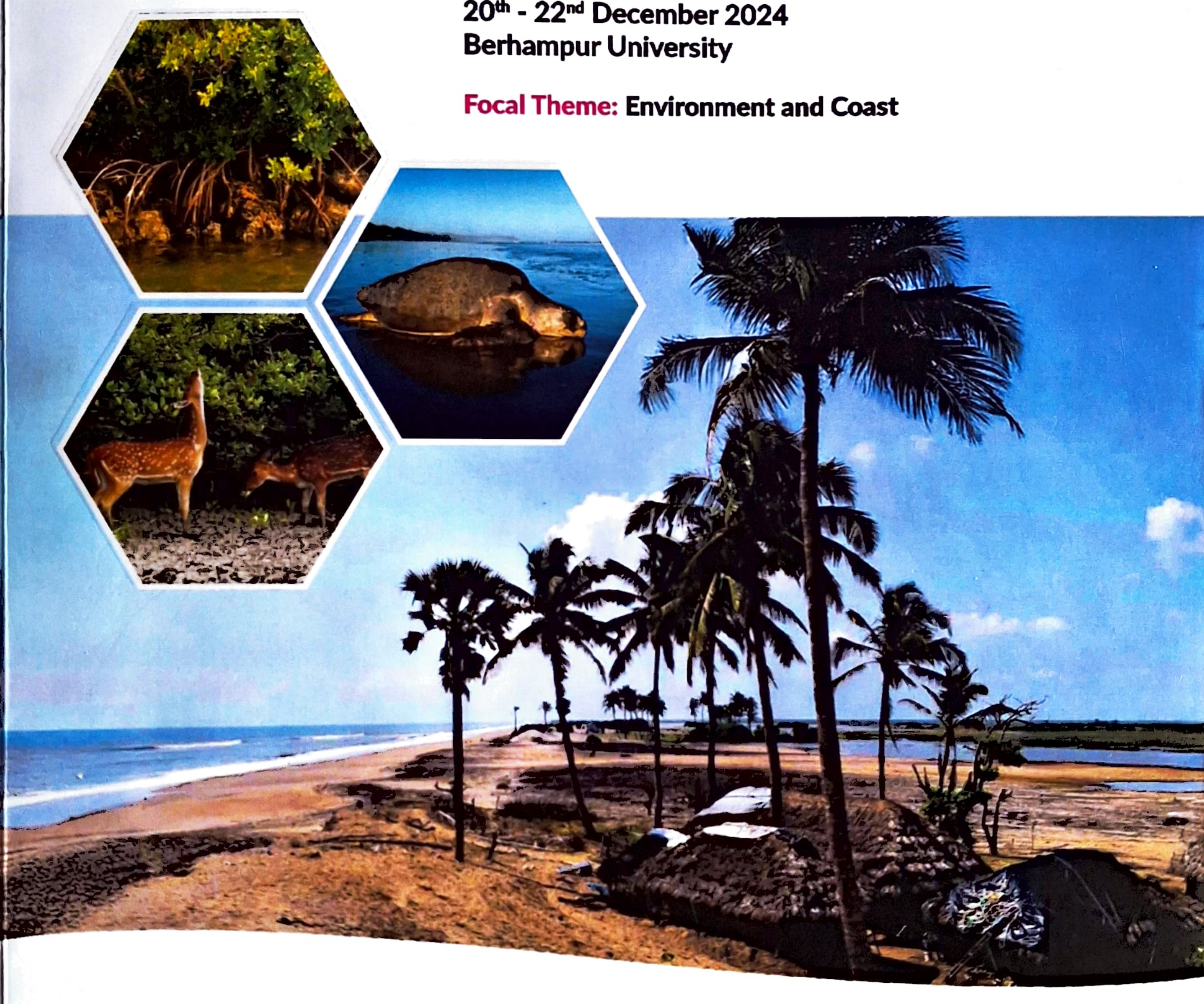
Proceedings

ODISHA ENVIRONMENT CONGRESS 2024

20th - 22nd December 2024
Berhampur University

Focal Theme: Environment and Coast



ORGANISED BY

CED

HDF·cDAR Centre for Development Action & Research

nmnh

OES

Proceedings of the
Odisha Environment Congress - 2024

Editorial Board

Organised by:

Human Development Foundation-cDAR
Centre for Environment and Development
Regional Museum of Natural History
Orissa Environmental Society
Berhampur University, Berhampur
&
Dakhina Odisha Unnayan Parishad

Publisher : Pakhighara Prakashani
32, Madhusudan Nagar
Bhubaneswar-1, Odisha, India.
Ph. +91 94385 59778
e-mail : pakshighara02@gmail.com

Design & Pre-press
Godfrey's Graphics
Sasthamangalam, Thiruvananthapuram

ISBN ; 978-81-19327-52-2
Price : ₹895.00

Expanded polystyrene microplastic is more cytotoxic to seastar coelomocytes than its nonexpanded counterpart: a comparative analysis

Arunodaya Gautam1,2

[1]*Aquatic Toxicology Laboratory, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700019, West Bengal, India.*
[2]*Estuarine and Coastal Studies Foundation, Howrah, 112, Deshapran Shasmal Rd, Kadam Tala, Howrah, West Bengal 711101*
Corresponding Author E-mail Address: arunodaya.ecotox@gmail.com

ABSTRACT

Microplastics (MPs) are established contaminants of coastal ecosystems. Present investigation is aimed to assess comparative toxicity of polystyrene microplastic (PS-MP) and expanded polystyrene microplastic (EPS-MP) in the coelomocytes of *Astropecten indicus*, a common seastar of Digha coast of Bay of Bengal, India. The coastal water of Digha, a tourist spot of attraction, bears the ecotoxicological risk of contamination by various agents, including expanded and nonexpanded microplastics of industrial origin. Coelomocytes of seastar perform multiple physiological functions, including pathogen engulfment, cytotoxicity, and respiratory gas exchange, and are considered immune effector cells in echinoderms. We report an adverse shift in total count, phagocytic response, cytotoxicity and oxidative potential of the coelomocytes of *A. indicus* under the exposures of 0.5 and 1 mg L^{-1} PS-MP and EPS- MP for 7 and 14 d. Experimental data suggested a higher level of cytotoxicity of EPS-MP in coelomocytes in comparison to that of PS-MP. Seastar is considered as a keystone species, which plays an important role in maintaining the functional homeostasis of coastal ecosystem. Unrestricted contamination of coastal water by MPs may lead to a persistent immunophysiological stress in seastar. Experimental endpoints may

be considered as effective monitoring tool to assess ecotoxicity of MPs in seastar and alike organisms sharing the same habitat.

Keywords: *Astropecten indicus*, Bay of Bengal, superoxide anion, NO, SOD.

INTRODUCTION

Microplastics (MPs) pollution have been identified as a serious ecotoxicological concern for marine environment. MPs originate from diverse human activities including beach littering, road run off sewage, waste dumping and marine fishery activity (Jambeck et al., 2015; Sebille et al., 2016). According to Guzetti et al. (2018), secondary MPs in marine environment are generated due to photolytic exposure, mechanical wave and leaching of plastic debris disposed in the ocean. Expanded polystyrene (EPS) is used in material packaging, production of lightweight concrete, buoys, floating docks, net floats and boat stand in coastal region (Sulong et al., 2014). Plastic debris have been reported along the shorelines of Northern hemisphere (Obbard, 2018), Pacific (Egger et al., 2020), Atlantic (Hidalgo-Ruz et al., 2012) and Mediterranean ocean (De La Fuente et al., 2021). Eriksen et al. (2014) estimated over 5 trillion tons of plastic pieces floating in sea globally.

Seastar, *Astropecten indicus* (Phylum: Echinodermata: Class: Asteroidea: Order: Paxillosida), a generalized feeder is geographically distributed along the coast of South-East Asian countries including the coast of Bay of Bengal, India (Loh and Todd, 2011). Coastline of India, including Bay of Bengal is reported to be contaminated by various types of MPs (Tiwari et al., 2019). According to these authors, human population size near the shoreline may be the cause of variable degree of MP pollution of coastal water. Being deuterostome, echinoderms bear evolutionary importance and exhibit a central role in maintenance of ecosystem integrity (Pinsino and Matranga, 2014). Coelomocytes, the immune cells of echinoderms, are free moving cells recorded in coelom and water vascular system (Smith, 2010). Pinsino and Matranga (2014) claimed coelomocytes of sea urchin as sentinels of environmental stress and sensitive 'cellular model' for immunotoxicological investigations. Purple sea urchin (*Paracentrotus lividus*) is also reported to be a novel cellular biosensor of environmental stress (Pinsino et al, 2008).

Echinoderms are ancient and successful invertebrates which are in constant exposure of environmental stresses like toxicants and emerging pollutants (Pinsino and Matranga, 2014). They are indicators of environmental toxicities of sediment metals and polychlorinated biphenyls (Coteur et al, 2003). Immune-associated parameters like superoxide anion, nitric oxide, phenoloxidase, superoxide dismutase, catalase and glutathione-*S*-transferase are reported as biomarkers of chemical toxicity in many aquatic invertebrates

(Mukherjee et al., 2015; Ray et al., 2017). Importance of density and phagocytic potential of immunocytes in assessment of environmental toxicity is in report (Chakraborty et al., 2021). We quantitated and analyzed the effects of sublethal exposures of PS-MP and EPS-MP on the total count and phagocytic potential of coelomocytes, an established immunocyte of seastar. Analysis of cytotoxic and oxidative stress along with detoxification potential were evaluated by estimating the generation of superoxide anion, nitric oxide (NO) and activities of phenoloxidase (PO), superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) in the coelomocytes of *A indicus* exposed to PS-MP and EPS-MP separately. Chemical contaminants of water often impaires the cytotoxic potential of its inhabitants and results in the state of immunocompromisation in them. Structural analysis of PS-MP and EPS-MP exhibited marked contrast. Morphological dissimilarity of these two particles led us to investigate their comparative cytotoxicity in coelomocytes, the chief immunoeffector cells of seastar. Currently, the entire stretch of Digha coast is in the risk of perennial contamination of microplastics of anthropogenic origin. I report a shift in total coelomocyte count, percentage of phagocytic efficacy, generation of superoxide anion, NO and activities of PO, SOD, CAT, and GST in the coelomocytes of *A. indicus* under sublethal concentrations of PS-MP and EPS-MP. Present experimental endpoints bear immunological significance in seastar, an evolutionary and ecologically important deuterostome of the coastal region of Bay of Bengal. Prolonged contamination of coastal water by PS-MP and EPS-MP is apprehended to cause dwindling of population of *A. indicus* in its natural habitat of Bay of Bengal.

2. MATERIALS AND METHOD

2.1. Physical characterisation of PS-MP and EPS-MP

PS-MP (CAS number: 9003-53-6) was purchased from HIMEDIA, India and EPS styrofoam was collected from local market. EPS-MP was prepared manually by rubbing the source material over sand paper of 80 grit (NH Brand, India). The determination of the hydrodynamic particle diameter of PS- MP, and EPS-MP were carried out.

2.2. Collection, transportation, and laboratory acclimation of experimental starfish

Adult healthy seastar *A. indicus* was collected from Digha coast (21.6222° N, 87.5066° E) of West Bengal, India, by net trawling method and transported to the laboratory with sea water. Seastars with average diameter of 2.4 ± 0.3 cm, were acclimatized in glass aquaria consisting artificial sea water (ASW) and sand sediments with adequate aeration and filtering system at a density of single specimen per 3 L of sea water at constant temperature range (15-22 °C), salinity (33 PSU) and pH (8.0-8.1) for 7 days.

2.3. Experimental exposure of PS-MP and EPS-MP

A number of 5 experimental replicates each consisted of 5 seastars per container were treated with 0.5 and 1 mg L^{-1} of PS-MP and EPS-MP separately for 7 and 14 days along with a parallel set of control without any toxin.

2.4. Collection, enumeration and determination of viability of coelomocytes of experimental seastar

Experimental seastar specimens were bled and the coelomic fluid was aseptically collected in precooled coelomocyte culture medium (CCM: 2 mM EGTA, 20 mM HEPES, 0.5 M NaCl and 5 mM MgCl2; pH 7.9) (Pinsino et al., 2007) to prevent anticoagulation. The total coelomocyte density was determined microscopically using Neubauer's haemocytometer. Coelomocyte viability was checked by staining the cells with trypan blue dye.

2.5. Determination of phagocytic response of coelomocytes

The phagocytic response was estimated by challenging the coelomocytes with yeast suspension in a prestandardised ratio of 1:10 over grease free slide and incubated at 27 °C in a humid chamber for 3 h (Gautam et al., 2020). Postincubated monolayer was rinsed with CCM and stained with Giemsa's stain and observed under microscope (Axiostar Plus, Zeiss). Number of adherent coelomocytes involved in phagocytosis were determined microscopically by considering at least 200 fields (Adamowich and Wojtaszek, 2001).

2.6. Estimation of antioxidant molecule generatiom

Superoxide anion generation was estimated by following the principle of reduction of nitroblue tetrazolium (NBT) (Takahashi et al., 1993). Nitric oxide (NO) generation in coelomocytes was estimated by reacting the cells with Griess reagent (solution of 0.1% naphthyl ethylenediamine dihydrochloride and 1% sulfanilamide in 5% concentrated orthophosphoric acid) (Green et al., 1982).

2.7. Preparation of coelomocyte lysate

Lysate of coelomocytes was prepared by incubating 10^6 number of cells mL^{-1} with 5 mL of 0.1

% Triton X-100 at 4 °C for 12 hours to ensure complete cell lysis. Suspension was centrifuged at 650 g for 15 minutes at 4 °C and the supernatant was collected in a prechilled glass vial. Activities of PO, SOD, CAT and GST were determined from the collected supernatant.

2.8. Estimation of PO activity

Activity of PO (EC 1.14.18.1) was estimated in coelomocyte lysate after Sung et al. (1998) using 1-3, 4-dihydroxy phenylalanine (L-DOPA) as substrate.

2.9. Estimation of proo

Estimation of the activity of SOD (EC 1.15.1.1) in the coelomocyte lysate was recorded after Song et al. (2009) with minor modifications. Activity of CAT (EC 1.11.1.6) was estimated in the lysates of coelomocytes of *A. indicus* after Xu et al. (1997) with hydrogen peroxide as substrate. GST (EC 2.5.1.18) activity in the lysate of seastar coelomocytes was determined (Habig et al., 1974) using the substrate 1-chloro-2, 4-dinitrobenzene (CDNB).

2.10. Statistical analyses

Obtained data were checked for normality and homogeneity using Bartlett's test (Mukherjee et al., 2016). As all the acquired data were found to be normal, parametric, one-way analysis of variance (ANOVA) (Tang et al., 2020) followed by Tukey's multiple comparison test (GraphPad Prism version 5 for windows, GraphPad Software, La Jolla California, USA) for each toxin concentration and after each experimental day for determining the significance between control and treated sets. Data were presented as mean ± standard deviation (S.D) (n=5). Asterisk (*) in the data represented significant difference from the control. Dissimilar letters between the data represented the significant difference between the PS-MPs and EPS-MP exposure sets. Differences were considered significant at $p^* < 0.05$.

3. RESULTS

3.1. Physical characteristics of PS-MP and EPS-MP

The average hydrodynamic size of PS-MP and EPS-MP were recorded as 653.3 d.nm and 7401 d.nm (Z-average) (Figure 1A, B). The peaks of DLS of PS-MP and EPS-MP at highest intensity (99 %) were recorded as 248.3 ± 38.9 d.nm and 398.6 ± 25.7 d.nm (Figure 1A, B). Surface charge of PS-MP and EPS-MP were recorded as -0.159 mV and -34.8 mV (Figure 1C, D).

FESEM images of PS-MP (Figure 1E) exhibited distinct spherical structure of MP having an average diameter of 2.04 ± 0.36 μm. The FESEM images of EPS-MP (Figure 1F) exhibited uneven porous structure with an average size of 1.54 ± 0.155 mm.

3.2. Total coelomocyte count

Adverse shift of total coelomocyte count of *A. indicus* was recorded under the exposure of 0.5 and 1 mg of PS-MP and EPS-MP per liter of ASW for 7 and 14 d

(Figure 2A). The lowest yield of total coelomocyte count was estimated as 7.8 ± 0.73 x 10^6 cells mL^{-1} and 4.2 ± 0.931 x 10^6 cells mL^{-1} upon exposure of 1 mg L^{-1} of PS-MP and EPS-MP for 14 d respectively.

3.3. Phagocytic response

Phagocytic response of coelomocytes of starfish *A. indicus* markedly decreased at each concentration of PS-MP and EPS-MP for 7 and 14 d (Figure 2B). Minimum phagocytic indices were recorded as 63.2 ± 1.82 and 55 ± 1.37 upon exposure of 1 mg L^{-1} of PS-MPs and EPS for 14 d respectively.

3.4. Superoxide anion generation

Coelomocytes of starfish *A. indicus* under the exposure of 0.5 and 1 mg of PS-MP and EPS- MP per litre ASW for 7 and 14 d exhibited significant increase in the generation of superoxide anion (Figure 3A). Coelomocytes exposed to 1 mg L^{-1} of PS-MP and EPS-MP for 7d resulted in maximum generation of superoxide anion, recorded as 0.01866 ± 0.00113 OD10^6 $cells^{-1}$ $minute^{-1}$ and 0.01968 ± 0.0009 OD 10^6 $cells^{-1}$ $minute^{-1}$ respectively.

3.5. NO generation

Detrimental shift in NO generation was recorded in the coelomocytes of seastar *A. indicus* under the exposure of 0.5 and 1 mg of PS-MP and EPS-MP per litre of ASW for 7 and 14 d (Figure 3B). Maximum generation of NO in the coelomocytes of seastar were recorded as 0.18936 ± 0.011 μM nitrite 10^6 $cells^{-1}$ $minute^{-1}$ and 0.2328 ± 0.0009 μM nitrite 10^6 $cells^{-1}$ $minute^{-1}$ upon exposure of 1 mg L^{-1} PS-MP and EPS-MP for 14 d respectively.

3.6. PO activity

Decrease in PO activity was observed in the coelomocytes of starfish *A. indicus* under the exposure of 0.5 and 1mg of PS-MP and EPS-MP per litre of ASW for 7 and 14 d (Figure 3C). Coelomocytes exposed to 1 mg L^{-1} of PS-MP and EPS-MP for 14d yielded lowest activity of PO, estimated as 0.24768 ± 0.011unit mg $protein^{-1}$ and 0.2157 ± 0.0129 unit mg $protein^{-1}$ respectively.

3.7. SOD activity

Inhibition in SOD activity was recorded in the coelomocytes of starfish *A. indicus* under the exposure of 0.5 and 1 mg of PS-MP and EPS-MP per litre of ASW for 7 and 14 d (Figure 4A). The highest inhibition in the activity of SOD in the coelomocytes of *A. indicus* were estimated as 0.0822 ± 0.00288 unit mg $protein^{-1}$ $minute^{-1}$ and 0.0642 ± 0.00204 unit mg $protein^{-1}$ $minute^{-1}$ under the exposure of 1 mg L^{-1} of PS-MP and EPS-MP per litre of ASW for 14 d respectively.

3.8. CAT activity

Coelomocytes of starfish *A. indicus* under the exposure of 0.5 and 1mg of PS-MP and EPS-MP per litre of ASW for 7 and 14 d yielded significant depletion of CAT activity (Figure 4B). Maximum depletion in the activity of CAT in the coelomocytes of *A. indicus* were recorded as 0.02572 ± 0.00091 k mg protein^{-1} and 0.02361 ± 0.00097 k mg protein^{-1} upon the exposure of 1 mg L^{-1} of PS-MP and EPS- MP for 14 d respectively.

3.9. GST activity

Decrease in GST activity was recorded in the coelomocytes of starfish *A. indicus* exposed to

0.5 and 1 mg of PS-MP and EPS-MP per litre of ASW for 7 and 14 d (Figure 4C). Highest depletion in the activity of GST in the coelomocytes of seastar were recorded as 0.075± 0.00307 μmole mg protein^{-1} minute^{-1} and 0.0528 ± 0.00362 μmole mg protein^{-1} minute^{-1} under the exposure of 1 mg L^{-1} of PS-MP and EPS-MP for 14d respectively.

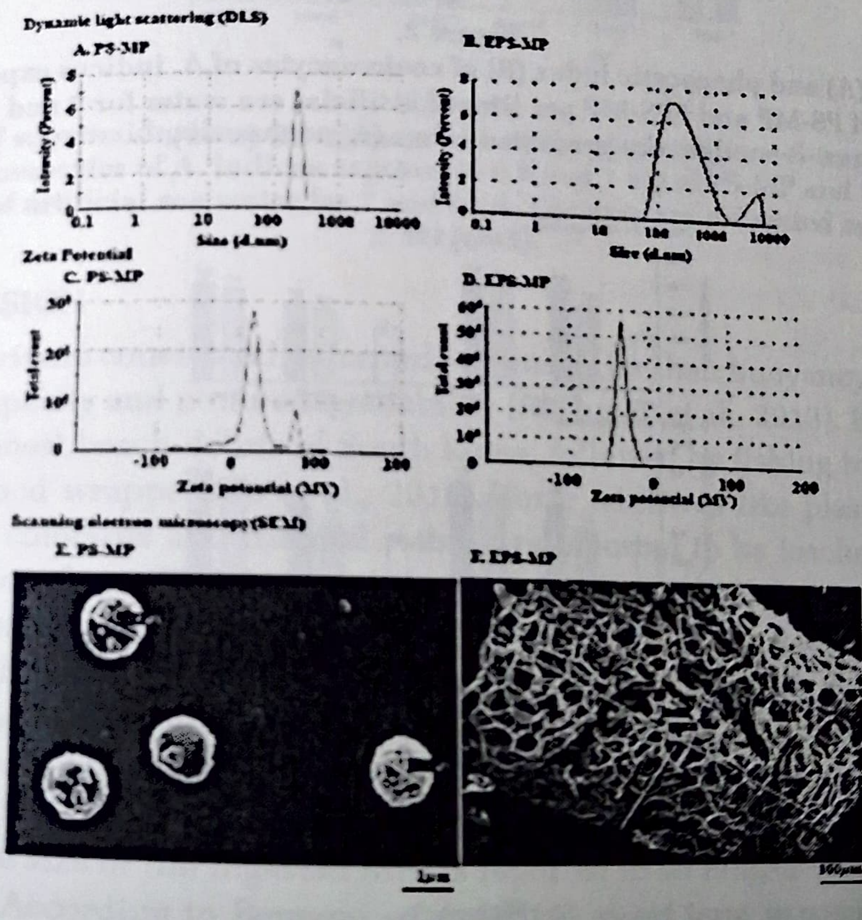


Figure 1.
Dynamic light scattering (A, B), zeta potential (C, D) and scanning electron microscopy (E, F) of PS-MP and EPS-MP.

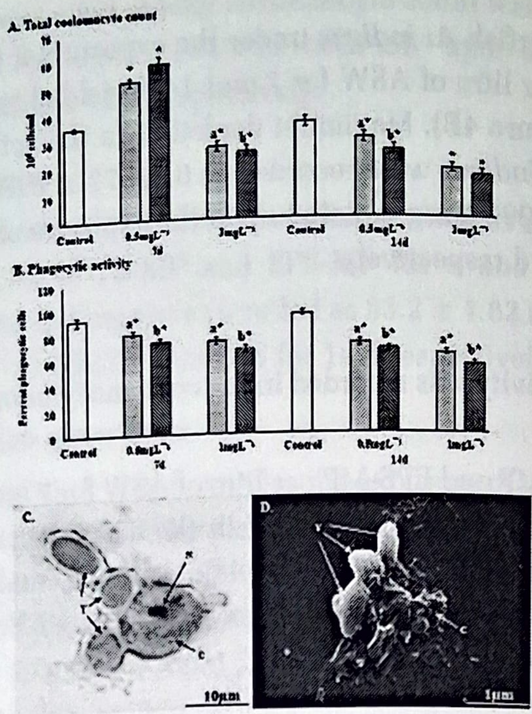


Figure 2.

Total count (A) and phagocytic index (B) of coelomocytes of A. indicus exposed to 0.5 and 1 mg of PS-MP and EPS-MP per litre of artificial sea water for 7 and 14 d. The data are presented as mean ± SD (n=5).

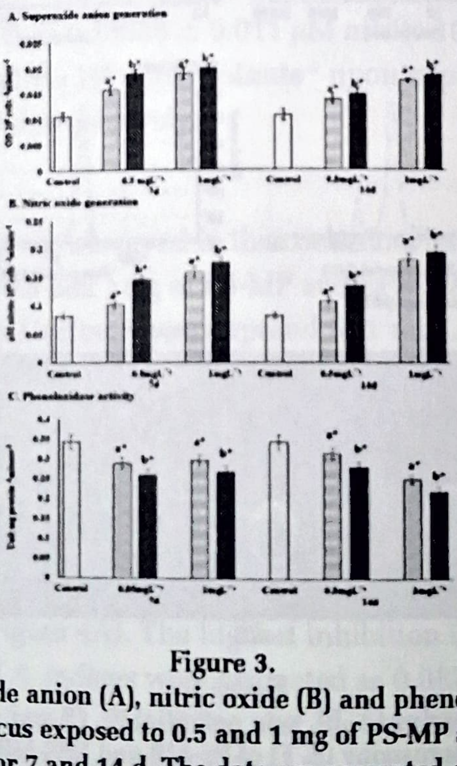


Figure 3.

Generation of superoxide anion (A), nitric oxide (B) and phenoloxidase activity (C) of coelomocytes of A. indicus exposed to 0.5 and 1 mg of PS-MP and EPS-MP per litre of artificial sea water for 7 and 14 d. The data are presented as mean ± SD (n=5).

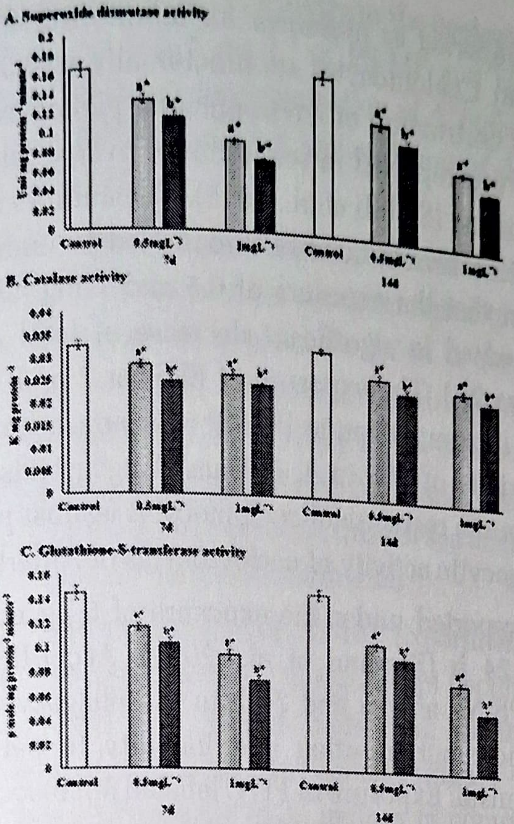


Figure 4.
Activity of superoxide dismutase (A), catalase (B) and glutathione-S-transferase (C) in the coelomocytes of A. indicus exposed to 0.5 and 1 mg of PS-MP and EPS-MP per litre of artificial sea water for 7 and 14 d. The data are presented as mean ± SD (n=5).

4. DISCUSSION

Plastic debris are considered as hazardous wastes for their buoyancy, durability, sorption capacity and nonbiodegradability (Rochman et al., 2013). EPS buoys is the commonest beach debris of South Korea, followed by fishing ropes, plastic bags and food wrapper (Eo et al., 2018). Toxic additives like plasticizers, UV stabilizers, colorants and thermal stabilizers reported to be leached in higher concentration from EPS (Andrady and Rajapakse, 2016) in comparison to those of non-expanded polystyrene (Thaysen et al., 2018). Feng et al. (2020) reported differential accumulation of MPs in the coelomic fluid, gonads and gut of 4 species of sea urchins of Bay of North China. According to them, gut accumulated highest number of MPs followed by coelomic fluid and gonad. This suggests ingestion as the major mode of particulate entry of plastic in sea urchins. The size of the ingested MPs is reported to be ranged between 2.7 µm to 4.7 mm. According to Bergami et al. (2019), short term exposure of PS NP affected the innate immune parameters of Antarctic sea urchin, *Sterenchinus neumayeri*.

Coelomocytes are reported as biosensor for monitoring environmental stress (Pinsino et al., 2008). Coelomocytes are functionally associated with cytotoxic molecule mediated destruction of environmental pathogens. Decrease in total coelomocyte count was reported in sea urchin *P. lividus* under exposure of 0.1 and 0.5 mg mL^{-1} lindane (Stabili et al., 2015). The authors concluded that two different subchronic concentrations of lindane led to immunosuppression in *P. lividus*. We report that the exposure of 0.5 and 1 mg mL^{-1} PS-MP and EPS for 7 and 14 d resulted in significant decrease of total coelomocyte count in *A. indicus* (Figure 2A). The exposure of EPS for 7 and 14 d yielded lower coelomocyte count in comparison to PS-MP exposure, which indicated higher level of immunotoxicity of EPS in *A. indicus*. Phagocytosis is considered as a classical innate immune response of coelomocytes against pathogen and toxin. Impairment of phagocytic activity of coelomocytes of Antartic sea urchin

S. neumayeri was reported under the exposure of 5 μg mL^{-1} fluorescent PS-COOH for 6 and 24 h (Bergami et al., 2019). According to the authors, internalization of PS-NP after 6 and 24 h in the phagocytes of *S. neumayeri* resulted in immunocompromisation and inability to pathogen destruction efficacy by the organism. Exposure of FITC labeled *Micrococcus leuteus* caused decrease in phagocytic activity in the coelomocytes of European starfish *Asterias rubens* (Coteur, 2002). Present investigation indicated impairment in innate immune activity of *A. indicus* under the exposure of PS-MP and EPS for 7 and 14 d. EPS induced phagocytic activity of coelomocytes was recorded to be lower in comparison to exposure of PS-MP. Result may indicate higher level of immunotoxicity of EPS-MP.

Gopalakrishnan et al. (2009) reported that generation of superoxide anion by the immunocytes is correlated with the oxidative burst in the phagocytic cells of marine gastropod *Haliotis diversicolor*. Superoxide anion upon conjugation with NO generates a strong bactericidal oxidant molecule, peroxynitrite (Torreilles and Guerin, 1999). Chemical stress induced shift in generation of superoxide anion and NO may lead to oxidative damage in organism. Hyperproduction of superoxide anion was reported in coelomic fluid and respiratory tree of sea cucumber *Apostichopus japonicus* exposed to desiccation related stress for 0, 3, 6, 12 and 24 h (Cui et al., 2020). We report increase in generation of superoxide anion and NO in the coelomocytes of *A. indicus* under the exposure of 0.5 and 1 mg L^{-1} of PS-MP and EPS-MP for 7 and 14 d (Figure 3A, B). Exposure of EPS-MP resulted in higher oxidative stress on the coelomocytes of *A. indicus* was indicative to immune suppression in the same species. PO plays a major role in fungistatic, bacteriostatic and antiviral activities in invertebrates (Johanson and Söderhäll, 1996). According to Nappi and Ottaviani (2000), PO is a potential cytotoxic enzyme capable of generating toxic quinoid intermediates for host defense. We recorded alteration in PO activity in the coelomocytes of *A. indicus* under the exposure of 0.5 and 1 mg L^{-1} of PS-MP and EPS-MP (Figure 3C). This is an indication of immunocompromisation of *A. indicus* in contaminated habitat. The exposure of EPS-MP yielded higher degree of shift in PO activity in comparison to that of PS-MP.

Markad et al. (2012), claimed that determination of antioxidation response may be considered as a tool to monitor environmental toxicity. According to Livingstone (2003), the excess of superoxide anion is chemically neutralised and detoxified by SOD and CAT. Dolmatova et al. (2018) reported that the exposure of lead downregulated CAT activity in the phagocytes of holothuroid *Eupentacta fraudatrix*. Increased activity of SOD and CAT were recorded in intestine, tentacles, peristome and coelomic fluid of sea cucumber *Apostichopus japonicus* by feeding chameleon plant *Houttuynia cordata* as food supplement (Dang et al., 2019). Experimental exposure of PS-MP and EPS-MP for 7 and 14 d resulted in significant decrease of SOD and CAT activities in coelomocytes of *A. indicus,* indicating MP induced suppression of antioxidant defence potential in same species (Figure 4A, B). Exposure of EPS-MP exhibited a higher toxicity in *A. indicus* in comparison to that of its nonexpanded counterpart.

GST is enzymatically involved in detoxification process of all eukaryotes (Nicosia et al., 2014). GST is physiologically associated with the reduction of oxidative stress (Hermes-Lima and Storey, 1993). Acute exposure of PS-MP caused decrease in GST activity in coral *Pocillopora demicornis* (Tang et al, 2018). Chloropyrifos induced shift in the activity of GST of digestive gland of bivalve *Scapharca inaequivalvis* was reported by Antognelli et al., (2006). We reported, suppression of GST activity in the coelomocytes of *A. indicus* under the exposure of 0.5 and 1mgL^{-1} of PS-MP and EPS- MP for 7 and 14 d (Figure 4C). Experimental findings suggested potential inhibition of detoxification potential in *A. indicus*. Exposure of EPS yielded higher grade of toxicity in *A. indicus* compared to that of PS-MP exposure.

PS-MP is a polymer of styrene conjugated with carboxyl group, whereas EPS is synthesized by treating polystyrene with air and chemical additives like benzene derivatives and HBCDD, a hazardous additive. Ultrastructural analyses of PS-MP and EPS-MP revealed that the PS-MP is a smooth surfaced round material with an average diameter of 2±0.042μm. Contrarily, EPS-MP are irregular shaped, large sized granules with extensive surface porosity (Figure 1E, F). Higher degree of cellular toxicity of EPS- MP may be correlated with its relative size, structural complexity, irregular morphology and leaching potential of toxic chemical additives. However, more research is to be carried out in this direction.

Unrestricted use and selling of diverse plastic-made items are characteristic to Digha coast. Plastic debris are often disposed directly to coastal water causing contamination. Use of large sized plastic nets for capture fishery is an age-old practice followed by the fishermen of Bay of Bengal. Tiwari et al. (2019) isolated polystyrene microplastics from beach sand samples of the shoreline of Bay of Bengal. This study also indicated that the beaches of India are contaminated with fibrous, granular and film shaped microplastic particles. This contamination may be corelated with various anthropogenic activities along this coastal area. Hossain et al. (2020) reported presence of fibrous, particulate and fragmented microplastics in the gastrointestinal tract of tiger and brown shrimps of northern Bay of Bengal. While enlisting the coastal fauna of Digha coast of Bay of Bengal, Sarkar et al. (2013) reported presence of *A. indicus* alongwith other seastars. Thus, this species is assumed to be exposed to both primary and secondary microplastics. Immunophysiological parameters like density count, phagocytosis, cytotoxic molecule generation and oxidative stress response are reported as effective markers of environmental contamination (Pinsino et al., 2008). Considering the even distribution of *A. indicus* and its physiological sensitivity towards microplastic, experimental parameters are assumed to be useful for ecotoxicological monitoring of plastic pollution in coast of Bay of Bengal. Moreover, echinoderms are coelomate deuterostome and bear evolutionary importance in the phylogeny. Present investigation would thus provide a useful information base for effective monitoring of ecotoxicity and immunophysiological status of seastar inhabiting the coast of Bay of Bengal.

Acknowledgement

Authors thankfully acknowledge the University Grant Commission, Government of India for sanctioning junior research fellowship to Abhinandan Barua (09/028(0967)/2016-EMR-I). Centre for Research in Nanoscience and Nanotechnology is also acknowledged for instrumental facilities.

References

Adamowicz, A., Wojtaszek, J. (2001) Morphology and phagocytic activity of coelomocytes of *Dendrobaena veneta* (Lumbricidae). *Zool. Pol.,* 16 (1-4:, 91–104.

Antognelli, C., Francesca, B., Andrea, P., Roberta, F., Vincenzo, T., Elvio, G. (2006) Activity changes of glyoxalase system enzymes and glutathione- S - transferase in the bivalve mollusc *Scapharca inaequivalvis* exposed to the organophosphate chlorpyrifos 86, 72–77. https://doi.org/10.1016/j.pestbp.2006.01.007

Bergami, E., Krupinski Emerenciano, A., González-Aravena, M., Cárdenas, C.A., Hernández, P., Silva, J.R.M.C., Corsi, I. (2019) Polystyrene nanoparticles affect the innate immune system of the Antarctic sea urchin *Sterechinus neumayeri*. *Polar Biol.*, 42: 743–757. https://doi.org/10.1007/s00300-019-02468-6

Brandts, I., Teles, M., Gonçalves, A.P., Barreto, A., Franco-Martinez, L., Tvarijonaviciute, A., Martins, M.A., Soares, A.M.V.M., Tort, L., Oliveira, M. (2018) Effects of nanoplastics on *Mytilus galloprovincialis* after individual and combined exposure with carbamazepine. *Sci. Total Environ.* 643: 775–784. https://doi.org/10.1016/j.scitotenv.2018.06.257

Burns, E.E., Boxall, A.B.A. (2018) Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. *Environ. Toxicol. Chem.*, 37: 2776–2796. https://doi.org/10.1002/etc.4268

Chakraborty, S., Ray, M., Ray, S. (2021) Bivalve haemocyte adhesion, aggregation and phagocytosis: A tool to reckon aresenic induced threats to freshwater ecosystem. Fish Shellfish Immunol, 114, 229-237. http://doi.org/10.1016/j.fsi.2021.05.008

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S. (2015). The Impact of Polystyrene Microplastics on Feeding, Function and Fecundity in the Marine Copepod *Calanus helgolandicus*. https://doi.org/10.1021/es504525u

Coteur, G., Gosselin, P., Wantier, P., Chambost-Manciet, Y., Danis, B., Pernet, P., Warnau, M., Dubois, P. (2003) Echinoderms as bioindicators, bioassays, and impact assessment tools of sediment- associated metals and PCBs in the North Sea. *Arch. Environ. Contam. Toxicol.* 45: 190–202. https://doi.org/10.1007/s00244-003-0199-x

Coteur, G., Warnau, M., Jangoux, M., Dubois, P. (2002) Reactive oxygen species (ROS) production by amoebocytes of *Asterias rubens* (Echinodermata). *Fish Shellfish Immunol.* 12: 187–200. https://doi.org/10.1006/fsim.2001.0366

Cui, Y., Hou, Z., Ren, Y., Men, X., Zheng, B., Liu, P., Xia, B. (2020). Effects of aerial exposure on oxidative stress , antioxidant and non-specific immune responses of juvenile sea cucumber *Apostichopus japonicus* under low temperature. *Fish Shellfish Immunol.*, 101: 58–65. https://doi.org/10.1016/j.fsi.2020.03.050

Dang, H., Zhang, T., Yi, F., Ye, S., Liu, J., Li, Q., Li, H., Li, R. (2019) Enhancing the immune response in the sea cucumber *Apostichopus japonicus* by addition of Chinese herbs *Houttuynia cordata* Thunb. as a food supplement. *Aquac. Fish.*, 4, 114–121. https://doi.org/10.1016/j.aaf.2018.12.004

De La Fuente, R., Drótos, G., Hernández-García, E., López, C., Van Sebille, E. (2021) Sinking microplastics in the water column: Simulations in the Mediterranean Sea. *Ocean Sci.*, 17, 431– 453. https://doi.org/10.5194/os-17-431-2021

Dolmatova, L.S., Dolmatov, I. Y. (2018) Lead Induces Different responses of two subpopulations of phagocytes in the Holothurian *Eupentacta fraudatrix* 17: 1391–1403.

Egger, M., Sulu-Gambari, F., Lebreton, L.(2020) First evidence of plastic fallout from the North Pacific Garbage *Patch. Sci. Rep.*, 10: 1–10. https://doi.org/10.1038/s41598-020-64465-8

Eo, S., Hong, S.H., Song, Y.K., Lee, J., Lee, J., Shim, W.J. (2018) Abundance, composition, and distribution of microplastics larger than 20 mm in sand beaches of South Korea. *Environ. Pollut.*, 238: 894–902. https://doi.org/10.1016/j.envpol.2018.03.096

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS One 9, 1–15. https://doi.org/10.1371/journal.pone.0111913

Feng, Z., Wang, R., Zhang, T., Wang, J., Huang, W., Li, J., Xu, J., Gao, G., 2020. Microplastics in specific tissues of wild sea urchins along the coastal areas of northern China. Sci. Total Environ. 728, 138660. https://doi.org/10.1016/j.scitotenv.2020.138660

Ferguson, J. C. 1990. Seawater inflow through the madreporite and internal body regions of a starfish *(Leptasterias hexactis)* as demonstrated with fluorescent microbeads. J Exp. Zool., 255(3), 262– 271. https://doi.org/10.1002/jez.1402550303

Gautam, A., Ray, A., Manna, S., Sarkar, M.P., Ghosh, A.R., Ray, M., Ray, S., 2020. Shift in phagocytosis, lysosomal stability, lysozyme activity, apoptosis and cell cycle profile in the coelomocytes of earthworm of polluted soil near a tannery field of India. Ecotoxicol. Environ. Saf. 200, 110713. https://doi.org/10.1016/j.ecoenv.2020.110713

Gopalakrishnan, S., Thilagam, H., Huang, W. Bin, Wang, K.J., 2009. Immunomodulation in the marine gastropod *Haliotis diversicolor* exposed to benzo(a)pyrene. Chemosphere 75, 389–397. https://doi.org/10.1016/j.chemosphere.2008.12.027

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal. Biochem. 126, 131–138. https://doi.org/10.1016/0003-2697(82)90118-X

Guzzetti, A.E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in Marine Organism: Environmental and Toxicological Effects. Environ. Toxicol. Pharmacol. https://doi.org/10.1016/j.etap.2018.10.009

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-Transferases. J. Biol. Chem. 249, 7130– 7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Hermes-Lima, M., Storey, K.B., 1993. In vitro oxidative inactivation of glutathione S-transferase from a freeze tolerant reptile. Mol. Cell. Biochem. 124, 149–158. https://doi.org/10.1007/BF00929207

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.

Hossain, M. S., Rahman, M. S., Uddin, M. N., Sharifuzzaman, S. M., Chowdhury, S. R., Sarker, S., Nawaz Chowdhury, M. S., 2020. Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal. Chemosphere, 238, 124688. https://doi.org/10.1016/j.chemosphere.2019.124688

Jambeck, J. R., Ji, Q., Zhang, Y.G., Liu, D., Grossnickle, D. M., & Luo, Z.X., 2015. Plastic waste inputs from land into the ocean. Science, 347(6223), 764–768. http://www.sciencemag.org/cgi/doi/10.1126/science.1260879

Johansson, M.W., Söderhäll, K., 1996. The Prophenoloxidase Activating System and Associated Proteins in Invertebrates, in: Rinkevich, B., Müller, W.E.G. (Eds.), Invertebrate Immunology. pp. 46–66. https://doi.org/10.1007/978-3-642-79735-4

Laha, D., Pramanik, A., Laskar, A., Jana, M., Pramanik, P., Karmakar, P., 2014. Shape-dependent bactericidal activity of copper oxide nanoparticle mediated by DNA and membrane damage. Mater. Res. Bull. 59, 185–191. https://doi.org/10.1016/j.materresbull.2014.06.024

Li, B., Lan, Z., Wang, L., Sun, H., Yao, Y., Zhang, K., Zhu, L., 2019. The release and earthworm bioaccumulation of endogenous hexabromocyclododecanes (HBCDDs) from expanded polystyrene. Environ. Pollut. 255, 113163. https://doi.org/10.1016/j.envpol.2019.113163

Livingstone, D.R., 2003. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Rev. Med. Vet. (Toulouse). 154, 427–430.

Loh, K.S., Todd, P.A., 2011. Diet and feeding in the sea star *Astropecten indicus* (Döderlein, 1888). Raffles Bull. Zool. 59, 251–258.

Markad, V.L., Gaupale, T.C., Bhargava, S., Kodam, K.M., Ghole, V.S., 2015. Biomarker responses in the earthworm, *Dichogaster curgensis* exposed to fly ash polluted soils. Ecotoxicol. Environ. Saf. 118, 62–70. https://doi.org/10.1016/j.ecoenv.2015.04.011

Mukherjee, S., Ray, M., Ray, S., 2015. Immunotoxicity of washing soda in a freshwater sponge of India. Ecotoxicol. Environ. Saf. 113, 112–123. https://doi.org/10.1016/j.ecoenv.2014.11.035

Mukherjee, S., Ray, M., Ray, S., 2016. Shift in aggregation, ROS generation, antioxidative defense, lysozyme and acetylcholinesterase activities in the cells of an Indian freshwater sponge exposed to washing soda (sodium carbonate). Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 187, 19–31. https://doi.org/10.1016/j.cbpc.2016.05.001

Nappi, A.J., Ottaviani, E., 2000. Cytotoxicity and cytotoxic molecules in invertebrates. BioEssays, 22, 469-480.

Nicosia, A., Celi, M., Vazzana, M., Alessandra, M., Parrinello, N., Agostino, F.D., Avellone, G., Indelicato, S., Mazzola, S., Cuttitta, A., 2014. Profiling the physiological and molecular response to sulfonamidic drug in *Procambarus clarkii*. Comp. Biochem. Physiol. Part C 166, 14–23. https://doi.org/10.1016/j.cbpc.2014.06.006

Obbard, R.W., 2015. ScienceDirect Microplastics in Polar Regions : The role of long range transport. Curr. Opin. Environ. Sci. Heal. 1, 24–29. https://doi.org/10.1016/j.coesh.2017.10.004

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels *Mytilus spp.* to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737. https://doi.org/10.1016/j.envpol.2016.06.039

Pinsino, A., Thorndyke, M. C., Matranga, V., 2007. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. Cell Stress Chaperones, 12(4), 331–341. https://doi.org/10.1379/CSC-288.1

Pinsino, A., Della Torre, C., Sammarini, V., Bonaventura, R., Amato, E., Matranga, V., 2008. Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: A field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy. Cell Biol. Toxicol. 24, 541– 552. https://doi.org/10.1007/s10565-008-9055-0

Pinsino, A., Matranga, V., 2014. Sea urchin immune cells as sentinels of environmental stress. Dev. Comp. Immunol. 49, 198–205. https://doi.org/10.1016/j.dci.2014.11.013

Pramanik, M., Tsujimoto, Y., Malgras, V., Dou, S.X., Kim, J.H., Yamauchi, Y., 2015. Mesoporous Iron Phosphonate Electrodes with Crystalline Frameworks for Lithium-Ion Batteries. https://doi.org/10.1021/cm5044045

Ray, S., Ray, M., Bhunia, A, S., Banerjee, P., Mukherjee, S., 2017. Prospects of molluscan immunomarkers in monitoring aquatic toxicity. In: Larramendy, M. L. (Eds.) Ecotoxicology and Genotoxicology: Non-traditional Aquatic Models, Royal Society of Chemistry, Cambridge, pp. 70-106.

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environ. Sci. Technol. 47, 1646–1654. https://doi.org/dx.doi.org/10.1021/es303700s

Sarkar, J.A.Y., Alukdar, S.T., 2003. Marine invertebrates of digha coast and some recomendations on their conservation. Rec. zool. Surv. India, 101 (3-4), 1-23.

Sebille, E. Van, Gilbert, A., Spathi, C., 2016. The ocean plastic pollution challenge: towards solutions in the UK. Grantham Institute Breifing Paper, 19(19), 3–4. www.imperial.ac.uk/grantham/publications

Smith, L. C., Ghosh, J., Buckley, K. M., Clow, L. A., Dheilly, N. M., Haug, T., Henson, J. H., Li, C.,

Lun, C. M., Majeske, A. J., Matranga, V., Nair, S. V., Rast, J. P., Raftos, D. A., Roth, M., Sacchi, S., Schrankel, C. S., & Stensvåg, K., 2010. Echinoderm immunity. Adv. Exp. Med. Biol. , 708, 260–301. https://doi.org/10.1007/978-1-4419-8059-5_14

Song, Y., Zhu, L.S., Wang, J., Wang, J.H., Liu, W., Xie, H., 2009. Soil Biology & Biochemistry DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia foetida*) induced by atrazine. Soil Biol. Biochem. 41, 905–909. https://doi.org/10.1016/j.soilbio.2008.09.009

Stabili, L., Pagliara, P., 2015. Chemosphere The sea urchin *Paracentrotus lividus* immunological response to chemical pollution exposure : The case of lindane. Chemosphere 134, 60–66. https://doi.org/10.1016/j.chemosphere.2015.04.006

Sulong, R.H.N., Mustapa, S.A.S., Rashid, A.K.M., 2019. Application of expanded polystyrene (EPS) in buildings and constructions : A review. J. Appl. Polym. Sci. 47529, 1–11. https://doi.org/10.1002/app.47529

Sung, H.H., Chang, H.J., Her, C.H., Chang, J.C., Song, Y.L., 1998. Phenoloxidase Activity of hemocytes Derived from *Penaeus monodon* and *Macrobrachium rosenbergii*. J. Invertebr. Pathol. https://doi.org/10.1006/jipa.1997.4703

Takahashi, K., Akaike, T., Sato, K., Mori, K., Maeda, H., 1993. Superoxide anion generation by pacific oyster (*Crassostrea gigas*) hemocytes: Identification by electron spin resonance spin trapping and chemiluminescence analysis. Comp. Biochem. Physiol. -- Part B Biochem. 105, 32–41. https://doi.org/10.1016/0305-0491(93)90166-3

Tang, J., Ni, X., Zhou, Z., Wang, L., Lin, S., 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral *Pocillopora damicornis*. Environ. Pollut. 243, 66-74. https://doi.org/10.1016/j.envpol.2018.08.045

Tang, Y., Rong, J., Guan, X., Zha, S., Shi, W., Han, Y., Du, X., Wu, F., Huang, W., Liu, G., 2020.

Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. Environ. Pollut. 113845. https://doi.org/10.1016/j.envpol.2019.113845

Thaysen, C., Stevack, K., Ruffolo, R., Poirier, D., Frond, H. De, Devera, J., Sheng, G., Rochman, C.M., 2018. Leachate From Expanded Polystyrene Cups Is Toxic to Aquatic Invertebrates (*Ceriodaphnia dubia*). Front. Mar. Sci. 5, 1–9. https://doi.org/10.3389/fmars.2018.00071

Tiwari, M., Rathod, T. D., Ajmal, P. Y., Bhangare, R. C., Sahu, S. K., 2019. Distribution and characterization of microplastics in beach sand from three different Indian coastal environments. Mar. Pollut. Bull., 140(January), 262–273. https://doi.org/10.1016/j.marpolbul.2019.01.055

Torreilles, J., Guerin, M.C., 1999. Production of peroxynitrite by zymosan stimulation of *Mytilus galloprovincialis* haemocytes *in vitro*. Fish Shellfish Immunol. 9, 509–518.

Xu, J.B., Yuan, X.F., Lang, P.Z., 1997. Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. Environ. Chem. 16, 73–76.