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Ingestion of weathered high density polyethylene microplastics-induced oxidative stress and modulation of antioxidant responses in post larval stages of Litopenaeus vannamei

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Introduction

Plastic debris in the marine environment is comprised of various synthetic organic polymers, including polypropylene, polystyrene, polyamide, polyvinyl chloride, and polyethylene (PE). Among these, polyethylene (PE) is the most abundant type of plastic debris (Tao et al., 2023). PE is the most prevalent form of plastics due to high production rate. The presence of PE-MP in marine environment significantly alters the potential toxicity to marine invertebrates and vertebrates through rapid ingestion post weathering (Gewert et al., 2021). Research has shown that exposure to polyethylene can lead to increased mortality rates in various vertebrate and invertebrate species (Kühn and van Franeker, 2018; Ziajahromi et al., 2018; El-Sherif et al., 2022);. MPs by themselves and also due to leaching of various toxic chemicals into the marine environment has adverse effects on organisms including stunted growth, decreased reproductive success (Rochman et al., 2013), histopathological changes, aberrant offspring development (Browne et al., 2013; Hariharan et al., 2022), intestinal blockages, altered behavior, decreased feeding, and elevated mortality (Browne et al., 2013; Lusher et al., 2013).PE is inert and capable of adsorbing pollutants in environment causing immense toxic effects to nontarget organism (Teuten et al., 2009). Their presence could alter the entire food web leading to deleterious impacts on human health.

In this study we used woven sac bags which are prevalent across various industries and exhibit high reusability due to their malleability, structural integrity, and resistance to corrosion. In the context of eliminating plastics, High-Density Polyethylene (HDPE) emerges as one of the most versatile polymers, characterized by its linear chain structure, which imparts increased density and strength. The processes of weathering, photodegradation, and biofouling contribute to an elevation in HDPE density, causing it to settle at the substrate and become more accessible to filter feeders (Ali Chamas et al., 2020). Weathering further exacerbates the fragmentation of microplastics (MPs), resulting in an augmented surface area that enhances their reactivity and susceptibility to degradation (Klein et al., 2018).

Modulation of antioxidants leads to oxidative stress that can damage DNA, cell components, and inactivate enzymes. Antioxidants are essential for preserving homeostasis and aquatic organisms have been demonstrated to upregulate their antioxidant systems including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), as well as non-enzymatic free radical scavengers, such as reduced glutathione (GSH) and biotransformation enzymes, such as glutathione-S-transferase (GST). Changes in these antioxidants' levels can therefore be used as markers of xenobiotic exposure (Singaram et al., 2013). It is helpful to identify possible exposure to hazardous chemicals by using a battery of biomarkers because many of the pollutants to which crustaceans may be exposed are frequently unknown (Gopalakrishnan et al., 2011).

Over the past ten years, marine shrimp farming has grown significantly in India due to rising domestic shrimp consumption and overseas exports, particularly in South India, which has the most shrimp farms on the country's east coast (Krishnan and Babu, 2022). Furthermore, the region's mariculture has become contaminated as a result of the significant industrial expansion and urbanization that have occurred in this same area. Shrimp can accumulate contaminants, microplastics which can have a variety of negative physiological and metabolic impacts. In a prior investigation, commercial fish collected from south India's coastal region were found to contain microplastics (Janardhanam et al., 2022; Frank et al., 2023; Harikrishnan et al., 2023).

Only a few studies have examined the bioaccumulation of MPs/ MFs in crab larvae, where these authors have investigated effects of MPs on survival, feeding and swimming behavior (Wang et al., 2020; Woods et al., 2020; Hodkovicova et al., 2022; Timilsina et al., 2023). Our previous studies show that high density poly ethylene induce proximate imbalance in the post larval stages of *Litopenaeus* vannamei (Leela et al., 2025). Until now to our knowledge there are no studies on the effects of weathered MPs concerning the changes in antioxidant level during the early growth. The current investigation was undertaken to evaluate the interactions between environmentally weathered high-density polyethylene microplastics (wHDPE-MPs) in L. vannamei under controlled laboratory conditions. L. vannamei was selected due to its increasing commercial significance, ecological relevance, and the substantial environmental variability it encounters. The primary objectives of this study were to (i) assess the accumulation of small-sized wHDPE-MPs in *L. vannamei* and (ii) examine the modulation of antioxidant responses in post larval stage of *L. vannamei* under chronic exposure to wHDPE-MPs. This research aims to enhance our understanding of the detrimental effects of wHDPE-MP particles on the developmental stage of invertebrates under coastal and aquaculture ecosystems.

Methodology

Post-larvae (PL-10) white leg shrimp (*Litopennaeus vannamei*) were obtained from commercial hatchery in Marakanam (Tamil Nadu, India) and transported to the laboratory and acclimated for a period of 5 days under laboratory condition in 100 L glass tank with continuous aeration, 20 PSU of salinity, and a 12:12 h light/dark photoperiod (800–1200 lx). The water was changed every day. Until the necessary salinity was reached, the original salinity of 25 PSU was steadily increased by 2 PSU every 24 hours (Hariharan et al., 2022). The shrimps were fed daily with micro particulated PL-shrimp feed considering the 10% of the biomass. Using precalibrated Hydro-Lab, USA-multi parameter water quality probes, physiochemical parameters such as pH, temperature, and salinity were frequently monitored.

High density weathered Microplastics (wHDPE) were prepared following the methods of Hariharan et al. (2022). Briefly, plastic woven sacks that had partly weathered were retrieved from the beaches along the Chennai coast for the current study and the gathered plastic woven sacks were thoroughly cleaned with distilled water. The material was characterized using Fourier Transform Infrared Spectroscopy-FTIR (PerkinElmer Spectrum Version 10.5.4) to identify the polymer type. The characterized materials were then promptly pulverized to fine grains in a ball mill sediment grinder (Fritsch Industrie Str. 8, Idar-Oberstein, Germany) after being frozen at -80°C. Later, the fine particles were sieved through a sequence of test sieves less than 45 µm and the particles retained in 45 µm test sieve were taken for the investigation. Multiple measurements were used to determine the concentration per microgram of microplastics, the number of dissolved wHDPE particles, and the size ratio of the particles. To ensure there were enough clean particles, the wHDPE microplastics were carefully combined with 1 ml milli-Q water in sterile tubes. To induce equal mixing of the particles in the water, the tubes were vigorously swirled. The particles were then immediately transferred to a Sedgewick-Rafter counting slide and counted under a microscope. The tube was examined for particles that had attached to their surfaces in order to standardize the number of particles in the prepared concentration.

Toxicity procedures were adopted from the protocol of USEPA, (1998). In the present study, *L. vannamei* were exposed to two concentrations of wHDPE microplastics. Triplicate tanks were maintained for each concentration. The concentration used for the present study was chosen from the previous literature (Hariharan et al., 2022; Leela et al., 2025). The larvae in Group I was reared in normal seawater without any microplastics as control

group. Group II larvae were exposed to 0.1 mg/L and Group III were exposed to 0.2mg/L wHDPE microplastic in seawater. The whole exposure experiment was conducted through static renewal test method for 45 days. 150 larvae were used per replicate per group for the chronic exposure. To avoid the settlement of suspended wHDPE-MP in the tanks mild aeration were provided. Seawater was changed once in two days. Following exposure the animals were maintained in the normal seawater for depuration for 15 days. The concentrations were selected based on environmental conditions and previous literature (Hariharan et al., 2022). The experimental shrimps were kept under a 12:12 h light-dark cycle. Moreover, physicochemical information including water temperature, salinity, pH, and dissolved oxygen was recorded twice daily using a multiparameter water quality probe (Hydrolab, USA). Dissolved oxygen (5± 2 mg/L), salinity (24 ± 1 ppt), temperature (28 \pm 1 oC), and pH (8.1 \pm 0.1) were maintained throughout the experimental period.

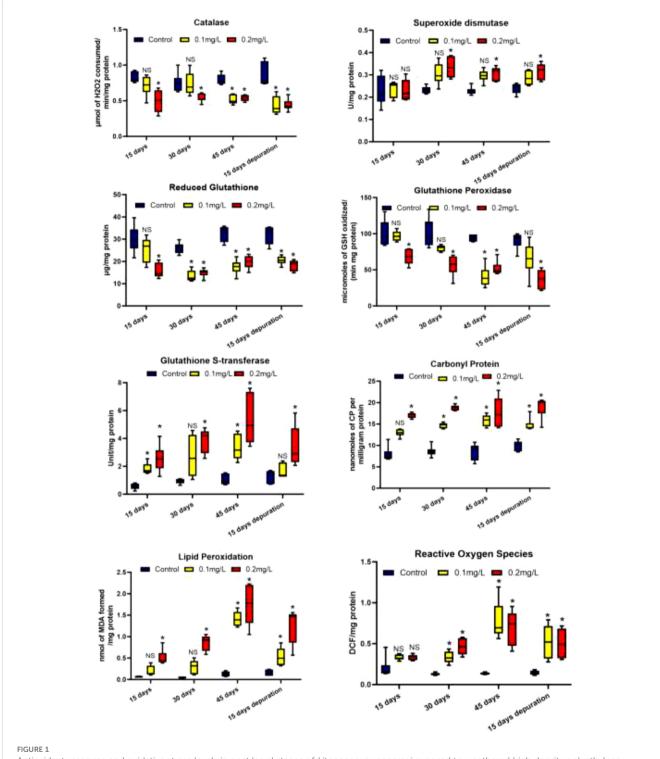
Sampling was done at every 15 days interval, so total 3 time points were fixed for exposure study and one time point was fixed for depuration study. At every time point 30 larvae from each tank were taken and homogenized in a buffer (pH 7.6) that contained 0.5 M sucrose, 20 mM Tris, 1 mM EDTA, 1 mM DTT, 0.15 M KCl, and 0.05 mM phenylmethylsulfonyl fluoride (PMSF). The samples were then centrifuged for 20 min at 4°C at 10,000×g, and the supernatants were utilized for testing the oxidative stress and antioxidant associated parameters. CAT activity was determined according to the method of Sinha (1972) and expressed as µmol of H₂O₂ consumed/min/mg protein. The Marklund and Marklund approach (1974) was used to measure the extent of inhibition of pyrogallol auto-oxidation at an alkaline pH in order to estimate SOD activity. GPx was calculated using the Rotruck et al. (1973) methodology, which involved measuring the amount of GSH consumed in the reaction mixture. According to Moron et al. (1979), GSH was determined by measuring the optical density of the yellow color produced when glutathione reduces 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 412 nm. Glutathione S-transferase (GST) activity was measured using the 1-chloro-2, 4-dinitrobenzene (CDNB) substrate following the conjugation of glutathione with the acceptor substrate (Habig et al., 1974). Malondialdehyde (MDA) level was expressed as nmol of MDA generated/mg protein, the color developed was measured at 532 nm, and LPO was computed using the Devasagayam and Tarachand (1987) methodology. According to Lushchak et al. (2005), the amount of protein carbonyl (CP) was determined by reacting with 2,4-dinitrophenylhydrazine (DNPH). CP was measured using spectrophotometry at 370 n, indicating a molar extinction coefficient of In the guanidine chloride solution, concentrations of CP were quantified as nanomoles of CP per milligram of protein (10³ M⁻¹ cm⁻¹). Total ROS was measured following Driver et al. (2000). Statistical analysis was carried out using SPSS (version 20). The results of the measured parameters are presented as the mean \pm SD after being examined for significance. The data was analyzed using one-way analysis of variance (ANOVA) and then post-hoc Tukey's multiplecomparison test. To ascertain how the parameters interacted with one another, the Pearson correlation coefficient was employed.

Results and discussion

The primary antioxidant and associated enzymes such as catalase, SOD, GPx, GST and GSH in the PL stage of L. vannamei were observed to be modulated as the concentration and the exposure periods increased (Figure 1). SOD activities increased as the exposure period and MP concentration increased. After 30 days of exposure the SOD values for control was 0.232 ± 0.014 and the same increased to 0.333 ± 0.040 when the PL were exposed to 0.2 mg/L. Similarly, during depuration the level of SOD in both exposed concentration was greater compared to respective controls however the lower concentration did not show significant difference. After 15 days of depuration period the SOD values in control group was 0.239 \pm 0.021 and the same was 0.316 \pm 0.032 at 0.2mg/L of MPs. The GSH and GPx activities decreased in the PL stage when exposed to MPs, however the highest concentration and the longest exposure periods showed significant decrease compared to the respective controls. After 45 days of exposure GSH value in control group was 32.36 \pm 3.52 and the same decreased to 19.76 \pm 2.82 at 0.2mg/L. Similarly after 45 days of exposure GPx value in control group was 93.97 ± 4.36 and the same decreased to 51.93 \pm 8.93 at 0.2mg/L Moreover the PL stage did not show any recovery for these two antioxidants during the depuration period. GST activities were induced significantly in PL stage due to MP exposure and this was observed to be dose dependent. Following 45 days of exposure, GST activity in control group was 1.05 \pm 0.33 and it increased to 5.33 \pm 1.58 at 0.2mg/L. Similarly during the depuration period the level of GST were greater compared to the respective control groups.

The first antioxidant enzyme that scavenges superoxide radicals (O_2^-) is SOD, while CAT converts H_2O_2 that is produced as a result of the reaction that SOD catalyzes. The lower levels of SOD and CAT activity seen in this investigation are in line with ROS production occurring in response to MPs. Increased formation of the superoxide anion radical, which has been shown to impede CAT activity, may also be the cause of the decreased CAT activity (Snezhkina et al., 2019). It is possible that the lower levels of enzymatic and non-enzymatic antioxidant activity found in the tissues of *L. vannamei* exposed to MPs represent an adaptive response of prawns to oxidative stress due to wHDPE exposure.

CP was found in greater levels in the PL stage of *L. vannamei* subjected to the highest wHDPE concentration in all the exposure periods (Figure 1). After 15 days of exposure, the CP levels in the control group was 7.96 ± 1.56 and it increased to 17.04 ± 0.56 .when the PL were exposed to highest concentration at 0.2 mg/L, Similarly, when the PL stage was exposed for 30 and 45 days to both MPs concentrations, CP were greater compared with the respective control group and, all these values were statistically significant (p<0.01). Lipid peroxidation levels in the PL stage were measured in the form of MDA. MPs at higher concentration caused a significant increase in oxidative stress in PL stage after all the exposure periods (Figure 1). After 15 and 30 days of exposure the LPO levels in PL stage were 0.06 ± 0.007 and 0.046 ± 0.009 in control group and it increased to 0.509 ± 0.162 and 0.859 ± 0.167 at 0.2 mg/L respectively, and these values are highly significant



Antioxidant response and oxidative stress levels in post larval stages of *Litopenaeus vannamei* exposed to weathered high density polyethylene microplastics. The line in each box represent median and whiskers represents the upper and lower 95% confidence intervals of the mean of six determinations using samples from different preparations. One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used. The significant (P < 0.05) differences between control and exposure groups were indicated by the asterisks (*); NS indicate no significant difference with respective control group.

compared to the respective control group. ROS levels in PL stage increased significantly after 30 and 45 days of MP exposure in case of both the MP concentration, however after 15 days the change was insignificant with respective control group (Figure 1).

Although xenobiotics can cause adaptive changes, such as phase I enzymes that oxidize them to make them more polar so they can be conjugated and eliminated, this process involves redox reactions that can produce free radicals (Gu and Manautou, 2012; Banaee

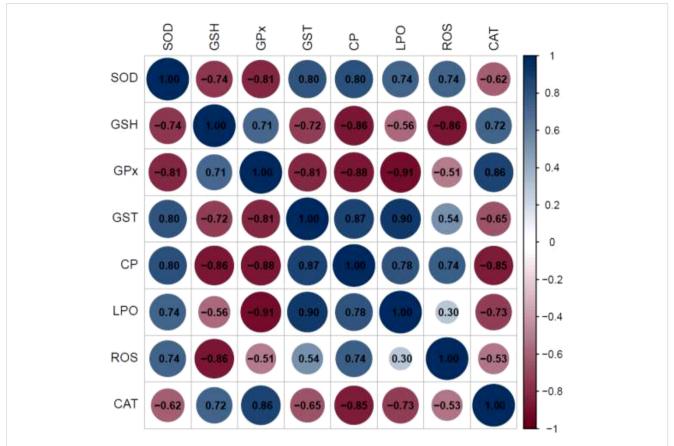


FIGURE 2

Depicts the correlation analysis between the oxidative stress and antioxidant response in post larval stages of *Litopenaeus vannamei* exposed to weathered high density polyethylene microplastics. The blue color represents the positive correlation and the pink tint the negative connection. The measured components' higher relation is shown by the dark color, and their lesser relation is shown by the light hue.

et al., 2025). The existence of several organic and inorganic pollutants might induce the generation of ROS. According to Almeida et al. (2007), ROS can cause oxidation of proteins, lipids, and DNA, and other biological components. Little evidence of differential ROS production was seen in the tissues of L. vannamei exposed to MPs in the PL stage in the current investigation. The findings indicating ROS damage was higher at the highest concentration tested for the PL stage are strikingly comparable to those obtained when fish fry were exposed to weathered MPs (Janardhanam et al., 2022; Páez-Osuna et al., 2024). Protein oxidation is the outcome of carbonylation and creation of irreversible protein carbonyls (Estévez et al., 2022), can lead to protein conformational changes, and this is one of the consequence of ROS production. As a result of the alteration, enzyme catalytic activity would decrease, which will ultimately lead to protein degradation (Zhang et al., 2008).

Induced carbonyl protein levels in PL stages exposed to MPs which may be a sign of exposure stress in PL stage of *L. vannamei*. A rise would probably be the more drastic of the two conceivable reactions. PL subjected to 0.2 mg of MPs had higher CP. Such levels, however, probably have no negative effects that could compromise the PL stages of *L. vannamei*. Numerous investigations have shown that aquatic organisms exposed *in vivo* to various pollutants exhibit increased levels of LPO in a variety of tissues (Almeida et al., 2007).

GPx and GSH are antioxidants which can be depleted during ROS scavenging, which is supported by the current study's findings that prawns exposed to MPs had greater MDA concentrations, reduced GPx activity, and lower GSH concentrations. Exposure to a variety of contaminants can deplete antioxidant defenses, increasing susceptibility to lipid peroxidation. Significant reduction in GPx activity and corresponding rise in MDA are in line with glutathione's function in electrophile detoxification. Greater use of GSH by GPx to catalyze the reduction of H₂O₂ to H₂O and O₂ is consistent with lower GSH concentrations. Lower glutathione concentrations are the cause of GPx's decreased action (Raffa et al., 2011). The increased GST activity after MP exposure indicates that detoxifying processes have been activated. These results imply that MPs interfere with at least one of the detoxification and lipid peroxidation processes, which are both impacted by GST. Since MPs have been demonstrated to induce oxidative stress in a number of species (Paul-Pont et al., 2016; Páez-Osuna et al., 2024), it is possible that the increased GST activity seen in prawns exposed to MPs is an adaptive reaction to oxidative stress.

The relationship between the measured antioxidant associated components in PL stage of *L. vannamei* exposed to wHDPE MPs for 45 d is depicted in Figure 2 by a correlation analysis; the majority of the measured components tested exhibit highly significant relation r values. After 45 d of exposure to wHDPE MPs, most of the

parameters showed significant correlation with other antioxidant and oxidative stress associated parameters. Notably, the several antioxidant parameters in PL stage of L. vannamei exhibited a negative correlation with large r values (Figure 2). GST have a negative correlation with CAT, GPx and GSH and all these (r) values were highly significant. Similarly LPO have a positive correlation with CP and ROS generated in the tissues of PL stage of L. vannamei. In the majority of these cases, the correlation was very significant. The majority of oxidative stress related indicators exhibit a negative correlation with antioxidants. These findings unequivocally demonstrate that increased stress-related parameters directly affect antioxidant related parameters. There is a positive and negative relationship between the parameters under study as a result of the effect of wPE MPs on antioxidant-associated measures. The majority of the measured components examined had extremely significant relation r values, according to the relationship between the measured oxidative stress and antioxidant responses in PL stage of L. vannamei exposed to wHDPE MPs for 45 d, moreover illustration of the correlation results demonstrated that the correlation coefficients were more than 0.700 in most cases.

In this investigation, exposure to MPs clearly caused an increase in SOD and a decrease in GPx activities in the PL stage of L. vannamei. The activities remained elevated and never returned to normal during the depuration phase. According to these findings, MPs exposure might cause SOD and reduce GPx activities; however, at lower MPs exposure concentrations, the activities would considerably decline following a period of depuration. Prior research have shown that stored electrons might be used to generate ROS utilizing whatever oxygen was left, increasing the generation of ROS under stress conditions brought on by MPs exposure (Li et al., 2016; Páez-Osuna et al., 2024).

Biological macromolecules like proteins, lipids, and DNA would be harmed when ROS concentrations were higher than the levels of antioxidant (Páez-Osuna et al., 2024). MDA was produced oxidatively when free radicals and polyunsaturated fatty acids reacted, and its quantity may serve as a marker of oxidative stress. MDA concentrations in the PL stages subjected to the greatest concentration of MPs in this investigation were higher than the control, and following a period of depuration, the concentrations were unable to recover to normal. This suggested that MPs increased oxidative stress, which can be difficult to recover from.

In conclusion, our data suggests that the oxidative stress produced may reveal information about the overall health of an organism exposed to MPs, and more specifically to wHDPEs. These findings lead us to hypothesize that, like in higher vertebrates and other invertebrates, the CAT, SOD, and glutathione-mediated antioxidant may play a significant role in shrimp's detoxification process. The increased SOD activity indicates that these processes are generating reactive oxygen species, which is supported by the rise in oxidative stress parameters, clearly states suggesting that the antioxidant system is modulated due to MPs exposure. Moreover when combined, these biomarkers may be essential instruments for determining the exposure and toxicity of MPs to marine life, and they can be applied in routine monitoring programs for coastal and marine environments.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

ML: Writing – original draft, Formal analysis. VP: Writing – original draft, Formal analysis. SS: Methodology, Writing – original draft. SA: Writing – review & editing, Project administration. SM: Methodology, Writing – original draft. SE: Formal analysis, Writing – original draft. HT: Writing – original draft, Validation, Supervision. RT: Validation, Writing – review & editing, Writing – original draft. RK: Conceptualization, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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