). Usually, ROS level rises, leading to a decline in the antioxidant enzyme activity and other enzyme activity and causes cellular damage. SOD is a cellular antioxidant defense enzyme that converts superoxide anion into hydrogen peroxide (H_2O_2). SOD activity end product of H_2O_2 undergoes by the action of CAT enzyme reduced into water and oxygen. This CAT enzyme is located in the peroxisomes of mitochondria. In this study, CAT enzyme activity increased in each concentration. This enzyme prevents the organisms from the production of ROS levels. In addition, GPx, GSH and GST, an important intracellular antioxidant enzyme, helps reduce the H_2O_2 and

lipid peroxide into less toxic hydroxyl compounds (Winston and Giulio 1991; Jiang et al. 2017), prevents the formation of intracellular free radicals and ROS level. Compared to control, our results showed increased SOD enzyme activity (Figure 5-i-a) in *D. similis* at 24 hours of exposure to polypropylene microplastics. This study indicates that polypropylene microplastic alters SOD enzyme activity, leads to higher ROS level production and induces oxidative stress in *D. similis*. Similar results revealed by Zhang et al. (2019a) 14th-day exposure of polystyrene microplastic increased the SOD activity in the liver of *Oreochromis niloticus* (Zhang et al., 2019b). In Zebrafish, CAT enzyme level increases due to 21-day exposure to polystyrene microplastics (Liu et al. 2019). Likewise, results showed that CAT enzyme activity (Figure 5-i-b) increases based on their concentration in this study. Likewise, GPx and GSH activity increases (Figure 5-i-c & 5e). This result denoted that polypropylene microplastic exposure in *D. similis* induces intracellular ROS levels. Yu et al. (2018) reported that polystyrene microplastic exposure in juvenile *Ericheir sinensis* leads to increased GPx and GSH activity in low concentrations, while exposure in high concentrations causes the decrease of GPx GSH activity. This GPx and GSH enzyme activity decreases due to microplastic exposure inducing oxidative stress's energy cost (Yu et al. 2018). In this study, GST activity also slightly increased (Figure 5-i-d). Nevertheless, not gave significant effects to compare to the control group due to slow degradation or loss of GST enzyme activity when exposure to polypropylene microplastic in *D. similis* (Revel et al. 2019). Paul- pont et al. (2016) reported that seven-day exposure to polystyrene microplastic causes decreased GST activity in the gills and digestive gland of marine mussels *Mytilus edulis.*

Correspondingly, the formation of free radicals in *D. similis* leads to cause the reactive oxygen species, oxidative stress, protein, lipid, DNA (Deoxyribonucleic acid), and modified enzyme activity (Alkimin et al. 2020). Our report shows that polypropylene microplastic induces ROS level and oxidative stress in *D similis*. In LPO assay (Figure 5-ii-f), their final product MDA (Malondialdehyde), causes injury to the lipid membrane. Our results denoted that exposure microplastic enters into the lipid droplet of *Daphnia* sp. Further, it can enter into cellular organelles and alter their functions (Rosenkranz et al. 2009). Carbonyl protein is an indicator of oxidative protein damage by organisms' exposure to different toxic substances. When animal exposure to different toxicants affects the carbonyl protein leads to oxidation. This oxidation leads to biological changes and deleterious effects in organisms. In our study replies that concentration increases the CP level increases (Figure-ii-5g). Similarly, polypropylene microplastic exposure in marine polychaete *Hediste diverticular,* carbonyl protein content increases. This result shows that oxidation of protein encouraged by ROS leads to cell damage and death (Silva et al. 2020)

AChE is a neurotransmitter that helps the transmission of impulses in organisms. Variation in AChE activity depends upon of organisms should react differently in response to external disturbance or stimulus. Our results show that polypropylene microplastic also reduces the choline esterase enzyme activity (Figure 5-iii-h) of *D. similis.* Previous reports show that microplastic polystyrene particles were inhibited the AChE activity in freshwater fish Tilapia (Ding et al. 2018). Likewise, Jemec et al. (2008) reported that in Daphnids, metal exposure causes diminished neurotransmitter activity and functionalized polystyrene nano plastic particles significantly reduced AChE activity (Lin et al. 2019). The above result

suggested that acute exposure (24 hours) of polypropylene microplastic particles in *D. similis* accumulates in their digestive tract and alters the important biochemical enzyme parameters. Further studies will find out the molecular mechanisms behind the influence of polypropylene microplastic exposure in *Daphnia sp*.

Conclusion

The present study explained, accumulation and effects of polypropylene microplastic in freshwater cladoceran *D. similis*. The work shows that 24 hours exposure of (1000mg/L) polypropylene microplastic particles causes immobilization of organisms, generating free radicals. Consequently, it turned on oxidative stress and further altered the physiochemical enzyme parameter such as SOD, CAT, GPx, GSH, GST, CP, LPO and AChE in *D. similis*. In the future, further study may be focussed on polypropylene microplastic toxic effects in different trophic organisms. In the freshwater ecosystem, prolonged exposure of polypropylene microplastic particles in *Daphnia sp* could cause ecological imbalance and toxic to higher architecture through the food web. Further research will shed light on the preventive measure of microplastic particles entering freshwater ecosystems and removing persisted microplastic particles by finding new potential remediation techniques.

Declarations

Ethical approval and consent to participate

Not applicable

Consent to publish

Not applicable

Author contributions

Jeyaraj Jeyavani: Conceptualization, Methodology, Software; **Ashokkumar Sibiya:** Formal analysis; **Narayanan Gopi:**Formal analysis; **Shahid Mahboob:** Resources, Validation; **Khalid A. Al-Ghanim:** Visualization, Validation; **Fahad Al-Misned:** Formal analysis; **Zubair Ahmed:** Visualization, Validation; **Mian Nadeem Riaz:** Formal analysis; **Balasubramanian Palaniappa:** Formal analysis; **Marimuthu Govindarajan:** Writing - Review & Editing; **Baskaralingam Vaseeharan:** Conceptualization, Visualization, Validation

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Availability of data and materials

Not applicable

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Declarations of competing interest

The authors declare that they have no known competing financial interests.

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Figures

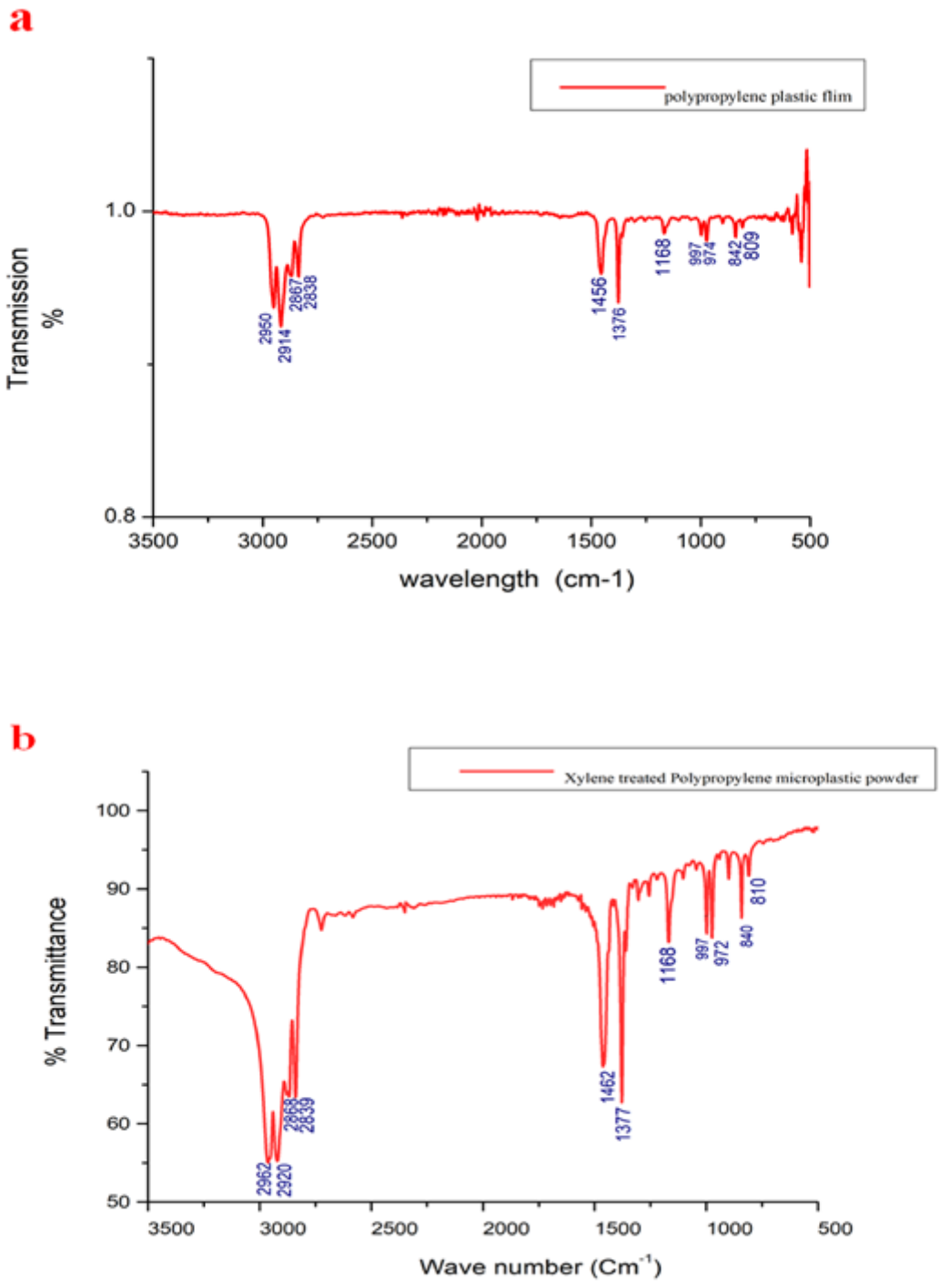
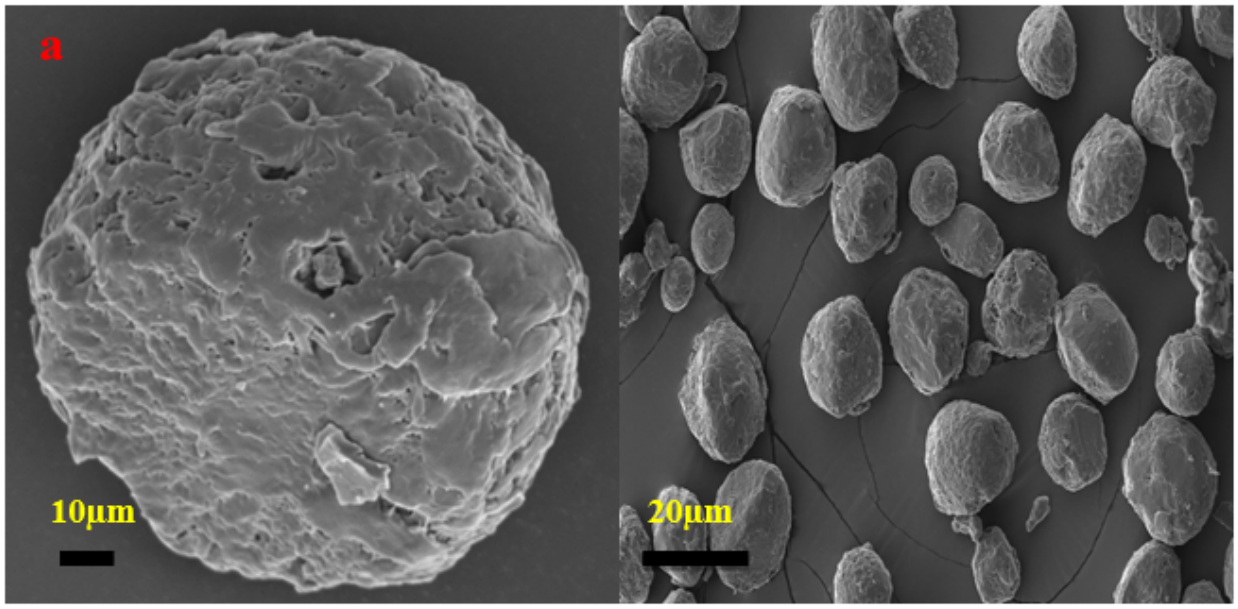


Figure 1

The FTIR spectrum (x-axis cm-1, Y-axis % of transmission) of polypropylene plastic a) ATR – FTIR mode of virgin polypropylene plastic film b) FTIR mode of polypropylene microplastic



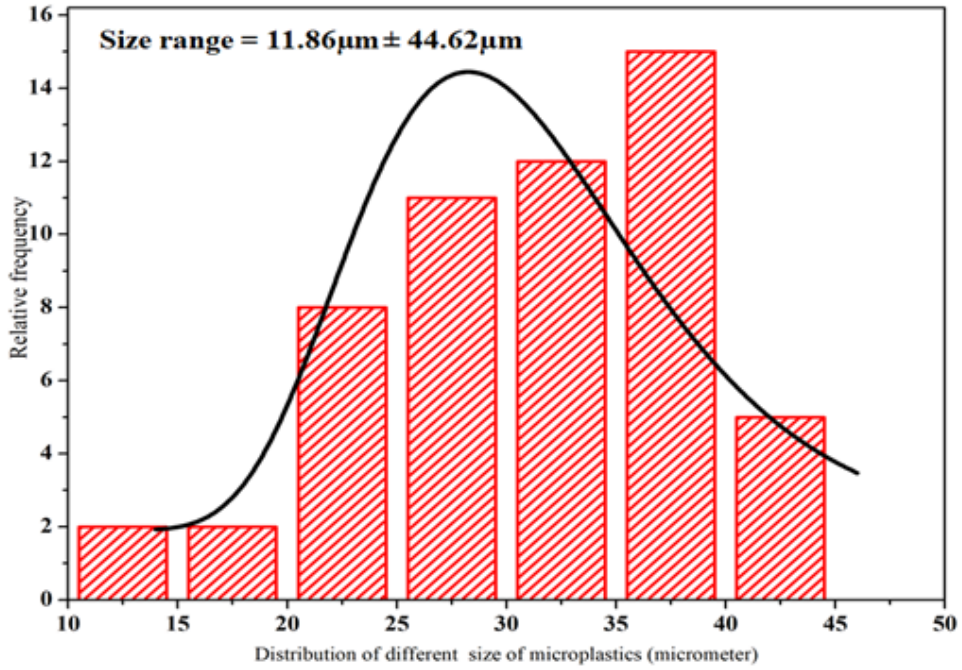


Figure 2

Physical characterization of polypropylene microplastic. a) SEM image of polypropylene microplastic (Spherical shape with irregular surface) b) Size distribution analysis of polypropylene microplastics size range from 11.86µm – 44.62µm.

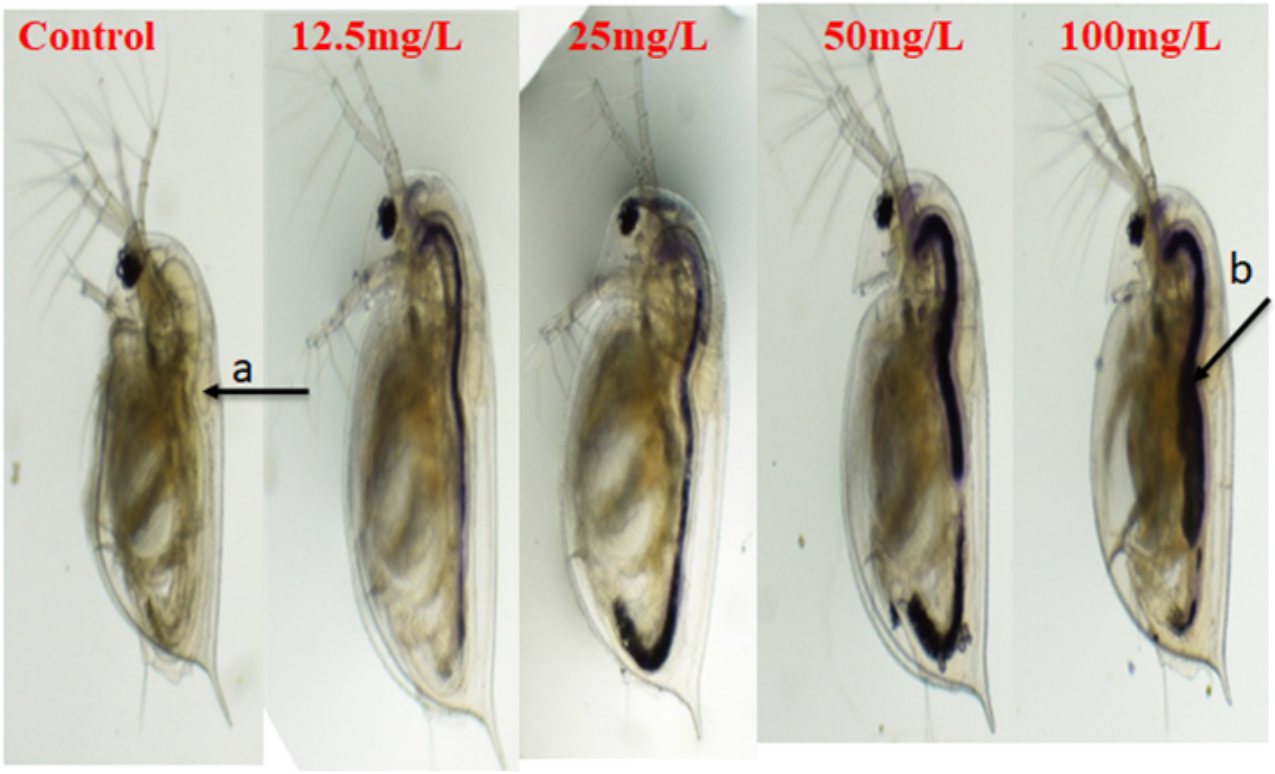


Figure 3

Accumulation of polypropylene microplastic in digestive track of D. similis. a-control group empty of digestive track; b- accumulation of microplastic in digestive tract

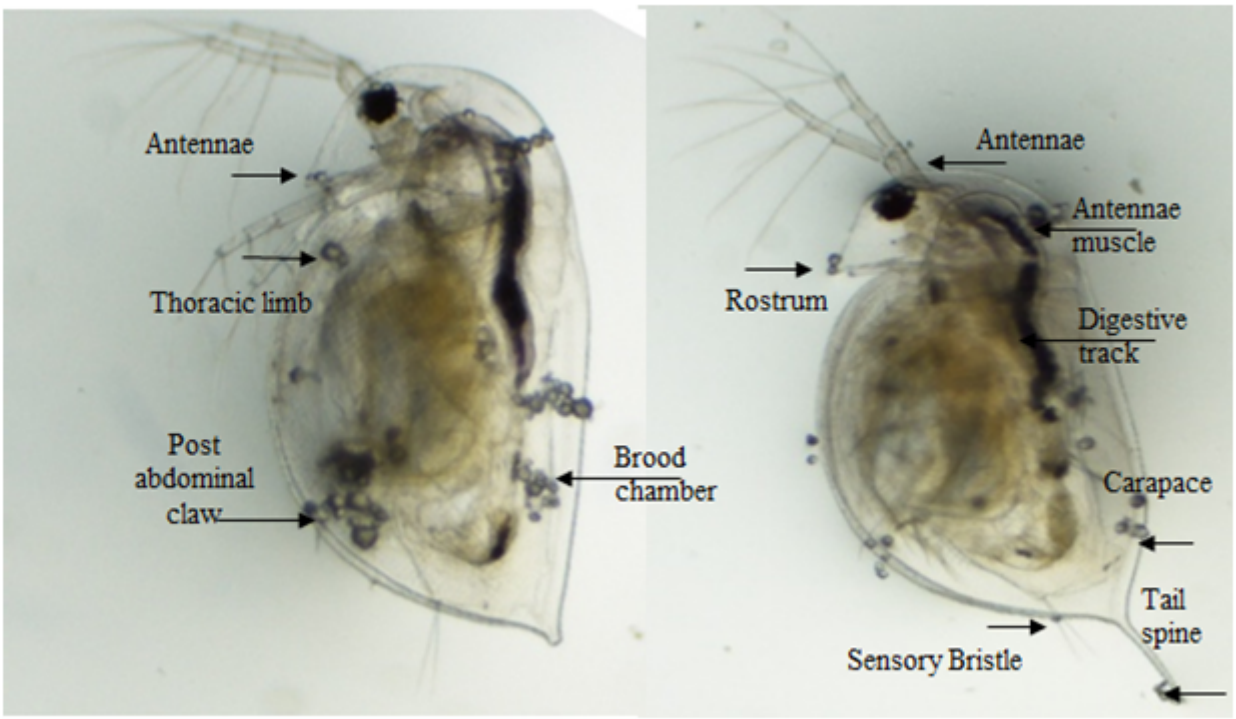


Figure 4

External adherence of polypropylene microplastic in D. similis

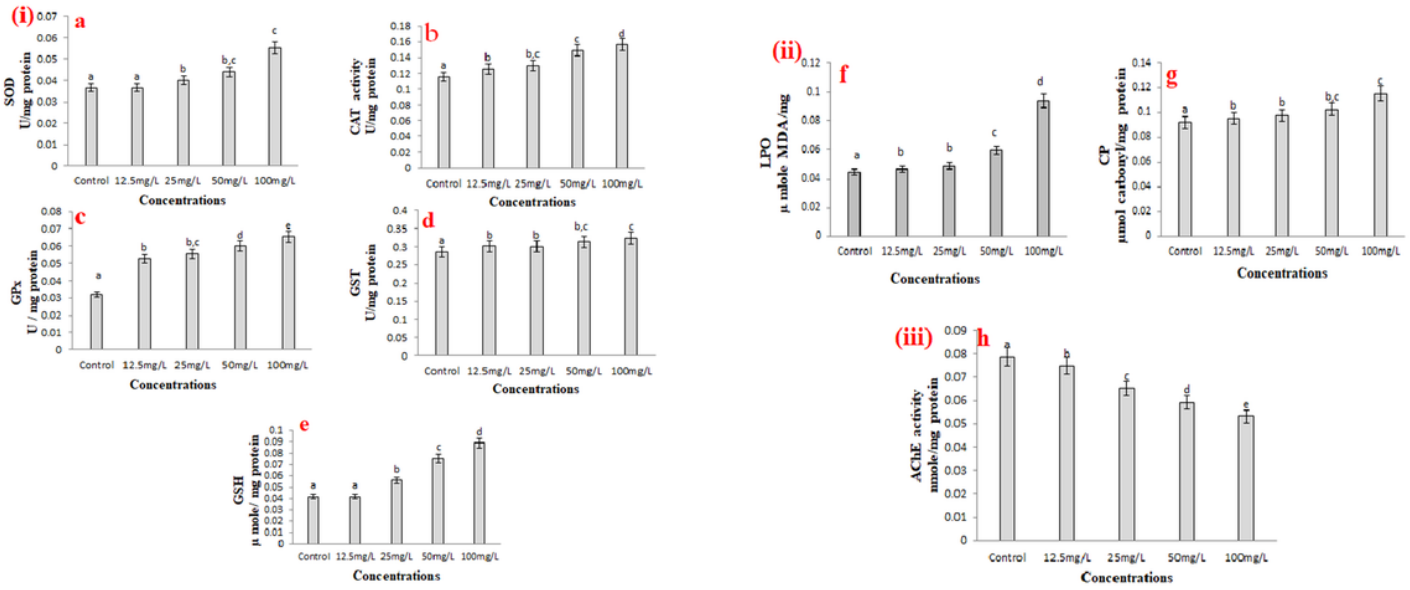


Figure 5

Biochemical parameter response in different concentration (0, 12.5, 25, 50, 100 mg/L)) (24 hours) exposure of polypropylene microplastic on D.similis. (i) Anti-oxidant and non-antioxidant enzyme activity

a) SOD, b) CAT, c) GPx d) GST e) GSH. (ii) Oxidative stress parameter f) LPO g) CP (iii) Neurotransmitter enzyme h) AChE

Supplementary Files

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