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TOXICOLOGICAL EFFECTS OF MICROPLASTICS AND PHENANTHRENE TO ZEBRAFISH (*DANIO RERIO*)

M.Swarnalatha, M.Ramaswamy
[1]Associate Professor, Department of Zoology, Emerald Heights College for Women Ooty, The Nilgiris, Tamil Nadu. India.
[2]PG & Research Department of Zoology, Government Arts College (Autonomous), Coimbatore, Tamil Nadu. India
Email id: swarnalathaslvm@gmail.com

Abstract:
The toxicology of microplastics in combination with other pollutants has attracted widespread attention. In this study, zebra fish were exposed to 3 mg/L polystyrene microplastic, 0.2 mg/L phenanthrene, and a combination of both were measured after 12 and 24 days of exposure. Phenanthrene and microplastic accumulation increased with exposure time and was also greater in the combined exposure group than in the single exposure group. Overall, our study demonstrates that microplastics enhance the toxicity of phenanthrene and that the two have a synergistic effect.

Keywords: Zebra fish (*Danio rerio*),polystyrene microplastic and phenanthrene

Introduction
The worldwide pollution caused by microplastics has become a subject of concern. As a pollutant of great concern in recent years, microplastics have been detected in air, soil and water environments (Klein and Fischer, 2019; Yan *et al*., 2019; Zhang and Liu, 2018). Microplastics were not only found in areas of high human activity but have also been detected in the remote Antarctic and Arctic (Kanhai *et al*., 2018; Waller *et al*., 2017). Microplastics that are widespread in the environment can be transferred to living organisms through a number of pathways. White, clear and blue plastic fragments are easily swallowed by planktivorous fish as food (Huerta Lwanga *et al*., 2016). Once microplastics appear in the ecosystem, they can be transported along the food chain to many organisms with different trophic levels (Farrell and Nelson, 2013; Setälä *et al*., 2014). Microplastics can be detected in the digestive tract or tissues of fish (Alomar and Deudero, 2017), crustaceans (Desforges *et al*., 2015), shellfish (Van Cauwenberghe and Janssen, 2014), birds (Tanaka *et al*., 2013) and other animals.

While some of the ingested microplastics are excreted in the feces (Jeong *et al*., 2016), some enter the tissues and cause toxic effects on related organs (Karami *et al*., 2016). The toxicological effects of microplastics on organisms manifest in various aspects. For low trophic levels, small plankton such as copepods, microplastics could accumulate in the digestive tract and even block the digestive tract. Increased intake of microplastics leads to decreased feeding rates, weight loss, growth suppression, reduced fecundity, longer reproductive time, decreased mobility, shorter lifespan, and increased mortality (Gambardella *et al*., 2018; Jaikumar *et al*., 2019; Jeong *et al*., 2016; Kokalj *et al*., 2018; Wang *et al*., 2019b). In addition, microplastics have several toxic effects. Wen *et al*. (2018)found that discus fish (*Symphysodon aequifasciatus*) experienced oxidative stress after 30 days of exposure to microplastics, and the activities of superoxide dismutase and glutathione peroxidase increased. Wan *et al*. (2019) found that polystyrene microplastics could cause changes in genes related to glycolysis and lipid metabolism in larval zebrafish. Greven *et al*. (2016) found that microplastics in the fathead minnow cause stress response of innate immune cells and interfere with the population's disease resistance.

In addition to their own toxicity, microplastics could also be used as carriers for other pollutants. The surface of the microplastic is negatively charged and could attract positively charged

materials, and hydrophobic or hydrophilic interaction forces also allow microplastics to adsorb organic chemicals (Tourinho *et al*., 2019). Wen *et al*. (2018) found that coexposure to microplastics and cadmium caused severe oxidative stress in juvenile discus fish (*Symphysodon aequifasciatus*). Trevisan *et al*. (2019) reported that coexposure to nanopolystyrene microplastics and polycyclic aromatic hydrocarbons (PAHs) reduced the absorption of zebrafish larvae and decreased PAH-induced developmental malformations.

Past studies have shown that a variety of environmental pollutants, such as microplastics and phenanthrene (PHE), exist in aquatic ecosystems, and aquatic organisms are also affected by these pollutant (Alimba and Faggio, 2019; Li *et al*., 2018; Tourinho *et al*., 2019). Zebrafish are model organisms that are often used to study chemical toxicity. This study evaluated the effect of microplastic and phenanthrene (PHE) coexposure on oxidative stress and immune function in adult zebrafish. The results of this study could provide a basis for the biological impact of the combined contamination of microplastics and PAHs.

MATERIALS AND METHODS FOR THE DATA COLLECTION AND ANALYSES

Online Study Sites

Data have been collected from many countries around the world. Results from the publications of various scientists and researchers are presented.

Data Collection

The first phase involved the identification of related studies. In order to conduct a systematic literature search, the following specifications were created for the database: Searching Database • Scopus, Web of Science, Google Scholar, PubMed, Dimension Searching Conditions • Journal articles written in English • Impact of MPs on fish and human-related journals, book chapters, conference proceedings • Accessible over the internet (No time limitation)

Phenanthrene (PHE) (purity > 99.5%, CAS number 85-01-8) was purchased from Hi-media, Coimbatore, Tamil Nadu, India. Polystyrene microplastics with an irregular particle shape (150 µm) were obtained fromHi-media, Coimbatore, Tamil Nadu, India.Adult Zebrafish (*Danio rerio*) were used for all exposure experiments. Fish were maintained in a controlled laboratory environment with a 12-hour light/dark cycle, and water temperature was maintained at 28 ± 1°C. Fish were acclimated to the laboratory conditions for at least 7 days prior to the experiments. The exposure groups included the following:Control (CTR): No exposure to polystyrene microplastics or phenanthrene.Phenanthrene (PHE): Exposed to 0.2 mg/L phenanthrene.Polystyrene (PS): Exposed to 3 mg/L polystyrene microplastics.Combined (CP): Exposed to both 3 mg/L polystyrene microplastics and 0.2 mg/L phenanthrene.

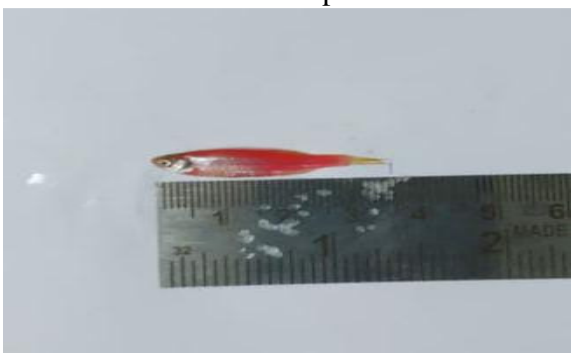

Figure:1. ZEBRAFISH (*DANIO RERIO*)

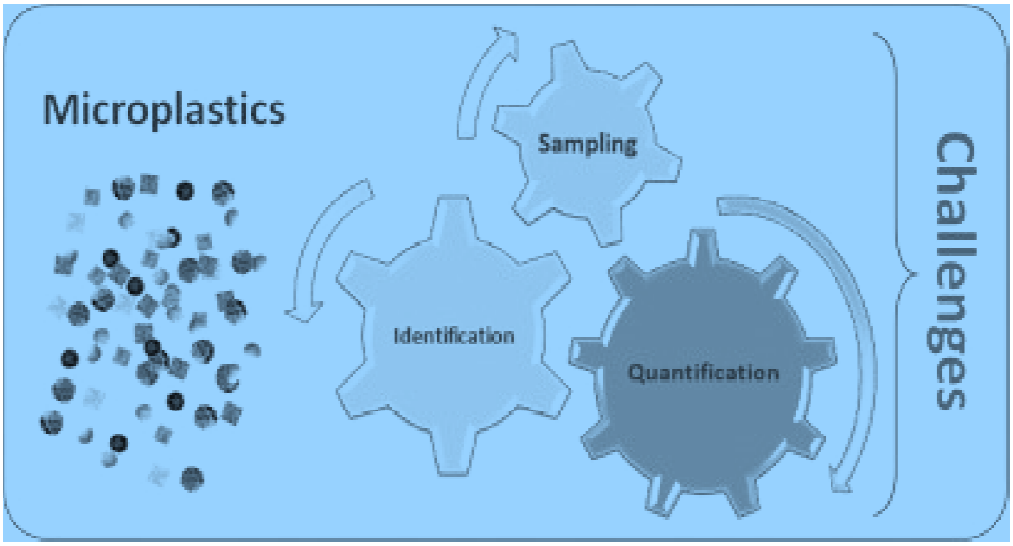

Figure: 2. Effects of microplastics and phenanthrene to Zebra fish (*Danio rerio*)

Tables

Table 1: Zebra fish Exposure Conditions

Treatment Group	Microplastic (Polystyrene)	Phenanthrene	Exposure Duration (Days)
Control	0 mg/L	0 mg/L	12, 24
Microplastic	3 mg/L	0 mg/L	12, 24
Phenanthrene	0 mg/L	0.2 mg/L	12, 24
Combined	3 mg/L	0.2 mg/L	12, 24

Table 2: Polystyrene Microplastic accumulation in Zebra fish

Treatment Group	Polystyrene Particles Accumulated (Mean ± SD)	12 days	24 days
Control (CTR)	0 ± 0	0	0
Phenanthrene (PHE)	0 ± 0	0	0
Polystyrene (PS)	1.79 ± 1.53	1.79	6.99
Combined (CP)	2.89 ± 1.82	2.89	9.66

Results

1.Zebra fish Exposure Conditions:

The zebra fish were exposed to different treatment conditions over 12- and 24-day periods, as outlined in Table 1. The exposure groups included the Control (CTR), Phenanthrene (PHE), Polystyrene (PS), and Combined (CP) groups, with corresponding concentrations of polystyrene microplastic and phenanthrene as specified(Figure:1).

2.Polystyrene Microplastic Accumulation in Zebrafish:

Table 2 summarizes the accumulation of polystyrene microplastics in zebrafish under different exposure conditions. No microplastics were observed in the Control (CTR) and Phenanthrene (PHE) exposure groups at either 12 or 24 days of exposure.In the Polystyrene (PS) exposure group, an average of 1.79 ± 1.53 polystyrene particles were accumulated per fish after 12 days, and this increased to 6.99 ± 3.59 particles per fish after 24 days. This increase in

particle accumulation over time suggests a gradual uptake of polystyrene microplastics.In the Combined (CP) exposure group, zebrafish accumulated 2.89 ± 1.82 polystyrene particles per fish after 12 days, with accumulation rising to 9.66 ± 7.41 particles per fish after 24 days. The combined exposure group showed a higher level of microplastic accumulation compared to the PS-only group at both time points, suggesting a potential synergistic effect of polystyrene microplastic and phenanthrene exposure (Figure:2).These findings demonstrate the increased accumulation of polystyrene microplastics in zebrafish, with a notable effect observed under combined exposure to both microplastics and phenanthrene.

Discussion

Most of the changes recorded in the liver transcriptome were already activated by exposing adult zebrafish for 20 days to the lower Microplastics concentration (100 µg/L), which is comparable to concentrations measured in highly polluted marine and freshwater environments(Zhao*et al*., 2014, Goldstein et al., 2012). Although it is not possible to deduce dose/effect relationship with the data here presented, which was not the aim of the study, we report that the transcriptomic alterations observed after exposure to the lower MPs concentration (100 µg/L) are substantially overlapped by those exerted by the higher concentration of MPs (1000 µg/L). The main effects on gene transcription were attributed to i) immune enhancement *versus* extracellular antigens and depression *vs*. intracellular ones, and ii) down-regulation of energy metabolism pathways.

In total, 41 differentially expressed genes (17 up- and 26 down-regulated) were attributed to immune response, most of them were differentially transcribed after both MPs treatments. Up-regulation was recorded for key role genes subserving MHCII processing and antigen presentation (*cd74a*, *cd74b*, *ciita*), lymphocyte activation (*ccr7*, *ccl19a*, *ccl19b*), innate immunity (*trim-35-23*, *trim35-24*, *trim35-25*, *chia.3*) and general activation of the immune response (*ccl38.6, lym6m3, tcirg1a, tcimb*) or cell proliferation (*mia, lect2l*). Down-regulation was found for gene transcripts involved in innate antimicrobial response (*marco, hsp60, hsp70*), antiviral defense (*ifit8*, *ifitm1*, *batf3*), ROS formation (*nox5*, *noxo1a*, *slc7a11*), angiogenesis, cell adhesion and maintaining of cell junctions (*lpar6a*, *fn1a*, *creld1b*, *cscl8a*, *cers2b*, *mmp14a*, *mmp14b*, *alox5b*). It is worth noting the down-regulation of *claudin b (cldnb)* e *crumbs homolog 3b* (*crb3b*) transcripts, due to the relevant role played by these gene products for epithelium integrity. The modulation of the expression for genes related to oxidative stress response and immune processes following MPs exposure have been previously documented(Choi *et al*., 2018), although the molecular and biological mechanisms activated, remain to be fully elucidated. For instance, according to transcriptomic analysis performed on *Pocillipora damicornis*, MPs were able to alter the JNK and ERK pathways, possibly undermining the immune response(Tang *et al*., 2018). In mussel, MPs affected the expression of important immunity genes of the PRRs and AMPs family, which have a central role in the immune functions of bivalves(Détrée *et al*., 2018). Fewer transcriptomic data are available for fish, but a recent study conducted on zebrafish larvae reported that PS MPs can lead to the alteration of complement system genes(Veneman *et al*., 2017). According to the transcriptional profile that we observed, the down-regulation of innate immunity and epithelial integrity genes, suggest that MPs can affect fish immune functions, with possible defeated control of pathogen entry at epithelial barriers and rising chances of infections at mucosal sites.

The alteration of the liver transcriptome is coherent with the histopathological signs detected in both GIT and gills epithelium. The lower number of detectable goblet cells in the GIT epithelium could be in part interpreted as due to mucous discharge, but is particularly relevant to consider that AB + mucus has been

linked to the higher occurrence of anti-microbial peptides, thus indicating impairment of a relevant first-line defense against pathogen penetration. Enhanced expression of immune genes involved in response against extracellular antigens (eg. MHCII, and markers of neutrophils and APC) is outlined by overt mucosal neutrophilia and it is coherent with an excessive entry of exogenous antigens. Zebrafish exposed to MPs also showed alterations of gill epithelium, enhanced occurrence of neutrophils, adhesion and partial fusion of secondary lamellae and mucous hypersecretion. The reduction of epithelial integrity observed is consistent with previous studies, which reported the capacity of MPs to induce epithelial damage in the GIT and gills tissue, such as epithelial detachment and loss of gills secondary lamellae(Karami *et al*.,2016). Interestingly, another study comprising zebrafish exposure to 1000 µg/L of PS MP, reported the augmented mucous secretion in the GIT, followed by the increase of pathogenic bacteria in the gut microbiota(Lu *et al*., 2018).

Conclusion

The combined exposure group exhibited a synergistic effect, leading to a higher level of polystyrene microplastic accumulation than observed in the Polystyrene (PS) exposure group alone. This suggests that phenanthrene exposure enhances the uptake of polystyrene microplastics in zebrafish, potentially altering their bioaccumulation and toxicity profiles.In conclusion, the study highlights the interaction between microplastics and environmental pollutants, such as phenanthrene, which may amplify the environmental and toxicological impacts of microplastic pollution on aquatic organisms. This finding warrants further investigation into the synergistic effects of microplastics and chemical pollutants, especially in the context of long-term ecological and health consequences.

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