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Metabolomics effects of microplastics on filtering organisms
Efectes metabolòmics dels microplàstics en organismes filtradors

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Sé el cambio que quieres ver en el mundo.

Mahatma Gandhi

Primer de tot, agrair a les meves tutores, Dra. Marinella Farré i Dra. Marta Llorca, no només per deixar-me formar part del projecte PLAS-MED, sinó també per aconsellar-me i guiar-me durant les etapes més difícils. A la Dra. Mònica Martínez, pels seus consells i la seva ajuda durant el transcurs d'aquest treball.

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Als meus pares pel seu suport i recolzament durant tot el recorregut.

REPORT

CONTENTS

1. SUMMARY

It is known that microplastic (MPL) litter causes negative effects on the environment and human health. Plastic particles enter in the marine food web and produce different adverse effects, among them can produce alterations on the metabolism of marine wildlife, and eventually reaching the human trophic chain.

Despite the research carried out during the last years, nowadays continue to be needed assessing the bioaccumulation of plastic particles in the aquatic biota according to their composition and size, their ability to be translocated to different tissues and to study their potential to produce sub-acute toxicological effects under realistic scenarios of exposure to establish the basis of realistic risk assessment.

This TFG was carried out within the PLAS-MED project devoted to the investigation of MPLs in the Mediterranean coast. This work was initially focused on the study of the metabolomics changes that can be produced on Mediterranean mussel (*Mytilus galloprovincialis)* exposed under controlled conditions to polyethylene (PE) MPLs with the antibacterial agent, triclosan (TCS), adsorbed onto their surface. The metabolomics response would be evaluated by comparing metabolomics profiles of exposed and non-exposed mussels from the Ebro Delta, analysed by high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC-HRMS). This study was planned to assess two different tissues of mussels: stomachs and haemolymphs.

However, due to the health crisis by COVID-19, was impossible to finish the experiments and carry out the analysis of the samples. For this reason, this work was finalised by review of several metabolomics studies focused on the evaluation of MPLs or plastic additives effects on organisms.

The main conclusion of this study is that MPLs and related contaminants (plastic additives) induce metabolomics changes in marine organisms due to oxidative stress and changes in gene expression, as well as other toxicological effects such as decrease of fecundity. A decrease of

particle size and non-spherical shape produces an increase of toxicity. Small particle size and polymeric amorphous structure provides better sorption of other contaminants.

Keywords: Microplastics, plastic additives, metabolomics effects, mussels

2. RESUM

Es coneix que la presència de micro-plàstics (MPLs) en el medi causen efectes negatius en el medi ambient i la salut humana. Les partícules de plàstic ingressen a la xarxa alimentària marina i produeixen diferents efectes adversos, entre ells poden produir alteracions en el metabolisme de la vida silvestre marina i, finalment, arribar a la cadena tròfica humana.

Malgrat la recerca duta a terme durant els últims anys, avui dia es continua necessitant avaluar la bioacumulació de partícules de plàstic en la biota aquàtica d'acord amb la seva composició i grandària, la seva capacitat de desplaçar-se a diferents teixits, i estudiar el seu potencial per produir efectes toxicològics aguts en escenaris reals d'exposició per a establir la base d'una avaluació real del risc.

Aquest TFG es va dur a terme dins del projecte PLAS-MED dedicat a la investigació dels MPLs a la costa mediterrània. Aquest treball es va centrar inicialment en l'estudi dels canvis metabòlics que poden produir-se en els musclos mediterranis (*Mytilus galloprovincialis*) exposats a condicions controlades de MPL de polietilè (PE) amb l'agent antibacterià triclosan (TCS), adsorbit a la superfície del plàstic. La resposta metabolòmica s'avaluaria comparant els perfils metabolòmics dels musclos del Delta de l'Ebre exposats i no exposats, obtinguts per cromatografia de líquids d'alta eficàcia acoblat a espectrometria de masses d'alta resolució (HPLC-HRMS). Aquest estudi va ser planejat per avaluar dos teixits de musclo diferents: hemolimfa i estómac.

No obstant això, degut a la crisi sanitària provocada pel COVID-19, va ser impossible acabar els experiments i analitzar les mostres mitjançant HPLC-HRMS. Per aquest motiu, aquest treball es va finalitzar mitjançant la revisió de publicacions d'estudis de metabolòmica focalitzats en l'avaluació dels efectes causats pels MPLs o additius plàstics en organismes.

La principal conclusió d'aquest estudi és que els MPLs i els contaminants relacionats (additius plàstics) indueixen canvis metabòlics en els organismes marins a causa de l'estrès oxidatiu i canvis en l'expressió gènica, així com altres efectes toxicològics com la disminució de la fecunditat. La disminució de la mida de la partícula de MPL i una forma irregular d'aquesta fa

augmentar la toxicitat. Les partícules més petites i les regions amorfes dels polímers faciliten la sorció d'altres contaminants.

Paraules clau: micro-plàstics, additius plàstics, efectes metabolòmics, musclos

3. INTRODUCTION

Plastics are typically mixtures of polymers and other substances called additives, used to improve plastic performance. Most polymers are usually synthetically produced from petrochemicals. However, a variety is made from renewable materials such as polylactic acid or cellulosic materials. Due to their ease of manufacture, versatility, and low cost, plastics are used in a multitude of products of different sectors including electronics, packaging, building materials, the automotive industry, among many others.

After their use, plastic enter in the waste stream to be recycled, incinerated or, lastly, discarded in landfills. Moreover, there are different direct sources of plastics to the aquatic environments including the action of the wind that can transport agricultural plastics or plastic residues from landfills, lost gear and ghost fishing nets, public littering, or the lack of efficient waste management in some world areas. According to a European plastic association called PlasticsEurope Market Research Group (PEMRG) and the Conversion Market & Strategy GmbH, plastic production achieved 61.8 million tonnes in Europe and 359 million tonnes around the world in 2018. It should be highlighted that, off the 61.8 million tonnes produced in Europe, only 9.4 million tonnes were collected to be recycled [1]**.** In addition, microplastics (MPLs) and textile fibbers are not retained or degraded in wastewater treatment plants (WWTP), therefore, reaching the aquatic systems.

According to their use and the type of polymer base, a percentage can be recycled by mechanical or chemical methods. In principle, all types of thermoplastics can be mechanically recycled. However, the replacement of virgin polymer to recovered plastic in the same application is not often possible. Intensive technology, selection, chemical processes of depolymerisation or thermal degradation are necessary to recover most of the plastic materials making recycling more difficult and wasteful. Despite the fraction of used plastics that are discarded in landfills and the fraction that cannot be recycled or incinerated for energy production, thanks to the EU strategies on prevention and recovery [2] [3], the proportion of plastic recycled has increased during the last years. In 2016, 27.1 million tonnes of plastic waste were collected to be treated in Europe. Among them, the percentages of plastic recycling and

those used for energy recovery were 31.1% and 41.6%, respectively [4]. However, due to the costs of collecting, sorting, reprocessing and the low market value of the recycled plastics, the reuse and recycling of end-of-life plastics remain lower compared to paper, metal or glass. Part of plastic, which cannot be recycled or discarded in safety landfills, ends up in open dumps or in the environment. When these materials are released to the environment, they rarely biodegrade**.** A non-negligible fraction of plastic wastes is dispersed into the natural environment as litter causing significant impacts on ecosystems and human health [5]. For these reasons, plastics are recognised by scientific community as an emerging risk for the environmental and human health [6]**.** The Figure 1 summarizes the life cycle of products with plastics.

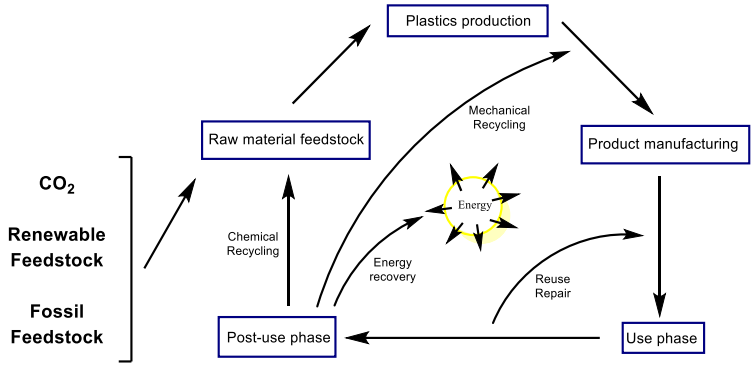


Figure 1. Life cycle of products containing plastics. Adapted to PlasticsEurope, 2017 [3].

The most abundant polymer constituents identified in the aquatic environment are Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET) and Polyvinyl chloride (PVC). Some studies have concluded that the high coastal concentrations of these plastics are closely correlated with the human population density. Nowadays, plastic contamination is of high concern because of the rapidly increasing of the world population and hence, the fabrication of plastics [7]**.**

Nevertheless, there is growing awareness about this issue and some companies are primarily interested in remove single-use plastics to be more sustainable and environmentally friendly. Besides, governments are taking remedial actions and adopting new measures in entering to care Earth's biodiversity and ecosystems. Some of the measures that will be implemented by the European Commission are mentioned below [8]**:**

- The Member State will be required to collect 90% of the single-used plastic from beverage bottles until 2025.

- If there are suitable and available alternatives, the single-use plastic products like cutlery, dishes or cotton swab will not be able to be marketed.

- It will be required to etiquette clearly some products composed of plastic, containing the documentation about the waste management system.

3.1. MICRO AND NANO-PLASTIC

MPLs are classified as primary or secondary. Primary MPLs are used during the process of fabrication of different cleaning and cosmetic products such as facial cleansers, toothpaste and textiles. They are also used in medicine, as vectors for drugs, for instance [9]. Secondary plastics are the consequence of further fragmentation of larger plastics once in the environment generating MPLs or even nano-plastics (NPLs) [9]**.** The main factors that can degrade plastics include UV-irradiation, mechanical forces and microbial action, whose lead to a fragmentation of those plastics to MPLs and NPLs. The ultraviolet radiation provident of the sunlight brings them the activation energy necessary to incorporate oxygen atoms in the polymer chain, causing degradation of this material until the ingestion of wide range marine species, accumulating in different tissues of these organisms [10]**.**

MPLs are referred to particles with less than 5 mm [11] while NPLs are those with less than 100 nm [12]. Table 1 summarizes the most accepted classification of plastics according to their size.

Table 1. Classification of plastics by their size according to the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection [13].

Type of plastic	Size
Megaplastic	> 1 m
Macroplastic	2,5 cm - 100 cm
Mesoplastic	5 mm - 2,5 cm
Microplastic	100 nm - 5 mm
Nanoplastic	< 100 nm

The characterization of MPLs and NPLs in the environment must cover not only the identification by size but also by shape, chemical composition, specific density and concentration. For megaplastics, macroplastics, mesoplastics and the larger microplastics, the characterization of the surface morphology is in general carried out by optical microscopy, while for smaller MPLs is in general performed by scanning electron microscopy (SEM). During recent years, SEM has been used coupled to other techniques to improve their performance such as scanning electron microscopy-energy dispersive X-ray Spectroscopy (SEM-EDX) [14]. Electronic Scanning Microscopy (SEM) allows visual observation of the sample while Energy Dispersive X-Ray Spectroscopy (EDX) can be used to obtain semi-quantitative information about its composition. High-magnification images obtained by advanced microscopy techniques facilitate the discrimination of microplastics from organic particles; however, their application for routine analysis of a large number of samples is not feasible [15]. For chemical characterization, one of the most commonly used analytical techniques is Fourier-Transform Infrared (FTIR) spectroscopy [16]**.** The variation of the permanent dipole moment of a chemical bond after the absorption of IR light is the fundamental physical principle on which the FTIR spectroscopy is based. Another technique is Raman spectroscopy, consisting of a laser beam falling on a particle resulting in different frequencies of back-scattered light depending on the molecular structure and atoms present. A unique spectrum for each polymer is provided and non-polar functional groups are well detected. Thermal analysis and other novel techniques such as pyrolysis coupled to gas chromatography ((Pyr)-GC-MS) are another useful group of techniques. Through these techniques, the chemical characterisation of several particles, which includes information on the type of plastic and the additives contained in it, can be carried out simultaneously in a single operation [17] [18]. The main limitation of these techniques is the lack of quantitative measurements or in some cases, the measurements are based on the average number of particles. To solve this problem recently, the advantages of liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) have been explored. Even so, to achieve the identification and detection of this type of plastics are required sophisticated multiple analytical techniques working simultaneously, which can leverage its unique advantages of each them [19]**.**

3.2. FATE AND BEHAVIOUR OF MPLS/NPLS IN AQUATIC MEDIA

As stated before, the environmental pollution due to plastic contamination is a concerning problem because of its widespread in aquatic media and its potential effects in biota. Rivers connect the overall land area to the marine environment. They are considered the major pathway for plastic litter transport, which originated in catchments, to oceans and seas. Especially secondary microplastics can release to aquatic media by this way [20].

Another problem associated to the presence of MPLs/NPLs in marine media is their interaction with other co-contaminants such as heavy metals, persistent organic pollutants (POPs) and hydrophobic organic chemicals (HOCs) which can adhere and accumulate on plastics surface [21] [22] [23]. This makes plastic to act as environmental passive carrier that could aggravate toxicological effects for biota. Some toxic effects associated to the presence of MPLs include intestine obstruction, local inflammations, lipid accumulation and induction of oxidative stress (that can create serious problems with energy assimilation and fertility). It could also cause chemical fatal consequences due to the different type of chemical contaminants that can be founded in plastics [24]. In addition, if MPLs have hydrophobic and lipophilic co-contaminants adsorbed on their surface, it could increase toxic effects in comparison to contaminant alone. Although little is known about these effects, there are few studies addressing this problem. For example, *K. Syberg, A. Nielsen et al.* [25] have found that the toxicity of antifungal triclosan (TCS) in copepod *Acartia tonsa* is increased when it is adsorbed on PE. Regarding secondary MPLs, those are formed by weathering effects that can alter plastic surface. This alteration can make an increase or decrease on plastic sorption' capacity for other co-contaminants. Some studies carried out by the hosting group in the PLAS-MED project have shown that some contaminants such as TCS and the herbicide glyphosate (Gly) could be more adsorbed by UV-irradiated plastic than by virgin plastic. In this context, it is important to evaluate the toxic effects of weathered MPLs with adsorbed co-contaminants.

3.3. ANALYTICAL TECHNIQUES FOR METABOLOMICS STUDIES

The aim of metabolomics studies is to analysis quantitatively or qualitatively the metabolites that are in a sample. Firs of all, before going into the subject of this section, it could be interesting understand the normal work process for quantitative metabolome profiling from sample collection to metabolite quantification. The Figure 2 shows a schematic diagram about it:

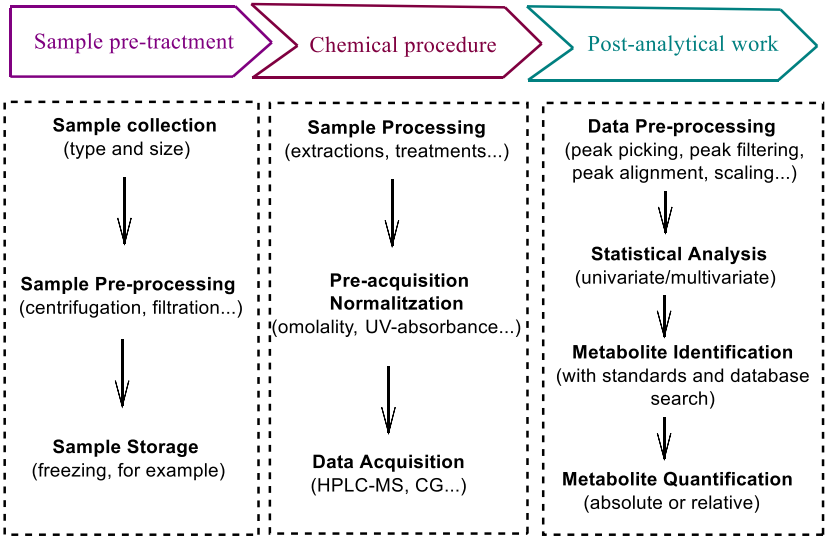


Figure 2. General workflow for quantitative metabolome profiling. Adapted to Y. *Wu and L. Li* [26].

Doing a bibliographic research, some interesting information about analytical techniques of metabolomics studies has been found. Most of them are based on liquid (LC) or gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS). If the study covers a wide range of metabolites, different separation techniques should be used simultaneously. For example, for the specific analysis of semi-volatile metabolites such as fatty acids, flavonoids or other volatile metabolites, GC-HRMS could be a complete technique. On the other hand, the complete analysis of peptides, nucleotides or lipids, for example, is carried out by means of HPLC-MS. HPLC is extensively used as a separation technique for metabolomics studies. In many works, HPLC is coupled to UV/Vis spectrometry or X-ray fluorescence spectrometry (XRF) for metabolites quantification, but it is not considerate an efficient choice, because, probably, the metabolites involved in the study don't absorb at the same wavelength, and it would be an impediment. Unlike in the previous case, when HPLC is coupled to Mass spectrometry (MS), this technique also provides structural information, an interesting advantage over UV/Vis and XRF. Better sensitive and accuracy are advantages of MS that could be important to consider [27].

The two analytical techniques most often used for this type of studies are Nuclear Magnetic Resonance (NMR) spectroscopy and liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS).

LC-HRMS

Among the different separation techniques, liquid chromatography (LC) is the most used during the last years coupled to high resolution mass spectrometric detectors. Although gas chromatography (GC) is robust, efficient and it is considered an excellent separation technique with a higher resolution chromatography, only identify volatile compounds with low molecular weight. Moreover, it frequently requires a large process of chemical derivatization to reduce polarity and to increase volatility and thermal stability. For these reasons, LC is most common used as a separation method than GC in the last few years [28].

The high-resolution mass analyser has become increasingly popular over the past few decades. The different mass analysers are a part of the instrument essential to distinguish one mass peak from another. In addition, the mass resolution and resolving power are important parameters to consider because they significantly affect the final marker identification [28]. There are different analysers such as quadrupole (q), magnetic sector, ion trap (IT), time-of-flight (TOF) and different types of Fourier transform (FT). They can be combined and became a complex high-resolution analyser like triple quadrupole (qQq) or q-TOF [29].

The analytical technique that would have been used was high resolution liquid chromatograph coupled to QExactive, a combination of quadrupole and Orbitrap MS. This technique has become popular since 2011 [29]. The quadrupole acts as selector of concrete ions of interest in order to identify by different narrow mass ranges and, then, these ions are conduced to Orbitrap analyser (depending on the scan mode (see Figure 3), a fragmentation chamber before Orbitrap is needed or not). Orbitrap technology consists of three electrodes, the central electrode and split outer electrodes. The ions trajectory assumes a spiral shape due to the combination of three cycle motions: axial oscillation and rotational motion around the central electrode and a radial motion [30]. A specific angular frequency (in axis *z)* of each ion gives a specific m/z value (see Equation 1) and the intensity information is determined by the amplitude of this frequency, applying a Fourier transformation operation [31].

Equation (1) $\omega_z = \sqrt{\frac{k}{m/z}}$

Where *k* is an instrumental constant, *m/z* is a mass/charge and w_z is an angular frequency of axis *z*.

High resolution MS analysers are a great choice due to its high mass resolution and mass accuracy. The main advantage of Orbitrap in comparison to other high mass resolution

analysers is that the first one can be used also as an accurate mass detector. Moreover, Orbitrap-MS have a higher m/z range and high mass accuracy. However, the high Orbitrap's resolution provides a slower scan speed compare to other analysers such as qTOF or qIT.

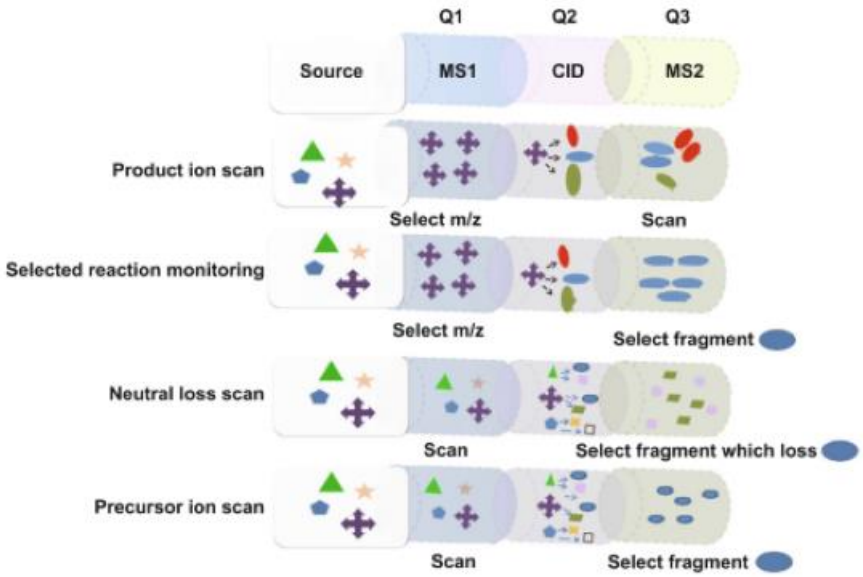


Figure 3. Mass spectrometry scan modes (Ref: *S. Amrani, Y. Halimi et al.* [32]).

NMR spectroscopy

This analytical technique is particularly useful for metabolomics research and commonly used in non-target metabolomics analysis [28]. NMR spectroscopy can identify compounds without the necessity of standards. NMR spectroscopy has important characteristics like great reproducibility, non-destructiveness and the capacity to quantify different types of metabolites simultaneously. In addition, the sample pre-treatment not requires a complex preparation [33] .

Different NMR active nuclei are used for metabolomics studies, but ones more than others. For example, the NMR active nuclei most used is ^{1}H, because of its abundance in metabolites from several biological samples. ^{31}P is also common in this type of studies because of its occurrence in metabolites that can be found in energy metabolism like Adenosine triphosphate (ATP), Adenosine biphosphate (ADP) and Adenosine monophosphate (AMP). In contrast, ^{15}N and ^{13}C are little used because their low natural abundance (0.4% and 0.11%, respectively) [27].

There are multiple NMR experiments (one-dimensional,1D, and two-dimensional, 2D). Although its low resolution, 1D proton is the most method commonly used in metabolomics research due to its shorter acquisition time. The longer acquisition time of 2D NMR is still a limitation and becomes an issue for metabolomics studies [34]. In addition, compared to MS, this analytical technique has low sensitivity and NMR spectroscopy cannot identify metabolites under a concentration of 1 μM [28]. Other disadvantage is that this technique needs higher sample mass than MS. The high maintenance cost of NMR is another limitation to consider in comparison to MS [34].

3.4. MUSSELS AS BIOFILTER-FEEDERS AND BIOINDICATORS

The mussels' mechanism of suspension-feeding consists in to filter the aquatic media water with the prospect of ingesting mainly some water nutrients. Nevertheless, the water contamination can affect their feeding, and this could be one of the main introduction pathways to marine food web for some contaminants as well as humans' exposition through seafood consumption [7].

Mussels can efficiently accumulate traces of chemical contaminants, like the ones mentioned in previous sections, in their tissues through feeding. Hence, those chemicals can be metabolized producing sometimes harmful metabolomics effects. To investigate metabolomics effects in mussels it is usual to analyse mussel's tissue to assess the occurrence of xenobiotics such as biocides, pharmaceutical and personal care products or small plastics as well as the alteration of metabolomics pathways [35]. In this way, mussels are used as biomonitoring organisms for research purposes in environmental pollution. In the specific case of MPLs, it has been demonstrated that plastic particles are accumulated in intestines while microfibers can be distributed in all organs. A study from 2018 [36] demonstrated that the mussel' tissue that contained the highest concentrations of MPLs are intestines. Moreover, the accumulation of microfibers was founded in all mussel' organs.

4. OBJECTIVES

Originally, the primary objective of this work was to study the metabolic impact of MPLs/NPLs contaminated with TCS on *Mytilus galloprovincialis*.

To carry out this work the specific objectives were:

- To evaluate the adsorption capacity of TCS to PE microspheres.
- To compare the influence of weathering in PE adsorption by comparison of the ability of UV-irradiated PE and non-irradiated PE to adsorb TCS.
- To study metabolomics responses of mussels exposed to PE with adsorbed TCS through feeding.

Due to the impossibility of access to the laboratory to carry out the last part of the experimental work, consisting on the analysis of the samples, the objectives of this work were reconducted, by collecting and comparing other similar metabolomics studies. Therefore, the final objectives of this work were:

- To evaluate if there are some trends about metabolomics disruption of marine organisms when they are exposed to contaminants such as MPLs or nanomaterials.
- To evaluate the influence of different shape and size MPLs in toxicology and their interaction with other contaminants.
- To compare the ability of MPLs to potentiate the effect of different co-contaminants.
- To compare the metabolomics responses between males and female mussels.

5. MATERIALS AND METHODS

5.1. STUDY AREA: THE EBRO DELTA

The Ebro Delta is in the province of Tarragona, Catalonia, in the mouth of the Ebro River (NE Spain). It covers an area of 320 km^2 [37]. An important part of the Ebro Delta is a Natural Park since 1983. The Ebro Delta has two semi-enclosed embayment: Alfacs (in the southern bay, with an area of approximately 50 km^2) and Fangar Bay (in the northern bay, with an area of approximately 10 km^2) [38]**.** Its typically Mediterranean climatology is characterized by thermal oscillation between 12-22 ºC throughout the year: frequent rain in late autumn and winter but severe drought in summer. The Ebro Delta activity is mainly dedicated to agriculture (rice crops), about 65% of the whole land, playing an important role in the economic sector [39]**,** tourism and aquaculture. The aquaculture exploitations are mainly located in Alfacs Bay [37]**.** The Ebro Delta is taking on the challenge of improving the conservation of the ecosystem despite the anthropogenic activities, taking in mind that the population density has increased in the last few years.

5.2. CHEMICALS AND REAGENTS

PE microspheres, ranging from 3 to 16 μm, were supplied by Cospheric (Santa Barbara, California, USA); TCS (Irgasan; <97% purity) was supplied by Sigma-Aldrich (provided by Merck KGaA, Darmstadt, Germany).

Solvents used include Acetonitrile (>=99.9%, Fisher Scientific, Loughborough, UK), Water HPLC Grade from J. T. Baker (Gliwice, Poland), and Methanol and Chloroform from CHROMASOLV®Plus (for HPLC grade provided by from Sigma-Aldrich, Steinheim, Germany).

The mussels' feeding was composed by a mixture of microalgal species supplied by Dieta compuesta Microalgal® - Acuinuga, n.d.

5.3. EXPERIMENTAL DESIGN

Mediterranean mussels were collected from different areas of the Ebro Delta and transported under cool conditions to the laboratory, at IDAEA-CSIC. Organisms were introduced in mesocosms of 500 L of capacity or in the 15 L aquariums (microcosms), depending on the experiment type. Seawater was taken from the area of Sant Feliu de Guíxols, Girona. Mesocosms and microcosms were exposed to day and night periods. Temperature and salinity were maintained stable along the experiment. PH, nitrates and nitrites were controlled along the whole exposure time to ensure that their values were around 7, 25 mg/L and 0, respectively.

With the goal to evaluate changes in the metabolomics pattern of the mussels, three experiments were performed (one express experiment (short duration) and two long metabolomics experiments), where mussels were exposed to UV-irradiated and non-irradiated PE microspheres and TCS.

5.3.1. Express experiment 1: non-irradiated PE and TCS

Express experiments consisted in to introduce 22 mussels in each aquarium (7 in total). In Figure 4, a picture of two microcosms is shown.

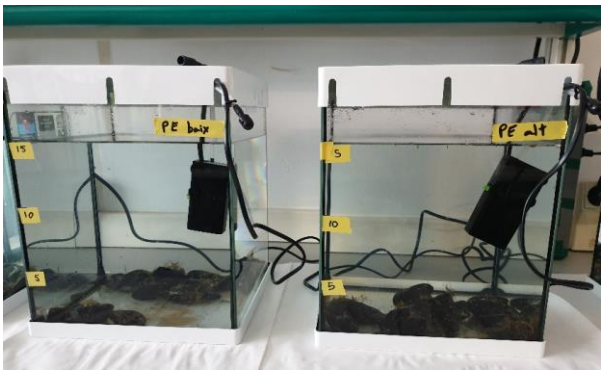


Figure 4: 15 L microcosms for express experiment.

After 2 acclimatization days, the exposure was initiated. One of the aquariums was the blank and the mussels were fed with clean algae, while the mussels of other aquariums were fed with PE, TCS and mixtures of PE and TCS according to the Figure 5.

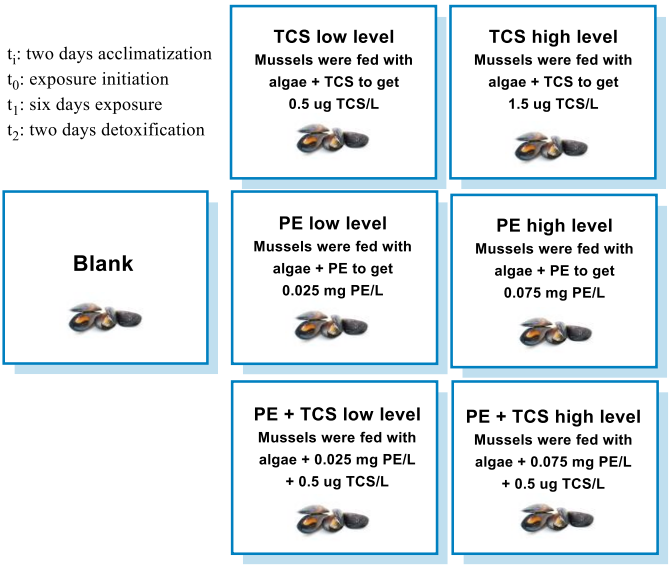


Figure 5: Summary of express experiment design.

Six mussels were collected from Control microcosm in t_0 (after acclimatization period and before to start the feeding experiment) to extract their stomachs and haemolymph. After 6 days of exposure (t_1) and after 2 more days of detoxification (t_2) in which mussels were fed with clean algae, 6 mussels were collected for each time per microcosm to extract their stomachs and haemolymph as well. Aquariums were cleaned and water was changed every day during the 6 days of exposure, before the feeding/exposure.

5.3.2. Long metabolomics experiment 1: UV-irradiated and non-irradiated PE

UV-irradiated PE solutions were prepared for long metabolomics experiments: first, 6.0000 g of Clear Polyethylene microspheres (3-16 µm) were weighted and rinsed with seawater. Then, they were exposed in a UV radiation for 24 hours (600 W) by SUNTEST CPS, simulating a solar irradiation of 28 days. Finally, 2 L of a solution of this irradiated PE (3000 mg/L) was prepared.

Four mesocosm of 500 L were used for long metabolomics experiments (see Figure 6). One of them was the blank mesocosm and the other 3 were used for the exposures. Fifty mussels were introduced in each mesocosm. After one week of acclimatization, the exposure was initiated during a period of 3 weeks. After 3 weeks of exposure, the experiments were

prolongated during one more week for detoxification. During detoxification, the mussels were fed with clean algae.

Ten mussels were collected from Control mesocosms in t_0 to extract their haemolymphs and stomachs. For times t_1 and t_2, 10 mussels were collected per mesocosm to extract their stomachs and haemolymphs as well.

In summary:

- one week of acclimatization.
- t_0: samples taken before to start the exposure and after one week of acclimatization.
- t_1: samples taken after three exposure weeks.
- t_2: samples taken after one detoxification week.

During the exposure weeks, mussels were fed and exposed to plastic mixture every two days. Furthermore, the mesocosms were cleaned daily including skimmers and pumps.

Figure 6: 500 L mesocosm for long metabolomics experiment.

After the acclimatization week, each mesocosm was under the following conditions:

(A) CONTROL: Mussels were fed with 1 mL of microalgae food.

(B, C) BIOASSAY: Mussels were fed with the corresponding dose of microalgae food and with UV-irradiated PE. In the case of mesocosm B at 0.3 ppm and in the case of mesocosm C, at 1.2 ppm.

(D) BIOASSAY: Mussels were fed with non-irradiated PE at low concentrations (0.3 ppm).

In Figure 7, a diagram of the second design experiment is shown.

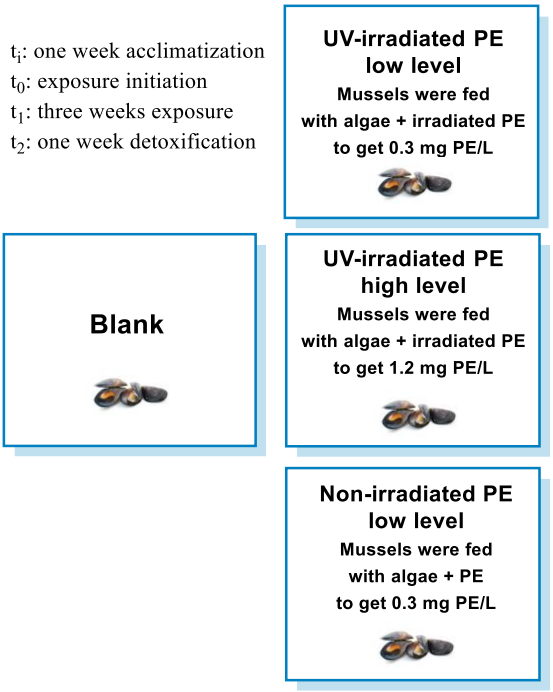


Figure 7. Experimental design of long metabolomics experiment 1 assay.

5.3.3. Long metabolomics experiment 2: UV-irradiated PE and TCS

UV-irradiated PE/TCS solution were prepared, weighting 0.0010 g of this pollutant (TCS) into the volumetric flask (250 mL). The solution was kept stirring in an orbital digester during 24 hours before the administration day.

After the acclimatization week, each mesocosm was under the following conditions:

(A) CONTROL: Mussels were fed with 1 mL of microalgae food.

(B, C) BIOASSAY: Mussels were fed not only with microalgae food, but also with UV-irradiated PE with TCS adsorbed after irradiation at low concentrations (0.5 ppb of TCS). In the case of mesocosm C, mussels were also exposed to a mix of UV-irradiated PE/TCS but at high concentrations (1.5 ppb of TCS).

(D) BIOASSAY: Mussels were fed with UV-irradiated PE at low concentrations (0.3 ppm), in this case, without TCS.

The following Figure (8) shows a diagram of the long metabolomics experiment.

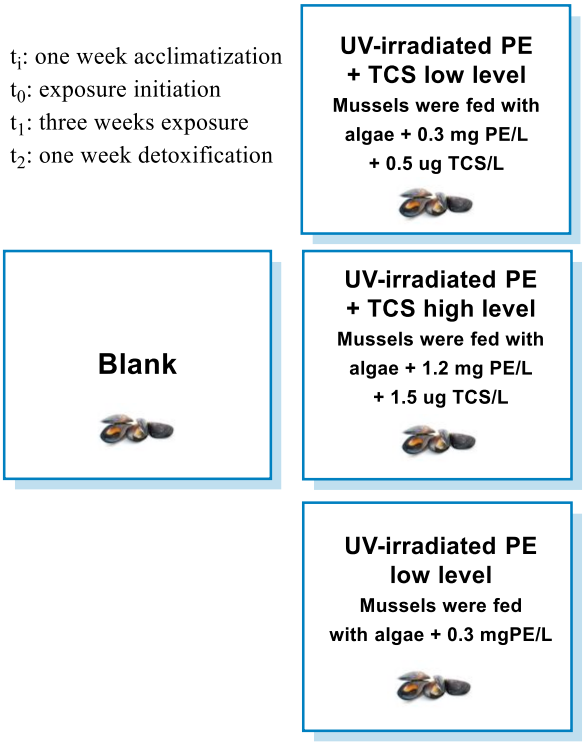


Figure 8. Experimental design of long metabolomics experiment 2 assay.

5.4. SAMPLE PREPARATION

5.4.1. Mussel's stomach

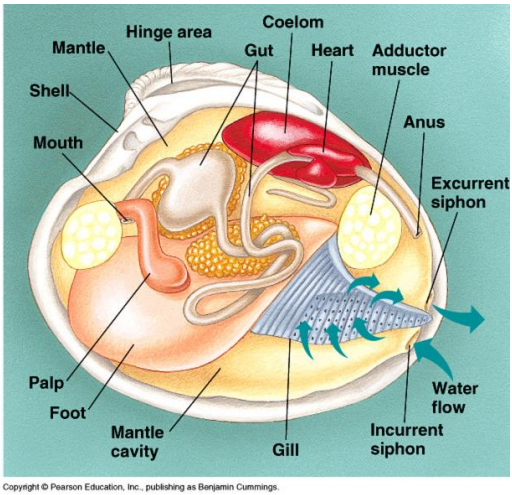


Figure 9. Parts of the mussel.

The mussels' stomachs were collected, inserting a small knife between two valves on the dorsal side and removing its anterior adductor to open it (see Figure 9). Stomachs were stored at -60 ºC until their extraction. To begin the extraction, first, the stomach was weighed. Then, it was cut into small chunks and transferred into a 20 mL vial. Six mL of a Methanol:Chloroform (2:1) solution were added to the mussel stomach and then, the extraction was achieved by Ultrasonic Assisted Extraction (UASE) during 15 minutes. After the extraction, 3 mL of HPLC-grade water and 3 mL of chloroform were added, and this mixture was centrifuged for 10 min at 3000 rpm at 20 ºC. The Methanol/Water fraction was remained on the top and the lower layer was the chloroform.

The analytes were in both water-methanol and chloroform phases, so after separating both phases, each of them was transferred into another vial and stored at -60 ºC until their analyses. Analyses would be performed by UHPLC-HRMS and GC-HRMS, respectively, in order to obtain the metabolomics profile.

5.4.2. Mussel's haemolymph

The mussels' haemolymphs were extracted doing a small hole in the shell, using a 1 mL needle to inject the mussel's tissue and extract the liquid, and stored at -60 ºC until their treatment. After defrosted the samples for around 1 hour, for each sample, 125 µL of mussel' haemolymph, 125 µL of acetonitrile and 1000 µL of HPLC-grade water were added to an Eppendorf Save-Lock Tubes and it was vortexed for 30 seconds (1/5 dil.). All tubes were centrifugated during 10 minutes under the following conditions: 4000 rpm at 20 ºC. Finally, the

supernatant was introduced into a LC vial up to 0.5 mL and stored at -60 ºC until its analysis. The analysis would be performed by UHPLC-HRMS.

5.5. ANALYSIS BY UHPLC-HRMS

The analysis would be carried out by UHPLC-HRMS using an Acquity LC (Waters, Milford, MA, USA) chromatograph system equipped with C_{18} column coupled to a QExactive (Thermo Fisher Scientific, San Jose, CA, USA) hybrid quadrupole-orbitrap mass spectrometer, equipped with an electrospray ionisation source operating in negative and positive ionisation mode.

The results obtained by stomach extracts would be compared with the results obtained from the haemolymph samples to get more evidence-based knowledge about metabolomics responses of the mussels during the experiments by statistical treatments.

5.6. BIBLIOGRAPHIC RESEARCH

Due to the impossibility to obtain experimental data about metabolomic responses of exposed mussels because of the pandemic induced by COVID-19, it was considerate as an alternative to do a research about prior metabolomics studies with different species and compounds to compare them. Some characteristics of compounds such as shape, size and hydrophobicity were being into account to find some trends by comparison between studies of different researchers' groups. The research has been done using two data bases: SciFinder and Scopus. Some of the keywords used to find articles and get several results were oxidative stress, microplastics exposure, metabolomics effects, size and shape-dependence, interactions between microplastics and co-contaminants, polar and non-polar contaminants and male and female mussels' metabolism.

The platform Mendeley has been used for bibliographic citation.

6. RESULTS AND DISCUSSION

6.1. MPLS SINGLE EXPOSURE

MPLs can alter the metabolome profile of species by their accumulation in the exposed tissues but without their internalisation. The most common external exposure is due to the particles that can pass across the gills, also in non-filter feeding marine organisms. However, the major negatively effect of MPLs is caused by ingestion and their accumulation in the digestive tract. Prior studies reported the accumulation of MPLs in marine biota in several tissues through feeding. According to *Cole et al.* [40], polystyrene microplastics exposures promote a decrease of algae ingestion in copepods. This can be reflected by conceptual energetic carbon budget. In comparison to the control group, these organisms suffer energetic depletion when they ingest polystyrene, reducing their feeding capacity and the ingest of carbon biomass. The first effects are due to their large retention time in digestive tract.

In Table 2, some metabolomics studies of different marine species including mussels and copepod exposed to microplastics and nanomaterials are summarized. All the studies listed in Table 2 have been carried out *in vivo* in laboratory marine mesocosms, as in the initial plan of the present work, where the experiments were conducted following this systematic approach: acclimatization period followed by expositions with different contaminant concentrations, in parallel to a control group. Nevertheless, the time of exposure is slightly different in each case, from days to weeks. As it is summarized in Table 2, the most commonly reported effect is the oxidative stress relying on antioxidant agents like superoxide dismutase (SOD) or catalase (CAT) activity, reactive oxygen species (ROS) effect, total oxidant status (TOC) and total antioxidant capacity (TAC), as well as changes on protein abundances and fecundity and gene expression disruption.

Concerning the oxidative stress, *I. Brandts et al.* [41] confirmed that it is produced an increase of antioxidant capacity in mussels when are exposed to 50 mg/L of PS. However, lower concentrations of PS (0.5 mg/L) provides an increase of oxidative status. In another study carried out in 2016 [42], observed that when marine rotifers were exposed to 0.1 mg/L nano- PS

an increase of ROS species were produced. Moreover, in a study carried out by *G. Magara, A. C. Elia et al.* [43], an increase of CAT activity was shown in mussels when they are exposed to PE MPLs. Knowing that enzyme catalase is an antioxidant agent, it provides a decrease of ROS production. The reaction that takes place is shown in reaction 1:

$$\text{Reaction (1)} \quad 2H_2O_2 \xrightarrow{\text{Enzyme CAT}} O_2 + 2H_2O$$

In summary, MPLs produce certain oxidative stress, but depending on the concentrations of exposure, means of exposure, the particle sizes and the polymers used, this perturbation can be compensated by the metabolism of exposed organisms by the activation of some metabolic routes. Therefore, in these studies, cannot be established any clear trend in oxidative status disruption on marine organisms because of the difficulty of comparing them. First, in these studies, different conditions were employed to carry out the experiments in the mesocosms. Besides, different responses were measured (see Table 2). Moreover, concentrations of exposure, route of exposure and the particles sizes and shape were as well different in the few studies already reported. Therefore, in our case cannot be hypothesized a priori the response, but our initial assumption when the experiment was planned was that the ROS effect will be produced.

As mentioned before, the particles shape and size particle are relevant factors ruling the effects of particles in biota.

Different studies concluded that the toxicity is inversely proportional to the particles size. In a study carried out by *K. W. Lee, W. J. Shim et al.* [44], where copepod (*Tigriopus japonicus*) were exposed to PS beads of three different sizes (0.05, 0.5, 6 μm), the most evident effects (an increase of toxicity and impact on survival and their development) were observed with 0.05 and 0.5 μm PS, the smaller ones. In another example*, C. B. Jeong et al.* [42] studied the PS size-dependent toxicity in the Monogonont Rotifer and the authors also concluded that small size particles can cause more negative effects (oxidative stress) in marine biota. Finally, in another study [45], confirmed that 1 μm PS cause more oxidative damage on *Daphnia magna* than 10 μm PS. With all these arguments, there is a clear tendency about particles size-dependent: little particle size produces more negative effects.

Regarding to shape, although no significant differences were observed by J*. S. Choi, Y. J. Jung et al.* [46] between spherical and non-spherical MPLs, some studies concluded that fibres

cause more toxic effects on aquatic organisms. For instance, an accumulation of MPLs fibres were observed into zebrafish gut by R. Qiao *et al.* [47] and, hence, more several intestinal toxicity were resulted. In addition*, A. D. Gray and J. E. Weinstein* [48] also concluded that PP fibres were significantly more toxic than beads in shrimp. A dissecting microscope were used to assess the number of particles that were found in shrimps' gut. They also recorded the mortality after exposures. Finally*, Sarah Y. Au, Terri F. Bruce et al.* [49] observed a greater toxicity of MPLs fibres when ingestion of amphipod *Hyalella azteca* were studied. It seems to be an increase of MPLs fibres toxicity in comparison to spheres particles.

Table 2. Selected metabolomics studies of MPLs or nanomaterials exposure in marine biota.

Compound exposure	Exposition	Analytical Techniques	Results	Ref.
PE in Blue mussel *(Mytilus edulis)*	Mesocosms, 96 hours feeding mussels with 100 MP/mL and 1000 MP/mL, T = 17 ± 1ºC.	Spectrophotometric analysis (Varian Cary 50 spectrophotometer at 25ºC)	This MPL provides an oxidative challenge in mussels. SOD activity was not significant altered in both tissues. In contrast, CAT activity increased. SeGPx decreased in digestive gland and increased in gills.	[43]
PS in Mediterranean mussel (*Mytilus galloprovincialis)*	14 acclimatization days in 80 L tanks without being fed at pH=7.9 and T=19ºC. 96 hours feeding mussels with PS from 0,5 to 50 mg/L in 3,5 L tanks.	Biochemical TOS and TAC were measured using an automatic analyser (AU 600 automated biochemical analyser, Olympus, Minneapolis, Minnesota). Glucose, aspartate aminotransferase (AST) and alanine transaminase (ALP) were determined using commercial kits (Biomérieux, France; Olympus Systems Reagents; Olympus life and Material Science Europe GmbH, Hamburg, Germany)	TOS increased in digestive glands in 0,5 mg/L PS exposure and TAC after 50 mg/L PS exposure. Inhibition of cholinesterase activity in haemolymph.	[41]

PS in marine copepod (*Calanus helgolandicus*)	9 days to gauge the sublethal impacts due to MPLs. Then, 3 acclimatization days and 24h exposure: 75 MP/mL and 250 ug C/L	Eggs production: dissection microscope. Mean eggs size: Olympus IX71; ×400 magnification Respiration Rate: optrode (Fibox 3 LCD trace) Algal size: Multisizer 3 coulter counter (Beckman). Carbon budget: 5 nL biovolume ≈1 µg C	Ingestion, fecundity and survival of copepods were decreased due to MPLs exposure. Prolonged exposures of MPLs decreased reproductive outputs.	[40]
Fullerene in Mediterranean mussel (*Mytilus galloprovincialis)*	Marine mesocosms for 35 days: 7 acclimatization days, 21 days of exposure and 7 days of depuration. Mussels were fed daily with microalgae and different fullerene concentration: 0,0; 1,1; 2,3 and 3,0 pg/L.	HPLC−HRMS using a Waters Acquity UPLC System (Waters, Milford, MA).	Seven free amino acids were altered. Alanine, Leucine and Isoleucine were increased. However, glutamine was decreased, causing an activation of facultative anaerobic energy metabolism. General increased of free fatty acids. These results induce hypoxia and oxidative stress.	[50]

6.2. INTERACTION BETWEEN MPLS AND OTHER CONTAMINANTS

Different studies have assessed if the particles size, particles shape, and the hydrophobicity of polymers have influenced the adsorption of contaminants from surrounding environments.

Concerning to size particle, *I. Velzeboer, C. J. A. F. Kwadijk et al.* [51] concluded that adsorption of Polychlorinated biphenyls (PCB's) onto nano-sized PS was stronger than micro-sized PE. In addition, the same trend was observed in another study [52], when the sorption of 3,6-dibromocarbazole and 1,3,6,8-tetrabromocarbazole to different size of PP microplastics (0.45 – 0.85 mm) was assessed. The authors confirmed that there was higher adsorption of contaminants in 0.45 mm microplastics than in 0.85 mm. *X. Zhang, M. Zheng et al.* [53] also studied the relation between the strong adsorption of chemicals to different size MPLs. The study consisted of comparing the sorption of three synthetic musk to five different range of PP size (2-5 mm, 0.85-2 mm, 0.45-0.85 mm, 0.15-0.45 mm) and they concluded that little size microplastics have more capacity to sorb the chemical contaminants in question. With all these pieces of evidence, it is reasonable to affirm that the size of the particles is a great influence in sorption dynamics. An increase of surface area relies on the enhancement of sorption ability.

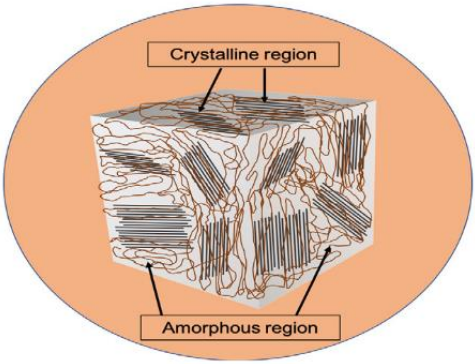


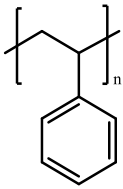
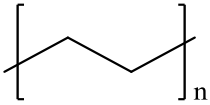
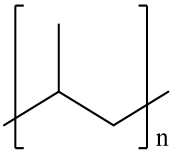
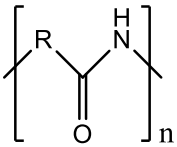
Figure 10. Semi-crystalline structure (Ref: *O. H. Fred-Ahmadu, G. Bhagwat et al.* [13]).

The importance of plastic polymer structure related to their chemical's sorption capacity is another characteristic to consider. Polymers can be amorphous, semi-crystalline or crystalline. The Figure 10 shows a semi-crystalline polymer structure and it can be observed crystalline regions and amorphous regions. Some studies compared the interaction between different types of microplastics (with different structure), with other contaminants. In Table 3 is shown the structure of several thermoplastic polymers.

For instance, *C. Goedecke et al.* [54] compare the different capacity to sorb chemicals between different types of microplastics (Polypropylene (PP), Polystyrene (PS) and Polyamide 6

(PA6)) with different structure. The study concluded that PS has more capacity to sorb metformin and difenoconazole than PP and PA6. Moreover, *T. Hüffer and T. Hofmann* [55] also concluded that PS has more capacity to sorb non-polar organic compounds than PE and PA. It is evident that an amorphous structure allows a greater interaction of the polymer chains with the environmental pollutants and provides better capacity to sorb contaminants. Therefore, the higher the percentage of amorphous structure in a polymer, the greater the adsorption of contaminants due to the more accessible superficial area and greater free volume. On the contrary, this ability decreased in semi-crystalline MPLs such as PE or PP. These plastics have amorphous and crystalline regions throughout their structure. Their crystalline areas hinder the chemicals' diffusion, due to the alignment of the chains and the ordered and compact structure.

Table 3. Structures of several thermoplastic polymers [54].

Polymer	Monomer	Structure
Polystyrene (PS)		Amorphous
Polyethylene (PE)		Semi-crystalline
Polypropylene (PP)		Semi-crystalline
Polyamide 6 (PA6)	 $R=C_5H_{10}$	Semi-crystalline

The octanol-water partition coefficient (K_{ow}) of different contaminants provides information about their hydrophobicity properties and it is a common parameter use to assess adsorption characteristics of each contaminant to particles such as MPLs or nanomaterials, which are non-polar. According to the study carried out by *S. Seidensticker, P. Grathwohl et al.* [56], several

compounds such as phenanthrene, diazinon, TCS and nonylphenol has stronger sorption to PE and PS than others co-contaminant like carbamazepine, benzotriazole and caffeine due to they are more hydrophobic. In Table 4, the Log of K_{ow} of different contaminants is shown. High values of log K_{ow} indicates less water solubility and high hydrophobicity and, therefore, more ability to be adsorbed by MPLs. Hence, non-polar compounds have a stronger interaction with MPLs than polar contaminants.

Table 4. Octanol-water partition coefficient logarithm of some organic compounds.

Organic Compound	**Log K_{ow}** [56]
Nonylphenol	5.76
Triclosan	4.76
Phenanthrene	4.46
Diazinon	3.81
Carbamazepine	2.45
Benzotriazole	1.44
Caffeine	0.07

Some collected studies also research the co-exposure between plastics and other co-contaminants and the probability to potentiate their impact to marine organisms. Their interaction can produce antagonistic effects (the mixture does not increase the negative effects to aquatic biota in comparison to exposure to toxicants only) or synergic effects (the MPLs or nanomaterial potentiate the toxicity of co-contaminant). According to *G. Magara, A. C. Elia et al.* [43], fluoranthene and polyethylene co-exposure not induce significant changes in oxidative challenges in mussels' metabolome, showing little impact. The impact is more evident when polyethylene or fluoranthene are acting alone. Hence, in this case, the mixture of PE and fluoranthene have antagonistic effects. In contrast, *S. Rainieri, N. Conlledo et al.* [57] concluded that MPLs, in this case, low-density polyethylene, potentiate the gene expression disruption in zebrafish when it is co-exposure with other POPs co-contaminants like PCB, brominated flame retardants (BFR), perfluorinated compounds (PFC) or methylmercury producing, therefore, synergic effects.

All contaminants in both studies are POPs and then, associated with non-polar compounds. A priori, it is reasonable to suggest a similar behaviour about the physical and chemical effects

in marine organisms, but it is evident from bibliographic research that these contaminants not always provide the same synergic or antagonistic effects. Different studies that are showed in the Table 5 demonstrate that there is no correlation between synergic or antagonistic effects with the hydrophobicity of each organic pollutant and their interaction with MPLs.

Table 5. Co-exposure effects on marine organisms.

MPLs and marine organisms	**Co-contaminant**	**Log K_{ow} (co-contaminant)** [58] [59]	**Synergic or antagonistic effects**	**Ref.**
LD-PE in zebrafish *(Danio rerio)*	PCBs	≈ 4-9	Synergic effects	[57]
PE in copepod (*Acartia tonsa)*	TCS	4.76	Synergic effects	[25]
PS in *Daphnia magna*	Phenanthrene	4.46	Antagonistic effects	[60]
PS in mussels (*Mytilus spp.)*	Fluoranthene	5.20	Antagonistic effects	[61]
PE in blue mussel (*Mytilus edulis*)	Fluoranthene	5.20	Antagonistic effects	[43]
PS in *Mylitus galloprovinviallis*	Carbamazepine	2.45	Antagonistic effects	[41]
PE in *Daphnia Magna*	Glyphosate acid	-3.4[(a)]	Synergic effects	[62]

[(a)]This value was taken from database PubChem.

6.3. INFLUENCE OF THE ORGANISM'S GENDER IN THE INDUCED EFFECTS

In metabolomics studies, the gender of organisms involved could be a relevant parameter, due to the different metabolic behaviour of different sex or the metabolic status according to their natural cycles. This is particularly interesting in the case of contaminants that can be related with endocrine disruptor effects. For example, *C. Ji, L. Wei, J. Zhao et al.* [63] demonstrated that the female and male mussels have different metabolomics responses in front of some contaminants such as bisphenol A (BPA). While female mussels have significant variations in some metabolites like an increase of acetoacetate, malonate or branched chain amino acids (BCAA), the male mussels did not show evident effects to BPA plastic additive exposures. Then, this research concluded that only female mussels have negative metabolome effects when they are exposed to this plastic additive. Nevertheless, although the other study carried out by the same research group [64] also concluded that female mussels are affected, 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) plastic additive expositions induce cell apoptosis and disturbance in energy metabolism in both genders. The difference was that female mussels also suffer a disturbance in osmotic regulation. With this data, it is deduced that male mussels can suffer alteration of metabolomics profile depending on the type of toxicant exposure and female mussels suffer the worst consequence in their metabolome when they are exposed to endocrine disruptors.

7. CONCLUSIONS

The present work has combined experimental and bibliography research. Due to the absence of experimental results, it was impossible to establish relevant conclusions regarding to metabolomics responses of mussels after being exposed to PE with adsorbed TCS. Nevertheless, gathering metabolomics studies in mussels and other marine species, led us to reach some interesting conclusions:

- Concerning MPLs exposure:
  - The most common effects to marine organisms are oxidative stress, changes on protein abundances and fecundity and gene expression disruption.
  - Size particle has a strong influence on toxicity: an increase of negative effects was observed for the small size MPLs.
  - It seems that irregularly shaped MPLs such as fibers cause the worst toxic responses in marine biota than spherical or fragments particles.
- Concerning MPLs and contaminant co-exposure:
  - Organic contaminants with high hydrophobicity have a higher interaction with MPLs. However, not always emphasize their negative effects: we cannot affirm that non-polar compounds always provide synergic or antagonistic effects due to the variability of responses that we found on several studies.
  - There is a clear relation between size particle and polymeric structure and their sorption capacity: NPLs and polymerics with amorphous structure have more ability to adsorb other contaminants than MPLs and semi-crystalline polymers.

- Concerning gender:
    - Female mussels have the worst consequence in their metabolism due to endocrine disruptors exposure than male mussels. Male mussels have different responses in each study, and they suffer metabolomics changes or not depending on the toxicant exposure.

The little literature about comparable studies it makes difficult to find evident trends due to the variability of types exposure. For example, it would be interesting to carry out studies with different marine organisms, but with the same mesocosms conditions and methods, in order to enhance the knowledge about the organisms' metabolomics profile and their responses after exposure.

Relative to identify and quantify metabolites, although the accuracy of the most common analytical techniques used in metabolomics studies is considerable, the improvement of their speed and resolution powder and the development of suitable optimization methods continues to be a challenge.

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9. ACRONYMS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BCAA	Branched chain amino acids
BDE 47	2,2',4,4' tetrabromodiphenyl ether
BFR	Brominated flame retardants
BPA	Bisphenol A
CE	Capillary electrophoresis
CEC	Capillary electro-chromatography
CLD	Chemiluminescence detector
ECD	Electron capture detector
GC	Gas chromatography
FID	Flame ionization detector
HOCs	High organic pollutants
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IT	Ion Trap
K_{ow}	Octanol-water partition coefficient
MPLs	Microplastics
MS	Mass spectrometry
NMR	Nuclear Magnetic Resonance
NPLs	Nano-plastics
PA6	Polyamide 6
PAHs	Poly aromatic hydrocarbons
PCA	Principal Component Analysis
PCB	Polychlorinated biphenyl
PE	Polyethylene
PEMRG	PlasticsEurope Market Research Group
PET	Polyethylene terephthalate
PFC	Perfluorated compound
POPs	Persistent organic pollutions
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
ROS	Reactive oxygen substances
SEM-EDX	Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy

SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TCS	Triclosan
TID	Thermionic ionization detector
TOC	Total oxidant capacity
TOF	Time-of-flight
UHPLC	Ultra-high performance liquid chromatography
UV/Vis	Ultraviolet-visible
WWTP	Wastewater treatment plants