

## EFFECTS OF PLASTIC ADDITIVES AND ORGANIC CONTAMINANTS IN MICROPLASTICS ON ZEBRAFISH

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Plastic pollution is an emerging threat with serious consequences for animal health and the environment. Among them, microplastics (MPLs) with a size below 5mm are the ones that could cause harmful effects to biota since they can be ingested by a wide variety of species. The risks associated with these small fragments come from the material itself and the chemical contaminants that are absorbed into it from the surrounding water. To assess bioaccumulation in tissues, a feeding study of 4 laboratory treatments was conducted with zebrafish for 60 days. In a preliminary study, MPLs collected from 3 beaches, Lambra, Porís and Famara, were analysed to assess those from more contaminated areas. The analysis showed that Lambra and Porís had the most contaminated MPLs, which were selected to perform separated experiments with zebrafish. Exposure experiments were carried out through the diet (10% of total). In parallel, two more experiments were carried out, a control experiment using clean pellets from a factory and a blank control experiment without MPLs in the fish diet. The analysis of polymers bioaccumulated by zebrafish consisted ultrasound-assisted extraction with toluene and analysis by size exclusion chromatography using an advanced polymer chromatography column coupled to high-resolution mass spectrometry with an atmospheric pressure photoionization source under negative ionization conditions (LC(APC)-APPI (-)-HRMS). For plastic additives and other organic contaminants adsorbed onto MPLs a suspected screening was applied based on UASE with methanol followed by ultra-performance liquid chromatography coupled to HRMS. The chromatographic separation was achieved using a C18 analytical column coupled to HRMS equipped with an electrospray ionisation source working in negative and positive ionization conditions. Data analysis was carried out by Compound Discoverer 3.0, where the first screening generated 130,539 tentative identifications at confidence level 5. After refining 23 plastic additives were confirmed at level 1. Among other contaminants potentially adsorbed from surrounding environments, polycyclic aromatic hydrocarbons (PAHs) were dominant, likewise, most of them were found in the Porís beach, between 300-550 ng/g, followed by polychlorinated biphenyls (PCBs) at 20 ng/g. Our main findings support the hypothesis that, in this real scenario, plastic additives and chemical contaminants adsorbed on environmental microplastics (EMPLs) bioaccumulate in the fish liver due to long-term ingestion of MPLs.

**Key words:** Microplastics, plastic additives, Bioconcentration, Zebra Fish experiment, HRMS

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