Uttar Pradesh Journal of Zoology

Volume 45, Issue 10, Page 135-143, 2024; Article no.UPJOZ.3459
ISSN: 0256-971X (P)

Chronic Toxicity of Microplastics on Fish (*Labeo rohita*) and Their Impact on the Freshwater Ecosystem: A Case Study of Gangasagar Pond, Darbhanga, India

Rakhi Kumari [a*]

[a] *P.G. Department of Zoology, LNM University, Darbhanga, India.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i104057

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3459

Original Research Article

Received: 19/02/2024
Accepted: 23/04/2024
Published: 29/04/2024

ABSTRACT

The present investigation was conducted to evaluate the chronic toxicity of microplastics on fish, *Labeo rohita* and isolation, identifications and estimation of microplastics in freshwater pond, Gangasagar Pond, Darbhasnga. traceable clinical, anatomical (morphological) and histopathological alterations. The data were analyzed and found that microplastic fibre (ranging between 12 to 56/m^3) were dominated followed by microplastic film (ranging between 10 to 38), fragment (ranging between 8-22). The number of foam, beads (spherical) and coloured were relatively low. The sources of these plastic contaminations are mainly domestic wastes, discarded packaging materials and medical wastes. Trace of microplastics in gill chamber as well as buccal cavity was well marked causing edematous changes. The Histopathological studies suggest that intestine and stomach were the most affected organs in response to microplastics abuse. Microplastics will ultimately affect the aquatic fauna of the pond adversely.

**Corresponding author: Email: rcharan00@gmail.com;*

Keywords: Labeo rohita; microplastics; toxicity; water pollution; histopathology.

1. INTRODUCTION

Microplastics are emerging contaminants in the aquatic ecosystem, which cause many harmful effects on aquatic organisms, especially on fish. MPs are microscopic plastic particles attend less than 5mm long in many shapes and sizes [1,2]. MPs have been traced to 728 fish species from different water sources of worldwide [3]. MPs induced fish showed several changes in behavior, including altered feeding, swimming, weakened predatory performance, foraging, and ventilation [4]. MPs exposure in fish can alter the physiology, resulted oxidative stress and inflammation. MPs first reached into gastrointestinal tract and after that it may affect a specific area entering the blood and affect several organs and tissues. Moreover, microplastics contaminated food ingestion causes bioaccumulate in the human body and exerts toxicological effects [5].

Darbhanga is a district of Bihar, due to its richness in ponds it is called a city of ponds. The district of Darbhanga has total area 2279 sq km. It is situated between 25.23' to 26.27' (North) latitude and 85.45' to 86.25' (East) longitude. The district as well as Darbhanga town is usually has no industrial complexes. The facts highlights that the sources of plastic contamination are mainly domestic wastes discarded packaging materials . The urbanization of this district increases with population and diminishing of water bodies. There is no solid waste treatment plant in district and neither sewage water treatment plant. There are four big tanks are situated at mid of Darbhanga city, among them Gangasagar pond is considered as more polluted with plastics wastes (Fig. 1 and Fig. 2).

Fig. 1. Gangasagar pond

Fig. 2. Plastic debris

Hence, present investigation includes isolation and identification of microplastics from fish culture pond of Darbhanga. Histopathological impacts of microplastics on different organs of fish, *Labeo rohita.*

2. MATERIALS AND METHODS

The present study site is Gangasagar pond, Darbhanga (Fig. 1). The 24 sites were selected to collect the samples of microplastics for once a month during a period (January, 2021 to December, 2021). Microplastics were collected by filtering the water through SOBO aquatic filter or alternatively, different sizes of plankton net with variable mesh size ranging 50-300 µm were applied. Simultaneously flow meter-10-100LPH, Ocean star Make was fixed at the net opening to get concentration of microplastics per unit water volume (item/gram). The collected samples were brought to lab for extraction of microplastics following Loder and Gerdts [6] by classic filtration set up, and NaCl solution used, microplastics are recovered by removing the supernatant. Another method also used included zinc chloride solution and sodium iodide solution [7]. Size fractionations, visual quantification and identification of microplastics were done. The microplastics have been categorized on the basis of their shape like fiber, film, foam, fragments and beads.

The fish specimen were collected from the pond and brought to lab and clinical morphological and anatomical study was carried out. The organs like gut content, gill chamber and buccopharynx selected for anatomical analysis. The organs like muscle, gill, stomach, intestine and pyloric caeca were dissected for histopathological examination, the tissues were fixed in alcoholic Bouins for 24 hrs and 5µ sections were stained in H&E.

3. RESULTS

The Gangasagar pond situated at mid of Darbhanga city (Fig. 1). The domestic and medical wastes release into the pond (Fig. 2). The visual quantification of microplastics in surface samples of pond waters has been presented (Table 1, Fig. 3). Altogether 20 samples were studied and Table 1 depicts of visually identified samples in pond. The samples were dominated by microplastic fibre (ranging between 12 to 56/m^3) followed by microplastic film (ranging between 10 to 38), fragment (ranging between 8-22). The number of foam, beads (spherical) and coloured were relatively low and could not be detected in many of the samples (Table 1).

Table 1. Visual quantification of microplastics identification in surface samples of Gangasagar Pond (January, 2021- December, 2021)

	Fibre	Film	Foam	Fragments	Beads	Coloured
In Per M^3						
1	40-43	33-37	0-2	12-14	1-2	0-2
2	47-50	5-10	1-4	6-8	1-3	3-4
3	45-47	22-27	2-5	8-10	0	1-2
4	35-43	32-36	0-1	16-18	0-2	1-3
5	42-48	18-20	1-3	16-20	0-1	0
6	50-56	22-28	0	10-13	0	1-3
7	32-36	15-20	2-4	12-15	0-1	0
8	20-25	18-21	1-2	20-24	1-3	1-2
9	40-44	11-15	1-4	12-14	4-5	2-5
10	21-27	33-38	0	16-19	0	0-2
11	30-33	21-25	2-4	20-22	1-3	0
12	40-42	32-36	0	12-14	1-3	0-2
13	21-27	21-25	2-5	16-18	0	2-4
14	40-44	32-36	1-2	14-18	2-3	3-5
15	21-27	11-15	2-6	12-15	1-2	0-3
16	22-29	6-10	0	12-17	2-4	0-1
17	33-38	8-12	2-3	7-10	0-1	0-1
18	30-32	11-15	3-6	11-14	0-2	0-2
19	10-12	9-12	0	10-13	0	0
20	13-19	6-10	1-2	16-19	0-1	0-1
Average	**31.85-35.7**	**16.5-20.6**	**0.95-2.65**	**12.85-16.15**	**0.7-1.85**	**0.65-2.2**

The fish, *Labeo rohita* exposed with microplastics were collected from the Gangasagar Pond. The test fish were subjected to clinical inspection of buccopharyngeal region (Fig. 4) and gill chamber. Traces of microplastics were found either in gill chamber or mouth (Fig. 5). At the sites of microplastic's particles deposition in gill showed edematous patches (Fig. 6).

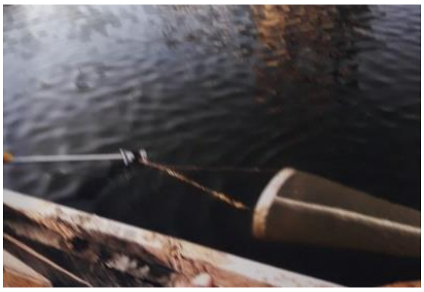


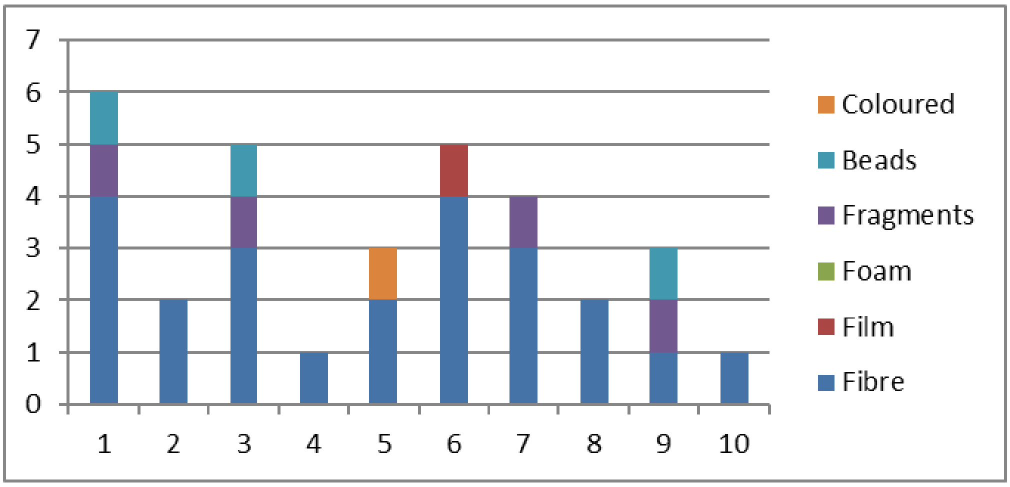
Fig. 3. Plankton net used for collecting microplastics

The visual quantification of microplastics in gill chamber and gut: The finding in the wild fish has been presented in Table 2 and Table 3. The tables reveal trace of fibre in almost all cases. The numbers of fibres varied between 1-6, in several other type of microplastics were rarely encountered. Similar was the findings in case of gill chamber.

Histopathological changes in gill and parts of gastrointestinal tract: The microplastics exposed fish groups were showed histopathological alterations. The gill showed clumping of gill lamellae and edematous tips (Fig. 7). The intestine showed pathological alterations in form of atrophy and clumping of villi (Fig. 8). The stomach showed clear spaces in serosa indicating atrophy (Fig. 9). No traceable histopathological alteration could be recorded in case of pyloric caeca, liver, kidney and ovary.

Table 2. Visual quantification of microplastics in gut of *Labeo rohita*

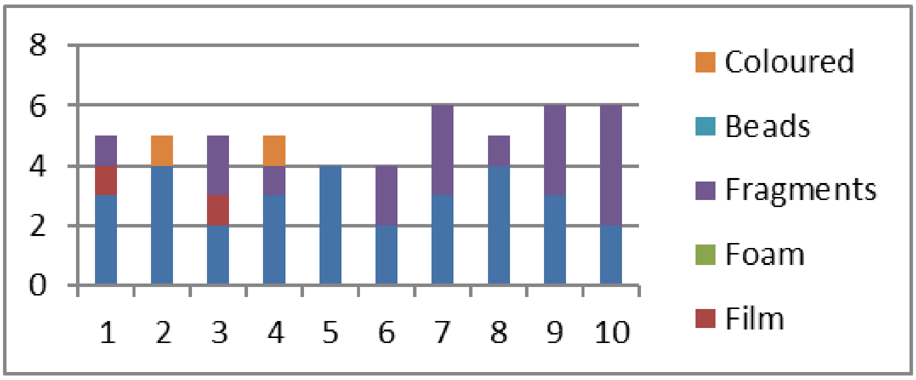
	Fibre	Film	Foam	Fragments	Beads	Coloured
In per M^3						
1	4	0	0	1	1	0
2	2	0	0	0	0	0
3	3	0	0	1	1	0
4	1	0	0	0	0	0
5	2	0	0	0	0	1
6	4	1	0	0	0	0
7	3	0	0	1	0	0
8	2	0	0	0	0	0
9	1	0	0	1	1	0
10	1	0	0	0	0	0



Graph 1. Bar graph showing Visual quantification of microplastics in gut of *Labeo rohita*

Table 3. Visual quantification of microplastics in gill of *Labeo rohita*

	Fibre	Film	Foam	Fragments	Beads	Coloured
In per M^3						
1	3	1	0	1	0	0
2	4	0	0	0	0	1
3	2	1	0	2	0	0
4	3	0	0	1	0	1
5	4	0	0	0	0	0
6	2	0	0	2	0	0
7	3	0	0	3	0	0
8	4	0	0	1	0	0
9	3	0	0	3	0	0
10	2	0	0	4	0	0



Graph 2. Bar graph showing Visual quantification of microplastics in gill of *Labeo rohita*

Fig. 4. Showing clinical inspection of buccopharyngeal region of *Labeo rohita*

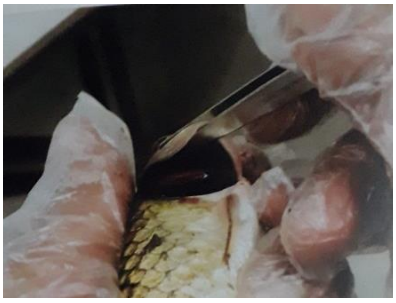


Fig. 5. Showing clinical inspection of gill chamber

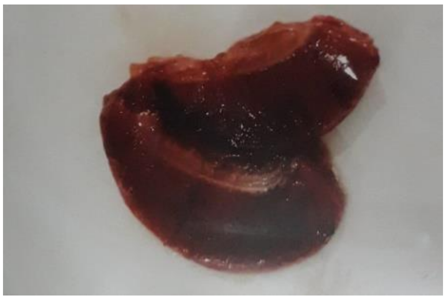


Fig. 6. Photograph of gill of microplastics exposed fish, *Labeo rohita*, H&E X 150

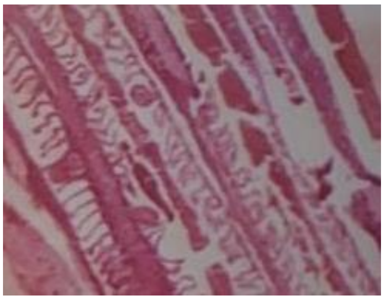


Fig. 7. Photomicrograph of T.S. of gill of exposed fish, *Labeo rohita*, H&E X 150

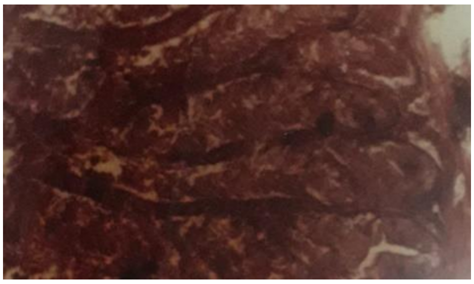


Fig. 8. Photomicrograph of T.S. of intestine of treated fish, *Labeo rohita*, H&E X 150

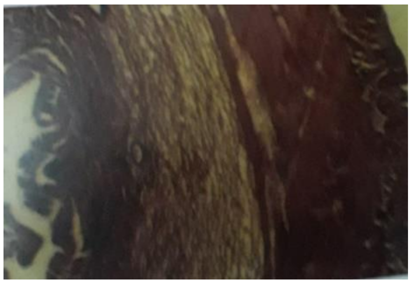


Fig. 9. Photomicrograph of T.S. of stomach of exposed fish, *Labeo rohita*, H&E X 150

4. DISCUSSION

The sources of plastic contamination in ponds of Darbhanga city are mainly domestic wastes and discarded packaging materials and medical wastes. Due to durability quality of plastics it persists in environment and causes harmful effects on aquatic flora and fauna [8]. These plastic wastes degrade slowly over time under the sun light (ultraviolet radiation) and high temperature [9]. Such degradation process resulted microplastics and nanoplastics [10]. Plastics in freshwater bodies are also prone to physical and environmental degradation [11]. Microplastics in the surface of freshwater water bodies reported by various authors of the worldwide [12,13,14,15,16,17] has conformity with present work.

The present method of visual shorting of microplastics present (Table 2) in water has been endorsed by Hidalgo- Ruz et al*.,* [18]. Perusal of the Table 2 suggest that the Harahi pond presently studied was reasonably infected with microplastics in different form/ shapes identified as fibre, film, fragment, foam and beads and coloured. Particle size is closely related to the migration property of microplastics in the environment (Charon *et al.,* 2019). The size of microplastic fractionated during present work has also a valid rational. The size of microplastics has been categorized following the method of GESAMP [2].

During present investigation wild samples as Indian major carp, *Labeo rohita* was selected. Trace of microplastics is quite evident in case of rohu in buccal cavity (Fig. 1 and gill chamber (Fig. 2). The isolated gut analysis showed presence of fibre and a few film/beads (Table 2). Similar, in gill chamber average count of fibre varied between 2-4. The present findings have confirmed with earlier workers reports [19,20,21,22,23]. Angsupanich, [24] suggested that the gastrointestinal tract is involved in the accumulation of microplastics. Microplastics ingestion in fish resulted producing obstructions across the digestive tract and limiting feeding owing to appetite [25,26]. Microplastics intake in fish could induce anatomical and physiological changes in the digestive tracts, causing dietary and growth retardation issues in fish [27,28]. The European sea bass show intestinal tract alterations and impaired intestinal function following 90-days exposure through feed pellets [29]. In freshwater fish, microplastics have been observed in gut and gill chamber of matket-purchsed Nile Perch (*Lates niloticus*) and Nile Tilapia (*Oreochromis niloticus*) [30]. Dawson et al., [31] suggested that fish exposed to microplastics can accumulate in organs and tissues. The studies have also been the conformity of present histopathological findings.

Prata [32] suggested that prolonged inflammation and irritation caused by microplastics exposure may induce cancer by causing DNA damage. Another study, Chang [33] suggested that nano plastics exposure caused oxidative stress and persistent irritation revealed evidence of pro-inflammatory agents, which stimulated vasculature, that led to the creation and development of cancers.

5. CONCLUSION

The Gangasagar pond contains a high level of microplastic contamination in form of fibre and film. The sources of these aquatic pollutants can be traced back to domestic and medical wastes. The concluding remark of present study is the fish, Indian major carp, rohu (*Labeo rohita*), was naturally exposed with microplastics (less than 5mm) reflect traceable clinical, anatomical (morphological) and histopathological alterations. The Histopathological studies suggest that intestine and stomach were the most affected organs in response to microplastics abuse. No detectable alterations could be marked in liver, kidney, pyloric caeca and ovary. Microplastic can be ingested by aquatic organisms, capable of causing adverse effects on aquatic organisms. Therefore, care should be taken to avoid or reduce their discharge into the aquatic environment.

ACKNOWLEDGEMENT

The authors are thankful to the PG Department of Zoology, LNM University, Darbhanga, Bihar for the provision of laboratory facilities used in this study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Cole M, Lindeque P, Halsband C, Gallow TS ay. Microplastics as contaminants in the marine environment: A Review. Mar. Pollut. Bull. 2011;62:2588–2597.
2. Group of Experts on the Scientific Aspects of Marine Environmental Protection. GESAMP: Sources, Fate and Effects of Microplastics in the Marine Environment: A Global Assessment; GESAMP: London, UK; 2015.
3. Hossain MA, Olden JD. Globalmeta-analysis reveals diverse effects of microplastics on freshwater and marine fishes. Fish Fish. 2022;23(6):1439–1454.
DOI: 10. 1111/faf.12701
4. Liang W, Li B, Jong MC, Ma C, Zuo C, Chen Q, et al. Process-oriented impacts of microplastic fibers on behavior and histology of fish. J. Hazard. Mater. 2023; 448:130856.
DOI: 10.1016/j.jhazmat.2023.130856
5. Ferrante MC, Monnolo A, Del Piano F, Mattace Raso G, Meli R. The pressing issue of micro-and nanoplastic contamination: Profiling the reproductive alterations mediated by oxidative stress. Antioxidants. 2022;11(2):193.
DOI: 10.3390/ antiox11020193
6. Loder M, Gerdts G. Methodology used for the detection and identification of microplastics-a critical apptaisal. Mar. Anthro. Litter. 2015;447.
7. Nuelle MT, Dekiff JH, Remy D, Fries E. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 2014;184:161-169.
8. FAO. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy; 2017.
9. Natural Resources Institute, NRI. Reducing ocean plastics-formative research leading to intervention planning in India. University of Greenwich, UK. 2019; 1-45.
10. Hoornweg D, Bhada-Tata P. What a waste. A global review of solid waste management. World Bank Urban Development Series. 2012;15:98.
11. Andrady AL. Microplastics in the marine environment. Mar. Pollut. Bull. 2011; 62:1596-1605.
12. Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H, Amato S. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin. 2013;77(1-2):177-182.
Available:http://dx.doi.org/10.1016/j.marpolbul.2013.10.007
PMID:24449922
13. Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. The Science of the Total Environment. 2017;586:127-141.
Available:http://dx.doi.org/10.1016/j.scitotenv.2017.01.190
PMID:28169032
14. Zhang K, Shi H, Peng J, Wang Y, Xiong X, Wu C, Lam PK. Microplastic pollution in China’s inland water systems: A review of findings, methods, characteristics, effects, and management. The Science of the Total Environment. 2018;630:1641-1653.
Available:http://dx.doi.org/10.1016/j.scitotenv.2018.02.300
PMID:29554780
15. Bordós G, Urbányi B, Micsinai A, Kriszt B, Palotai Z, Szabó I, Hantosi Z, Szoboszlay S. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. Chemosphere. 2019;216:110-116.
Available:http://dx.doi.org/10.1016/j.chemosphere.2018.10.110
PMID:30359912
16. Bilal M, Hassan HU, Siddique MAM, Khan W, Gabol K, Ullah I, Sultana S, Abdali U,

Mahboob S, Khan MS, Atique U, Khubaib M, Arai T. Microplastics in the surface water and gastrointestinal tract of Salmo trutta from the Mahod and Lake, Kalam Swat in Pakistan. Toxics. 2023;11(1):3.
Available:http://dx.doi.org/10.3390/toxics11010003
PMID:36668729

17. Khan W, Hassan HU, Gabol K, Khan S, Gul Y, Ahmed AE, Swelum AA, Khooharo AR, Ahmad J, Shafeeq P, Ullah RQ. Biodiversity, distributions and isolation of microplastics pollution in finfish species in the Panjkora River at lower and upper DIR districts of khyber pakhtunkhwa province of Pakistan. Brazilian Journal of Biology = Revista Brasileira de Biologia. 2024;84: e256817.
Available:http://dx.doi.org/10.1590/151969 84.256817
PMID:35293545

18. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Miroplastic in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 2012;46: 3060e3075.

19. Dos Santos J, Jobling M. Gastric emptying in code, *Gadus morhua* L. Emptying and retention of indigestible solids. J. Fish Bio. 1991;38(2):187-197.

20. Cadervall T, Hansson LA, Lard M, Frohm B, Linse S. Food chain transport of nanoparticles affects behavior and fat metabolism in fish. Plos One. 2012; 7(2);32254.

21. Olivera M, Ribeiro A, Hylland K, Guilhermino L. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecological Indicators. 2013;34: 641-647.
Available:http://dx.doi.org/10.1016/j.ecolind.2013.06.019

22. De Sa LC, Luis LG, Guilhermino L. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with pray, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ Pollut. 2015;196:359-362.

23. FAO. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy; 2019.

24. Angsupanich S, Somsak S, Phrommoon J. Stomach contents of the catfishes *Osteogeneiosus militaris* (Linnaeus, 1758) and *Arius maculatus* (Thunberg, 1792) in the Songkhla lake. Warasan Songkhla Nakharin (Sakha Witthayasat lae Technology); 2005.
Available:https://agris.fao.org/agris-search/search.do?recordID=TH2008001875

25. Wright SL, Thompson RC, Galloway TS. The PhysicalImpacts of MPs on marine Organisms: A Review. Environ. Pollut. 2013;178:483–492.

26. Jabeen K, Li B, Chen Q, Su L, Wu C, Hollert H. Effects of virgin MPs on Goldfish (*Carassius auratus*). Chemosphere. 2018;213:323–332.

27. Huang JN, Wen B, Xu L, Ma HC, Li XX, Gao JZ. Micro/ nano-plastics Cause Neurobehavioral Toxicity in Discus Fish *(Symphysodon aequifasciatus*): Insightfrom Brain Gut Microbiotaaxis. J. Hazard. Mater. 2022;421:126830.
DOI: 10.1016/j.jhazmat.2021.126830

28. Borrelle SB, Rochman CM, Liboiron M, Bond AL, Lusher A, Bradshaw H. Opinion: Why we need an international agreement on marine plastic pollution. Proc. Natl. Acad. Sci. USA. 2017;114(38):9994–9997.
DOI: 10.1073/ pnas.1714450114

29. Peda C, Caccamo L, Fossi MC, Gai F, Andaloro F, Genovese L, Perdichizzi A, Romeo T, Maricchiolo G. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environ. Pollut. 2016;212:251-256.

30. Biginagwa F, Mayoma B, Shashoua Y, Syberg K, Khan FR. First evidence of Microplastics in the african great lakes: Recovery from lake Victoria Nile perch and Nile tilapia. J. Gt. Lakes Res. 2016;42:1146-1149.

31. Dawson AL, Kawaguchi S, King CK, Townsend KA, King R, Huston WM. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nat. Commun. 2018;9(1): 1001.
DOI: 10.1038/s41467018-03465-9

32. Prata JC. Airborne MPS: Consequences to human health? Environ. Pollut. 2018;234: 115–126.
DOI: 10.1016/j.envpol.2017.11.043

33. Chang C. The immune effects of naturally occurring and synthetic nanoparticles. J. Autoimmun. 2010;34(3):J234–J246.
DOI: 10.1016/j.jaut.2009.11.009

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3459