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IMPACT OF MICRO PLASTICS EXPOSURE ON VITELLOGENESIS AND SEX HORMONES OF FEMALE MOLE CRAB *EMERITA ASIATICA*

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ABSTRACT

The marine ecosystem now considered as the major concern due to the contamination of micro plastics that affects the marine organisms by accidental ingestion and causes physiological, morphological and genetic profiles. The organisms also effect on endocrine system that leads to reproductive damage, hence the present investigation was focused on the effect of micro plastics exposure on reproductive system of female crab *Emerita asiatica*. After 24 and 48 days of the experimental periods, the female crabs then estimated for the hormonal changes in the ovaries of mussels by estimating vitellogenin, 17β-estradiol, and testosterone and some biochemical parameters such as protein and glycogen content to observe for toxic stress. The results showed that significant decrease in the vitellogenin and 17β-estradiol besides elevated level of testosterone was observed in the animals exposed for 28 days with 1000 particles mL^{-1}. In addition to that, level of protein and glycogen level was significantly debited in the hemolymph due to toxic stress. Hence, the present study found that exposure of micro plastics would reduce the reproductive hormones of female crab and affect their reproductive system. Overall, the present study provides the importance of usage and discarding the plastics in the environment. The micro plastic contamination in the environment not only affect the aquatic crustacean's species, which can ultimately be affecting the Human beings through the collapsing of food chain.

Keywords: *Emerita asiatica;* Vitellogenin; 17β-estradiol; Micro plastics

1.0 INTRODUCTION

Over the past few decades, the amount of plastic trash in the oceans has expanded globally by several orders of magnitude as production keeps outpacing available resources for reuse, recycling, or disposal (Rochman et al., 2013). Studies on microplastic waste have

shown that these tiny pieces can be discovered in sediments, the water column, and are consumed by invertebrate animals (Horn et al., 2019).

Microplastics of size less than 5mm, are becoming the major concern that raises environmental health issues due to its effect on ecosystem as well as on physiological, morphological, and gene and proteomic profiles alterations Sendra et al. (2021). The recent statistics also stated that for about 8 million tons of microplastics enters the marine environment annually and it also affect for about 690 marine species. Liu et al. (2020).

Contamination of marine ecosystems estimated the annual loss of about 5% in ecosystem services. In addition, Macroplastic (<1 m), mesoplastic (2.5 cm–5 mm), microplastic (5 mm–1 μm) and nanoplastic (<1 μm), that interacts with aquatic organisms also showed the major harmful effect upon ingestion. Capolupo et al. (2020). Microplastics also reduce the reproductive growth. Ingestion or exposure of microplastics by marine organisms poses a serious change in feeding, reproduction and energy metabolism Anbumai and Kakkar (2018).

Inorganic chemicals such as phthalates, polybrominated diphenyl ethers, and bisphenol A are added to plastics as plasticizers, flame retardants, stabilizers, and fungicides in plastic production causes serious damage on endocrine system that disrupts development and reproduction Mao et al. (2022).

Generally, MPs adsorb various persistent organic pollutants (POPs) and metals due to their, large surface area, which is consumed by marine organisms leads to produce toxic levels in biological tissues at different toxic levels. Addition to this, three plastic additives (Bisphenol A, phthalates and polychlorinated biphenyl 153) were identified as causing infertility and they are also known as endocrine-disrupting chemicals (EDCs), since they interfere endocrine system, by mimicking hormonal active agents that decreases the fertility rate and reproductive failure. Urli et al., (2023).

Emerita is a small genus of decapod crustaceans also known as sand crabs or mole crabs. Along the East coast, *E. asiatica* is actively reproducing from April to June. The larvae that are free to swim sink to the bottom and develop into tiny examples of the adult form. The present study was designed to study the impact of micro plastics exposure on the reproductive system of female crab *E. asiatica*.

2.0 MATERIALS AND METHODS

A total of 300 female crabs (*Emerita asiatica*) were collected and constantly circulated with sea water to maintain its physiological environmental conditions. The assay was conducted by separating the specimens into four different concentrations such as 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 mussels were quantified for hormonal markers such as vitellogenin content, 17β-estradiol and testosterone, and the biochemical parameters such as estimation of protein and glycogen content on the ovaries of female crabs.

2.1 Quantification of Vitellogenin content

Vitellin and vitellogenin provides carbohydrate, protein and lipids for maturing oocytes the source for the development of embryos. The hemolymph of female crab exposed at different levels of microplastics were estimated for Vitellogenin by ELISA. Revathi et al. (2013).

2.2 Quantification of 17β-estradiol hormone

The 17β-estradiol hormone was estimated for steroid content of exposed microplastics at different concentrations in female crabs by Radio immuno assay (RIA) by Oreczyk et al. (1974).

2.3 Quantification of Testosterone

The hormone testosterone was estimated in different concentration of microplastics exposed female crabs by RIA explained by Revathi et al. (2013).

2.4 Quantification of Protein

The samples were exposed to different concentrations of microplastics were estimated for the concentration of protein by Coomassie Brilliant blue explained by Bradford, (1976).

2.5 Quantification of glycogen content

The sample was treated with different concentration of microplastics were estimated for glycogen content followed by the method of Dezwann and Zandee (1972).

3. 0 RESULTS

3.1 Quantification of vitellogenin content

Vitellogenin content was significantly decreased in the different concentration of micro plastics exposure animals compared to the control animal. In the control, the vitellogenin content was observed as 1.8 & 3.1 µg/mg respectively in the Hepatopancreas and Hemolymph. On the other hand, the vitellogenin content was significantly debited in the higher concentration of micro plastics (1000 Particles mL-^{1}) exposure animals to 0.3 &1.2 µg/mg respectively in the Hepatopancreas and Hemolymph. Likewise, 100 Particles mL-^{1} exposure showed 0.7 &1.8 µg/mg and 10 Particles mL-^{1} exposure showed 0.8 &2.2 µg/mg in Hepatopancreas and Hemolymph after 28 days of experimental period (Fig 1).

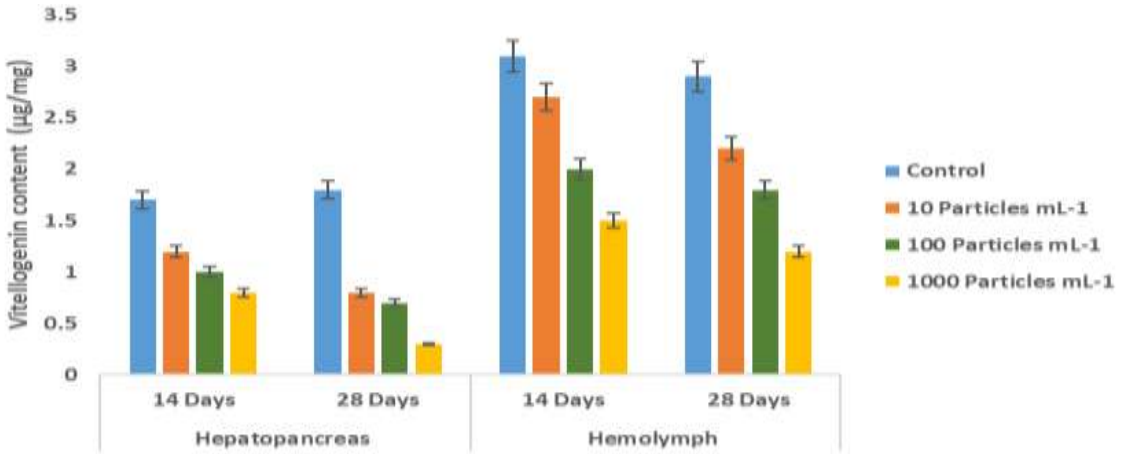

Fig. 1: Effect of different concentration of micro plastics exposure in vitellogenin content in Hepatopancreas and Hemolymph.

3.2 Quantification of 17β-estradiol hormone

17β-estradiol hormone plays a crucial role in the reproduction of female crabs. Our study results represented in the Figure 2 clearly indicated that, the exposure of micro plastics has remarkably reduced the 17β-estradiol hormone level in the hemolymph compared to the control animals. Different concentration of micro plastics such as 1000, 100 and 10 Particles mL-^{1} exposure animals showed reduced amount of 17β-estradiol hormone level as 13, 104

and 68 pg/g, whereas the control animals showed 165 pg/g of 17β-estradiol in the hemolymph*E. Asiatica.*

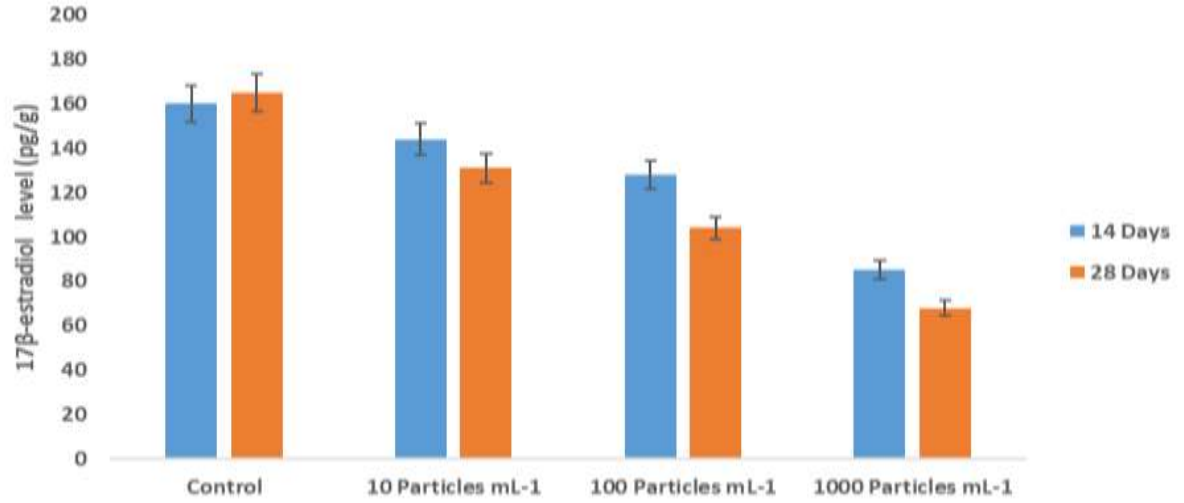

Fig. 2: Effect of different concentration of micro plastics exposure in 17β-estradiol hormone in Hemolymph.

3.3. Quantification of protein content

Protein content in the hemolymph of the experimental group animals measured and results demonstrated that, the protein content was potentially affected in the micro plastics exposure animals compared to the control animals (Fig. 3). Different concentration of micro plastics (1000, 100 and 10 Particles mL^{-1}) exposure has dose dependently decreased the protein content to 138.8, 115.0 and 73.2 pg/g respectively. The hemolymph protein concentration in the control animals, in contrast, was 159.2 pg/g.

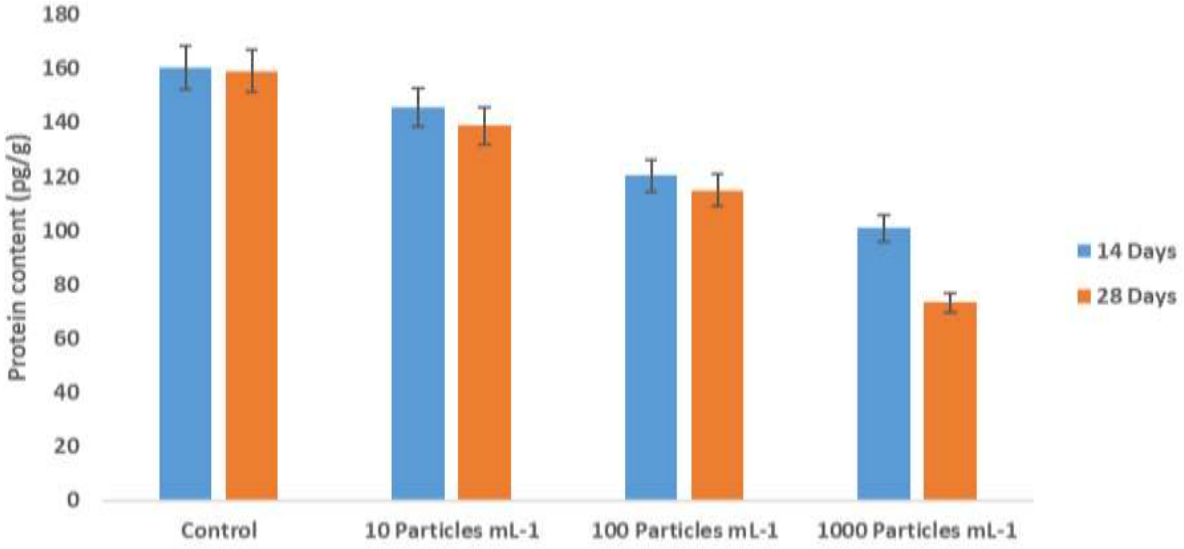

Fig. 3: Effect of different concentration of micro plastics exposure in Protein content in Hemolymph.

3.4 Quantification of glycogen content

The glycogen content in the hemolymph of micro plastics exposure animals showed significant level of depletion in the glycogen compared to the control group animal. Our study results showed that, the different concentration of micro plastics (1000, 100 and 10

Particles mL-^{1}) exposure animals showed gradual decrease in the glycogen content to 40.2, 25.5 and 15.3 pg/g, whereas the control animals showed 48.6 pg/g (Fig. 4).

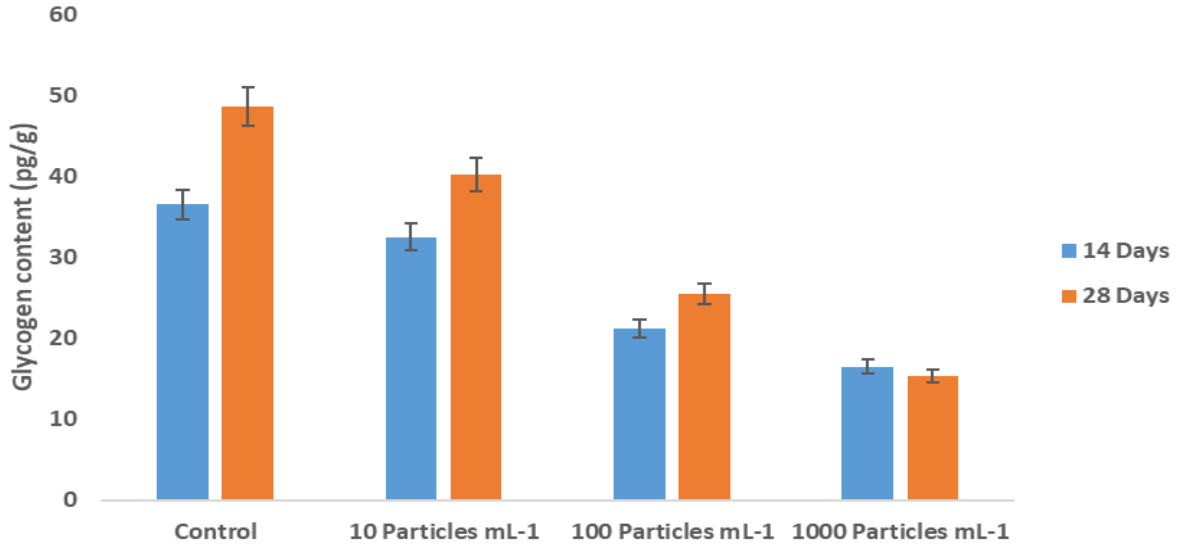

Fig. 4: Effect of different concentration of micro plastics exposure in Glycogen content in Hemolymph.

4.0 DISCUSSION

Contaminants of micro plasticsis a new threat to the marine environment, and it is important to understand the interactions and possible toxicity to the marine crustaceans. Consumption of microplasticsby invertebrates leads to the oxidative stress and inhibits their somatic and reproductive growth. This might be brought on by energy reserves being diverted away from growth processes and given to life-sustaining maintenance processes (Trestrail et al., 2020; Gonçalves and Bebianno, 2023).

The yolk-precursor lipoprotein vitellogenin is a widespread recognition as a biomarker for detecting estrogenic activity in the crab in water-borne chemical contaminants. Vitellogenin the precursor of Vitellin, plays a key role in ovarian development. Our study results on Vitellogenin content was significantly debited in the micro plastics consumed animal.It was previously described that, the microplastic could have decreased the activity of vitellogenin and choriogenin in the liver of *Oryzias latipes*through the down regulation of transcription of gonadotropin-releasing hormone (GnRH) in hypothalamus (Rochman et al., 2014; Karami et al., 2016).

Our study results indicated that *Emerita asiatica*have showed elevated level of testosterone and decreased level of 17β-estradiol in the micro plastics exposure animals. Since, a hormone 17β-estradiol is plays a crucial role in the reproductive system of female crabs. Moreover, both male and female crabs can produce testosterone and 17-estradiol, even though based on sex the production will vary significantly. Our study findings are correlated with Revathi et al. (2013) reported that, the inhibition of gonadal maturation is associated with the sex hormone testosterone and 17β-estradiol. Since, the irreversible amount of both testosterone and 17β-estradiol levels were observed in the ovary of prawns exposed to Tributyltin (TBT), and its leads to the impact on the ovarian development of prawn. Another study on, consumption of micro plastics significantly reduced the production of sex hormone testosterone in Male prawn and induced the reproductive toxicity (Sun et al., 2022). Hence,

the changes of sex hormone levels ideally affect the ovariandevelopment of small crustaceans. In the contrast of our study, Zhang et al. [21] reported that environmentally realistic concentrations of TBT had an adverse effect on ovarian development in *S. marmuratus*.

Our present study results showed that decreased level of total protein and glycogen content in the hemolymph of micro plastics exposure animals. The reason behind that, increase in glycogenolysis under the toxic stress of microplastic ingestion may be the cause of the decreased glycogen content. Similar to our study, reduction of glycogen content in hepatopancreas of *M. kistnensis* was observed against a organotin compound TBTCL exposure (Kharat et al., 2009). Another study on, exposure of dibutyltinchlorine by the marine crustaceans *Anodonta anatine*, showed remarkable decrease in carbohydrate content (Holwerda and Herwig, 1986). Similar to the glycogen, total protein level also depleted in the micro plastics exposure animals. Reduction in protein content of animal hemolymph is the points to an increase in proteolysis and the potential use of the end product of their degradation for metabolic purposes.This was due to, during the oxidative stress conditions animals need moreenergy for proteolysis may be mobilized into the trichloroacidic acid cycle through amino acid metabolism.Increased protein catabolism and decreased protein anabolism may be to blame for the drop in protein content when exposed to pollutants. Our study results are supported by Kharat et al. (2009) reported that, depletionof protein content in different tissues of prawn *Macrobrachium kistnensis*post exposure to TBTCL.

5.0 CONCLUSION

Our present study results showed that, the consumption of micro plastics was potentially down regulated the production of vitellogenin content and which can ultimately affect the vitellogenesis of the female crab *Emerita asiatica*. On the other hand, the sex hormones level also significantly affected as well as the total protein and glycogen content was remarkably decreased upon the micro plastics exposure. Overall, the present finding is concluded that, the exposure of micro plastics can lead to the severe impact on the reproductive system of marine female crab *Emerita asiatica.* Further, our study results provide an important awareness to the public to use and discard of plastics in a safest way to save the small crustacean species.

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