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Sub-acute toxic effects of polystyrene microplastics in a freshwater teleost juvenile fish *Heteropneustes fossilis*

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Abstract- Pollution of the water bodies caused by microplastics (MPs) is one of the serious environmental issues that gain worldwide attention. MPs ingested by fish accumulates in different tissues of fishes causing toxic effect of MPs on overall health of fish that ultimately impact on human health because fish is an important and cheap source of animal protein in the worldwide. The aims of this study were to examine the ingestion, behavioral changes, tissue accumulation and toxic effects of polystyrene (PS) MPs in juvenile *Heteropneustes fossilis* fish. Test fish were exposed to different concentration of PS MPs for 10 days and Mortality was monitored. More than 70% mortality was observed after 10 days exposure to 130 µg/L of MPs while 120 µg/L of MPs caused only 60% mortality. 100 µg/L of microplastics dosing resulted in 40% mortality of fish. Sub-acute exposure to PS MPs resulted in 10-day LC_{50} of 108.01µg/L. Abnormal swimming behavior, erratic movement, decrease in swimming activity, swimming velocity and vertical swimming, gradual increase in resting time, increased breathing rate, decrease in surface visiting frequency and spending most of time in resting state were observed as behavioral changes after exposure of polystyrene microplastics. These findings suggested that *Heteropneustes fossilis* intake of significant levels of microplastics seriously affected the overall health of fish.

Key words: Microplastics, Pollution, Mortality, Toxicity, Fish, *Heteropneustes fossilis*

INTRODUCTION

Over the past decades, a dramatic increase in the world´s plastic production has received an increasing concern about its toxicity in the environment. Microplastics (MPs) are small particles or debris sized between 0.1 to 5000 µm of plastic which are synthetic organic polymers extracted from petroleum and other products.[1,2] Nowadays, there is involvement of plastics in almost all aspects of modern life including construction, furniture, textiles and agricultures and so on. This increase in plastic production and application has caused enormous increase in release of plastic waste into the environment. Plastics are continuously fragmented and degraded in the environment by various physical or chemical processes that changes plastics into microplastics.[3] This leads to production of secondary microplastics. Microplastics are of two types: primary microplastics and secondary microplastics. Primary microplastics are purposefully manufactured for particular applications like facial cleanser, cosmetics and air blasting technology.[4-7] Secondary microplastics are derived from breakdown of larger plastic debris present at both sea and on land.[8-10] Microplastics are vectors for various chemical compounds (such as persistent organic pollutant, heavy metals and pesticides) in the environment by absorption of these chemicals on the plastic particle surface. Fishes easily ingest microplastics in incidental or intentional ways due

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to their small size and resemblance of microplastics to natural food items. Fish can readily ingest different types of MPs like Polystyrene (PS), Polyethylene (PE), Polypropylene (PP), Polyvinylchloride (PVC) and Polyamides (PA).[12-15]

The stinging catfish *Heteropneustes fossilis* popularly known as Singhi (Bloch 1974), a fresh water teleost fish species of Heteropneustidae family is a species of air sac catfish found in India, Bangladesh, Pakistan, Nepal, Sri Lanka, Myanmar and Bhutan. This is enriched with high amount of protein, iron (226mg/100g) and calcium and occupy higher price in the market.[16,17] This fish has very high nutritional value as it is rich in high amount of protein, iron and calcium. It has high economic importance and there is a great demand due to their medicinal value.[18] They are recommended for patients after recovering from malaria due to their invigorating qualities.[19] Although consumption of once a week is recommended, there are some contaminants such as MPs in aquatic medium have raised many concerns regarding the benefits of fish consumption. Due to dominance in the natural environment, omnivorous and bottom feeding behavior, *H. fossilis* is considered as a good model for evaluating ecotoxic effect of pollutants. Pollution of aquatic ecosystems by different types of microplastics has been reported in several previous studies. As a result, significant level of MPs has been found in various tissues of fish. A significant level of MPs was accumulated in gastro-intestinal tract of exposed fish to MPs.[20] Physical and chemical properties of MPs significantly affect their bioavailability and toxicity. Their toxic effect depends on the type of polymer present in them due to different characteristics of additive chemicals like phthalates, heavy metals and stabilizers. Polystyrene is a polymer made from monomer Styrene; a liquid hydrocarbon commercially manufactured from petroleum. PS was first manufactured by BASF (Badische Anilin und Soda Fabrik) in 1930 and is used in numerous plastic products. Because of its low cost, clarity and excellent processability general purpose polystyrene is being extensively used in labware for diagnosis, analysis and packaging of medical devices. In manufacture of medical parts, their components and applications such as bottles and containers, high impact polystyrene is used, which are more resistant plastics. Microplastics are ingested or consumed by invertebrates and fish intentionally or non-intentionally due to their small size and resemblance with planktons.[21,22] Ingestion of microplastics cause physical effects to fish (mechanical damage of digestive tract) as well as adverse physiological effects that affecting feeding, respiratory activity, behavior, inhibit growth and development, reproductive toxicity, oxidative stress[23], immune toxicity, genetic damage[24] and even death of fish.[25-27] Assessment of environmental and health hazards of plastic polymers depending on their chemical composition has put the PS in the top rank of hazardous polymers in the environment.[28]

Several previous studies reported toxic effect of other MPs on different living organisms.[14] They observed the toxic effect of five common types of MPs on zebra fish, showed no or low acute lethality but caused intestinal damage including cracking of villi and splitting of enterocytes. It was found that there is very less study analyzing toxic effect on *H. fossilis.*

The median lethal concentration is the usual method of reporting acute toxicity results. It is a convenient reference point for expressing the acute lethal toxicity of a given toxicant to the test animals. The present study aimed to assess responsiveness of *H. fossilis to* PS MPs through determination of sub-acute 10 days LC_{50} value and behavioral response induced from exposure to different concentration of microplastics.

MATERIALS & METHODS

Fish acclimatization

The juvenile fish (*Heteropneustes fossilis*) procured from fish market, Bazaar Samiti, Patna. Fish were acclimatized for 2 weeks in four 40 L glass aquaria under laboratory conditions, keeping 20 fishes each aquarium before experiment. The fish were maintained at 24±1ºC temperature with 12 hours light /dark photo period. Common tap water was used in acclimatization process. The pH of water, dissolved oxygen (DO) and hardness of water were observed as 7.2±0.4, 6.6 ±0.5 mg/L and 185± 10 mg/L respectively. The continuous aeration was maintained in each aquarium by using an aerator. Fish were fed daily with commercial feed.

PS MPs –characterization

MPs used were Polystyrene MPs, purchased from Shivay Enterprises, a dealer of plastic- powder and granules, Vadodra, Gujarat. The size and morphology of gold- coated PS MPs were evaluated by Scanning Electron Microscopy (SEM, S4800, Hitachi). For preventing charging of PS MPs with Electron beam, samples were first subjected to gold coating (~5nm) before being examined with SEM. Size of PS MPs was calculated by using image J software. Average size of PS MPs calculated was 161.66µm (Figure 1).

Preparation of stock solution

Stock solution of PS MPs was prepared by dissolving the PS MPs in distilled water and sonicated it for 15 minutes. Working solution was prepared by dilution of stock solution and it was sonicated before each treatment.

Exposure of fish to PS MPs

For the experiment, acclimatized juvenile *H. fossilis* were distributed in six 40 L glass aquarium filled with 15 L water (one aquarium for control fish and 5 aquaria for each replicate per treatment, 10 fish per aquarium). During the whole period of experiment, aquaria were gently aerated. The fish were exposed to different concentration of PS MPs (0, 80, 90,100,110,120 and 130 µg/L) for 10 days. Fish were fed with commercial feed daily along with PS MPs. For maintaining dispersion of MP particles in water, aquaria were continuously aerated.

Data analysis

In this study, Excel software was used to evaluate the mean ± standard Error (Mean ± SE). One-way analysis of variance (ANOVA) was used to compare the means between the treatment and control groups. The level of significance was set at $p < 0.05$. Log dose/probit regression line method was used for statistical analysis of observed data.[29]

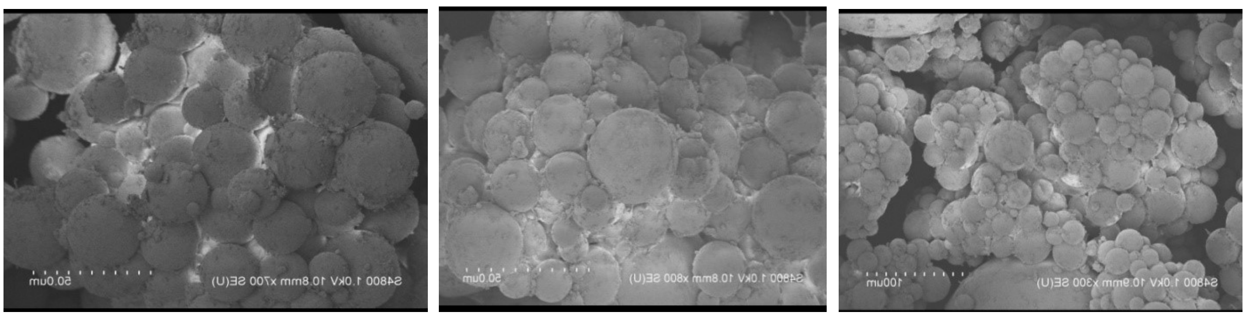

Figure 1: Scanning electron microscope images of investigated PS-MPs

RESULTS & DISCUSSION

For evaluation of sub- acute toxicity of PS MPs in *H. fossilis,* 10 days water toxicity was conducted. Effect of dose of PS MPs was analyzed. Mortality of fishes were monitored and recorded during 10 days of exposure. No mortality was observed among controlled fishes. Sub-acute exposure to PS MPs particles resulted in a time and dose-dependent way where increase in dose concentration increases fish mortality. Exposure to 130µg /L of PS MPs killed >70% of fish after 10 days exposure. Mortality of fish exposed to 100µg/L of PS MPs was 40% after 10 days exposure. 80µg /L exposure of PS MPs killed only 26.67% of fish. It indicated that toxicity occurred in a dose and time dependent pattern. Table 1 and 2 show a comparison of fish mortality at different dosing concentration for different time intervals in terms of mean (SE) using ANOVA test. By using Probit Analysis, sub-acute exposure of PS-MPs to *H. fossilis* resulted in a 10-day LC_{50} of 108.01 µg/L. Figure 2 signifies probit mortality of fish resulted from the PS MPs at different concentration after 10 days of acute exposure.

Table1: Fish mortality during the 10 days of exposure at different concentrations of PS MPs.

PS MPs (µg/L)	Days of exposure										
	1	2	3	4	5	6	7	8	9	10	Sum
0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	1	0	1	2
90	0	0	0	0	0	0	1	1	0	1	3
100	0	0	0	0	0	0	2	0	2	0	4
110	0	0	0	0	1	0	2	1	1	0	5
120	0	0	1	1	0	1	2	0	1	0	6
130	0	0	1	1	0	2	0	1	0	2	7

Table 2: Mean mortality of fish after 10 days exposure of PS-MPs at different concentration

Concentration of PS-MPs (µg/L)	Total number of test fish	Mean mortality%
0	10	0
80	10	26.67
90	10	27
100	10	40
110	10	50
120	10	60
130	10	73.33

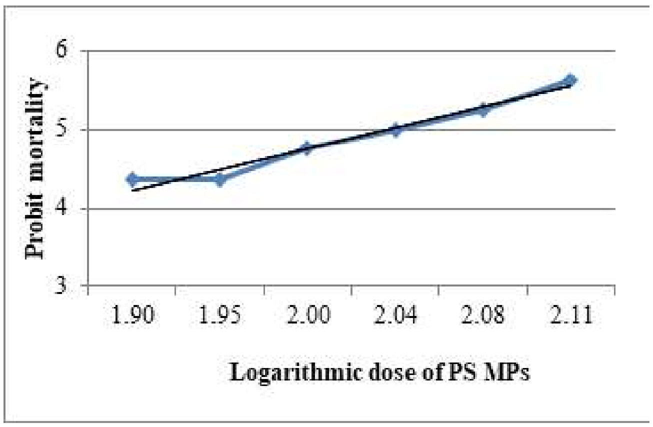

Figure 2: (%) mortality of fish resulted from the PS MPs at different concentration after 10 days of acute exposure.

Past ponders detailed about ingestion of different sorts of MPs by diverse species of fishes.[12-14] Fish have a tactile gustatory framework and have capability to isolate nourishment from unpalatable things affecting upon oral uptake, instep of that MPs is ingested altogether by nearly all species of fish.[30] The mechanism which causes fish not to segregate unpalatable plastics from food items, is not completely known. It is recommended that MPs and food co-occurring in fish mouth cavity have capacity to influence gustatory system of fish, diminishes perceptibility of unpalatable items, hence allows MPs to be gulped incidentally. Bigger MPs have less chance to be gulped. Plastics are determined for hundreds of long times in the environment and bigger plastic debris is degraded into smaller and smaller pieces by distinctive physical and chemical processes. Different types of MPs of size measuring from 1-500 µm counting PS have been watched in fresh water bodies at concentration run from few units to hundreds of particles per liter.[31-34]

Exposure of PS-MPs significantly affected behavioral and morphological characteristics of *H. fossilis*. Significant alterations were observed in behaviors and morphology of test fish exposed to 130µg/L of PS-MPs, hence only the results of behavioral and morphological alterations in these exposed fishes are reported in figure 3. These changes began around 4-5 days of exposure. Changes in behavior and morphology of *H. fossilis* were monitored during the PS-MPs experiment. Observation was conducted for 60 minutes every day and the observed changes were recorded. Behavioral changes including resting time, swim activity, erratic movement i.e. sharp changes in direction, vertical or sideways swimming were identified. Morphological changes including bent tail was the dominant in exposed fish which increase significantly by dose and time of exposure.

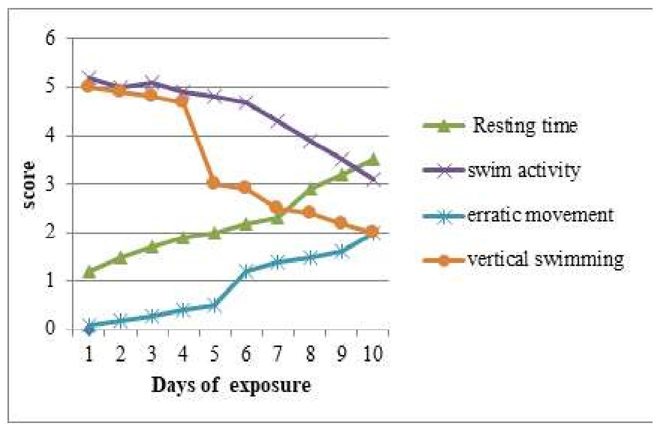

Figure 3: Behavioral alteration in test fish exposed to 130µg/L of PS-MPs for 10 days exposure

CONCLUSION

These results indicated that toxicity was occurred in a dose dependent pattern. On the basis of above results, it is concluded that fish exposed to PS MPs alter less or more energy requirements in the fishes which were unable to tolerate the stressed condition and this can lead negative effects on growth and survival of fish and behavioral alterations. This consequence has negative effects on fish biomass and yields of commercially important fish. Impacts on yields of fishes can possibly have both ecological and economic consequences. These findings help in the establishment of regulatory framework by policymaker to combat these emerging pollutants present in the water bodies.

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DECLARATION OF COMPETING INTEREST

The authors declare that there are no known conflicts of interest associated with this work. No part of this paper has been published or submitted elsewhere.

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