Uttar Pradesh Journal of Zoology

Volume 45, Issue 24, Page 328-335, 2024; Article no.UPJOZ.4490
ISSN: 0256-971X (P)

A Study of the Toxic Effect of Polystyrene Microplastics on Oxygen Consumption in a Freshwater Fish *Heteropneustes fossilis*

Ramesh Yadav [a] and D. K. Paul [a*]

[a] *Department of Zoology, Patna University, Patna, Bihar, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i244737

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/4490

Original Research Article

Received: 27/10/2024
Accepted: 30/12/2024
Published: 30/12/2024

ABSTRACT

Microplastics (MPs) are new emerging environmental pollutants in aquatic environments that attract widespread attention due to their small size and potential toxic impacts on aquatic organisms. In the present study, we investigated the toxic effects of short-term exposure to polystyrene microplastics at different doses on the oxygen consumption of *Heteropneustes fossilis*. The fish, after acclimatization, was exposed to polystyrene microplastics at 10.79µg/L, 17.98 µg/L, and 35.96 µg/L for 12 hours.The control group (first group) of fishes showed oxygen consumption of 72.86± 5.05 mg/kg/hr, the second group 69.21±3.64 mg/kg/hr, the third group 57.64±4.04, and the fourthgroup 51.92±4.95 in the closed chamber while 38.99 ± 2.34 mg/kg/hr, 33.47± 1.28 mg/kg/hr, 33.38± 2.40 mg/kg/hr, 29.36± 1.24 mg/kg/hr in open chamber. The decreasing trend of oxygen uptake in the fish was found in both cases after intoxication. The trend may be due to stress conditions of fish

**Corresponding author: Email: dkpaul.pat31@gmail.com;*

Cite as: *Yadav, Ramesh, and D. K. Paul. 2024. "A Study of the Toxic Effect of Polystyrene Microplastics on Oxygen Consumption in a Freshwater Fish Heteropneustes Fossilis". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (24):328-35. https://doi.org/10.56557/upjoz/2024/v45i244737.*

correlated with low RBC values and hemoglobin levels due to microplastic intoxication. Alterations in oxygen consumption may be due to respiratory distress due to impaired oxidative metabolism.

Keywords: Oxygen consumption; microplastics; pollutants; environment; fish.

1. INTRODUCTION

Microplastics (MPs) are small particles or debris whose diameter is ≤ 5mm with a size range between 0.1 to 5000 µm (Thompson, et al., 2009, EFSA, 2016) of plastic, which are synthetic organic polymers extracted from petroleum and other products. Due to their lightweight, inexpensive, and durable materials, plastics can easily be molded into various products used extensively in modern life (Rao, 2019). The increasing demand for plastic resulted in intensely enhanced yearly production from 1.5 million tons in the 1950s and reached more than 360 million tons in 2019 (Plastic Europe, 2020; Boucher, et al., 2020). India contributes around 0.09 to 0.24 million tons annually (Jambeck, et al., 2015). Plastic debris goes into the aquatic environment and contaminates it due to massive production and improper waste management practices (McDevitt, et al., 2017), such as unplanned plastic dumping, domestic and industrial runoff, recreational activities, and commercial activities (fishing, cargo, drilling) (Jambeck, et al., 2015) where it resides for years (Sridharan, et al., 2021).

Microplastics are categorized into polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), and ethylene vinyl acetate (EVA) based onthe type of polymer (Kim, et al., 2021). Based on the production of MPs, they are classified into primary and secondary (Li, et al., 2018). Primary MPs are plastic particles produced directly in a factory and used in medicines, synthetic micro -fibers in textiles, personal care products (as scrubbing agents in cosmetics in face wash) and air blasting pellets etc. (Barnes, et al., 2009) which are released and can be transported into the aquatic environment (Cole, et al., 2011; Gall & Thompson, 2015) via surface runoff, of effluents of wastewater treatment plants, domestic and industrial drainage systems (Murphy, et al., 2016). Secondary microplastics formed due to the progressive physical, chemical, and photodegradation of large plastic debris such as fishing nets, disposable plastic (Singh & Sharma, 2008, Eerkes-Medrano, et al., 2015), plastic bottles, etc. (Boucher & Friot, 2017) present in the aquatic ecosystem (Arthur, et al., 2009).

Fish are often used as bioindicators of water pollution by assessing the physiological state of fish, which helps evaluate the susceptibility or resistance potentiality and is also useful in correlating the behavior of the fish. Oxygen consumption is a suitable tool in the assessment of stress due to toxicants on aquatic organisms and also provides an index of energy expenditure mechanisms or metabolic status for environmental changes (Franklin, et al., 2010; Sornaraj, et al., 2005; Neelima, et al., 2016).

Microplastics are ingested or consumed by invertebrates and fish (Tanaka & Takada, 2016), intentionally or non-intentionally, due to their small size and resemblance with plankton (Crawford & Quinn, 2017). Ingestion of microplastics causes physical effects to fish (mechanical damage of digestive tract) as well as adverse physiological effects that affect feeding, respiratory activity (Tongo & Erhunmwunse, 2022), behavior, inhibit growth and development, reproductive toxicity, immune toxicity, and genetic damage (Harmon, 2018) and even death of fish (Derraik, 2002; Moore, 2008; Wright & Kelly 2017).Thus, the present study aims to evaluate the toxicity of polystyrene microplastics at different concentrations on oxygen consumption of the freshwater teleost fish *Heteropneustes fossilis*.

2. MATERIALS AND METHODS

2.1. Microplastics and Chemicals

Microplastics (MPs) were raw powders with irregular-shaped particles (size of microplastics 1-160 µm) purchased from Sivay Enterprises Pvt. Ltd.,a plastic- powder and granules dealer in Vadodara, Gujarat, India.

2.2. Microplastic Stock Preparation

The stock solution of MPs (1gm/L) was prepared with distilled water and sonicated before every use. The stock solution was diluted further for test concentrations immediately before the beginning of each experiment.

2.3. Fish and Microplastics Exposure

Singhi fish (*Heteropneustes fossilis*) were obtained from the local market of Patna, Bihar (India) and transported to the Fish Biology and Ecotoxicology Laboratory, Department of Zoology, Patna University, Patna, Bihar, India.The weight and size of fishes were 16.73± 4.23 grams and 8.4 ± 3.2 cm, respectively. These fishes were disinfected with $KMNO_4$ solution and acclimatized for 15 days in the laboratory before experimentation. Fishes in each tank were fed 4% of their body weight with pelleted feed. The physicochemical properties of test water (pH 7.4; Dissolved oxygen 6.1 mg/L, temperature 28.5 ºC, photoperiod 12L: 12D). Four groups (30 fish each group) were divided into triplicates for each treatment. The 1st group was the control; the 2nd group was exposed to (10.79µg/Lof MPs), the 3rd group was exposed to (17.98 µg/L of MPs), and the 4th group was exposed to (35.96µg/Lof MPs) for 12 hrs of exposure.

2.4. Oxygen Consumption

An experiment on the oxygen consumption of the fish was carried out in respiratory chambers (sealed glass chambers of volume 56 L contain three openings with air-tight seal). Four fish from each treatment were kept in respiratory chambers. The water sample was taken to estimate DO from the respiratory chamber at 3 hours. The Modified Azide of Winkler Method determined the DO concentration in water samples. The concentration of DO consumption was calculatedas mg per Kg body weight per hour.

2.5. Statistical Analysis

All data were presented as means ± standard error (SE). Statistical evaluations and graph generation were conducted using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Independent data analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons to assess the variation between and within the experimental groups. These statistical analyses were conducted using SPSS version 29 (IBM Corp., Armonk, NY, USA). A P-value of less than 0.05 ($P < 0.05$) was considered to indicate statistical significance.

3. RESULTS AND DISCUSSION

The results obtained from the experiments demonstrate the impact of PSMPs on the oxygen consumption of aquatic organisms. The study presents important findings regarding the physiological effects of microplastic exposure by measuring oxygen consumption across various concentrations of PSMPs in closed and open chamber systems. The oxygen consumption rate (mg/kg body weight per hour) is a reliable indicator of metabolic activity, and significant declines were observed as PSMP concentrations increased.

The results from the closed glass chamber reveal a clear reduction in oxygen consumption with increasing PSMP concentrations. The control group recorded an oxygen consumption of 72.86 mg/kg/hr, which decreased progressively with higher concentrations of microplastics. At 10.79 µg/lit, the consumption dropped to 69.21 mg/kg/hr, followed by a steeper decline at 17.98 µg/lit (57.64 mg/kg/hr) and 35.96 µg/lit (51.92 mg/kg/hr) (Fig. 1). The standard deviations (SD) ranged between 3.64 and 5.05, indicating some variability in the data but consistent reductions across all treatments.

When ingested or adsorbed onto respiratory surfaces such as gills, microplastics can physically obstruct gas exchange. Previous studies have demonstrated that microplastic exposure reduces filtration efficiency in aquatic organisms, leading to diminished oxygen intake. For instance, Wright, et al., (2013) showed that mussels exposed to microplastics experienced a significant reduction in filtration rates, affecting their oxygen consumption. This mechanism may explain the observed decline in the closed chamber setup, where the confined environment exacerbates the organism's inability to extract oxygen due to physical obstructions caused by microplastics efficiently.

In the open glass chamber setup, the control group exhibited a lower baseline oxygen consumption (38.99 mg/kg/hr) than the closed chamber. Exposure to 10.79 µg/lit of PS MPs led to a reduction in oxygen consumption (33.47 mg/kg/hr), with further decreases at 17.98 µg/lit (33.38 mg/kg/hr) and 35.96 µg/lit (29.36 mg/kg/hr) (Fig. 1). The results indicate that microplastic exposure negatively affects oxygen consumption, even in open systems with greater environmental oxygen availability. This supports the hypothesis that PSMPs directly impact the organism's respiratory system, as seen in other studies.

The open system, with its less restricted oxygen availability, still showed significant reductions in oxygen consumption across treatments. This suggests that the negative impacts of microplastics are not solely a function of environmental constraints but also the physiological stress induced by the particles themselves. According to research by Von Moos et al., (2012), microplastic ingestion in marine mussels led to oxidative stress and energy depletion, compromising normal respiratory functions. In this study, it is plausible that microplastics in the open chamber similarly induced oxidative stress, reducing the organism's metabolic rate.

The results from both chambers consistently show that oxygen consumption decreases as PSMP concentrations increase. However, oxygen consumption was generally higher across all concentrations in the closed chamber than in the open chamber. In the closed system, oxygen is gradually depleted, and organisms must exert greater effort to obtain oxygen, especially in the presence of PSMPs. Conversely, oxygen is more freely available in the open system, which may reduce the effort required for respiration. Despite this, both systems demonstrated a clear trend of reduced oxygen consumption with higher PSMP concentrations, indicating that the microplastics are the primary stressor. At 10.79 µg/lit, the consumption dropped to 69.21 mg/kg/hr, followed by a steeper decline at 17.98 µg/lit (57.64 mg/kg/hr) and 35.96 µg/lit (51.92 mg/kg/hr).

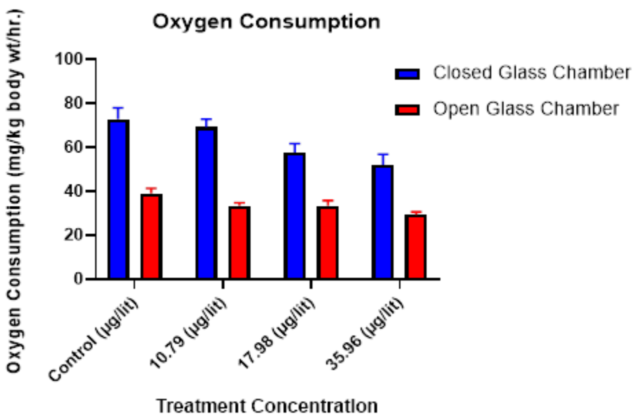

Fig. 1. Oxygen Consumption (mg/kg/hr) of *Heteropneustes fossilis* after treatment with three different concentrations of PSMPs

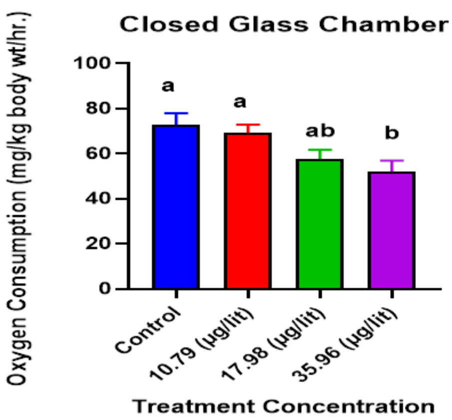

Fig. 2. Oxygen consumption in a closed chamber was measured across different treatment groups, with significant differences ($P < 0.05$) in the graph. Statistical analysis was performed using Tukey's post-hoc test to determine pairwise differences between the groups. Values are expressed as mean ± standard error (SE), with a sample size of n = 3

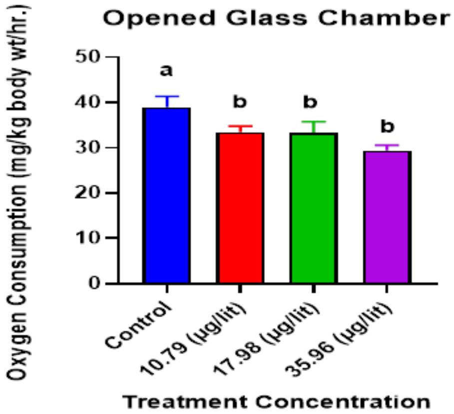

Fig. 3. Oxygen consumption in the open chamber was measured across different treatment groups, with significant differences (P < 0.05) in the graph. Statistical analysis was performed using Tukey's post-hoc test to determine pairwise differences between the groups. Values are expressed as mean ± standard error (SE), with a sample size of n = 3

Oxygen consumption of fish treated with concentrations 10.79 µg/lit and 17.98 µg/lit are not significantly different,but concentrations of 35.96 µg/lit treated considerably differ from control. Among the three treatments, 35.96 µg/lit significantly differs from 17.98 µg/lit treated (Fig. 2). The oxygen consumption of all three treatment concentrations of PSMPs is substantially different from the control. Among the three treated concentrations of PSMPs oxygen consumption is not significantly different (Fig. 3). Statistical analysis using Tukey's test revealed significant differences between the control group and the highest concentration (35.96 µg/lit) in both chambers. The 95% confidence intervals show that PSMP exposure at higher concentrations (17.98 µg/lit and 35.96 µg/lit) significantly affects oxygen consumption compared to the control and 10.79 µg/lit group. This statistical significance confirms that the reduction in oxygen consumption is not due to random variation but is a direct consequence of increased PSMP concentrations. According to Jovanovic (2017), higher microplastic concentrations correlate with greater metabolic stress in aquatic species, supporting the findings of this study.

Several potential mechanisms may explain the observed decline in oxygen consumption with increasing PSMP concentrations. PSMPs may accumulate on aquatic organisms' gills or other respiratory surfaces, blocking normal gas exchange. The physical presence of these particles can reduce the efficiency of oxygen diffusion, leading to lower oxygen consumption. Studies such as those by Wright, et al., (2013) have demonstrated that microplastic ingestion leads to similar outcomes in marine species. Microplastic exposure often triggers a stress response in organisms, requiring energy to be redirected from normal metabolic processes towards dealing with the stressor (e.g., detoxification or immune response). This energy reallocation reduces metabolic activity, as evidenced by decreased oxygen consumption. Von Moos et al., (2012) noted that microplastic exposure induces oxidative stress, depleting energy reserves and compromising respiratory function. PSMPs may release toxic chemicals or absorb other environmental pollutants, which can further impair respiration and metabolic processes. Research by Bakir et al., (2016) highlighted that microplastics could act as vectors for toxic substances, increasing the physiological burden on organisms.

The significant reduction in oxygen consumption at higher PSMP concentrations suggests broader ecological consequences. As metabolic rates decline, affected organisms may experience reduced growth, reproductive success, and survival rates, potentially disrupting entire ecosystems. The effects observed in this study are consistent with findings from other research on the ecological impacts of microplastic pollution. For example, Cole et al., (2011) reported that microplastic ingestion in planktonic organisms led to reduced feeding and growth rates, which could have cascading effects through the food chain. Zheng et al., (2024) reported that alteration in oxygen consumption in tilapia fish (*Oreochromis niloticus*) on the exposure of polystyrene micro(nano) plastics

(MNPs) to at an environmentally relevant concentration of 100 µg/L for 28 days due to respiratory damage. Kama et al., (2024) reported that MPs exposure significantly affects the oxygen consumption rate at a concentration of 10mg/lit exposed to Oreochromis niloticus.

The comparison between closed and open chamber results also highlights the potential for environmental context to influence the severity of microplastic impacts. In more confined or polluted environments, the effects of microplastics on oxygen consumption and metabolic function may be more pronounced, leading to greater ecological disruption.

4. CONCLUSION

The study demonstrates a clear, concentration-dependent decline in oxygen consumption due to PSMP exposure. Both closed and open chamber environments showed significant reductions in oxygen consumption, though the effects were more pronounced in the closed system. The statistical significance of these reductions, as confirmed by Tukey's test, indicates that the impact of microplastics on organismal respiration is both real and significant. The findings align with existing research on the physiological and ecological consequences of microplastic pollution, including the work of Wright et al., (2013); Von Moos et al., (2012) and Jovanovic (2017). The implications of these results are broad, suggesting that microplastic pollution could severely impact aquatic ecosystems by impairing the metabolic functions of key species. Further research is needed to explore the long-term effects of microplastic exposure on different species and environments, as well as potential mitigation strategies to reduce the ecological burden of this emerging pollutant.

ETHICAL APPROVAL

All experimental procedures complied with the standards prescribed by the Ethical Approval Committee of the Post Graduate Research Council of Patna University, Patna, Bihar.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

Department of Zoology Patna University Patna Bihar, India supported this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Arthur, C., Baker, J., Bamford, H. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.

Bakir, A., O'Connor, I.A., Rowland, S.J., Thompson, R.C. (2016). Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. *Environmental Pollution*, 219, 56-65.

Barnes, D. K., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical transactions of the royal society B: biological sciences, 364(1526), 1985-1998.

Boucher, J and Friot, D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources. IUCN, Gland, Switzerland, pp. 43.

Boucher, J., Billard, G., Simeone, E., Sousa, J. (2020). The Marine Plastic Footprint. The Marine Plastic Footprint. IUCN, Gland, Switzerland. https://doi.org/10.2305/iucn. ch.2020.01.en.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., (2011). Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588- 2597.

Crawford, C.B. and Quinn, B. (2017). Biol. Impacts Eff. Contam. Micro 159–178.

Derraik, J.G. (2002). The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44 (9), 842–852.

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C. (2015). Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63-82.

EFSA (European Food Safety Agency), (2016). Presence of Microplastics and Nanoplastics in Food, with Particular

Focus on Seafood. EFSA Panel on Contaminants in the Food Chain (CONTAM). http://doi.org/10.2903/j.efsa.2016.4501.

Franklin, R.K., Loo, H.S., Osumanu, H.A. 2010. Incorporation of Bentazone with Exserohilum rostratum for controlling Cyperus iria. American J. Agri. Biol. Sci., 5: 210–214.

Gall, S.C., and Thompson, R.C. (2015). The impact of debris on marine life. Mar. Pollut. Bull. 92, 170- 179.

Harmon, S.M. (2018). Chapter 8 - The effects of microplastic pollution on aquatic organisms. In: Zeng, E.Y. (Ed.), Microplastic Contamination in Aquatic Environments. Elsevier, pp. 249-270.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R. and Law, K. L. (2015). Plastic waste inputs from land into the ocean. Science, 347: 768-771.

Jovanovic, B. (2017). Ingestion of microplastics by fish and its potential consequences from a physical perspective. *Integrated Environmental Assessment and Management*, 13(3), 510-515.

Kama, N. A., Djawad, M. I., & Yaqin, K. (2024). Effects of Microplastics on the Oxygen Consumption and Histological Changes of the Cultured Nile Tilapia Oreochromis niloticus. *Egyptian Journal of Aquatic Biology & Fisheries*, *28*(5).

Kim, J.H., Yu, Y.B., & Choi, J.H. (2021). Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. Journal of Hazardous Materials, 413, 125423.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., & Zeng, E. Y. (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. *Water research*, *142*, 75-85.

McDevitt, J.P., Criddle, C.S., Morse, M., Hale, R.C., Bott, C.B., Rochman, C.M. (2017). Addressing the issue of microplastics in the wake of the Microbead-Free Waters Actda new standard can facilitate improved policy. Environ. Sci. Technol. 51, 6611-6617.

Moore, C.J., (2008). Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res. 108 (2), 131–139.

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater treatment works (WWTW) as a source of microplastics in the aquatic environment. Environmental science & technology, 50(11), 5800-5808.

Neelima, P., N. Gopala Rao, G. Srinivasa Rao and Chandra Sekhara Rao, J. 2016. A Study on Oxygen Consumption in a Freshwater Fish Cyprinus carpio Exposed to Lethal and Sublethal Concentrations of Cypermethrin (25%Ec). Int.J.Curr.Microbiol.App.Sci.5(4): 338-346.

Plastic Europe, 2020. Plastics-the Facts 2019, An Analysis of European Plastics Production, Demand and Waste Data. Plastic Europe, Association of Plastic Manufacturers, Brussels-Belgium. https://www.plasticseurope.org/ en/resources/publications/4312-plastics-facts-2020.

Rao, B. M. (2019). Microplastics in the aquatic environment: implications for post-harvest fish quality. Indian J. Fish. 66 (1), 142–152

Singh, B., & Sharma, N. (2008). Mechanistic implications of plastic degradation. Polymer degradation and stability, 93(3), 561-584.

Somaraj, I.R., Ranjitsingh, A.J., Pushparaj, A., Ramathilagam, G. 2005. Pesticidal stress influenced respiratory alterations in the fresh water fish, Mystus vittatus. Indian J. Environ. Ecoplanning, 10: 803–806.

Sridharan S., Kumar M., Bolan N.S., Singh L., Kumar S., Kumar R. and You S. (2021). Are microplastics destabilizing the global network of terrestrial and aquatic ecosystem services? Environ Res. 198: 111243. DOI:10.1016/j.envres.2021.111243.

Tanaka, K. and Takada, H. (2016). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6, 34351.

Thompson, R. C., Swan, S. H., Moore, C. J., &Vom Saal, F. S. (2009). Our plastic age. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1973-1976.

Tongo, I., & Erhunmwunse, N. O. (2022). Effects of ingestion of polyethylene microplastics on survival rate, opercular respiration rate and swimming performance of African catfish (*Clarias gariepinus*). *Journal of Hazardous Materials*, *423*, 127237.

Von Moos, N., Burkhardt-Holm, P., Köhler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis L.* after an experimental exposure. *Environmental Science & Technology*, 46(20), 11327-11335.

Wright SL and Kelly FJ. (2017). Plastic and human health: a micro issue? Environ. Sci. Technol. 51, 6634–6647.

Wright, S.L., Thompson, R.C., Galloway, T.S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, 178, 483-492.

Zheng, S., Tang, B. Z., & Wang, W. X. (2024). Microplastics and nanoplastics induced differential respiratory damages in tilapia fish Oreochromis niloticus. *Journal of Hazardous Materials*, *465*, 133181.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4490