EFFECTS ON ZEBRAFISH OF CHEMICAL CONTAMINANTS AND ADDITIVES PRESENT IN MICROPLASTICS

Arianna Bautista Gea

Curso 2021-2022

Tutoras:

May Gómez

Alicia Herrera

Marinella Farré

Trabajo Fin de Máster para la obtención del título en Oceanografía

Effects on zebrafish of chemical contaminants and additives present in microplastics

Personal details

Arianna Bautista Gea

Final master memory (TFM)

Course: 2021/2022

Faculty Marine Science

Universidad de Las Palmas de Gran Canaria

Tutor details

Dr. May Gómez Cabrera

Marine Ecophysiology Group (EOMAR),

Iu-ECOAQUA

Universidad de Las Palmas de Gran Canaria

Tutor details

Dr. Alicia Herrera Ulibarri

es

Marine Ecophysiology Group (EOMAR),

Iu-ECOAQUA

Universidad de Las Palmas de Gran Canaria

Co-Tutor details

Dr. Marinella Farré Urgell

Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Research group: Environmental and water chemistry for human health (ONHEALTH)

IDAEA-CSIC, Barcelona

_____

Arianna Bautista Gea

Dr. May Gómez	Dr. Alicia Herrera	Dr. Marinella Farré

Las Palmas de Gran Canaria, July 28, 2022

ACKNOWLEDGEMENT

I would like to thank the two research groups for hosting me and teach me so much. To EOMAR for accept me in their group and present me an innovative project. To Ico, Alexandro and Alberto for all the months of preparation and duration of the experiment. To Alicia for her patience and help in all aspects. To May for being a reference and for all the pushes she gives to be better scientists.

To ONHEALTH for these months of hard work and for welcoming me as a member of their group and training me, not only for this study but also for all the projects that I have been able to participate and help. To Xavi for patiently teach me such a strict methodology when it comes to analysis. To Albert, Maria and Katerina for always being willing to help me in any situation and guide me through this journey. To Marta for her advices in times of stress and for being a fundamental pillar in this study. And to Marinella for showing and teaching me a completely new world in science, for trusting me, giving me her support and for being an example to follow.

I would like to make a special mention to my family and friends, to push me to be better, for their support in good and tough moments and for bring me a fresh air when I needed it.

ABSTRACT

Plastic pollution is an emerging threat with serious consequences for animal health and the environment. Among them, microplastics (MPs) 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 treatments was conducted with zebrafish for 60 days. Exposure experiments were carried out through the diet (10% of total) and two more experiments, one using clean pellets from a factory and a blank control experiment without MPs in the fish diet. The analysis of chemical pollutants was by liquid chromatography coupled to a high-resolution mass spectrometry (LC-HRMS).

Our results verify the bioaccumulation of chemical pollutants in zebrafish tissues, also over the time. In addition, in some cases, pollutants have more tendency to adsorb to microplastics instead of being desorbed. The family of plasticizers show most of the compounds in level 2 of identification, while plastic synthesizers were quantified as the highest concentration in zebrafish tissues, followed by plasticizers.

Our main findings support the hypothesis that, in this real scenario, plastic additives and chemical contaminants adsorbed on environmental microplastics (EMPs) bioaccumulate in the fish tissues due to long-term ingestion of MPs.

CONTENTS

1. Introduction

In the last 70 years, the plastic role has increased its relevance in the whole world economy; as a result, its production has also increased. For example, in 2006, the annual production was 245Mt, and in 2020 it was 367Mt (Plastics Europe, 2006; 2021). The increase in plastic production has elevated its waste, which is one of the biggest parts of the world's litter in all environments, whether marine o terrestrial (Ferraro and Failler, 2020). Several types of polymers are used in packaging: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyvinyl chloride (PVC). These, in particular, are highly likely to end up in the marine environment. Land-based sources, including litter found on beaches, contribute about 80% of plastic waste. Furthermore, resin granules or "pellets" are common components in debris introduced into the oceans as losses during marine transport or through runoff from processing facilities (Andrady, 2011).

Plastic pollution in the ocean is becoming more and more extensive and harmful to marine ecosystems and to human health (Herrera et al., 2022). These reach the ocean, where degradation and other processes of the ocean environment itself cause this waste to fragment and erode into smaller pieces, defined as microplastics (MP) (Andrady, 2011; Ferrero and Failler, 2020; Frias and Nash, 2019, Herrera et al., 2022; Llorca et al., 2020). The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) defines microplastics as plastic particles with a diameter <5mm, including nanoplásticos (NPLs) that are below 1 nm (GESAMP, 2015; 2016). Due to ocean currents and subtropical gyres, these particles accumulate, where they can persist for hundreds of years. In addition to agglomeration in closed bays, gulfs and seas surrounded by populated coasts and watersheds (Eriksen et al., 2014: Ferrero and Failler, 2020).

Greenpeace International (2018) estimated that around 267 animal species are affected by plastic debris. This interaction with plastics involves entanglements, including abrasion, collisions, ingestion, and obstruction. As well, microplastics generate negative impacts on the marine biota, from zooplankton to large cetaceans, as well as birds and reptiles, as they accumulate in the stomach and get introduced to the food web (Eriksen et al., 2014; Murray, 2009; Ugwu et al., 2021). Besides plastic composition, which includes polymers and additives, the additives are used to improve the performance of the plastics and are flame retardants, UV filters, plasticizers, and antioxidants, among others. Plastic additives are not covalently bound to the polymer chains and can leach into living tissues or the environment (Amborgi et al., 2017; Llorca et al., 2020), and different groups have been related to environmental impacts such as endocrine disruption. Also, plastics adsorption properties can play an essential role in the transport to the biota of other organic contaminants, such as persistent organic pollutants (POPs) and contaminants of

emerging concern (CECs), contributing to the bioaccumulation and biomagnification of organic chemicals (Wurl and Obbard, 2004; Andrady, 2011). In particular, POPs such as polychlorinated biphenyl (PCBs), and polybrominated diphenyl ethers (BDEs), have a very high water-polymer distribution coefficient, K_p [l/kg], in favour of plastics (Andrady, 2011). Therefore, micro and nanoplastics act as vectors of contaminants throughout the marine biota, thus affecting human health due to their ingestion by species of commercial interest such as fish and bivalves (Andrady, 2011; Herrera et al., 2022; Llorca et al., 2020).

The Canary archipelago is affected by the massive arrival of plastics due to the Canary Current that transports plastics from the open Atlantic Ocean to the coast of these islands, especially to those beaches that have N-NE orientation (Baztan et al., 2014; Herrera et al., 2018, Rapp et al., 2020). Recent studies address the presence of microplastics on the beaches of Lanzarote (Baztan et al., 2014; Herrera et al., 2018), La Graciosa (Batzan et al., 2014; Herrera et al., 2018), Fuerteventura (Baztan et al., 2014), Gran Canaria (Herrera et al., 2018; Rapp et al., 2020; Santana-Viera et al., 2021), Tenerife (Álvarez-Hernández et al., 2019; González-Hernández et al., 2020; Reiold et al., 2020; 2021; Santana-Viera et al., 2021) and El Hierro (Hernández-Sánchez et al., 2021; Santana-Viera et al., 2021), while for the islands of La Palma and La Gomera only the study of Santana-Viera et al., 2021, has been documented. Likewise, there are very few studies on the pollutants associated with these microplastics in this archipelago (Camacho et al., 2019; Herrera et al., 2022).

This study analyzes the bioaccumulation of contaminants and additives associated with plastic on zebrafish tissues in different treatments: Control, virgin plastic “pellets”, and environmental plastic from Lambra and Porís beaches, La Graciosa and Tenerife islands, respectively.

2. Materials and methods

2.1 Microplastics and plastic additives

The microplastics in pellet form were referred to as virgin or synthetic plastics composed of low-density polypropylene (LDPP) (Sigma-Aldrich, ref 328116). Furthermore, they were classified as free of bioaccumulative toxic additives, as they had no contact with potential environmental contaminants.

The environmental plastics were collected on the beaches of Lambra, La Graciosa and Porís, Tenerife (Fig 1). Once in the laboratory, microplastics were grounded and then sieved with a 500µm mesh.

In order to analyze plastic additives, standards are needed to make a calibration line to quantify the concentrations. Therefore, Savva et al., 2022 methodology was followed.

Figure 1. Beaches where plastics were collected for treatments.

2.2 Ethical statement

To proceed with the experimental an ethical committee was requested where all procedures where fish and involved comply with the rules of the Council of the European Union (2010/63/EU) and Spanish legislation (RD 53/2013) in addition to being approved by the ethical committee of Bioethics of the University of Las Palmas de Gran Canaria (Ref. 06/2021 CEBA ULPGC).

2.3 Experimental design

2.3.1 Diet and experimental conditions

To analyze the additives of microplastics composition and the contaminants adsorbed onto microplastics surfaces that can be transferred to biota, zebrafish (*Danio rerio)*, were exposed to four different treatments: Control (A), virgin pellets (B), environmental plastics from Lambra (C) and Porís (D). Each treatment was carried out working in triplicates, and 12 treatments were carried out with 36 initial zebrafish per aquarium (Fig. 2).

Microplastics were prepared taking into account a percentage of 70% fragments and 30% pellets. They were grounded with a commercial coffee grinder and sieved with 500µm pore size mesh. The final product was mixed with the commercial feed (OMEGA ONE, 41%, fat 11%, ash 8% and fibre 2%) to obtain the MPs diets. The ingestion was one per day.

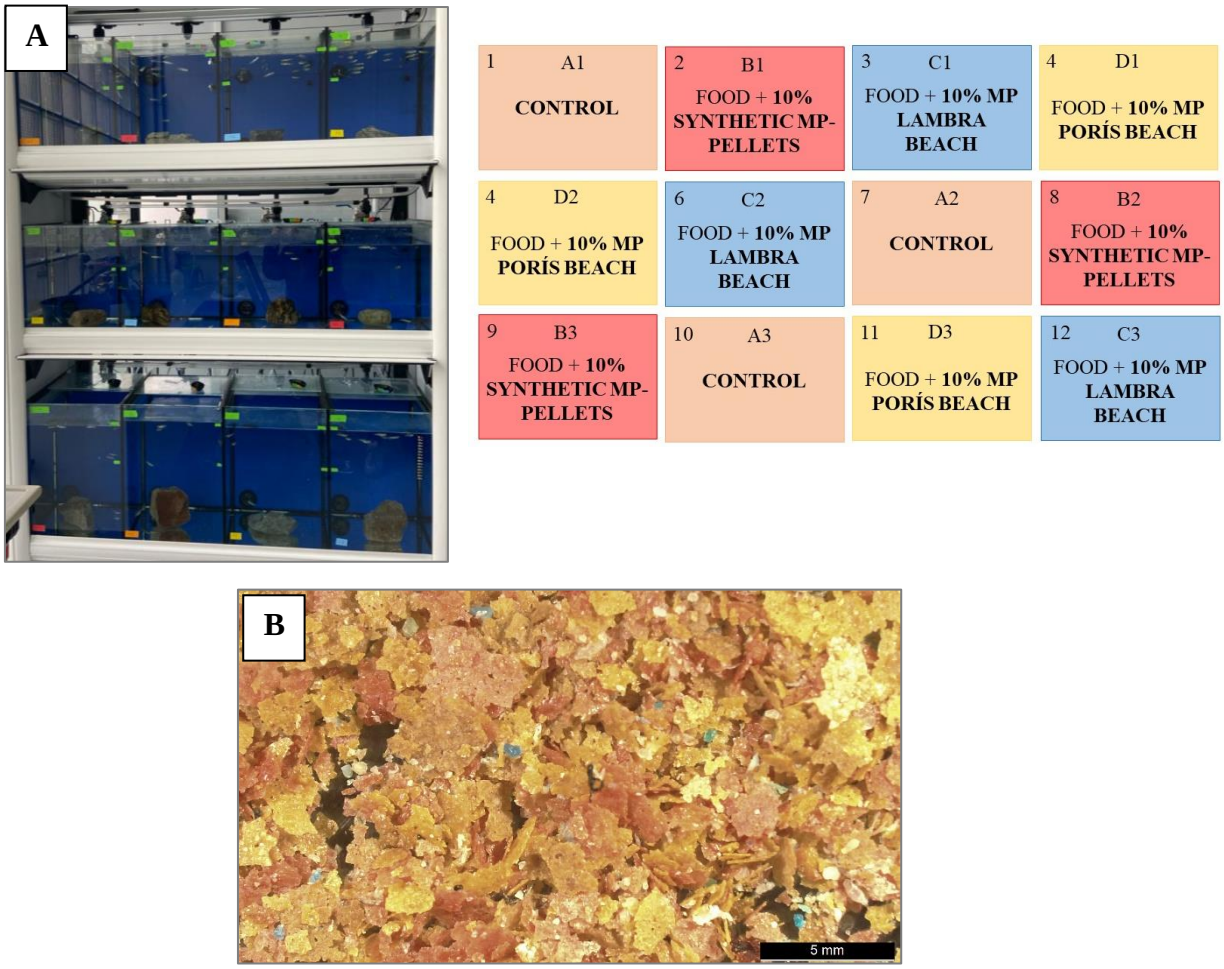


Figure 2. A) Organization of the different treatments. Treatments: A control, B Food with a 10% of synthetic fresh from factory, C environmental plastic from Lambra beach, La Graciosa and D environmental plastic from Porís beach, Tenerife. B) Example of diet D, plastics and food-flakes.

The experiment lasted two months, from February to April 2022, where the fish were cultured in the facilities of the ECOAQUA Institute, in the faculty of marine sciences at the ULPGC.

Zebrafish individuals were incubated in 30-40L tanks. The distribution of the aquariums was based on the treatments, as shown in Figure 2, was randomized to avoid possible effects due to position since, the lab was lighter coming from the left side than the right side; and temperature. In addition, the tanks contained air pumps to provide enough oxygen, thermostat and an own filtration system with sponges and bio balls. Also, once a week were checked temperature between 25-28ºC, pH 7-7.5, conductivity at 500-600 µS, nitrites with a maximum of 25 mg/L, nitrates at 0 mg/L and ammonium at 0-0.5 mg/L.

2.3.2 Samplings

Two samplings were performed on day 30 (T30) and day 60 (T60), i.e. at the middle of the experiment and at the end. At T30, 21 fish were caught per treatment, seven per tank, and euthanized at 300 mg/L of MS-222 (Matthews and Varga, 2012). In contrast, for T60, due to mortality during the experiment, the number was variable, and between 3-10 fish per tank were sampled.

The fishes were weighed and measured (Fig 3). Subsequently, the head and digestive system were removed since tiny microplastics were found retained in the tissues, which could alter the results in the analysis of contaminants and additives. Finally, the fish were grouped and stored in glass and/or aluminum tubes, where they were kept in the freezer at -80 ºC until analysis.

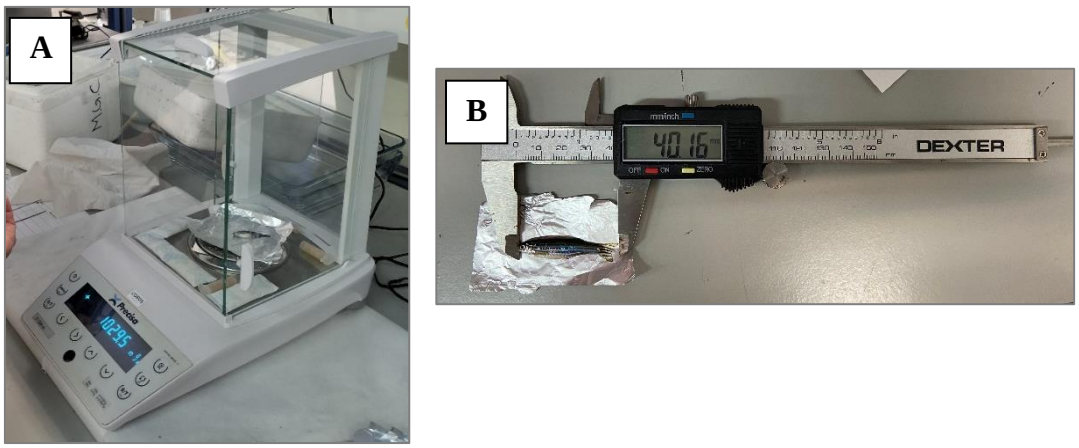


Figure 3. A) Analytical scale and B) digital meter. Weighing and measuring individuals in the two samplings

2.4 Analysis of plastic additives of plastic compositions and contaminants adsorbed onto microplastics surfaces

2.4.1 Extraction and pre-concentration processes

The samples were frozen and homogenized in an agate mortar. Subsequently, approximately 1g per sample were weighted working in triplicates. Then, the samples were extracted by ultrasound-assisted extraction (UASE) with 10ml of methanol for 15 minutes. This process was repeated three times per sample and the extracts were combined. Then, the combined extract per sample were dried with nitrogen at 30 ºC for 3 hours, to a final volume of 1-2ml, where they were centrifuged in order to separate the sample into solid and liquid phases. Finally, the methanol extracts were reconstituted in LC-vials to obtain a final volume of 1ml (Fig 4).

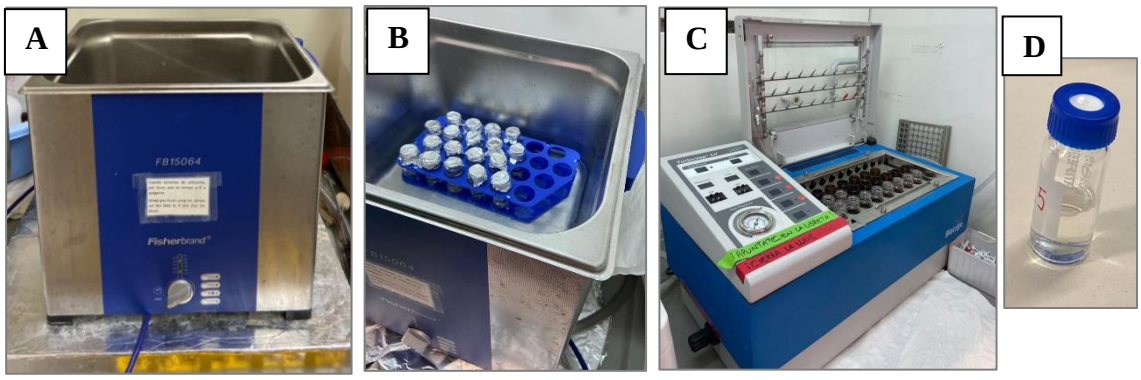


Figure 4. Extraction and pre-concentration processes. A) UASE, B) samples inside UASE, C) Evaporator with nitrogen and D) Final LC-Vial of 1 ml.

2.4.2 Analysis by liquid chromatography coupled to high resolution mass-spectrometry (LC-HRMS)

In the assessment of the transfer to the biota of plastic additives and organic contaminants adsorbed on the surface of real beached microplastics, the methanol extracts were analyzed by liquid chromatography coupled to a high-resolution mass spectrometer (LC-HRMS) (Fig 5). The chromatographic separation was achieved with an Acquity LC chromatograph, equipped with a Purospher© STAR RP-18 (5µm, 2 ×125mm) analytical column from Merck.

The mobile phase consisted of (A) HPLC-water and (B) acetonitrile (in negative mode) or HPLC-water acidified with 0.05% formic acid (in positive conditions). The elution gradient conditions for the LC mobile phase started with 90% eluent A holding for 2 min and decrease to 10% in 8 min, holding for two more min and raising to initial conditions (90% A) in one min and, finally, the re-equilibration of the system was achieved in 2 min.

First, the equilibrating process for this column was performed with water and acetonitrile at a flow rate of 0.05mL/minute. Blanks of methanol were inserted along each chromatographic batch to ensure the cleaning of the system and avoid carry overs. At the end, two solvents, water at 90% and acetonitrile at 10% were used for each batch. The injection volume was 20 µL sample and each chromatographic run took 15 minutes.

The chromatographic system was coupled to a QExactive Orbitrap equipped with an electrospray ionization (ESI) source operated in negative and positive ionization conditions in two different injections.

Data was acquired in full scan (50-1500 Da) with an FWHM of 70,000 and, in parallel, in data-dependent scan at a resolution of 35,000 FWHM where the 10 most intense ions

from the first full scan were further fragmented with an isolation of 1.0 Da and with a collision energy of 30 a.u.

The whole data was processed by means of Compound Discoverer 3.1.

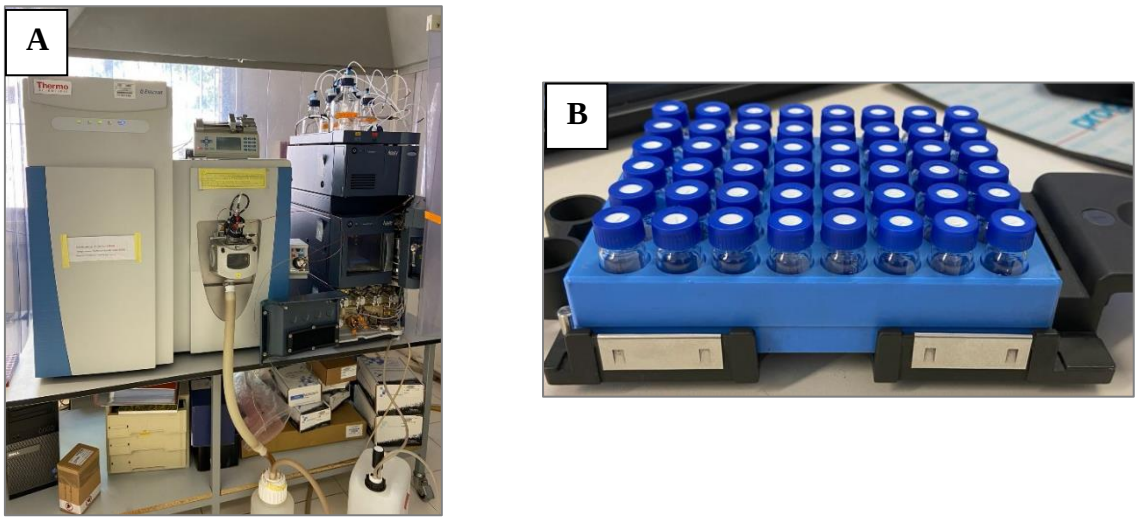


Figure 5. A) LC-HRMS, B) finals LC-vials.

2.4.3 Data processing and suspect screening

In figure 6, the general workflow is summarized. The row data of LC-QExactive analysis was processed using an automated screening with Compound Discoverer version 3.1 (Thermo Fisher Scientific). The first screening steps included peak picking, retention times (RT), alignment and grouping of isotopes and adducts, as well as grouping compounds across samples and predicting structures (Identification at confidence level 5). The first list of suspects compounds was filtered compared with ChemSpide and mz Cloud databases (confidence level 4). The subsequent filtering compared of isotopic patterns, ionization efficiency and fragmentation pattern (confidence level 3). The subsequent filtering was based on comparing the product-ions, obtained from the MS/MS spectrum, of a suspect compound (confidence level 2), using the information in the online databases. The confirmation or quantification was only possible when a reference standard was available, and confidence level 1 was achieved.

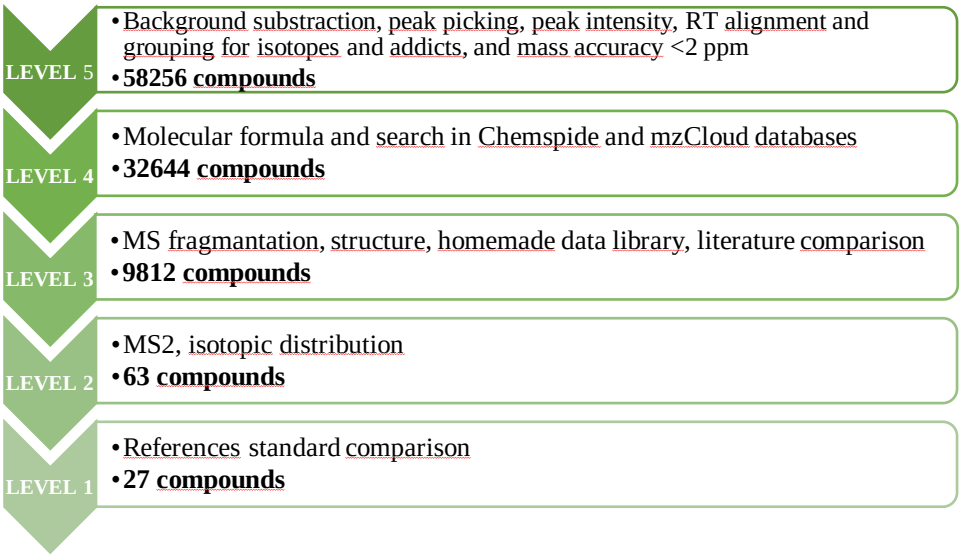


Figure 6. Methodology workflow

2.5 Statistical analysis

Statistical analysis was performed using the R Version 4.1.2. with RStudio Version 1.4.1106. Shapiro-Wilk's test checked the normality of data, but as the data were not normal (p-value<0.05), Kruskal-Wallies test was applied. Also, Conover post-hoc test was done to verify significant differences between treatments. Finally, a one-way *ANOVA test* was applied to determine if there were significant differences (*p-value <0.05*). Graphics were performed in RStudio using gglpot2, FactoMineR packages.

The compounds that were identified at level 2 of confidence were normalized according to the peak area of the chromatogram for each compound divided by the corresponding sample weight, in g, and then normalized for the highest relation for each compound. Then, multivariate analyses using Principal Component Analysis (PCA) were carried out to understand further the variations among treatments and the contribution of the several chemical compounds identified at level 2. Correlation among variables and PCs was quantified as the square cosine of their angle in the loading graph, which may range from 0 ($\alpha = 90°$: orthogonal, totally uncorrelated) to 1 ($\alpha = 0°$: parallel, perfectly correlated) (Vega-Herrera et al., 2021).

3. Results

3.1 Plastic additives and organic contaminants adsorbed onto beached microplastics used for exposure experiments

Before starting the feeding-experiment, the presence of chemical pollutants and plastic additives that can leach from microplastics collected in Canary Island beaches were analysed (Fig 7 and 8).

Plastic additives that were identified included, plasticizers, UV-filters, and plastic synthesizers were identified at confidence level 2. Most common plastic additives identified were plasticizers such as benzoic acid. In addition, a series of organic contaminants not related to plastic additives were as well identified in the microplastics used for the different exposure experiments. For example, lubricants and pesticides were present in the treatments B and D.

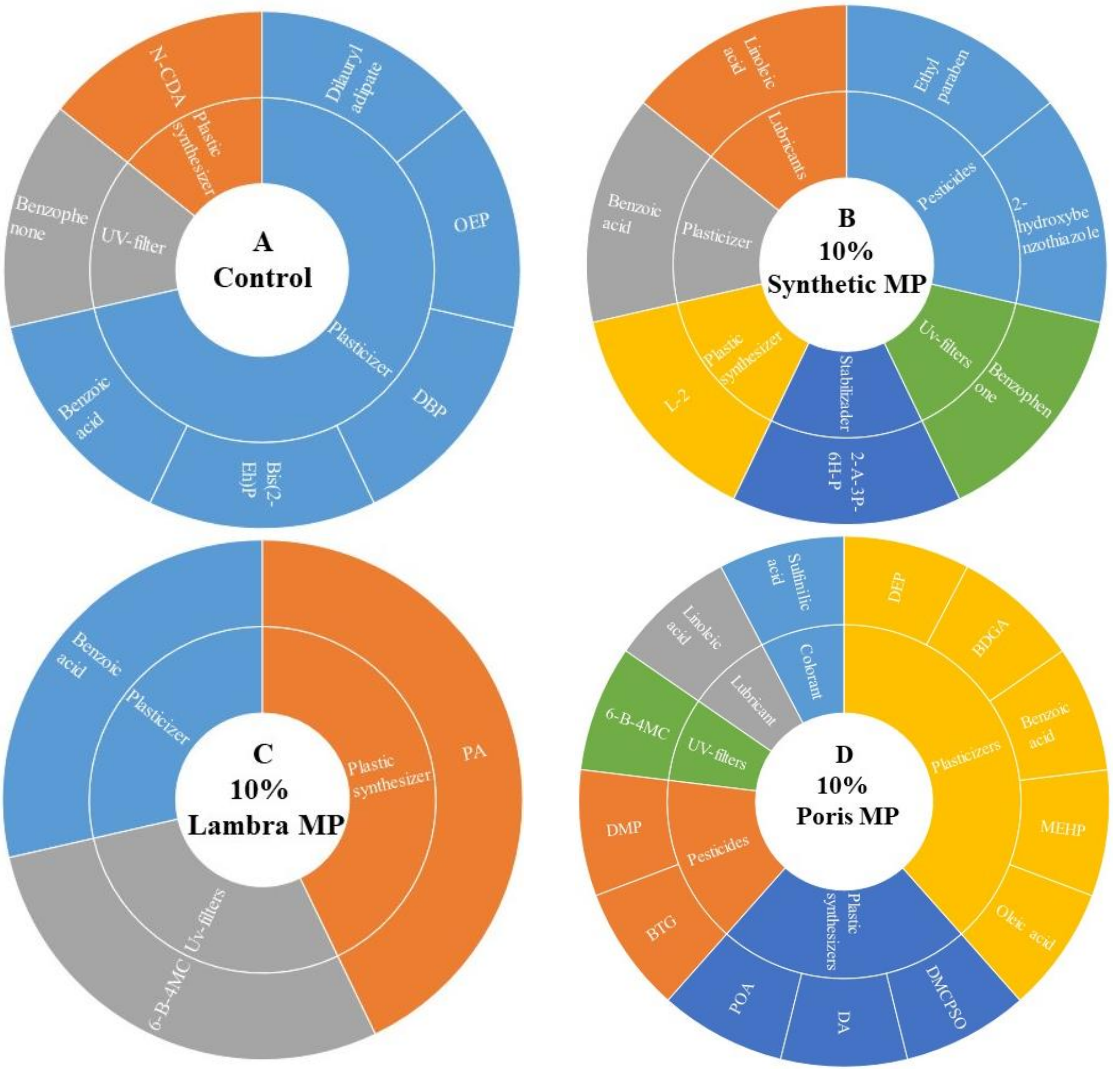


Figure 7. Presence of contaminants in the four treatments before the feeding-experiment.

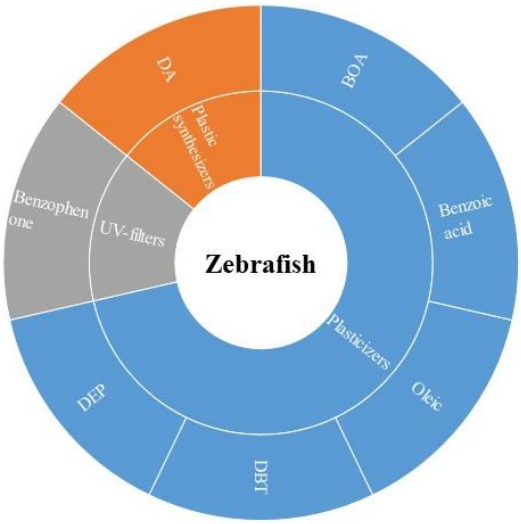


Figure 8. Presence of contaminants in zebrafish tissues before the feeding-experiment.

3.2 Chemicals transferred to zebrafish tissues

Table 1, shows all the chemical contaminants that reached level 2, a total of 63 compounds. Of these, only 27 could be quantified, while the remaining 36 compounds were normalized by the peak area of the chromatogram in weight and in percent per one.

Table 1. Contaminants present in zebrafish tissues. In green those chemical pollutants, which could be quantified.

Pollutant	Family	Pollutant	Family	Pollutant	Family
POA	Plastic synthesizer	11-ADA	Plastic synthesizer	M-2(TA)EP	Plasticizer
2Ms-3AN	Plastic synthesizer	HEMA	Plastic synthesizer	Bis(2-Eh)P	Plasticizer
DEAL	Plastic synthesizer	DA	Plastic synthesizer	D4	Plastic additive
Searic acid	Plastic synthesizer	N-CDA	Plastic synthesizer	N-B-2HE-DA	Plastic additive
V-70	Plastic synthesizer	Sorbic acid	Plasticizer	DL-Histidine	Plastic additive
Styrene	Plastic synthesizer	Oleic acid	Plasticizer	Benzophenone	Plastic additive
PA	Plastic synthesizer	Sorbic acid	Plasticizer	Benzaldehyde	Plastic additive
n-BMA	Plastic synthesizer	Linoleic	Plasticizer	5-PR	Plastic additive
MS	Plastic synthesizer	PAh	Plasticizer	4-BBP	Plastic additive
MSPB	Plastic synthesizer	OEP	Plasticizer	6-B-4MC	Plastic additive
LA	Plastic synthesizer	N-5cA(1-A)-BA	Plasticizer	9-OA	Plastic additive
L-2	Plastic synthesizer	N-1,4-BA	Plasticizer	Naphthalene	Pesticide
Lauramide	Plastic synthesizer	DNAP	Plasticizer	E-2-BCHO	Pesticide
HMCTSO	Plastic synthesizer	DEP	Plasticizer	DMP	Pesticide
DEGE	Plastic synthesizer	DBP	Plasticizer	BTG	Pesticide
DMCPSO	Plastic synthesizer	Coumarone	Plasticizer	2,6-DCHO	Pesticide
BDGA	Plastic synthesizer	BOA	Plasticizer	2-A-3P-6H-P	Stabilizader
BPA	Plastic synthesizer	Benzoic	Plasticizer	Nadic Anhydride	Paint
4-PCH	Plastic synthesizer	4-DBS-BA	Plasticizer	Coumarin	Paint
4-NP	Plastic synthesizer	2,6-DMN	Plasticizer	6-B-4MC	UV-filters
2-MN	Plastic synthesizer	2-A-3HP-2(TA)EP	Plasticiszer	Sulfanilic acid	Colorant

The results show of the sum of pollutants by family at day 30 show that the plastic family with the highest concentration in treatments A and D are plastic synthesizers (5290.9±4180.3ng/g, 6130.6±1653.8ng/g, respectively), while in treatments B and C are plasticizers (2922.4±1019, 2576.7±2288.5 ng /g). On the other hand, at day 60, the control group (A), showed the plasticizers with the highest concentration (3494.5±885.8 ng/g), while in the rest of the treatments were plastic synthesizers (B, 5177.4±2725.6 ng/g; C, 5404.3±2299.6 ng/g; D, 2996±1250 ng/g) (Table 2, Figure 9).

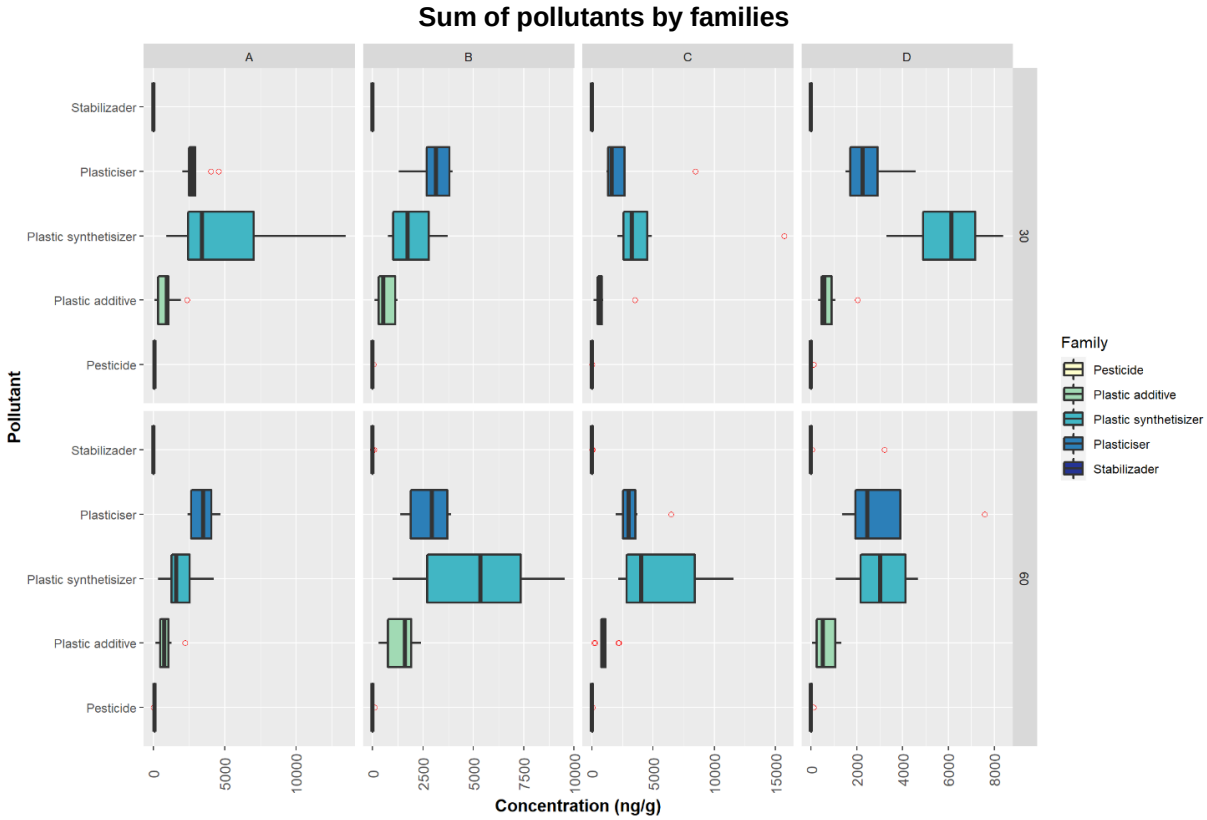


Figure 9. Concentration in (ng/g) of sum of chemical pollutants by families in both days 30 (top) and 60 (down). The central thick line of each boz designates the median, the box height shows the interquartile range, the whiskers indicate the lowest and the highest values and the circles point the values of outliers.

Only plastic synthesizers and pesticides showed significant differences between treatments (Kurskal-Wallies tests p-value<0.05). On the other hand, pesticides concentrations were significantly higher in treatment A in both days 30 and 60 (Conover PostHoc test p<0.05). However, plastic synthesizers have the highest mean concentration of chemical pollutants in treatment D (Conover Post-Hoc test p<0.05) containing environmental MP from Poris beach, with 6130.6 ± 1653.8 ng/g. Followed by control treatment, A, with 5290 ± 4180.3 ng/g, both at day 30. In the case on day 60, the highest mean concentration is in treatment C with 5404.3 ± 3399.6 ng/g, followed by treatment B with 5177.4 ± 2725.6 ng/g (Figure 10 and Table 2).

Table 2. Concentration (ng/g) of pollutants grouped on plastic families, present on the four treatments (A, B, C and D) and in both days (30 and 60) (mean, standard deviation, median, minimum and maximum values). A color scale was used, ranging from yellow for the lowest values, followed by green to blue to highlight the highest values.

Pollutants in Zebrafish tissues

Mean, sd and median, minimum and maximum values of sum by group

family	time	treatment	mean	sd	median	min	max
Pesticide	30	A	68.7	37.0	84.0	0.0	121.2
Pesticide	30	B	3.9	11.7	0.0	0.0	35.0
Pesticide	30	C	7.5	14.8	0.0	0.0	34.7
Pesticide	30	D	12.4	37.1	0.0	0.0	111.3
Pesticide	60	A	81.1	39.7	95.2	0.0	139.5
Pesticide	60	B	20.1	39.9	0.0	0.0	92.1
Pesticide	60	C	7.7	23.2	0.0	0.0	69.7
Pesticide	60	D	11.0	33.1	0.0	0.0	99.4
Plastic additive	30	A	960.5	768.2	948.3	102.4	2378.2
Plastic additive	30	B	690.5	475.8	544.3	97.6	1261.0
Plastic additive	30	C	909.0	1000.7	704.2	142.1	3494.1
Plastic additive	30	D	783.9	533.2	594.4	312.4	2035.0
Plastic additive	60	A	882.3	630.2	780.4	165.3	2238.1
Plastic additive	60	B	1439.8	738.4	1620.7	302.1	2403.3
Plastic additive	60	C	1052.0	717.8	878.2	170.2	2221.4
Plastic additive	60	D	597.4	470.4	518.7	32.2	1332.4
Plastic synthetisizer	30	A	5290.9	4180.3	3404.5	935.1	13477.6
Plastic synthetisizer	30	B	2027.9	1171.6	1735.1	768.6	3747.9
Plastic synthetisizer	30	C	4762.1	4204.1	3279.2	2100.5	15689.3
Plastic synthetisizer	30	D	6130.6	1653.8	6145.2	3296.5	8397.6
Plastic synthetisizer	60	A	2012.2	1144.2	1616.8	324.4	4263.0
Plastic synthetisizer	60	B	5177.4	2725.6	5346.6	994.5	9532.5
Plastic synthetisizer	60	C	5404.3	3399.6	4001.6	2159.3	11579.4
Plastic synthetisizer	60	D	2996.0	1250.3	3022.5	1069.0	4679.7
Plasticiser	30	A	2953.2	809.2	2714.8	2045.6	4562.2
Plasticiser	30	B	2922.4	1019.0	3143.2	1304.9	3959.4
Plasticiser	30	C	2576.7	2288.5	1601.0	1202.4	8453.9
Plasticiser	30	D	2531.9	1008.3	2246.3	1502.6	4575.6
Plasticiser	60	A	3494.5	885.8	3497.3	2417.9	4712.2
Plasticiser	60	B	2744.4	958.6	2947.2	1375.2	3892.6
Plasticiser	60	C	3220.4	1365.2	2996.7	1942.2	6469.4
Plasticiser	60	D	3093.4	1902.0	2458.5	1344.3	7574.2
Stabilizader	30	A	0.0	0.0	0.0	0.0	0.0
Stabilizader	30	B	0.0	0.0	0.0	0.0	0.0
Stabilizader	30	C	0.0	0.0	0.0	0.0	0.0
Stabilizader	30	D	0.0	0.0	0.0	0.0	0.0
Stabilizader	60	A	0.0	0.0	0.0	0.0	0.0
Stabilizader	60	B	7.4	20.7	0.0	0.0	62.5
Stabilizader	60	C	13.2	24.0	0.0	0.0	68.9
Stabilizader	60	D	360.1	1068.6	0.0	0.0	3209.7

The rest of the chemical groups did not show significant differences (Kurskal-Wallies tests p-value>0.05). For plasticizers on day 30, if we look at the median, treatment B has the highest concentration with 2922.4 ± 1019 ng/g, and on day 60 was treatment A with 3494.5 ± 885.8 ng/g. In plastic additives, the highest mean concentration was on treatment A with 960.5 ± 768.2 ng/g on day 30, while on day 60 was treatment B with 1439.8 ±738.4 ng/g. Finally, stabilizers only showed a mean concentration in treatment D with 360 ± 1068.6 ng/g, followed by treatment C with 13.2 ng/g, both at day 60 (Figure 10 and Table 2).

Table A2 (Annex) show the mean, sd, median minimum and maximum of the sum of pollutants by chemical groups, where the pollutants are grouped by chemical groups present in zebrafish tissues. Thus, it is found that on both days, the chemical groups with the highest concentration are cyclohexenes with 5071.4 ± 4249 ng/g on treatment A and phosphates, with 1638.2 ± 663 ng/g, in treatment D at day 30, as well as 5619.9 ± 3373.4 ng/g and 3031.1 ± 872.5 ng/g on treatments B and A at day 60.

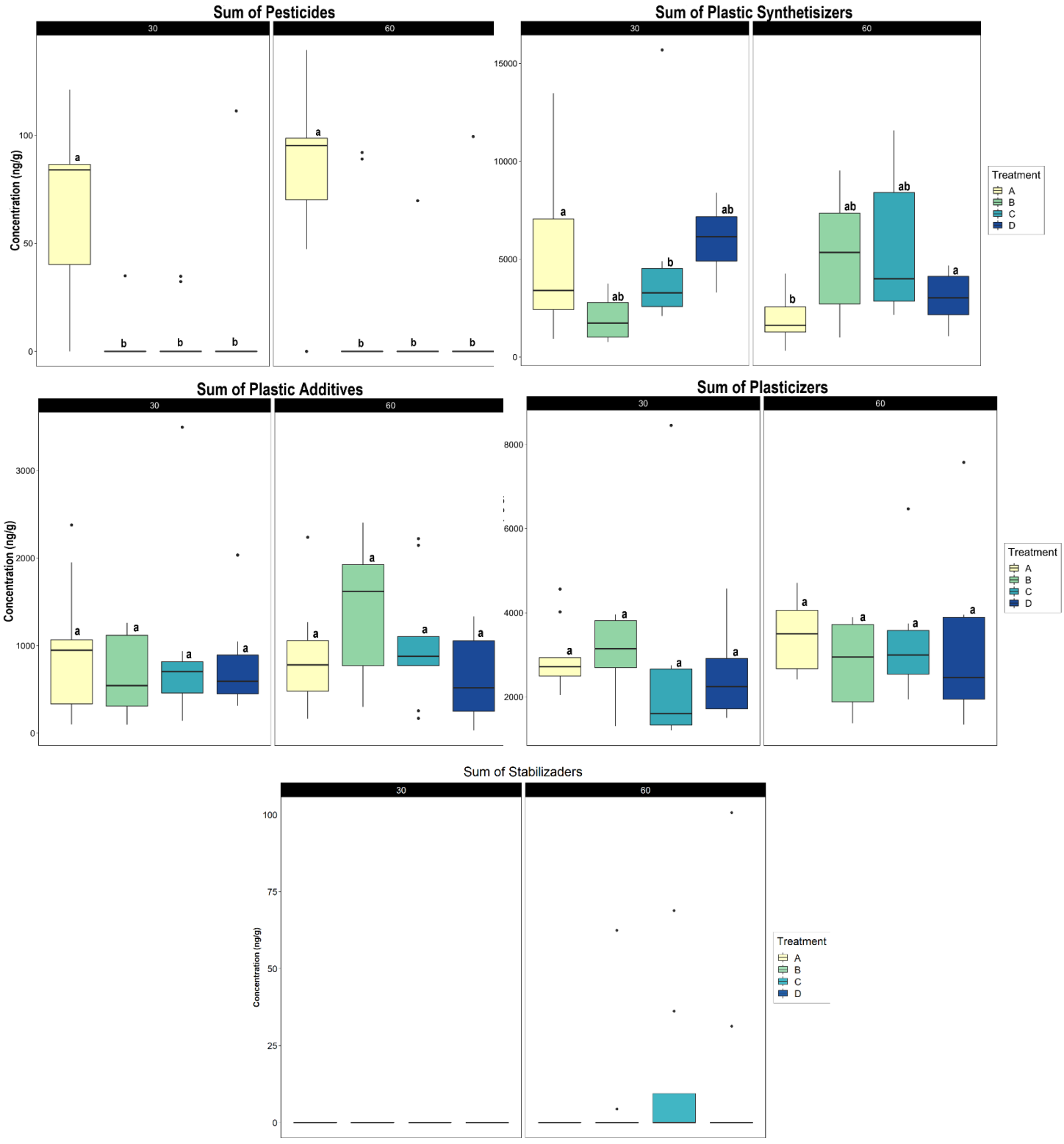


Figure 10. Concentration of chemical pollutants (ng/g) in zebrafish tissues at day 30 and 60. The central thick line of each box designates the median, the box height shows the interquartile range; the whiskers indicate the lowest and the highest values and the circles point the values of outliers. Different letters indicate significant differences in Kruskal-Wallies analysis and Conver post hoc test at day 30 and 60.

Regarding the chemicals identified at confidence level 2, PCA was built according to statistics descriptions for different treatments and days. Figure 11 shows the PCA scores on days 30 and 60 for the four treatments. A PCA is a statistical method that simplifies the complexity of high-dimensional sample spaces while preserving their information. In the case of the score plot, the axes PC1 and PC2 are the different linear combinations that give the most significant variation of samples. The first two principal components explain 25.1% and 8.9% for treatments on day 30, while 21.5 and 13.3% explain the treatments at day 60 of the data variance (data not shown), thus indicating the high dimensionality and complexity of the data.

The statistical study shows how the 37 tentative compounds were distributed among treatments. As seen in Figure 11, the feeding with different plastic types or origin (B, C and D) generates the leaching of different chemical pollutants once the plastic particles are ingested by zebrafish. In addition, the control treatment, A, had a different profile compared to the fishes fed with plastics.

In addition, the chemical groups show significant differences between treatments (Kruskal-Wallis p-value < 0.05), except the family of UV-filters and lubricants at day 60.

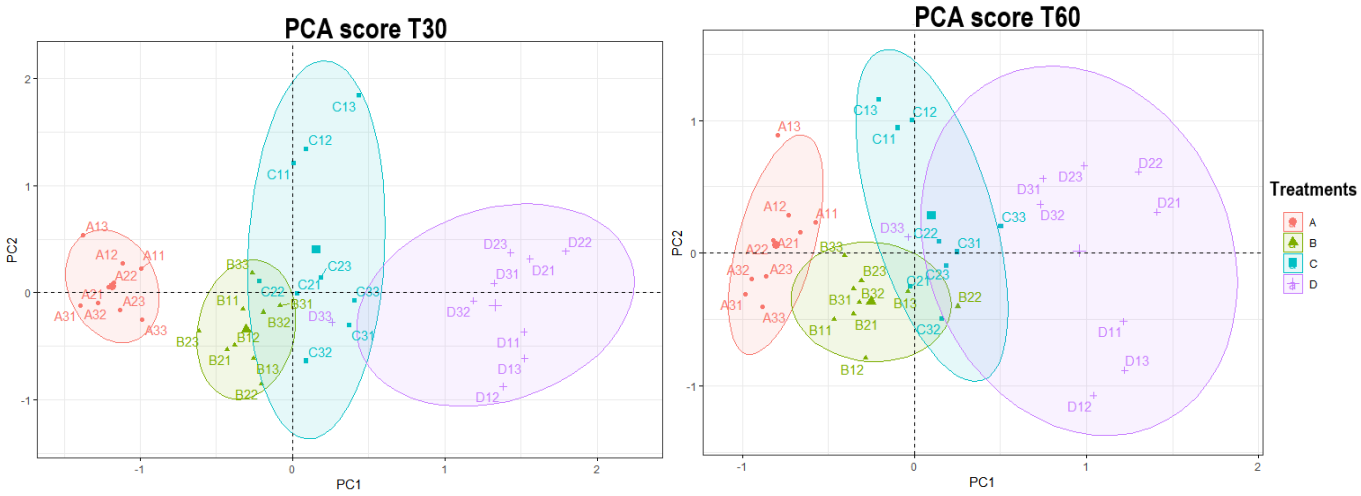


Figure 11. Scores T30 and T60 plot made by Principal Component Analysis (PCA) and applied to the treatment samples.

4 Discussion

Microplastic pollution is one of the biggest dangers to the aquatic environment and affects marine organisms and the entire food chain. The present study contributes to the already long list of adverse effects on biota. It provides new insights about long-term effects and bioaccumulation of microplastic pollution in which organic contaminants adsorbed onto particles surfaces, and the leaching of plastic additives coming from plastic formulations was studied using as a model organism the zebrafish.

There are scarce studies on exposure to microplastics on zebrafish in a feeding experiment (Qiang and Cheng, 2021; Lei et al., 2018; Tarasco et al., 2022) and fewer where chemical pollutants are analyzed (Rainieri et al., 2018).

This work is one of the few studies where a long-term experiment is carried out, where fish are fed with pristine microplastics, and environmental plastic collected from different beaches of the Canary Islands, which are considered hotspots of massive plastic arrivals from the Atlantic. In addition to the analysis all the pollutants in the tissues of zebrafish using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Also, all the pollutants were analyzed by means of a non-target screening to describe the fingerprint of contamination of microplastics and their potential to be transferred to biota and bioaccumulated.

In regard to organic contaminants in microplastics, it can be seen that their maximum means concentration is found in treatment D, with 10% of microplastics from Poris beach, at day 30, on plastic synthesizers with 6130.6 ± 1653.8 ng/g; and at day 60 with 5404.3 ± 3399.6 ng/g. They were followed by plastic additives of plastic formulations such as plasticizers in treatment A, control, at day 60 with 3494.5 ± 885.8 ng/g and on day 30, with 2953.2 ± 802.9 ng/g. However, significant differences were found between treatments only in the pesticide and plastic synthesizers families each time.

In the case of pesticides, it can be seen that the control treatment, A, also plastic-free, has the highest concentration of pollutants. In addition, the highly non-polar compounds present a low bioavailability due to their high *n*-octanol-water partition coefficient (K_{ow}), consequently they have more affinity to remain adsorbed on plastic surface instead of being desorbed once the plastics are ingested by zebrafish. In several studies, show that the pollutants like polychlorinated biphenyls (PCBs) even perfluoro sulphonates and sulphonamides, have more affinity to be comparted onto the microplastics. When bioavailability decreases, even if higher amounts are ingested, fewer concentrations can be accumulated in the tissues (Llorca et al., 2018 and 2020).

Bioaccumulation over time is also appreciated. It is worth pointing out that before the feeding experiment, the fishes presented some pollutants like their food. The previous analysis was still level 2, tentative identification, when few compounds are before and after the experiment. Therefore, it indicates that more prolonged exposure to these microplastics increases the concentration of pollutants in zebrafish tissues (Figure 7, 8 and 9; Table 2, A1). In another study using seabass (*Dicentrarchus labrax)* as a test specie *(*Herrera et al., 2022), the plastic additives accumulated in liver; while in the study by Asgba et al., 2008, showed that the bioaccumulation of cadmium was preferential in the liver and kidney, and during the detoxification phase, was detected in the bloodstream. Moreover, since these organs are the major organs of metabolic activities, including detoxification, the pollutants might be transported into these organs from other tissues like the muscle and gills. In our study, we used all the fish except the head and the digestive tract to avoid any microplastics clogged in the system, so the tissue translocation was not studied, but the bioaccumulation potential was assessed.

On the other hand, in level 2, tentative identification, on suspect screening, plasticizers family is the one with the highest number of compounds in zebrafish tissues, followed by plastic synthesizers. Plasticizers group is usually found in high concentrations in plastic formulation, in general are the plastic additives used at higher concentrations, as a consequence in water samples, sediments, are as well the plastic additives more frequently found (Teo et al., 2015). Despite plasticizers were those more frequently found in the samples, in this work, a higher number of plastic synthesizers were quantified because the available standards in the lab among those that were detected in these samples.

The effect of different polymers of microplastics in their transfer of contaminants and the analysis of visible effects on zebrafish were not analyzed in this study. However, other studies have shown that the physicochemical properties of the different plastics can affect the adsorption, release and transport of chemical pollutants, and different types of additives are added depending in the type of polymer (Gouin, 2021; Llorca et al., 2022).

We consider this study preliminary but important in terms of the way the experiment was conducted as the plastic was collected from the environment where it has been floating over the ocean and has been affected by UV radiation. These plastics are the ones to which marine organisms are exposed. For example, in the study by Tarasco et al., 2022, they demonstrate that long-term dietary exposure to contaminating polyethylene microplastics has the potential not only to jeopardize fish growth and reproductive performance but also to produce an intergenerational effect. Also, present the first evidence of the adverse effects on axial fish skeleton and bone compartment upon the ingestion of microplastics.

The present study provides evidence that chemical pollutants in environmental microplastics have been transferred to the tissues after 60 days of feeding experiment with 10% of EMPs. In addition, to present that pollutants are adsorbed to microplastics which indicates that zebrafish food has higher bioaccumulation than plastic diets.

5 Conclusion

- The bioaccumulation of chemical pollutants in zebrafish tissues is verified. In addition to increasing their concentration over time.

- The plastic-free treatment, control, showed higher concentrations in pesticides. Because pollutants had more tendency to adsorb to microplastics instead of being desorbed.

- In the level 2 tentative identification, there are more compounds from the family of plasticizers followed by plastic synthesizers.

- Plastic synthesizers were the plastic additives that were identified and quantified at the highest concentration in zebrafish tissues, followed by plasticizers, but the availability of standards should be considered because many of the plasticizers identified at level 2 were not quantified because the lack of standards.

- The unquantified data show that contaminants of the plastic treatments correlate with each other, while the control group does not.

Annexes

Table A1. All the chemical compounds found on level 2, tentative identification; with their abbreviation, chemical group and their family.

Pollutant	Abbreviation	Chemical group	Family plastic
Palmoleit acid	POA	Acid	Plastic synthesizer
2-(Methylsulfonyl)-3-(pyrazin-2-ylamino)acrylonitrile	2Ms-3AN	Nitrile	Plastic synthesizer
Diethanolamine laurate	DEAL	Amine	Plastic synthesizer
Stearic acid	Searic acid	Acid	Plastic synthesizer
V-70	V-70	Nitrile	Plastic synthesizer
Styrene	Styrene	Benzene	Plastic synthesizer
Phthalimide	PA	Amide	Plastic synthesizer
n-butyl methacrylate	n-BMA	Acrylate	Plastic synthesizer
Myristyl sulfate	MS	Sulfate	Plastic synthesizer
Myristamidopropyl betaine	MSPB	Betaine	Plastic synthesizer
Lily aldehyde	LA	Aldehyde	Plastic synthesizer
Laureth-2	L-2	Benzonate	Plastic synthesizer
Lauramide	Lauramide	Amide	Plastic synthesizer
Hexamethylcyclotrisiloxane	HMCTSO	Cycloxane	Plastic synthesizer
Diethylene glycol n-butyl ether	DEGE	Glycol	Plastic synthesizer
Decamethylcyclopentasiloxane	DMCPSO	Cycloxane	Plastic synthesizer
Butyldiglycol acetate	BDGA	Acetate	Plastic synthesizer
Bisphenol A	BPA	Phenol	Plastic synthesizer
4-Phenylcyclohexanone	4-PCH	Cycloxane	Plastic synthesizer
4-Nonylphenol	4-NP	Phenol	Plastic synthesizer
2-Methylnaphthalene	2-MN	PHA	Plastic synthesizer
11-Aminoundecanoic acid	11-ADA	Acid	Plastic synthesizer
(Hydroxyethyl)methacrylate	HEMA	Acrylate	Plastic synthesizer
Dodecanedioic acid	DA	Acid	Plastic synthesizer
N-Caprylyldiethanolamine	N-CDA	Amine	Plastic synthesizer
Oleic acid	Oleic acid	Acid	Plasticiser
Sorbic acid	Sorbic acid	Acid	Plasticiser
Linoleic acid	Linoleic	Acid	Plasticiser
Phthalic anhydride	PAh	Anhydride	Plasticiser
Oleoyl Ethanolamide Phosphate	OEP		Plasticiser
N-{5-carbamimidamido-1-[(naphthalen-2-yl)amino]-1-oxopentan-2-yl}benzamide	N-5cA(1-A)-BA	Amide	Plasticiser
N,N'-Bis(4-methyl-2-pentanyl)-1,4-benzenediamine	N-1,4-BA	Amine	Plasticiser
Di-n-Amyl phthalate	DNAP	P	Plasticiser
Diethyl phthalate	DEP	Phthalate	Plasticiser
Dibutyl phthalate	DBP	Phthalate	Plasticiser
Coumarone	Coumarone	Benzfuran	Plasticiser
Benzyl octyl adipate	BOA	Adipate	Plasticiser
Benzoic acid	Benzoic	Acid	Plasticiser
4-Diisobutylsulfamoyl-benzoic acid	4-DBS-BA	Acid	Plasticiser
2,6-dimethylnapthalene	2,6-DMN	PHA	Plasticiser
2-(Acryloyloxy)-3-(hexadecyloxy) propyl 2-(trimethylammonio) ethyl phosphate	2-A-3HP-2(TA)EP	Phosphate	Plasticiser
(2-Pentadecyl-1,3-dioxolan-4-yl) methyl 2 (trimethylammonio) ethyl phosphate	M-2(TA)EP	Phosphate	Plasticiser
Bis(2-ethylhexyl) phthalate	Bis(2-Eh)P	Phthalate	Plasticiser
Octamethylcyclotetrasiloxane	D4	Cycloxane	Plastic additive
N,N-Bis(2-hydroxyethyl)dodecanamide	N-B-2HE-DA	Amide	Plastic additive

DL-Histidine	DL-Histidine	Aminoacid	Plastic additive
Benzophenone	Benzophenone	Cetone	Plastic additive
Benzaldehyde	Benzaldehyde	Aldehyde	Plastic additive
5-Pentylresorcinol	5-PR	Resorcinol	Plastic additive
4-(t-Butyl)benzophenone	4-BBP	Phenone	Plastic additive
9-Octadecenamide	9-OA	Amide	Plastic additive
Naphthalene	Naphthalene	PHA	Pesticide
E-2-Benzylidenecyclohexanone	E-2-BCHO	Cycloxane	Pesticide
Dimethyl phthalate	DMP	Phthalate	Pesticide
Butoxytriglycol	BTG	Glycol	Pesticide
2,6-Di(adamantan-1-yl)cyclohexanone	2,6-DCHO	Cycloxane	Pesticide
2-Acetoxy-3-(octadecyloxy) propyl 6-(trimethylammonio) hexyl phosphate	2-A-3P-6H-P	Phosphate	Stabilizader
Nadic Anhydride	Nadic Anhydride	Anhydride	Paint
Coumarin	Coumarin	Benzofuran	Paint
6-t-Butyl-4-methylcoumarin	6-B-4MC	Benzofuran	UV-filters
Sulfanilic acid	Sulfanilic acid	Acid	Colorant

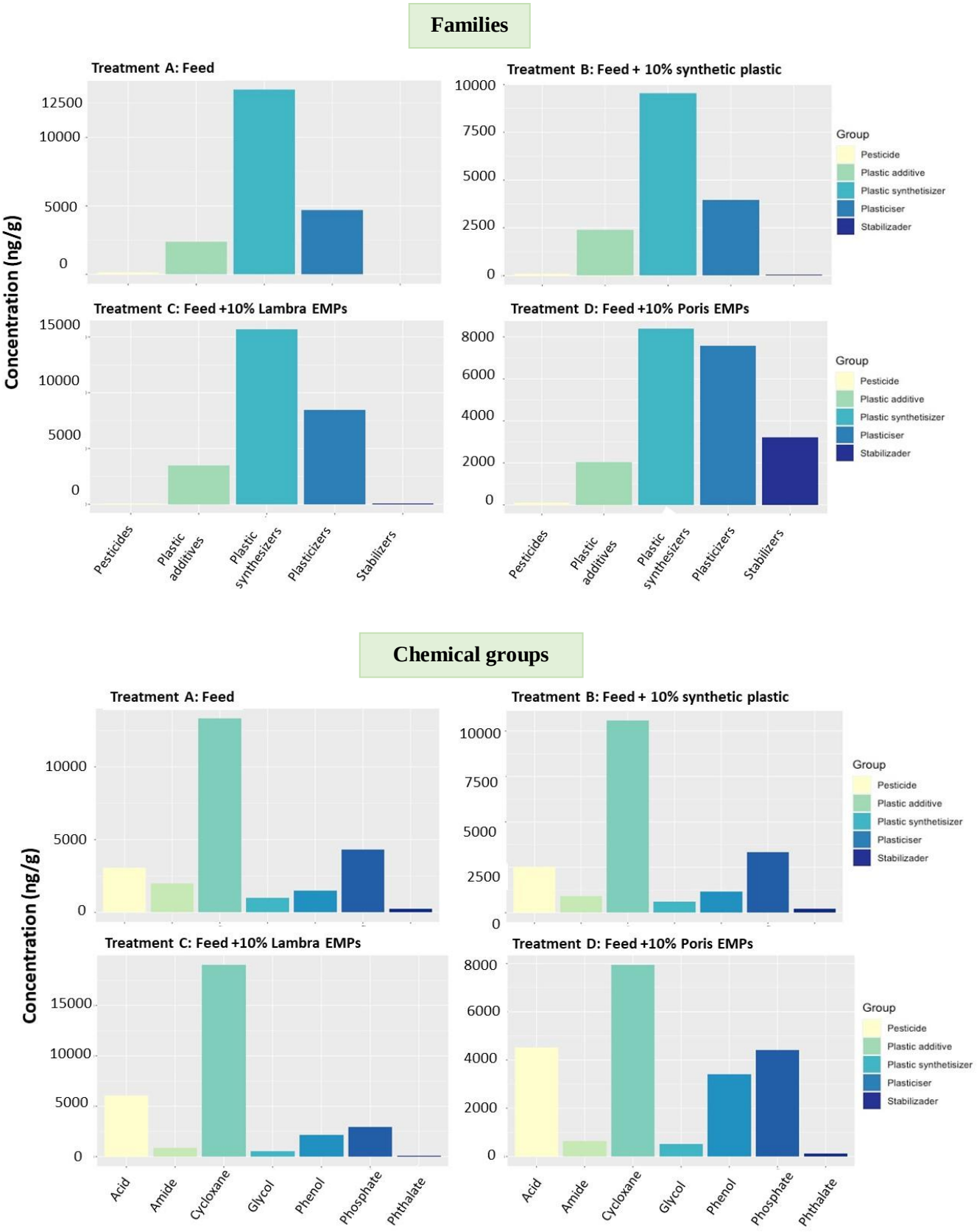


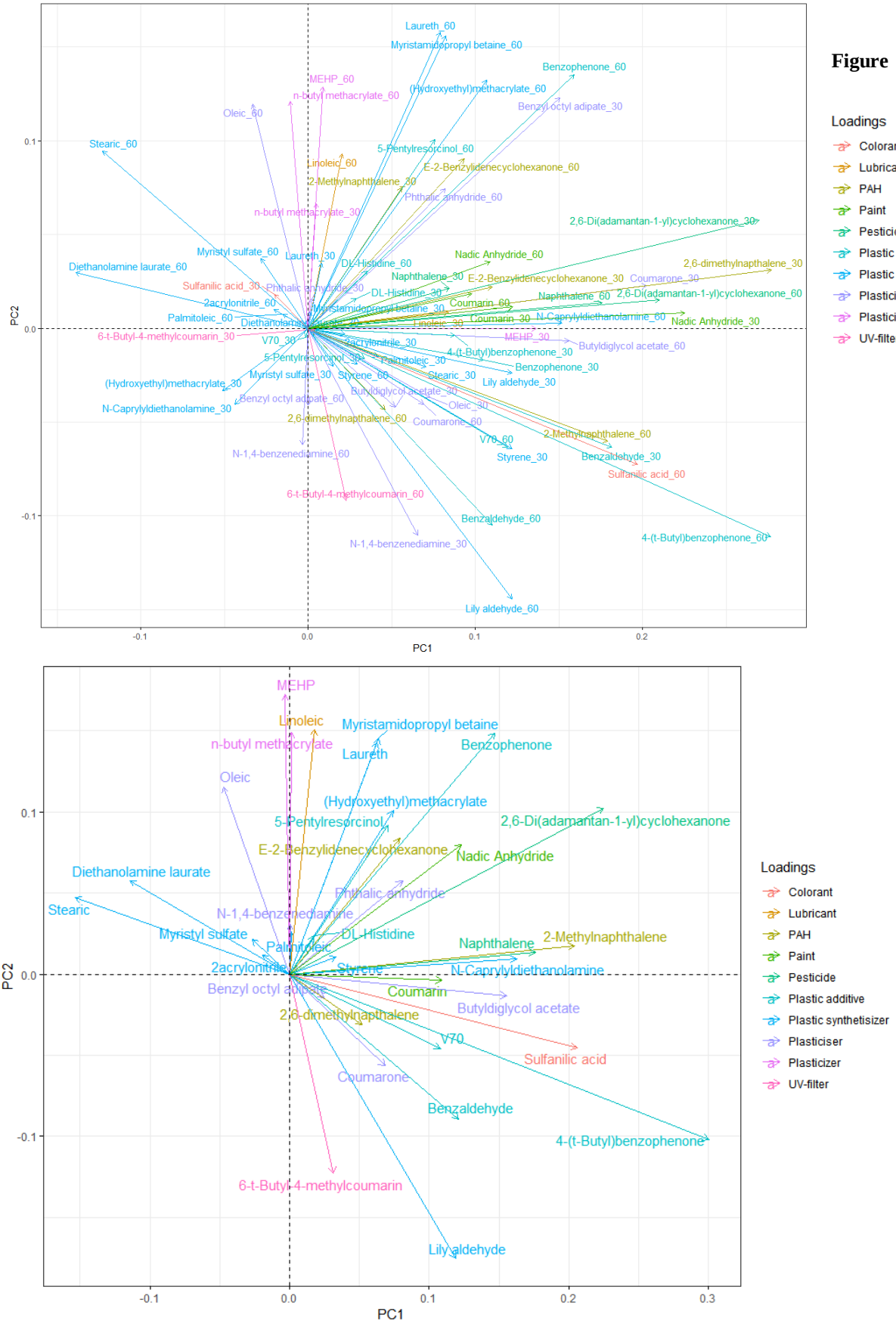
Figure A1. Average of the sum by family of plastic and chemical groups.

Pollutants in Zebrafish tissues

Mean, sd and median, minimum and maximum values of sum by group

group	time	treatment	mean	sd	median	min	max
Acid	30	A	1651.8	833.4	1733.5	533.6	3060.7
Acid	30	B	1569.8	672.5	1897.0	347.3	2523.4
Acid	30	C	1495.1	1747.6	778.7	475.6	6045.4
Acid	30	D	871.1	389.1	735.6	449.8	1591.1
Acid	60	A	377.0	342.8	254.2	120.8	1071.7
Acid	60	B	275.8	105.8	244.6	124.6	459.9
Acid	60	C	845.9	1012.8	468.9	254.7	3448.3
Acid	60	D	896.2	1388.8	440.5	117.3	4523.1
Amide	30	A	247.8	171.8	171.5	109.6	680.2
Amide	30	B	132.1	72.6	102.5	50.2	271.1
Amide	30	C	97.4	43.5	97.6	53.3	165.1
Amide	30	D	109.2	54.1	132.8	21.8	166.0
Amide	60	A	891.0	616.2	830.8	192.1	1993.9
Amide	60	B	526.9	256.1	493.9	166.1	905.0
Amide	60	C	464.5	298.6	366.7	121.6	871.3
Amide	60	D	359.4	175.3	339.9	143.9	635.1
Cycloxane	30	A	5071.4	4349.0	3094.2	664.3	13334.9
Cycloxane	30	B	2246.9	1688.4	2100.7	403.0	4660.4
Cycloxane	30	C	4570.9	5484.4	3045.9	1280.3	19018.6
Cycloxane	30	D	4071.6	2071.2	3020.1	1616.0	7940.2
Cycloxane	60	A	946.1	996.9	716.3	0.0	3128.7
Cycloxane	60	B	5619.9	3373.4	5214.0	355.8	10586.4
Cycloxane	60	C	4254.9	3737.2	3416.7	516.8	11138.1
Cycloxane	60	D	2058.8	1513.8	2125.7	0.0	4225.2
Glycol	30	A	513.0	255.1	467.4	193.8	996.8
Glycol	30	B	301.1	175.4	317.0	0.0	562.4
Glycol	30	C	145.9	143.1	127.5	0.0	356.9
Glycol	30	D	215.4	207.8	177.3	0.0	526.6
Glycol	60	A	212.6	175.2	260.8	0.0	461.0
Glycol	60	B	242.3	197.3	256.6	0.0	609.1
Glycol	60	C	217.5	176.9	205.3	0.0	522.2
Glycol	60	D	123.2	110.8	125.7	0.0	285.2
Phenol	30	A	419.2	357.9	577.8	0.0	818.4
Phenol	30	B	0.0	0.0	0.0	0.0	0.0
Phenol	30	C	861.7	618.2	848.7	0.0	2003.4
Phenol	30	D	2528.0	908.1	2699.9	425.2	3410.7
Phenol	60	A	862.5	359.5	871.8	291.8	1506.3
Phenol	60	B	273.0	398.5	0.0	0.0	1176.2
Phenol	60	C	1513.6	391.1	1557.6	991.9	2171.3
Phenol	60	D	1130.6	271.7	1033.9	990.9	1836.8
Phosphate	30	A	1316.9	407.8	1368.1	555.0	1892.8
Phosphate	30	B	1394.8	792.5	1324.6	199.1	2757.6
Phosphate	30	C	1071.1	740.3	865.1	268.3	2407.8
Phosphate	30	D	1638.2	663.0	1450.3	967.0	2944.5
Phosphate	60	A	3031.1	872.5	2885.9	1948.6	4324.3
Phosphate	60	B	2361.3	879.3	2625.7	1091.4	3338.5
Phosphate	60	C	2366.5	455.4	2470.3	1631.6	2937.6
Phosphate	60	D	2460.0	1046.3	2151.2	1051.9	4408.2
Phthalate	30	A	53.3	46.8	42.0	0.0	115.0
Phthalate	30	B	0.0	0.0	0.0	0.0	0.0
Phthalate	30	C	13.2	9.0	13.5	0.0	31.3
Phthalate	30	D	25.1	36.7	13.1	8.2	122.6
Phthalate	60	A	149.8	66.5	146.9	42.4	260.6
Phthalate	60	B	89.9	58.8	83.6	32.6	209.8
Phthalate	60	C	34.7	29.7	23.3	14.0	86.7
Phthalate	60	D	29.6	33.7	18.8	9.9	118.1

Table A2. Concentration (ng/g) of pollutants grouped on chemical groups, present on the four treatments (A, B, C and D) and in both days (30 and 60) (mean, standard deviation, median, minimum and maximum values). A color scale was used, ranging from yellow for the lowest values, followed by green to blue to highlight the highest values.



A2. Loading T30 and T60 plot made by Principal Component Analysis (PCA) and applied to the types of plastics.

References

Alvarez-Hernández, C., Cairós, C., López-Darias, J., Mazzetti, E., Hernández-Sánchez, C., González-Sálamo, J., Hernández-Borges, J., 2019. Microplastic debris in beaches of Tenerife (Canary Islands, Spain). *Marine Pollution Bulletin*, 146, 26–32. https://doi.org/10.1016/j.marpolbul.2019.05.064.

Andrady, A.L., 2011. Microplastics in the marine environment *Marine Pollution Bulletin*, 62, 1596–1605.

Ambrogi, V., Carfagna, C., Cerruti, P., & Marturano, V., 2017. Additives in polymers. In *Modification of polymer properties* (pp. 87-108). William Andrew Publishing.

Asagba, S. O., Eriyamremu, G. E., & Igberaese, M. E., 2008. Bioaccumulation of cadmium and its biochemical effect on selected tissues of the catfish (Clarias gariepinus). *Fish physiology and biochemistry*, *34*(1), 61-69.

Baztan, J., Carrasco, A., Chouinard, O., Cleaud, M., Gabaldon, J. E., Huck, T., & Vanderlinden, J. P., 2014. Protected areas in the Atlantic facing the hazards of micro-plastic pollution: first diagnosis of three islands in the Canary Current. *Marine pollution bulletin*, *80*(1-2), 302-311.

Eriksen, M., Laurent C., Lebreton, H., Caron, M., Moore, C., Borerro, J., Galgani, F., Ryan, P., Resser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, *9-12*, 111913.

Ferraro, G., Failler, P., 2020. Governing plastic pollution in the oceans: Institutional challenges and areas for action. *Environmental Science & Policy*, *112*, p. 453-460.

Frias, JPGL., Nash, N., 2019. Microplastics: finding a consensus on the definition. *Marine pollution bulletin*, *138*, p. 145-147.

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment (part 1). Available: http://www.gesamp.org/publications/reports-and-studies-no-90.
GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment (part 2). Available: http://www.gesamp.org/publications/microplastics-in-the-marineenvironment-part-2.

González-Hernández, M., Hernández-Sánchez, C., González-Sálamo, J., López-Darias, J., Hernández-Borges, J., 2020. Monitoring of meso and microplastic debris in Playa Grande beach (Tenerife, Canary Islands, Spain) during a moon cycle. *Marine Pollution Bulletin*, 150, 110757. https://doi.org/10.1016/j.marpolbul.2019.110757.

Greenpeace International, 2018. Plastic Debris in the World's Oceans. *Greenpeace International Retrieved* 5 May 2018.

Hernández-Sánchez, C., González-Sálamo, J., Díaz-Peña, F. J., Fraile-Nuez, E., & Hernández-Borges, J., 2021. Arenas Blancas (El Hierro island), a new hotspot of plastic debris in the Canary Islands (Spain). *Marine Pollution Bulletin*, *169*, 112548.

Herrera, A., Asensio, M., Martínez, I., Santana, A., Packard, T., & Gómez, M., 2018. Microplastic and tar pollution on three Canary Islands beaches: An annual study. *Marine pollution bulletin*, *129*(2), 494-502.

Herrera, A., Acosta-Dacal, A., Luzardo, O. P., Martínez, I., Rapp, J., Reinold, S., Montesdeoca-Esponda, S., Montero, D., Gómez, M., 2022. Bioaccumulation of additives and chemical contaminants from environmental microplastics in European seabass (*Dicentrarchus labrax*). *Science of the Total Environment*, *822*, 153396.

Llorca, M., Schirinzi, G., Martínez, M., Barceló, D., & Farré, M., 2018. Adsorption of perfluoroalkyl substances on microplastics under environmental conditions. *Environmental pollution*, *235*, 680-691.

Llorca, M., Ábalos, M., Vega-Herrera, A., Adrados, M. A., Abad, E., & Farré, M., 2020. Adsorption and desorption behaviour of polychlorinated biphenyls onto microplastics' surfaces in water/sediment systems. *Toxics*, *8*(3), 59.

Llorca, M., Vega-Herrera, A., Schirinzi, G., Savva, K., Abad, E., & Farré, M., 2021. Screening of suspected micro (nano) plastics in the Ebro Delta (Mediterranean Sea). *Journal of Hazardous Materials*, *404*, 124022.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Geng, J., Ding, L & Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. *Environmental science & technology*, *50*(7), 4054-4060.

Matthews, M., & Varga, Z. M., 2012. Anesthesia and euthanasia in zebrafish. *ILAR journal*, *53*(2), 192-204.

Murray, GR., 2009. Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364-1526, p. 2013-2025.

Plastics Europe, 2006. Plastics-the fact 2006. Plastics Europe.

Plastics Europe, 2021. Plastics-the fact 2021. Plastics Europe.

Qiang, L., & Cheng, J., 2021. Exposure to polystyrene microplastics impairs gonads of zebrafish (Danio rerio). *Chemosphere*, *263*, 128161.

Rainieri, S., Conlledo, N., Larsen, B. K., Granby, K., & Barranco, A., 2018. Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (Danio rerio). *Environmental research*, *162*, 135-143.

Rapp, J., Herrera, A., Martinez, I., Raymond, E., Santana, Á., & Gómez, M., 2020. Study of plastic pollution and its potential sources on Gran Canaria Island beaches (Canary Islands, Spain). *Marine Pollution Bulletin*, *153*, 110967.

Santana-Viera, S., Montesdeoca-Esponda, S., Sosa-Ferrera, Z., & Santana-Rodríguez, J. J., 2021. UV filters and UV stabilisers adsorbed in microplastic debris from beach sand. *Marine Pollution Bulletin*, *168*, 112434.

Savva, K., Borrell, X., Moreno, T., Pérez-Pomeda, I., Barata, C., Llorca, M., Farre,M ., 2022. Cytotoxicity assesment and suspectd screening of plastic additives in bioplastics of single-use household ítems. *(Submitted to Chermosphere)*

Tarasco, M., Gavaia, P. J., Bensimon-Brito, A., Cordelières, F. P., Santos, T., Martins, G., Daniela, T., Silva, N., Cabrita, E., Bembiano, MJ., Stainier, YR., Cancela, ML., & Laizé, V., 2022. Effects of pristine or contaminated polyethylene microplastics on zebrafish development. *Chemosphere*, 135198.

Teo, T. L., McDonald, J. A., Coleman, H. M., & Khan, S. J., 2015. Analysis of organophosphate flame retardants and plasticisers in water by isotope dilution gas chromatography–electron ionisation tandem mass spectrometry. *Talanta*, *143*, 114-120.

Ugwu, K., Herrera, A., Gómez, M., 2021. Microplastics in marine biota: a review. *Marine Pollution Bulletin*,169, 112540. https://doi.org/10.1016/j.marpolbul.2021.112540.

Vega-Herrera, A., Llorca, M., Savva, K., León, V. M., Abad, E., & Farré, M., 2021. Screening and quantification of Micro (Nano) plastics and plastic additives in the seawater of Mar Menor Lagoon. *Frontiers in Marine Science*, *8*, 697424.

Wurl, O., Obbard, J.P., 2004. A review of pollutants in the sea-surface microlayer (SML): a unique habitat for marine organisms *Marine Pollution Bulletin*, 48 (11–12), 1016–1030.

Valoración TFM

- Descripción detallada de las actividades desarrolladas durante la realización del TFM

La preparación del experimento con los peces cebra empezó en noviembre-diciembre, ya que teníamos que acondicionar los peces y tener un control. Por lo que, de noviembre hasta febrero las actividades que realicé eran limpiar los acuarios una vez a la semana y hacer un seguimiento dos veces por semana de los parámetros químicos de las peceras. También preparamos las dietas de los cuatro tratamientos, triturando los microplásticos y transformándolos en polvo para mezclarlo con la comida de los peces y tener una mejor ingestión. En febrero empezó el experimento y duro dos meses, hasta abril. En esos dos meses había que darles de comer todos los días a la misma hora, verificar los parámetros y limpiar los acuarios. Todas estas actividades fueron divididas entre el grupo de investigación

Después se realizaron dos muestreos, en dos días diferentes uno a mitad de tratamiento y otro al final, donde los peces eran eutanasiados y guardados para su posterior análisis para los diversos trabajos de investigación. También esta labor participamos algunos del grupo de investigación.

Posteriormente, las muestras fueron hacia Barcelona al centro IDAEA-CSIC donde yo realizaría el final del trabajo. Ahí hicimos toda la preparación de las muestras para ser analizada con un espectrómetro de masas de alta resolución mediante una cromatografía liquida. Para ello las muestras se homogeneizaron, se hicieron extracciones con metanol, se evaporaron, etc para obtener un volumen específico, 1 ml, para poder analizarlo. Toda esta parte de metodología duró una semana desde las 8 de la mañana hasta las 20 de la tarde. El espectrómetro de masas tarda 3 días en hacer en análisis, además que se analizó en positivo y negativo, haciendo un total de 6 días.

Para el tratamiento de datos, se usó el programa de *Compound Discoverer versión 3.1* donde los datos se cargaban para que el programa hiciera sus análisis con la literatura y demás para luego hacer el "target-analysis" que es buscar todos los contaminantes presentes en las muestras. Esto duró 4 días en finalizar, con un total de 8 días en positivo y negativo.

Con el mismo programa se aplicaron unos filtros y se analizó compuesto por compuesto los picos de los cromatogramas. Después se seleccionaron los válidos y se cuantificaron mediante rectas de calibrado en base a los patrones de los compuestos. Este proceso duró aproximadamente unas tres semanas – un mes.

- Formación recibida (cursos, programas informáticos)

Como curso no realicé ninguno, exceptuando el de riesgos laborales del centro CSIC para poder realizar las prácticas.

Como programa informático aprendí, de cero, a analizar los datos con el *Compound Discoverer versión 3.1* y el programa *Thermo Xcalibur 3.1*. Además, los gráficos se realizaron con RStudio y Excel.

- Nivel de integración e implicación dentro del departamento y relaciones con el personal

En ambos grupos de investigación mi nivel de integración ha sido completo. En el caso de EOMAR, había que trabajar en equipo para que el experimento saliera bien, nos turnábamos entre nosotros para dividir los trabajos y cuando había que trabajar todos juntos nos coordinábamos bien para tener una buena optimización del tiempo y del espacio. Además, la relación con todos ellos ha sido buena, profesionalidad, ante todo, pero también había momentos más informales. Además, participé junto con el resto del grupo en el congreso ISMS 2022, realizado en Gran Canaria este mismo Julio.

En cuanto al grupo ONHEALTH, mi integración con el equipo también fue buena y rápida. Me asignaron un espacio habilitado para poder trabajar todos los días desde el 25 de abril hasta 29 de Julio de lunes a viernes, junto con una compañera y una de las tutoras. Con ellos pude hacer todo el resto del trabajo que me faltaba. Además de participar en algunas actividades el grupo como, por ejemplo, ir a muestrear al Delta del Ebro o participar en el congreso XR –SEEM (Sociedad Española de Espectrometría de Masas) en Córdoba con un poster del trabajo, preliminar, final de máster.

- Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFM.

Como aspectos positivos todo el aprendizaje que he tenido durante estos meses. He aprendido muchísimo, sobre todo en la parte de química analítica y lo muy cuidadosa que es. Pero también he aprendido a tener más responsabilidades, a trabajar en equipo, a gestionar todas las emociones que se tienen en un trabajo de investigación, ya que es como una montaña rusa.

Como aspectos negativos, realmente no tengo ninguno.

- Valoración personal del aprendizaje conseguido a lo largo del TFM.

Todo el proceso ha sido increíble. He aprendido muchísimo y valoro mucho todo lo que me han enseñado cada uno de los grupos de investigación. Estoy muy contenta de haber participado en este proyecto. En estos 8 meses he crecido mucho como científica y me siento más profesional. He disfrutado mucho del proceso, incluyendo los momentos de estrés con los programas informáticos…pero al final he salido adelante, sobre todo gracias a la ayuda de mis tutoras y compañeros.