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Investigating the Acute Toxicity, Median Lethal Concentration of Polystyrene Microplastic to an Indian Air-Breathing Murrel, *Channa punctata* (Bloch, 1793)

Aarti Kumari, D. K. Paul

Department of Zoology, Patna University, Patna- 800 005, Bihar, India
*Corresponding author Email: aarti30597[at]gmail.com

Abstract: ***Contamination of microplastic in water is a worldwide issue which is increasing day by day due the different types of anthropogenic activities. These tiny particles of plastic pose a serious health risk to aquatic organisms. These tiny particles can originate from a variety of primary and secondary sources, such as the fragmentation of larger plastic items and the shedding of microfibers from textiles. One such kind of tiny particle is polystyrene microplastic (PS-MS). The present study aims to determine 96 hrs LC_{50} value of polystyrene microplastic for the snakehead fish, Channa punctata. A kind of computational and graphical technique can be applied to obtain an LC_{50} (median lethal concentration) from the response of concentration - mortality data produced by an acute mortality experiment. The data was statistically analysed using SPSS software based on Finney's probit analysis method. The result revealed the LC_{50} value to be 4.8 mg/l respectively. With the increase of the concentration of PS-MS, the response of the fish mortality increased gradually. The results indicate that PS-MS revealed acute toxicity to fish when exposed for 96hrs and this could liberate the fact that exposure of PS-MS may result in deleterious toxic effects to fish that influence the overall health of the aquatic environment.***

Keywords: Acute toxicity, LC_{50}, PS-MS, *Channa punctata*

1.Introduction

Production of massive and imperfect waste management, lead to discard of all the plastic into the aquatic environment [1]. Plastic waste in the aquatic ecosystem degrades into numerous tiny plastic fragments /fibres/ spheroids / granules /pellet / flakes/beads ranging between 1 nanometer (nm) to 5 millimeter (mm) in size; these particles are defined as microplastics (MPs) (EFSA, 2016). As emerging environmental contaminants, MPs have received increasing attention from both the scientific and public communities [1-2]. Over the past decade, the presence of MPs in aquatic environments has been reported worldwide and more recently [3] the importance of MPs pollution in freshwater environments has been emphasized from the available literature, MPs appear to be widespread in the waters [4].

Microplastics were confined as tiny plastic particles under 5 mm in diameter as per standards set by US National Oceanic and Atmospheric Administration (NOAA), which has the highest potential hazard because of the tiny surface and higher accumulation in the environment [5]. Study on microplastic document that the deleterious effect which caused by microplastics mainly focus on mortality, fecundity, and including intestinal health, immune regulation, behavioural alterations, the dosage-effect relationship with physiological stress, metabolism abnormity, and changes in energy budgets [6-8].

This fish has very high medicinal and nutritional value as it is rich in high amount of iron, protein and calcium. They are suggested for patients after recovering from malaria due to their invigorating qualities [5]. Although consumption of once a week is recommended, there are some contaminants such as MPs in aquatic medium that have raised many concerns regarding the benefits of fish consumption. Due to dominance in the natural environment, carnivorous and bottom feeding behaviour, *Channa punctata* is considered as a good model for evaluating the ecotoxic effect of pollutants. Pollution of aquatic ecosystems by different types of microplastics has been reported in several previous studies. As an outcome, significantly number of MPs has been found in different tissues of fish. A significant level of MPs was accumulated in the gastro-intestinal tract of exposed fish to MPs [9-10]. 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. Due to relatively inexpensive and easy processability, general purpose polystyrene is being extensively used in labware for diagnosis, analysis and packaging of medical devices. In the 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 [8]. Ingestion of microplastics cause physical effects to fish (mechanical damage of digestive tract) as well as adverse physiological effects that affecting feeding, respiratory activity, behaviour, inhibit growth and development, reproductive toxicity, oxidative stress [10], immune toxicity, genetic damage [10] and even death of fish. 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 [11].

Several previous studies reported the toxic effect of other MPs on different living organisms. They observed the toxic effect of five common types of MPs on zebrafish, showed no or low acute lethality but caused intestinal damage including cracking of villi and splitting of enterocytes [12]. It was found that there is very little study present in the area of toxic effects of *Channa punctata.*
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 *Channa punctata to* PS MPs through determination of sub-acute 96h LC_{50} value and behavioural response induced from exposure to different concentrations of microplastics.

2.Material and Method

2.1 Test Chemical:

For the present research work, polystyrene microplastic beads of 100nm size is purchased from Sigma-Aldrich (Product Number:43302; Batch Number/Source: BCCH1665) were used. The polystyrene micro particles came in the form of an aqueous solution with 10% concentration (solid). The particle size was 100nm with a standard deviation in size ≥ 0.01 μm and a density of 1.05g/cm^3.

2.2 Preparation of stock solution:

Parent stock purchased in the form of an aqueous solution with the 10%w/v (solid concentration), 1000 mg/l stock was prepared and rigorously homogenized using ultrasonication. All other working concentrations for the experiment were prepared from the 1000 mg/l stock by serial dilution using Milli-Q water. The stock solution was stored in a refrigerator and the temperature was maintained within the range 2-8℃ as mentioned by the manufacturer.

3.Data Analysis:

All experiments were repeated three times and performed in triplicate. Data were analysed with SPSS statistical analysis software (Version 22.0) using Probit Analysis Statistical Method (Finney, 1971) [18]. The LC_{50} values (with 95% confidence limits) were calculated. Differences among the results were considered to be statistically significant when P value was < 0.05. Also, the MS Excel 2021 was used to find a regression equation (Y=mortality; X=concentrations), the LC_{50} was derived from the best-fit line obtained.

4.Toxicity Bioassay:

The 96-hour toxicity bioassay of polystyrene microplastic with *Channa punctata* was carried out in the laboratory in a semi static condition following the standard guidelines of APHA and OECD [16-17]. Over 100 juvenile specimens of the *Channa punctata* (Order: Periformes, Family: Channidae) weighing 17-20g, length 12-14cm were collected from clean and unpolluted local freshwater, washed with 0.1%KMnO4 solution to remove dermal infection, and were acclimatized to laboratory condition for 20 days prior to experimentation. They were maintained in a glass aquarium containing seasonal tap water under natural photoperiod (13L:11D) and ambient temperature (18-21℃) including physico-chemical parameters (Table:1). The total amount of feed provided was not less than 2-3% of their body weight per day. Fish were fed ad libitum with commercial dry pellets (Taiyo Plus Discover). If in any batch, mortality exceeded 5% during acclimatization, that entire batch of fish was discarded. Moreover, selected water quality parameters have been monitored at regular intervals of time. After the 20 days of acclimatisation healthy active fishes were selected for the exploratory range finding test for the selected toxicants. Then after a set of ten healthy juvenile individuals of *Channa punctata* were transferred to a 40-litre glass aquarium. Fishers were exposed to a concentration of 2, 3, 4, 5, 6 mg/l of polystyrene microplastic (Table:2). Meanwhile, a control tank was maintained at similar laboratory conditions. The behavioural changes, and mortality was carefully recorded at every 24 h, 48 h, 72 h and 96 h of the exposure durations. The dead fishes were removed immediately. The data obtained were then subjected for the estimation of 96 h LC_{50} value for the polystyrene microplastic.

Table 1: The statistical summary of analysed physico-chemical parameters during the toxicity assay.

Parameter	Average	Standard deviation	Unit
Dissolve Oxygen	6.05	1.03	mg/l
pH	7.98	0.12	
Turbidity	7.08	2.85	NTU
Electrical Conductivity	0.69	0.08	Micro Siemen/cm
Total hardness	210.73	2.46	mg/l
Total alkanity	238.6	2.63	mg/l

Table 2: Percentage cumulative mortality at different exposure level of PS-MS during the bioassay.

Concentration of PS-MSs		Probit of Kill	% Cumulative mortality after time.			
in mg/l	Log concentration		24 h	48 h	72 h	96 h
Control (0.0)	0	0	0	0	0	0
2	0.301	3.72	0	0	0	10
3	0.477	4.16	0	0	0	20
4	0.602	4.48	0	0	10	30
5	0.699	5	0	10	30	50
6	0.778	5.52	0	20	30	70

3.Result and Discussion

In our present investigation, mortality caused by PS-MS showed a clear significant positive correlation between dose and mortality. The positive correlation between dose and mortality in all cases was noted because increased concentration of PS-MSs in aquarium water resulted in more intake or entry of active moieties in the bodies of fishes. The percent mortality observed for each concentration was calculated and converted to probit by means of SPSS software. Acute toxicity test i.e.LC_{50} values show 50% susceptibility of fish to particular pollutants and reflect their survival potential. The present finding reveals that LC_{50} values of PS-MS for snake headed freshwater fish, *Channa punctata* for 96 hrs exposure is 4.8mg/l, respectively. However, fishes exposed to de-chlorinated tap-water in the control condition were observed to be healthy and normal. In this study the result concluded that the mortality rate increased with increasing concentration of PS-MSs. This result is in conformity with the positive correlation between concentration and mortality of *C. punctata* when exposed to the PS-MSs.

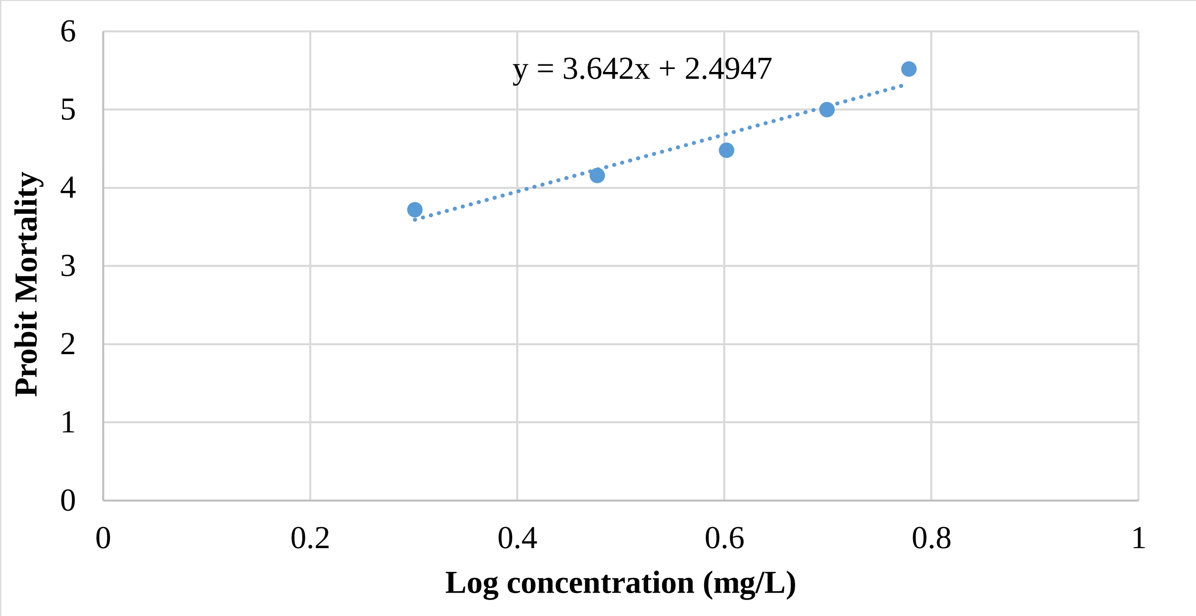

Figure 1: Graph determining LC_{50} value of PS-MS.

To the best of our knowledge, there's no similar study in the literature probing the poisonous effect of PS MPs in vitro or in vivo and this is the first study in this field (despite the wide use of polystyrene lathers and their significant release to the environment). The results of this work easily showed that *Channa punctata* readily intake PS MPs. Ingestion of other types of MPs similar as PS, PE, PP, PVC, and polyamides by different species of fish was reported in former studies, and ingestion of PS- MSs by *Channa punctata* verified in this study. These attestations indicate that fish ingest PS- MPs. Fish have a sensitive gustatory system and can insulate food from indigestible particulars efficiently upon oral uptake. Still, despite such advanced sense of taste, MPs are ingested significantly by nearly all fish species [19]. It is not clear what mechanisms beget to grope couldn't distinguish indigestible plastics from food patches. It's suggested that co-circumstance of MPs and food in the oral depression of the fish may affect the gustatory system of fish and decreases the detectability of indigestible particulars, and allows the MPs to be swallowed accidentally. Tiny particles of plastic has high injurious goods on living organisms and larger plastic patches have lower injurious goods on living organisms, larger plastic debris is degraded into lower and lower pieces by different mechanisms but unfortunately plastics are patient for hundreds of time, in the ecosystem. Environmental monitoring and further studies on chronic or acute toxicity of PS MP on freshwater fish are necessary. GI tract is the most important fish tissue which could be affected by the MPs directly. It has been reported that ingestion of MPs has obtrude with the normal functioning of the digestive systems of fish [20].

Channa punctata is a suitable model for the study of actions changes due to chemical toxins. In the present study, the chance of fish with abnormal behavioural changes increased with the attention of PS MPs. Behavioural changes (abnormal swimming, gradational increase in resting time and erratic movements, and drop in syncope exertion and perpendicular swimming) in *Channa punctata* due to PS- MP exposure and toxin have been verified for PS MP by our results. According to our review, many studies are in the literature regarding the circumstance and toxin of polystyrene microparticles and particular confines around 1 µm [21]. MPs (from 1 to 500 µm) including PS have been detected in freshwater and got attention from many units to hundreds of p/L and polystyrene microparticles at attention between 5.84 and 13 ng/l [22]. From a toxicological point of view, the median immobilization of Daphnia dulex was set up by [22] after 48 h exposure, at attention of polystyrene nanoparticles (75 nm) equal to 76.69 mg/ L (LC_{50}), a value within the confidence limits of that attained in this study. An analogous study on the acute toxicity of PS nanoplastics was carried out for 48 h by Lin and collaborators in 2019 in the cladoceran crustacean D. magna with an LC_{50} in the order of mg/ L units, under lining an increase in toxin in relation to a time of longer exposure. Since organisms in the natural environment are generally exposed to lower attention for a longer time. The submarine organism most affected by the PS-MP was the crustacean C. dubia with EC_{50} after 7 d- exposure in the order of units of µg/ L (106 p/ L) and a LOEC value in the order of tenths of µg/l (105 p/ L) [23-24] which is at effective attention close to those of environmental concern.

4.Conclusion

Present study concluded that microplastics mixed in the aquatic environment have caused significant toxicity on fish after trophic impurity. These results indicated that PS-MS was passed in a cure dependent pattern. On the basis of above results, it's concluded that fish exposed to PS MPs alter less or more energy conditions in the fishes which were unfit to tolerate the stressed-out condition and this can lead to negative effects on growth and survival of fish and behavioural differences. This consequence also has negative effects on fish biomass and yields of commercially important fish. Impacts on yields of fishes can conceivably have both ecological and profitable consequences. This exploration has handed solid information on the toxicological counter accusations of the 0.1 μm polystyrene, potentially considerable border line size between micro- and nanoplastic patches, demonstrating that this adulterant poses a serious dangerous threat to aquatic organisms in the trophic chain. Since plastic patches could fluently bypass conventional treatment plants, contaminate aquatic environments, and enter the food chain, they could potentially act also as carriers of poisons, pathogens, dangerous substances, and adulterants. Since there are presently no effective results for the junking of microplastic patches from the terrain, it would be necessary to borrow useful strategies to stimulate sustainable conduct that help further plastic pollution.

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