EFFECT OF MICRO PLASTICS ON ANTIOXIDANT BIOMARKERS OF *EMERITA ASIATICA*– AN *IN-VIVO* STUDY

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Abstract

Over the past few centuries, micro plastics in aquatic environment being the most serious problem among worldwide, ingestion of these materials <5mm arrests the somatic growth and causes serious physiological effects. The micro plastic also enters the food web which is also a major concern among all the invertebrates. Hence, the study was designed to evaluate the effects of micro plastics on antioxidant biomarkers in the mole crab*Emerita asiatica*. A total of 300 animals were collected and exposed to three different concentrations of micro plastics in a beaker such as 10, 100 and 1000 particles $mL^{-1}$for about 28 days and the sampling was taken after 14^{th} and 28^{th} day for the investigation of anti-oxidant biomarkers like lipid peroxide (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), Glutathione Peroxidase activity (GPx) and catalase (CAT). The results showed that the antioxidant enzymes were significantly decreased, whereas elevated level of LPO content was observed in the micro plastics exposed group animals.Overall the present findings concluded that micro plastics induces severe oxidative stress and damage the antioxidant system in mole crab*Emerita asiatica*.

Keywords: Micro plastics; *Emerita Asiatica*; Antioxidant

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1.0 Introduction

Globally, the micro plastics were spread in both terrestrial and aquatic environments that is being a major threat especially for marine environment. Micro plastic pollution by direct exposure or by ingestion causes serious health issues in most of the invertebrates, since the micro plastics measures only about <5mm, they can also move across the food web (Lambert et al., 2017).

Every year, a huge number of micro plastics enter the marine environment through anthropogenic activities such as fishery and mariculture (Su et al., 2022). Micro plastics have chemically active surfaces that absorb large number of heavy metals and transfer them along the food web. Direct consumption of micro plastics causes serious problems in physiology of invertebrates; the researches also proved that micro plastics have negative effects on invertebrates (Arif et al., 2022).

Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamides (PA), and polyvinyl chloride (PVC) have caused physical damage and adverse effects on the intestine, enterocytes, digestive tracts, and liver of marine organisms (Trestrail et al., 2020). It also causes Endocrine disorders, immune responses, oxidative stress, and alterations. It includes the reduction in somatic growth, delayed metamorphosis and lowered reproductive output (Capo et al., 2021).

In recent years, there are numerous studies have been conducted on the effect of heavy metals against biochemical and physiological properties of aquatic organisms, but there is lag in the investigation of antioxidants. Hence it would be interesting in investigating the effect of antioxidants against the mole crab Emerita asiatica.

The mole crab Emerita asiatica found in most of the Eastern coast of India. The crab belongs to the family Hippidae and the phylum Arthropoda, which is also known as sand crab. These sand crabs usually found on beaches during spring season. The Emerita has only short life span of 2 to 3 years. The larvae live as plankton for 4 months and these larvae carried to a long distance by the ocean waves and it exhibits tidal migration (Samantara et al., 2016).

The mole crabs do not feed when it is submerged under sand and extends their antennae for microscopic food from the water are collected. Generally, Mole crab feed upon the plankton mostly dinoflagellates and diatoms. The importance of this study investigates the species that consumes the microplastics to monitor their dietary habits and its changes in the biomarkers of antioxidants (Samantara et al., 2016).

2.0 Materials and Methods

A total of 300 healthy crabs (*Emerita asiatica*) were collected in a flow-through tanks containing filtered ad constantly recirculated seawater. The assay was conducted by separating the specimens into four different concentrations of beakers filled with filtered

seawater such as Control and three different concentrations of polystyrene smooth shaped micro plastics particles (10, 100 and 1000 particles mL^{-1}). The mussels were highly aerated and always contacted with micro plastics. The bioassay was calculated in duplicate after 14^{th} and 28^{th} day sampling.

The oxidative stress markers like lipid peroxide, reduced glutathione, superoxide dismutaseand catalase (CAT) in the different tissues were analysed.

2.1 Determination of lipid peroxidase activity

The lipid peroxidase activity was quantified by (Reilly and Aust, 1999), the 400µl of samples were collected and mixed with 1.6ml of 0.15M Tris Kcl of pH 7.0, 500µl of 30% Trichloroacetic acid and 500µl of 0.75% of Thiobarbituric acid, the mixture was then incubated 80°C for 1 hour and cooled down to observe the colour formation. After centrifugation, the absorbance was read at 532nm.

2.2 Determination of reduced Glutathione (GSH)

The GSH levels were analysed by the method of Moron *et al.*, 1979. In this the sample taken were mixed with equal volume of 10% trichloroacetic acid (TCA) for precipitation and centrifuged at 10,000 rpm for 10 minutes at 4°C to remove the precipitation. Then the supernatant was mixed with 2 ml of 0.6 mM 5,5'-dithiobis-2-nitro benzoic acid (DTNB) in 0.2 M sodium phosphate and the volume of the content was made up to 3 mL using 0.2 M phosphate buffer at pH 8.0. The absorbance of the content was read at 412 nm.

2.3 Determination of superoxide dismutase (SOD)

The SOD was determined by the method of Misra and Fridovich (1972), the 300µl of sample was added to 0.05M of carbonate buffer pH 10.0, then 300 µl of freshly prepared substrate adrenaline 0.3mM was added to initiate the reaction, Similarly the blank was also prepared with 200µl of distilled water in the place of specimen and the absorbance was read at 480nm for every 30s upto 150s.

2.4 Determination of catalase activity (CAT)

The catalase activity was determined by the method described by Sinha et al., 1972, 100 µl of different concentration of samples followed by 1 ml of hydrogen peroxide incubate at 37°C for 3 minutes and after that add 2ml of dichromate acetic acid and incubate at 100°C

for 10 min, then cooled and centrifuge to remove the precipitate to read the absorbance at 570nm against blank.

2.5 Determination of Glutathione reductase

The reaction mixture consists of 100µl of NADPH, 200µl of GSSG, 200µl of EDTA, 2.5 ml buffer and 100µl of different concentrations of sample as enzyme source to a total volume of 3.0 ml. The addition of NADPH initiated the reaction. Similarly, the blank was also prepared and absorbance was read at 340 nm for every 30s upto 150s (Moron *et al.,* 1979).

2.6 Determination of Glutathione peroxidase

The reaction mixture consisting of 50 mm potassium phosphate buffer, EDTA (1 mm), NaN3 (1 mm), NADPH (0.2 mm), GSSG-R (1 E.U) and GSH (1 mm) was setup in a total volume of 2.5 ml. Samples as enzyme source was added to the above mixture and incubated for 5 min at room temperature. The reaction was initiated by adding H2O2 (0.25 mm). Absorbance at 340 nm was recorded for 5 min after every 30 s interval (Moron *et al.,* 1979).

3.0 Results

3.1 Lipid Peroxidase activity (LPO)

Formation of malondialdehyde (MDA) was measured to analysis the level of LPO activity. The results showed that, elevated level of LPO activity was observed in the HP tissue (27.8 and 41.5 nmol) followed by Muscles (15.5 and 24.7 nmol) and Gills (7.5 and 12.4 nmol) after 14 days and 28 days of the experimental period. Since, the LPO level significantly increased from 10 Particles mL^{-1} dosage to 1000 Particles mL^{-1} dosage level in HP tissue and Muscle compared to the control animals. Moreover, the level of LPO in the Gills were slightly elevated than the control group (Figure 1).

3.2 Superoxide Dismutase Activity (SOD)

SOD and CAT are the important enzymes in the antioxidant enzymes to maintain the ROS generation. Our study results represented the Fig.2 revealed that there is remarkable level of decrease in the SOD activity was observed in the HP tissue (2.9U), Muscle (1.3U) and Gills (0.5U) tissue of 1000 Particles mL-1 treated animals compared to the control animals.

3.3 Catalase Activity (CAT)

Similar to the SOD activity CAT enzyme activity also potentially decreased in the dose dependent consumption of micro plastics. Compared to the control animals the micro plastics (1000 Particles mL^{-1}) treated animals showed 18U in Gills, 380U in HP and 260U in Muscle tissue of *Emerita asiatica* (Figure 3).

3.4 Glutathione Peroxidase activity (GPx)

The results illustrated in the figure 4 clearly demonstrated that, significant reduction in the antioxidant enzyme GPx was observed the experimental animal upon the treatment with different concentration of Micro plastics, which was observed that 48, 42, 40 nmol respectively in Muscle, Gills and HP tissues (Figure 4).

3.5 Reduced glutathione activity (GSH)

Similar to the GPx activity GSH level also significantly reduced in the Micro plastics animals. GSH level of experimental animals post 14 and 28 days of exposure of Micro plastics (1000 Particles mL^{-1}) showed that 3.2, 3.5 and 8.5 nmol respectively in the Gills, HP and Muscle tissues (Figure 5).

3.6 Glutathione reductase activity (GR)

GR activities were potentially decreased in the crap *Emerita asiatica* post exposure of different concentration of micro plastics. The results illustrated in the Figure 5 showed that, the Gills tissue showed 8.2nmol, HP showed 5.2nmol and 5.3nmol in muscle tissue post exposure of 28 days, whereas control animal showed 14.7, 13.1 and 10.1nmol of GR activity in different tissues.

4.0 Discussion

Variability in biomarkers is caused by the experiment's exposure settings. Over time as they become acclimated to the lab environment, antioxidant enzyme activity can alter(Jemec et al., 2008).Most of the studies revealed that parameters such as SOD, CAT, GPx, GST, GSH, and LPO provide suitable biomarkers for the monitoring oxidative conditions of any animals (Dafre et al., 2004; El-Shenawy et al., 2012; Oliva et al., 2012). In the present investigation, there were significant effect on biochemical parameters such as SOD, CAT, GPx, GST, GR, GSH, and MDA were analysed in the *Emerita asiatica*with the different concentration of micro plastics exposure.

Lipid peroxidation was eventually elevated during the condition of high level of Reactive oxygen species (ROS) production in the oxidative stress. This conditions can lead to the damaging of cells and cell membrane of the tissues (Porter, 1984).Increased reactive oxygen species react with polyunsaturated fatty acids during LPO enzyme activity, causing the generation of toxic and reactive aldehyde metabolites like MDA (Arif et al., 2022). In our present study, high level of LPO was observed in different tissues of the micro plastics consumed mole crab *Emerita asiatica*. Similar to our study, Arif et al. (2022) reported that, consumption of nanoplastics and heavy metal significantly elevated the level of LPO production in juvenile tri-spine horseshoe crabs.

In our present investigation both SOD and CAT activity was remarkably decreased in the different concentration of micro plastics consumed animals post 14 and 28 days of experimental time. One of the cell's most powerful antioxidant enzymes is SOD an CAT. O_2- and H^+ are converted to O_2 and H_2O_2, respectively by SOD (Alscher et al., 2002). In the next process, H_2O_2 is further broken down into water by CAT. In order to properly eliminate ROS brought on by environmental stress, SOD and CAT work together to protect cells from oxidative stress and damage. Similar to our study results,Liu et al. (2014) reported that, fresh water crap *Sinopotamon henanense* exposed to the different concentration of heavy metal lead was induced the oxidative stress and decreased the antioxidant enzymes such as CAT, SOD GR and GST.

GPx utilises GSH as a cofactor to break down H_2O_2 into water and ethanol. Animal GPx has the ability to operate as a prooxidant that clears cells of damaging peroxide metabolites. (Hu et al., 2015). Meanwhile, the excessive H_2O_2 accumulation in the mitochondria and peroxisomes/cytosol, which resulted from the decreased level activity of CAT and GPx, might consequently decrease the activity of SOD as in the case of oxidative stress conditions (Sivaprasadet al., 2002).GSH, the most prevalent cellular antioxidant and non-protein thiol in living things, is essential for reducing oxidative stress (Wang et al., 2008). A numerous study on reported that, decreased level in the GSH content of different tissues of aquatic animals (Chelomin et al., 2005; Ivanina et al., 2008; Wang et al., 2008) during the oxidative stress conditions, which is consistent with our results. In the contrast with our study results, the GPx and GSH content was remarkably decreased and significantly with the increase of Pb concentration as well as time of exposure. Our study result was supported by Liu et al. (2014), stated that the key antioxidant enzymes GPx and GSH was

gradually decreased in *Sinopotamon henanense*tissue such as HP, Gills and Muscle against exposure of increasing concentration of heavy metal Pb.

Our results on GR activity clearly demonstrated that, the level of GR activity was greatly debited in the micro plastics exposure animals than the control animals.In the contract with our results, *Solea senegalensis* collected from the heavy metal polluted area explored reduced level of GR activity and elevated level of LPO activity (Oliva et al., 2012).In addition to that, a decrease in GR activity may also reduce the production of GSH contents. A substantialreduction of GSH ultimately induced the reduction in the cellular scavenge ability against the production of ROS and free radicals and it's leads to the increasing the general oxidative potential in the cells. The GSH contents depletion also suggested indication of animals were suffering from serious oxidative damage (Wang et al., 2008).

5.0 Conclusion

Overall, the present findings demonstrated that, the exposure micro plastics has induced the severe oxidative stress and potentially damaged the antioxidant system of marine crap *Emerita asiatica*.

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Result Image

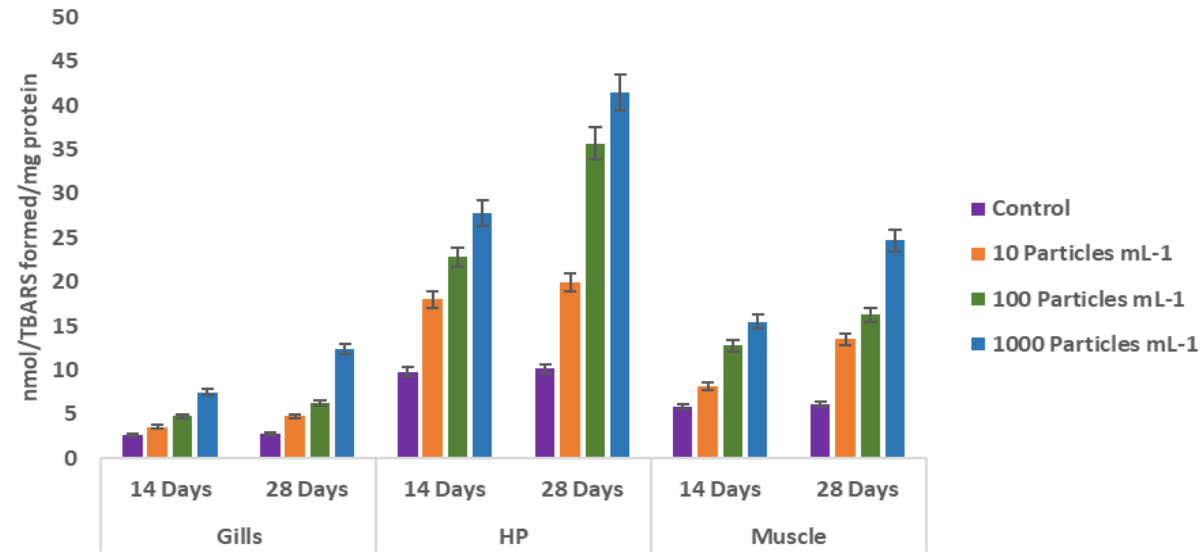

Figure 1: Effect of different concentration of micro plastics exposure on Lipid Peroxidase activity (LPO) in different tissues of *Emerita asiatica.*

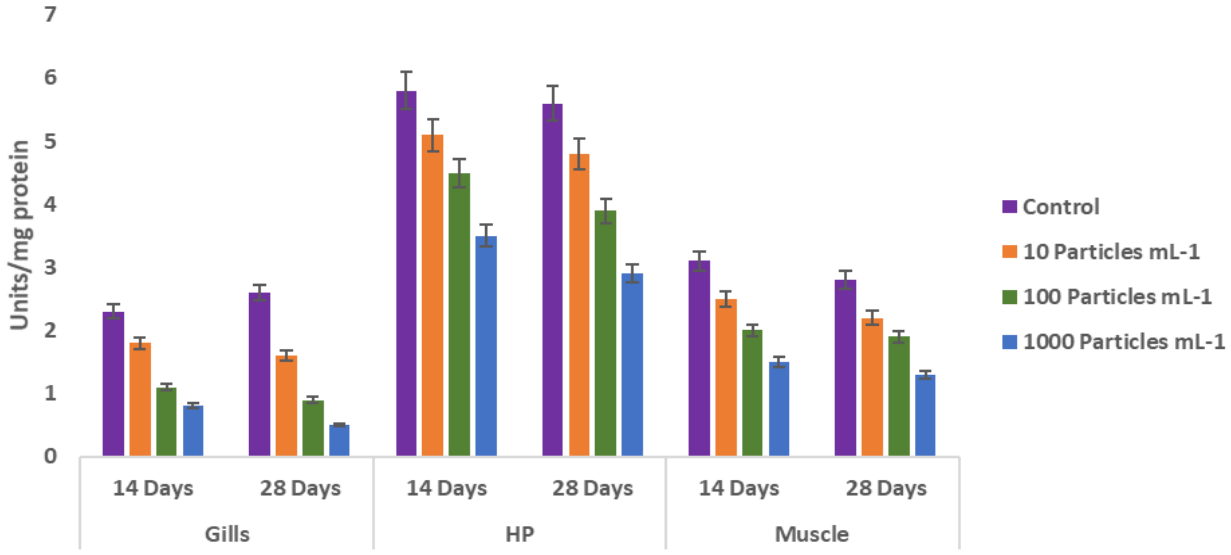

Figure 2: Effect of different concentration of micro plastics exposure on Superoxide Dismutase activity (SOD)in different tissues of *Emerita asiatica.*

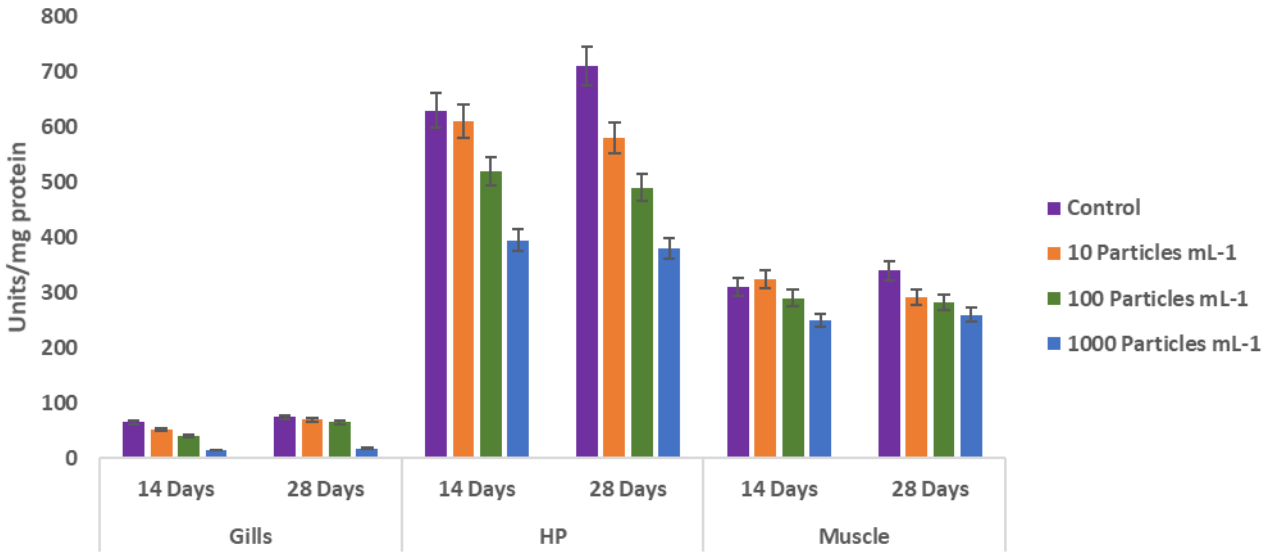

Figure 3: Effect of different concentration of micro plastics exposure on Catalase activity (CAT)in different tissues of *Emerita asiatica.*

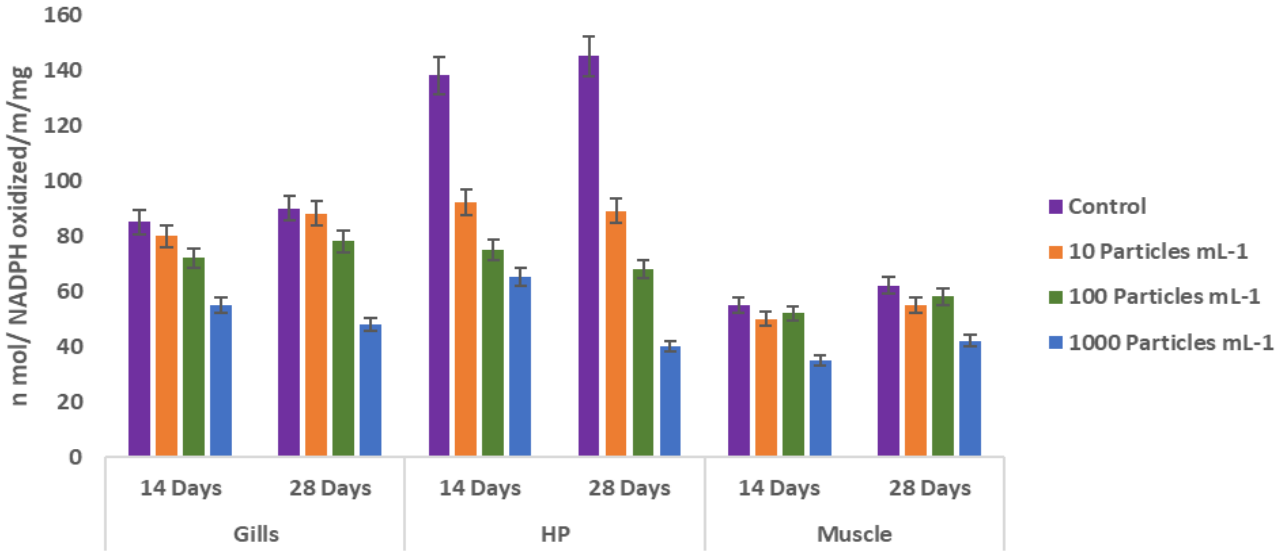

Figure 4: Effect of different concentration of micro plastics exposure on Glutathione Peroxidase activity (GPx)in different tissues of *Emerita asiatica.*

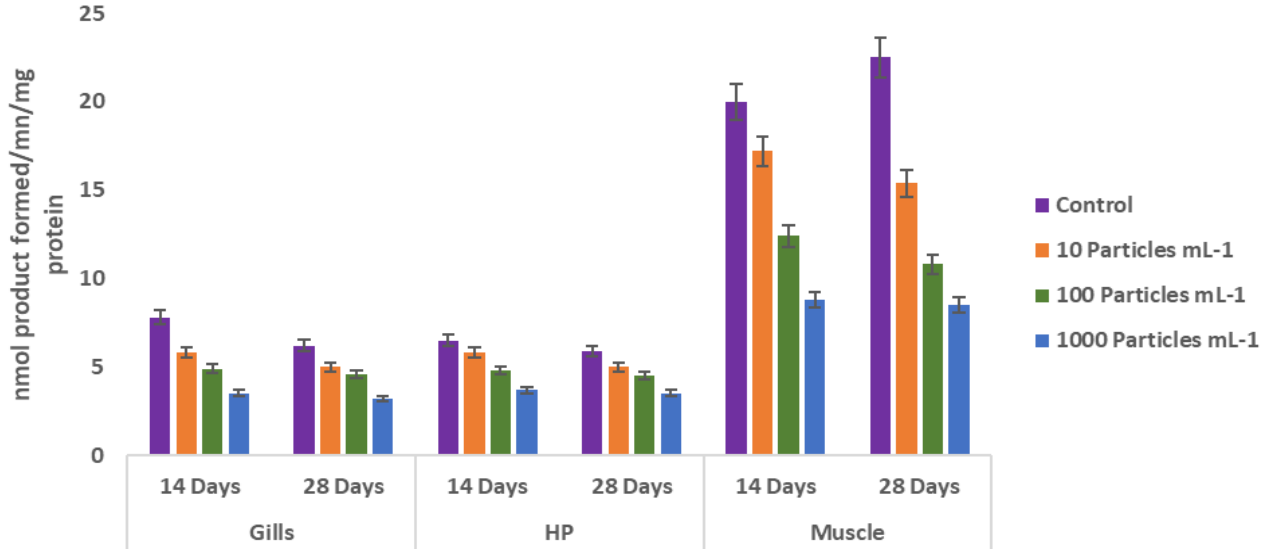

Figure 5: Effect of different concentration of micro plastics exposure on Reduced glutathione activity (GSH)in different tissues of *Emerita asiatica.*

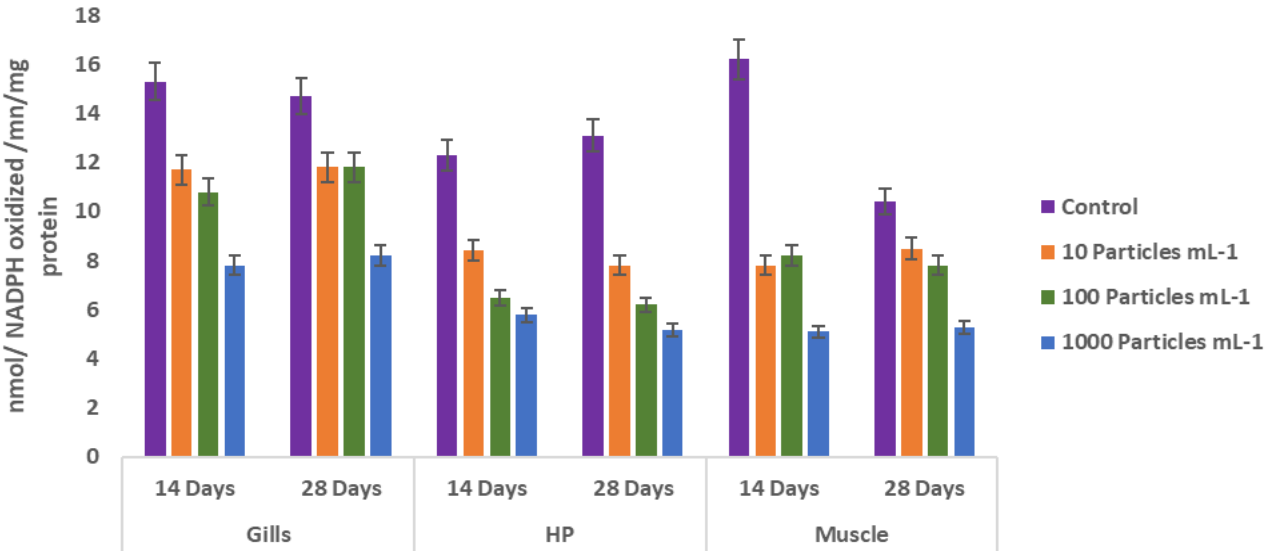

Figure 6: Effect of different concentration of micro plastics exposure on Glutathione reductase activity (GR)in different tissues of *Emerita asiatica.*