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Chronic toxicity of microplastics have lethal effects on common carp (*Cyprinus carpio* Linnaeus, 1785)

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Abstract. The 21st century has seen the realization that people have overused plastic. Unfortunately, plastic pollution is a widespread environmental problem in many of the world's rivers and seas. Plastic litter is well distributed in shallow as well as in deep-waters, and therefore it is inevitably present in the world's ocean which is in turn the final sink of plastic debris of all sizes, alongside other various anthropogenic toxicants (for example, heavy metals and persistent organic pollutants). The negative effects of microplastics (MPs) which are produced by the breakdown of larger plastic debris on aquatic organisms, including both freshwater and marine fish have been already well documented. However, there is a gap in the knowledge from studies conducted in the field or under laboratory conditions in Bulgaria.

Key words: microplastics, pollution, negative effects, fish, Bulgaria.

Introduction

In the past 60 years, plastic production has rapidly risen with the result of an accumulation of plastic debris all over our planet (Bouwmeester *et al.* 2015; Lei *et al.* 2018). Over time, plastic debris tend to breakdown into smaller particles called microplastics (MPs) (Phothakwanpracha *et al.* 2021). MPs which are defined as small plastic particles with a size lower than 5 mm in diameter have emerged as a global environmental problem (Alimba & Faggio 2019; Sanots *et al.* 2020). They can be divided into 2 main categories based on their sources of origin: primary MPs and secondary MPs. Primary MPs are plastics that are artificially produced directly in a microplastic form, while secondary MPs are formed by the fragmentation of larger sized plastic debris by various factors (Leja & Lewandowicz 2010; Li *et al.* 2016; Paço *et al.* 2017). It is estimated that at least 35540 tons of MPs float in the global ocean (Zhang *et al.* 2020). MPs fragments can pose further toxicological risks to aquatic organisms, owing to leaching of chemical additives (Schrank *et al.* 2019; Na *et al.* 2021). Furthermore, plastic additives are not chemically bound to plastic matrices which increases their leaching potential in the aquatic environments (Hahladakis *et al.* 2018).

In order to clarify the long-term effects of MPs, we assessed the accumulation in tissues and a battery of biomarkers in common carp, such as histopathological and histochemical lesions (PAS and SUDAN III reaction) as well as biochemical changes (activity of metabolic enzymes, i.e. LDH, AChE, ASAT and ALAT and antioxidant enzymes, i.e. CAT, GR and GPx) in gills and liver were followed under laboratory conditions. Here we present the first results from the experiment on the lethality rate of common carp after 30 days' exposure to MPs.

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Material and Methods

Healthy juvenile common carps (n = 120) were purchased from the Institute of Fisheries and Aquaculture in Plovdiv, Bulgaria. The fish were acclimatized for 1 week in 100-L glass tanks under constant aeration at the vivarium at the Faculty of Biology, Plovdiv University, Bulgaria. A regime of 12 dark : 12 light was provided. After acclimatization the fish were transferred in another 3 test aquariums, including a control (n = 20 fish in each tank). The most common types of MPs reported in field studies are polyethylene (PE; 28%) and polypropylene (PP; 19%), followed by polyvinylchloride (PVC; 10%) and polystyrene (PS; 7%) (De Sá *et al.* 2018). That is why, in our experiment raw and recycled high-density polyethylene (HDPE) pellets were obtained from the company Diko, Plovdiv, Bulgaria which produces various products from thermoplastic polymers. The pellets were provided from the manufacturer with a size of 1 mm x 1 mm, therefore it was not necessary to further cut them with metal scissors in pieces of less than 5 mm x 5 mm as explained by Li *et al.* (2022). According to Phothakwanpracha *et al.* (2021) the tested MPs were categorized as large (small MPs (<30 µm), medium MPs (30–300 µm), large MPs (300–1000 µm)). We prepared 2 concentrations of 100 mg HDPE pellets per litre. The pellets were weighed and transferred in 1 L beakers filled with double-distilled water which were left on a shaker for 72 hours at the lowest speed of 20 rpm. After the leaching period the water was filtered over 0.7 µm membranes using a vacuum pump. This MPs water was used to treat the exposed fish which were fed daily; thus the water was renewed every week with redosing of the tested MPs. The physico-chemical properties of the water, such as pH, T, O_2 and conductivity were recorded every week. After 30 days the fish was dissected and both fish gills and liver, and water samples were collected for different future analyses. Behaviour and mortality rate were also recorded.

Results and Discussion

Mortality of common carps was observed in the tanks subjected to the tested raw and recycled HDPE MPs during the second and third week of exposure. We recorded in total 6 dead fish in the aquarium subjected to raw HDPE and 4 dead fish in the aquarium subjected to recycled HDPE. This makes 30% and 20% mortality rate for the tested MPs, respectively. No mortality rate was recorded in the control group. Prior to mortality the swimming of the fish was slowed and lethargic with intense mucus secretion in both tested aquariums compared to the fish in the control group. The physico-chemical properties of the water remained relatively constant and no statistical differences were found between the tested tanks and the control during the 30 days' exposure ($p > 0.05$; GraphPad Prism 7, USA).

MPs can adversely affect aquatic organisms (Cole *et al.* 2011); both physical and chemical detrimental effects including gut damage, growth inhibition, fecundity reduction and mortality have been reported in various aquatic organisms, including fish (Ferreira *et al.* 2016; Cormier *et al.* 2019). We agree with Gonçalves *et al.* (2022) that plastic pollution in the marine environment, even at a nanoscale is prominent, and the toxicity of these particles on biota is highly worrying. Taking this into account, we also aimed to assess the histopathological, histochemical and biochemical changes, including oxidative stress in the tested fish after chronic exposure to MPs. Common carp is both a sentinel freshwater fish species and probably the most important freshwater species in aquaculture worldwide (Yancheva *et al.* 2022). Our results indicated a lethal outcome which is the final endpoint in aquatic toxicology. We therefore will further determine the different biomarker alterations at tissue and cellular level in order to better study the negative effects of MPs on common carp which will undoubtedly be significant.

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