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WYDZIAŁ CHEMICZNY



Imię i nazwisko studenta: Elena Portoles
Nr albumu: 174991

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dr hab. inż. Błażej Kudłak	prof. dr hab. inż. Jacek Namieśnik

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ABSTRACT

As a result of the growth of plastic production in recent years, there is a massive growth of plastic waste. Most of this plastic waste ends up in the environment, resulting prejudicial to it. For this reason, in recent years, there are many studies on how these plastics damage the environment. This work focuses on microplastics, which are plastic particles of a size smaller than 5 mm. These micro particles are present in most waters, including oceans, seas, rivers, lakes, sewage treatment plants, and even tap water. Through this review, it has been studied what microplastics are, how can they be classified and which is their history. To make it, the history of plastics is studied, since it is important first to know plastics, to finally understand what they are and where the microplastics come from. In addition it is studied what are the effects that these microplastics can have in the environment, focusing first on the effects on ecosystems, and then the effects on living organisms. This study focuses mainly on the aquatic environment. It is also studied which are the methods of analysis of microplastics, following a procedure, from taking of samples, the sampling processing, how to measure their shape and size, as well as find their color, and studying how to find the chemical composition of these microplastics. Finally, it has been done a research of studies about microplastics, mainly in aquatic media.

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1. INTRODUCTION

In recent years, the consumption of plastic has greatly increased. This material has allowed us many advances, since it is relatively inexpensive to manufacture, and you can obtain multitude of plastic variants with different properties. These advantages have caused the industry to massively generate plastic. Unfortunately, these materials are causing burden to the environment. Most of the plastics that have been generated are no longer used, so they have become waste.

These residues contaminate the earth, since plastic can take up to 1000 years to degrade, time in which it can cause multiple damages in the ecosystems.

Water pollution is one of the most serious. Currently several large islands of garbage are detected in the oceans around the world, they are in movement and contaminating enormous extensions of coasts and seas. Helen Briggs has published in 2018 on the BBC an alarming article (1) about plastics in the oceans. The article highlights that garbage accumulates in all oceans but the largest spot is the one in the Pacific, between Hawaii and California. According to the study, this waste area expands by 1.6 million square kilometres and contains nearly 80,000 tons of plastic.

In addition, plastic is one of the main factors of pollution of the air, that is, the environment. The plastics manufacturing system releases a large variety of toxins in the environment, moreover, a significant proportion of plastic ends up being burned or incinerated, thus generating enormous air pollution.

2. REVIEW ON TYPES OF MICROPLASTICS

2.1. HISTORY

The term plastic appeared for the first time in the 1630s, at that time, it was used to describe substances that could be moulded or could be shaped. The term derived from Ancient Greece, from the term *plastikos*, and from the Latin term *plasticus*. The new modern use of the term began in 1909 by Leo Hendrick, using it as a generic term to describe a large amount and variation of materials.

Plastic substances are composed of long chains of molecules, called macromolecules. These long molecules are composed of many smaller molecules connected in a sequence, which form a polymer. The German chemist Hermann Staudinger demonstrated the existence of these macromolecules and the relationship with the term polymer in the 1920s (2). Furthermore, he formed the first polymer journal in 1940 and received the Noble Chemistry Prize in 1953. Smaller molecules are called monomers, and these monomers can form longer chains through polymerization. Typically, in commercial plastics, there are between 10,000 and 100,000,000 monomers per chain, depending on the type of plastic.

The invention of plastics has not been a coincidence and it is due to many years of studies and product development.

Charles Goodyear produced the first known polymer in 1839. Goodyear managed to modify the mechanical properties of natural rubber by mixing it with sulphur and heating it. What was achieved with this mixture was that the obtained rubber was kept dry and flexible at any temperature. Before the discovery, when it was heated, it softened and became sticky. Goodyear patented this product that became known as vulcanization and that soon found many applications and it was transformed into a commercial product. Among other applications, it gave rise to the wheels for cars.

Alexander Parkes worked for a time in the production of natural rubber. Parkes looked for substances that could give results similar to those of rubber. Studying cellulose nitrate, Parkes obtained a new material in 1861 that could be used in its solid state, plastic or fluid, which was sometimes rigid like ivory, opaque, flexible, water resistant, colourable, etc. Parkes called this material Parkesina, patented it and, although it did not have much commercial success due to its high production cost, it was a definitive step in what we can consider the plastic material from which a large family of polymers has developed, those we know today.

In 1868 the company Phetan and Collander, a US company that produced billiard balls, promised a prize of \$ 10,000 to anyone who could develop a product capable of replacing ivory in the manufacture of balls since natural raw material was in short supply. John W. Hyatt began to investigate in this field and around 1868 improved the product developed by Parkes and obtained an economically viable product. This product was called celluloid, patented in 1870.

E. Baumann, in 1872, studied the polymerization process of vinyl chloride and paid attention to the importance of the thermoplastic product that was possible to obtain. But before they could start with the industrial production of vinyl polymers, it was necessary to wait for the deepening of knowledge about the synthesis of vinyl chloride due to F. Katte and the polymerization mechanism that the Russian chemist Ivanovic Ostromislenski made. In 1927 the American Union Carbide Chemicals produced the first vinyl chloride-acetate copolymers that were manufactured on an industrial scale from the year 1939.

The discoveries of parkesin and celluloid represented the beginning of a new material but the chemical structures of their molecules were totally unknown. The first hypothesis of the existence of macromolecules was developed in 1877 by Friedrich A. Kekulé, when he warned of the possibility that these natural organic substances could be made up of very large molecules and have special properties.

In 1893, Emil Fisher, suggested that the structure of natural cellulose could be made up of chains made up of glucose units, while the polypeptides would be large chains of associated polyamino acids. In 1907, Leo H. Baekeland perfected the formaldehyde resin that had been developed a few years earlier by Adolf Von Bayer. The substance he obtained was a rigid and low-flammable resin that he named Bakelite.

Hermann Staudinger, director of the institute of chemistry of Freiburg, began in 1920 the theoretical studies on the structure and property of polymers and synthetics. He formulated the hypothesis that polyesters and natural rubber were constituted by linear, independent and very long chemical structures and he proposed to name them as macromolecules. Staudinger's theories were not positively accepted all over the world. The experimental demonstrations showed that he was right, especially after the systematic investigations to the X-rays of the different polymers and the works of the synthesis of W.H. Carothers who demonstrated in experimental mode, the linear structure of macromolecules. Staudinger received the Nobel Prize in Chemistry in 1953. Between 1930 and 1942 other polymers were discovered such as styrene-butadiene copolymer (1930), polyurethanes (1937), polystyrene and poly (tetrafluorous-ethylene) (1938) or unsaturated politicians (1942), among others. In 1938, nylon was started by Dupont. Around this time, in Germany, P. Shlack made the first polymerization by opening the ring, of a cyclic organic compound, producing nylon. After the Second World War, the manufacture and commercialization of polymers had a great impulse with the appearance of epoxy resins in 1947 and ABS in 1948, which consists in an amorphous thermoplastic, which is very tenacious, and which has high resistance to impact, widely used in automotive and other domestic and industrial uses.

Another significant step in the studies of polymer chemistry occurred in 1953, with the discovery of stereoselective polymerization by the researchers Karl Ziegler and Giulio Natta. For these investigations they received the Nobel Prize in Chemistry in 1963.

This decade was marked by the birth of new polymers such as linear

polyethylene, polypropylene, polycarbonate, feline polyoxide, as well as new copolymers.

During the 1960s, plastics replaced many other products such as wood, cardboard or glass in packaging. In the 1970s plastics replaced some light alloys, taking the place of some metals. During the 1980s the production of plastics intensified and diversified becoming one of the main industries in the world. The needs of these materials and new developments were increasing, many research centres in industries and universities maintained and increased the research in these fields obtaining constant developments of polymers with the most varied chemical and physical properties.

From the old and appreciated celluloid of Hyatt, substitute material of noble substances that burned and sometimes exploded, we have arrived in more or less a hundred years to these polymers in many aspects superior to metals, ceramics and the traditional materials, and therefore already irreplaceable in the most advanced uses of modern technology.

The term microplastic first appeared in 1968 when the US Air Force Materials Laboratory made a publication about the study of microplastic properties and dimensional stability of materials. At that time, the term microplastic was used to explain the deformation of a plastic material. Exactly at the same time, the term macroplastic appeared in order to explain the deformation of a plastic material too. Since that publication, those two terms were not used again, until scientist adopted those two terms, generally to refer to the physical size of a piece of plastic. In the 1970s was when scientists discovered smaller pieces of plastic in the aquatic environment. It was discovered that large amounts of small plastic particles were floating on the surface of the Sargasso Sea (3)

Generally, at that time, these micro-sized pieces of plastic were simply called as “plastic particles”, and it was not until a publication in 2004 (4) that the modern use of the term microplastic was introduced. Within the publication the researchers used the term microplastics to describe small pieces of plastic that

had been collected from beaches in Plymouth, United Kingdom. The term of “microplastic” was adopted by the scientific community, and after that, the Steam Committee of the Marine Debris Program of the National Oceanic and Atmospheric Administration (NOAA) defined a microplastic: a piece of plastic smaller than 5mm throughout its longest dimension.

2.2. TYPES OF PLASTICS

Plastics are materials that come from petroleum treatment, which are combined with different additives. These additives are the responsible for the different properties of each type of plastic, such as its texture, resistance to temperature, malleability, brightness, stability, brightness, etc.

The different combinations of these compounds with different additives give us multitude of types of plastics.

Plastics can be divided into two main categories: thermostable plastics and thermoplastics.

- Thermostable plastics. These types of plastics are solidified or formed irreversibly when they are heated; they tend to ignite when they are subjected to very high temperatures. Also, they have high durability and resistance, and they are generally non-recyclable.
- Thermoplastic plastics. These types are soften when they are exposed to heat, and return to their original form at room temperature. There is no change in the chemical or physical characteristics of the polymer, but they can easily be moulded into a wide variety of products, such as detergent containers, milk containers, floors, credit cards, etc.

In conclusion the most important difference between thermostable and thermoplastic plastics are that thermoplastics can be easily moulded, while they have the same polymer structure.

The code of identification of plastics and resins is important for the identification of the different types of plastic and to know how to differentiate the recycling of plastics.

The plastics are differentiated according to a Code of Identification of Plastics, which is an international system in the industrial sector to distinguish the composition of resins in containers and other plastic products. This Code of Identification was done by the Society of the Plastics Industry (SPI) in 1988, in order to promote and have a more efficiency recycling.

The most abundant and most used polymers are:

1. PET (Polyethylene terephthalate). PET is mainly used in the production of bottles for beverages. Through its recycling, we mainly obtain fibres for sleeping bags, carpets, lines and pillows.
2. HDPE (High density polyethylene). HDPE is normally used in milk containers, detergent, motor oil, etc. HDPE after recycling is used for flowerpots, garbage containers and detergent bottles.
3. PVC (polyvinyl chloride). PVC is used in shampoo bottles, cooking oil containers, service items for fast food to take away, etc. PVC can be recycled as drainage and irrigation tubes.
4. LDPE (Low density polyethylene). The LDPE is in supermarket bags, bread bags, etc. The LDPE can be recycled as supermarket bags again.
5. PP (Polypropylene). The PP is used in most yogurt containers, bottle caps, etc. PP after recycling is used as steps for drainage records, car battery boxes, etc.
6. PS (Polystyrene). PS is found in disposable cups of hot drinks and meat trays. PS can be recycled into plastic joists, flowerpots, etc.

7. OTHERS. It generally indicates that it is a mixture of several plastics. Some of the products of this type of plastic are: bottles of ketchup, dishes for microwave ovens, etc. These plastics are not recycled because it is not known certainly what type of resins do they contain.

We can also classify plastics according to the type of degradation of each plastic. According to this classification, we can find three types of plastic: the ones known as conventional plastics, Oxo-degradable plastics and bio plastics. All these types of plastics can have the same composition, the only difference is that in the last two type of plastics (Oxo-degradable and bio plastics), some other compounds are added, such as vegetable fats, in order to accelerate degradation, making that the time these plastics are in the environment reduces.

After we know about types of plastics, we are going to differentiate in size categories for pieces of plastic. There are many ways to categorized sizes of plastic. A standardised size categories of pieces of plastic is:

Table 1. The standardised size categories of pieces of plastic (5)

Category	Abbreviation	Size	Size definition
Macroplastic	MAP	≥25mm	Any piece of plastic equal to or larger than 25 mm in size along its longest dimension
Mesoplastic	MEP	<25mm-5mm	Any pieces of plastic less than 25 mm to 5 mm in size along its longest dimension
Plasticle	PLT	<5mm	All pieces of plastic less than 5 mm in size along their longest dimension

Microplastic	MP	<5mm-1mm	Any piece of plastic less than 5 mm to 1 mm in size along its longest dimension
Mini-microplastic	MMP	<1mm-1µm	Any piece of plastic less than 1 mm to 1 µm in size along its longest dimension
Nanoplastic	NP	<1µm	Any piece of plastic less than 1 µm in size along its longest dimension

Matt Flowers on November 2016, made a classification of microplastics (6):

- Fibres: microfibers are non-biodegradable plastic, which come from items like fleece clothing, diapers, or one of the more common ways, from using the washing machine. He stated that fibres account for 71 % of the total microplastic pollution in the Great Lakes (USA).
- Microbeads: they are non-biodegradable plastic particles with less than one millimetre of diameter. We can find this type of microplastic, for example, in facial cleaners, exfoliating soap products or toothpaste.
- Fragments: these types of microplastics are smaller pieces of plastic that come from bigger pieces of plastic. Some examples are pieces of cutlery, lids, or single-use products.
- Nurdles: nurdles come from small plastic pallets used to manufacture plastic goods. These plastic pallets can easily spill out of transportation vehicles, and after that, due to storms of weather conditions they can go to the water.

- Foam: styrofoam is used for packing material, in food containers, coffee cups, etc. Styrofoam breaks down into smaller pieces, and they can be in food and beverages, affecting human health.

In another classification we can find primary microplastics and secondary microplastics.

- Primary microplastics

Primary microplastics are those that have already been manufactured with microscopic size. On the one hand we can find microspheres (<500 µm), which are internationally manufactured by the plastics industry for use in cosmetics, personal care products, dermal exfoliators, cleaning agents etc.

The purposes of different types of plastic in cosmetics and personal care products according to the Review of microplastics in cosmetics, by Leslie HA is according the next table (7):

Table 2. Purposes of plastics in cosmetics (7).

Plastic	Use in cosmetics and personal care products
Polyethylene terephthalate	Hair fixative; adhesion; film formation; viscosity control; aesthetic agent
Polyethylene isoterephthalate	Bulking agent
Polybutylene terephthalate	Viscosity control; film mormation
Polypropylene terephthalate	Emulsion stabiliser; skin conditioner
Polypentaerythrityl terephthalate	Film formation
Polyethylene	Film formation; viscosity control; abrasive; binding agent
Polypropylene	Viscosity control; bulking agent
Polystyrene	Film formation

Polyurethane	Film formation
Poly (methyl methacrylate)	Sorbent material for active ingredient delivery
Nylon 6	Viscosity control; film formation
Nylon 12	Viscosity control; bulking agent; opacifying agent
Polytetrafluoroethylene	Bulking agent; binding agent; slip modifier; skin conditioner
Ethylene/acrylate copolymer	Film formation; gelling agent
Ethylene/methacrylate copolymer	Film formation
Styrene/acrylate copolymer	Aesthetic agent (colour)

On the other hand, the pellets, which are plastics particles between 2 and 5 mm. These small coloured pellets of plastic are manufactured globally by the plastics industry for the purpose of being melted down and moulded to form larger plastic artefacts. They are usually composed of polymers such as polyethylene and polypropylene. These materials are usually concentrated in urban areas; however, significant accumulations have been found in numerous beaches, forming an important part of the marine litter that reaches the coast.

- Secondary microplastics:

Secondary microplastics are those plastic products from a larger size, which once they have been manufactured, whether on the sea surface, on beaches or in other areas, are exposed to external conditions such as solar ultraviolet radiation (and others), which will cause the degradation of plastics. This degradation can be different depending on the cause that generates it, and also causes the discoloration of the plastics, erosions on their surface, and an increase in their fragility. The fragmentation of larger plastics is therefore an additional source of entry of microplastics into the oceans. This fragmentation can take place before the plastics are discarded in the environment, as in the

case of the fragmentation of synthetic fibres when washing clothes, or the degradation can happen once these plastics of greater size already comprise of the environment and they are degraded by different chemical, biological and physical processes. As already advanced in the study of fibres, the washing machine produces a lot of contamination of microplastics. According to a study by Browne in 2011 (8), in a single wash of synthetic garments, more than 1,900 microplastic fibres can be released, which would reach the oceans through wastewater effluents.

The microplastics found in the aquatic environment have all kinds of shapes, colours and sizes; some of them have spherical appearance, while others have a fibrous or random shape. The primary microplastics usually have a spherical or fibrous shape, with a uniform surface. By contrast, secondary microplastics have a more random appearance and, therefore, they are more difficult to categorize. In addition, microplastics can have a variety of different colours. These different colours are used as part of the standardized system to classify the microplastics recollected in the environment. In many cases, the colour of the microplastics tells us about the degree to which they are contaminated with chemical contaminants.

3. IMPACT OF MICROPLÁSTICS

3.1. IMPACT ON ECOSYSTEMS

It has been estimated that, of the total plastic waste that we generate, 10% ends up in the oceans (9), where they can represent a danger for the environment. The entrances to the marine environment are produced from different sources and in a wide range of sizes, from microns to meters. The interactions that occur between marine litter and the marine environment are diverse. On one hand, plastics not only affect visual value of ecosystem but they can also cause economic and social damage due to interactions with fishing or other maritime activities. Likewise, there are more and more impacts on the marine biota as a whole, which will be discussed in the next section (3.2 Impacts on living organisms). On the other hand, with this rhythm of production of plastic of a single use and of a half life so short, the microplastics are nowadays one of the factors of marine pollution more extended and chronic. These small particles enter the oceans in very different ways and are distributed throughout the oceans by marine currents, resulting in a global distribution of these materials. Its presence in the diversity of oceanic habitats, and aquatic in general, only increases the number of ecosystems and organisms vulnerable to its exposure.

The ubiquity of marine litter and in particular of plastics (macroplastics and microplastics) in the different aquatic compartments is well known and is documented extensively (10).

Nowadays plastics have been found in almost all aquatic habitats on the planet, since open ocean, rivers, seas, surface waters and also in the water column and even sediments, both beaches and depths (10). Knowing the distribution and quantification of microplastics, given their size compared to the seas, is complex and has many inconveniences. On the one hand, there are multiple forms of entry of these particles into the oceans that, because they are not dated, make it impossible to calculate the degradation time of them. In addition,

the combination of currents and seasonal variations in the oceans makes it even more difficult to address a temporal and spatial distribution for these tiny particles. All this, together with the added difficulties of sampling and extraction of these microplastics, as well as the variety of techniques used for their quantification so far, makes knowing the abundance of these materials and their distribution is a very complex task. Despite everything, recent research shows us some figures. In a study by Cózar at 2014 (11) it was estimated that there are between 7,000 and 35,000 tons of plastic floating in the oceans. Another study of the same year affirms that the 5 trillions of plastic pieces estimated total a total of more than 250,000 tons (12).

When microplastics enter the marine environment, they persist in it, and their distribution is linked to the interactions of these materials with oceanographic and environmental conditions. Some of these interactions are the ocean currents, the transport generated by the wind and the processes of vertical and horizontal mixing derived from it. On the other hand, the own physical and chemical characteristics due to the composition of the material itself also affect the distribution of these particles in the aquatic environment. In this way, plastics composed of lighter polymers will remain in the water column or surface water, while higher density plastics sink to the seabed. The fragmentation and the state of degradation of the particles are factors that also influence its distribution. For this reason, it is useful and necessary to know the input sources of plastics to the ocean and temporality, so that the degree of degradation can be estimated according to the time they have in the system. In the same way, it is also interesting to know the concentrations of plastic on the beaches, since the larger articles, through their degradation, suppose an entrance of microplastics to the oceanic system.

Once the microplastics enter the oceanic circulation, they tend to accumulate in the regions of the oceanic turns (13). As a result, they may be suspended in the water column, in surface waters, estuaries, rivers, as well as in beaches and deep sediments.

In 2014, a comparative study of the distribution of floating plastic of all sizes between the different ocean basins was published. This study, published by

Eriksen at 2014, includes the data from 1751 sampling stations, covered in a total of 24 expeditions carried out between 2007 and 2013 in the oceanic turns (North and South Pacific, North and South Atlantic, Indian Ocean), the Mediterranean Sea, the Gulf of Bengal and the coastal waters of Australia. In all of them, macroplastics or microplastics were found, with a greater tendency in areas of convergence, but with similar percentages of abundance between the northern and southern hemispheres.

The marine sediments range from the bottoms of the ocean to the bottom to the beaches, and therefore they encompass a wide variety of environments in which plastics can be found, both in their macroscopic and microscopic forms. Once they reach the sea, a large part of the plastics float on the surface and are transported for long periods of time, far from their original sources. Finally, a large proportion of these floating debris are accumulated on coasts around the world, generating negative impacts on wildlife. This subject requires attention and detailed studies, due to the fact that green areas and coastal environments are part of the most important ecological and socioeconomic zones in the world (14).

In Europe, about 50% of the articles of the plastic industry packaging is single use (15), which accumulates rapidly in waste management facilities and as garbage in the environment. Even though some steps have been taken in the direction of manufacturing biodegradable plastics, there is no evidence that these suffer an effective degradation in the marine environment.

3.1. IMPACT ON LIVING ORGANISMS

Microplastics are present in almost all marine habitats throughout the planet, and due to the density of these elements, along with the characteristics of ocean currents, there is an important effect in their distribution, because, due to the different densities, and depending on their composition, the microplastics are distributed occupying different areas of water. Knowledge about the possible effects of microplastics on marine biota is still under development, much information is being developed in recent years.

The presence of these microplastics can affect living organisms in different ways, but the most important are: being ingested, or being transferred along the food chain.

However, according to various authors like Amy Lusher in 2015 (10), the relative impact of microplastics is closely related to their size: microplastics that measure between 1 and 5 mm would be more likely to affect food and digestion of certain organisms, while those of micron size can be actively ingested by small invertebrates, but also excreted. An important case is that of nanoplastics, which in some cases can even permeate the cell membranes of organisms, altering their structure, activity, and therefore their function.

A wide variety of marine organisms, including corals, invertebrates, molluscs, crustaceans, fish, birds, turtles or cetaceans, can ingest microplastic directly, or incorporate them by ingesting dams.

This can lead to disorders in diet and digestion, as well as in reproduction, among other effects, such as blocking the appendages used to obtain food or obstructing the passage through the intestinal tract of some organisms, as well as limiting the intake of food and therefore reduce the amount of energy available, as occurs with the macroplastics in other organisms such as seals, birds and turtles.

In some cases, the feeding mechanisms of the organisms do not allow them to distinguish between their prey and the microplastics and ingest them indistinctly, or they intentionally ingest them by confusing them with prey, for example, in the case of plankton.

The routes of incorporation of microplastics in the organism do not have to be limited only to intake, but, in some cases, as in the case of crabs, these can also be incorporated through the gills into the circulatory system.

Some studies that have investigated the colour and size of microplastics in relation to their confusion with dams, have found that microplastics of certain colours or shades are, in some cases, more easily confused with dams. Since

there is a great variety of microplastics whose density keeps them floating close to the surface, they are available for a wide range of organisms that interact with plankton, such as the larvae of important species of commercial interest.

The ingestion of microplastics by organisms of the lower links of the trophic chain (phytoplankton and zooplankton) can be an entry route for higher levels of the trophic chain through the consumption of prey previously contaminated by these elements. In addition, the fact that part of the zooplankton makes daily migrations at different depths, makes it serve as a transport of microplastics to greater depths than these elements usually are, being available for various food chains. Studies carried out in recent years have shown the trophic transfer of microplastics between fish and Norway lobster, and between copepods and macrozooplankton.

Although there are not many trophic transfer studies to the upper links of marine birds and marine mammals, some works, like for example the one carried out by Ericksson and Burton in 2003 (16), have already demonstrated this transfer.

Some studies made about loggerhead turtles and green turtles have indicated that because of the consumption of plastic litter by these species, the results are nutritional deficiencies due to the fact that plastic material cannot be digested and has no nutritional value (17). Besides, in a more recently study (18), juvenile green turtles were studied along the Brazilian coastline, and it was found that only small amounts of ingested plastic litter were required to produce intestinal blockage or death.

There is not much information regarding the effects of microplastics on humans. However, in 1988, in the British Medical Journal (19) there was a study in which a few humans were fed with 15 g of microplastics, smaller than 2 mm in diameter. The researchers observed that, compared to the volume of ingested microplastics, the resulting faeces were typically 3 times larger in volume. In addition, there was a significant increase in transit through the gastrointestinal system. In another study (20), published in 1997 by McIntyre et al. other healthy

human volunteers ingested 15 g of polyethylene microplastics that measured between 1 and 2 mm in diameter. As in the previous study, they also detected that the microplastics accelerated the transit time of the material through the human gastrointestinal system. An increase in gastrointestinal transit has also been demonstrated when ingestion of microplastics in pigs and dogs, published in British Journal of Nutrition in 1985. That indicates that this effect does not occur only in humans. A consequence of this increasing of the time of gastrointestinal transit can reduce the absorption of nutrients and maybe produce in someone nutritional deficiencies. In addition, many plastics contain chemical additives, such as phthalates, which can be toxic, and the monomers of polyester and poly (methyl methacrylate) are mutagens and respiratory irritants.

A recent study, published in February 2016 by Miranda DD in the Marine Pollution Bulletin (21), concluded that humans are ingesting microplastics, through a fish that contains microplastics.

It is important to highlight that microplastics have been found in tap water. This is a problem, because if there are microplastics in tap water, there may also be microplastics in daily foods, such as bread, pasta, or soups.

A scientific investigation carried out by "Orb Media" (22), an international journalism organization, located in Washington, which has had the collaboration of researchers from the State University of New York and the University of Minnesota, has detected microplastic in 83% of the 159 samples of tap water collected in five continents. For example, microplastics have been found in New York (United States), Beirut (Lebanon), New Delhi (India), Kampala (Uganda), Jakarta (Indonesia) and Quito (Ecuador).

Muhammad Yunus, winner of Nobel Peace Prize in 2006 warns of how worrying these events are.

A new and more recent research conducted by Orb Media (23), shows that a single bottle of drinking water can contain a significant amount of microscopic

particles. The existence of microplastics in bottled water is even more alarming, says Orb Erik Solheim, executive director of the United Nations Environment Program.

In particular, the exclusive analyses carried out by Orb Media on more than 250 bottles of 11 leading brands reveal widespread contamination with microplastics waste, such as polypropylene, nylon and polyethylene terephthalate (PET).

The world average stands at 314 particles per litre of bottled water and plastic was found in 93 per cent of the samples, which came from 19 locations in 9 countries on five continents.

Up to 90 per cent of ingested microplastics can be excreted by the intestine without problems, but the remaining 10 per cent can pass from the bloodstream to the kidneys or the liver, as also warns a report of the United Nations.

However, bottled water manufacturers stressed that their products met all government requirements.

4. METHODS OF MICROPLASTICS ANALYSIS

In the environment there is a great variety of microplastics, which are in different shapes, sizes, colours, and with different origins.

These characteristics are very important, since they determine the distribution and impact of them. For example, denser particles spend more time in contact and collide more strongly with sediment particles, thus affecting the degradation of microplastics.

The processing of the laboratory and the classification of microplastics is very important for the characterization of them. We can differentiate four fundamental steps during the processing of samples: sampling methods, sampling processing and characterization of microplastics. All these steps have been developed from a review of 68 studies (24).

4.1. GENERAL SAMPLING METHODS

Sampling of microplastics in the main marine environments requires more work than taking samples of tap water or bottled water. It is carried out through three different approaches: the samples can be selective, bulk, or volume-reduced.

Selective sampling refers to samples in which the extraction is done directly from the environment, where the elements are recognizable to the naked eye, usually on the surface of the sediments. This type of card can be accomplished when the range of sizes is between 1 and 6 mm in diameter (24), so that they are easily recognizable. However, when microplastics are mixed with other elements or do not have characteristic shapes (they have for example irregular shapes), special care must be taken when selectively sampling.

Bulk samples consist of taking the entire volume of the sample without reducing it during the sampling process. These type of samples are more appropriate for

when the microplastics can no be easily recognized, as they are covered by sediment particles, or because they can be found in small quantities and require filtration with large volumes of sediment or water, or because they are too small to be identified with a naked eye (24).

The volume-reduced samples are referred to the samples in which the volume of the bulk sample has been reduced during sampling. In this type of sampling only a portion of the sample is preserved, which is of interest for later review.

4.2. SAMPLE PROCESSING

In the case of bulk samples and volume-reduced samples, laboratory processing and classification of microplastics is essential (24). During the processing of samples in the laboratory, four main steps are distinguished: density separation, filtration, sieving and visual sorting.

Density separation. The specific density of the plastic particles can vary greatly depending on the type of polymer and depending on which was its manufacturing process. The density values for plastics range from 0.8 to 1.4 g cm⁻³, specifically for polypropylene from 0.85 to 0.94 g cm⁻³, polyethylene from 0.92 to 0.97 g cm⁻³, and for polystyrene from <0.05 to 1.00 g cm⁻³. These values refer to the resins, without taking into account the effect on the density of several additives that could be added during the manufacture of the product. The typical densities for sand or other sediments are 2.65 g cm⁻³. This difference is used to separate the lighter plastic particles from heavier sediment grains by mixing a sediment sample with a saturated solution and stirring it for a certain period of time (24). After mixing, the sediment is expected to settle quickly to the bottom, while the low density particles remain in suspension or float on the surface of the solution. After that, the supernatant with the plastic particles are extracted and subsequently processed.

Filtration. The plastic particles are separated from the supernatant obtained

from the density separation by passing the solution containing the plastic particles through a filter, generally aided by vacuum. The filter papers usually have pore sizes from 1 to 2 μm (24). In order to separate the larger particles before the filtration step, the water samples can first be sieved on a sieve with a mesh size of 500 μm .

Sieving. Microplastics can be separated from the samples using sieves of varying mesh sizes. The materials retained in the sieve are collected and sorted, while those that pass are generally discarded. The use of sieves with different mesh sizes allows us to distinguish categories of microplastic sizes.

Visual sorting and separation. The visual examination of the remains of concentrated samples is a mandatory process. A careful visual classification of the waste is necessary to separate the plastics from other materials, such as organic remains (like fragments of shells, parts of animals, of seaweed) and other elements (like coatings of metallic paints, tar, or glass). This process is carried out by direct examination of the sample with the naked eye or with the help of a dissecting microscope. Visual sorting and separation is adequate for microplastic for large microplastics, with sizes greater than 1 mm of diameter (25).

The previously isolated plastic fragments can also be washed to remove other substances that adhere to their surface (such as sand), washing, for example, by ultrasonic cleaning in a liquid medium or deionized water.

4.3. CHARACTERIZATION OF MICROPLASTICS

Microplastics can be found in a very varied way, with multiple shapes, sizes and origins.

4.3.1. SIZE

The minimum size of the microplastics that are collected depends directly on the sampling and processing methods. Thus, the samples are separated according to the mesh size during sieving. However, sieves with a smaller mesh size are usually 0.5 to 2 mm (24). Therefore, the minimum size of the collected microplastics depends on the mesh of the net when collecting samples, which could vary, for example, from 50 μm to 3mm.

A minimum size of microplastics has not been defined. The smallest size reported during a review of 68 studies by the article mentioned before (24) was 1 μm in diameter and 20 μm in length in sediment samples. During most of the studies, values of size greater than 500 μm were obtained for sediment samples and a size of 300 μm for seawater samples. This difference depends mainly on the tools used during sampling, and the steps followed during processing.

For the comparison of the methods used to quantify microplastics in the environment, it is useful to distinguish between two main sizes (24): particles with a smaller size than 500 μm , and particles with a size between 500 μm and 5mm. The quantification of the smallest particles, is restricted by the technological constraints of the identification equipment, these nanoplastics will require new innovative methods. Larger particles, between 500 μm and 5 mm, can be quantified using sieves.

4.3.2. SHAPE AND COLOUR

The number of categories that are used to classify microplastics depends on the multiple criteria of the respective authors.

Mainly, the microplastics are analysed from microscopy (24).

Microplastics have very varying shapes; we can find from irregular fibres, to spherical or elongated and thin. The shape of these plastic frames depends largely on the fragmentation process and the residence time in the environment. Thus, the shape of the microplastics can provide us with information. The attached edges are indicators of having entered to the sea recently or that they come from the largest plastics. Plastics with smooth edges are usually older plastic fragments. Larger particles tend to have more elongated shapes with irregular surfaces, while smaller particles are more circular (24).

The degradation and erosion of the surface of plastic particles are due to degradation, chemical wear or physical forces such as waves or wind. This is what causes plastics to have cracks in its surface, for this reason there are many varieties of particle shapes.

This degradation also causes oxidative aging of the particles, by ultraviolet rays and infrared rays of solar radiation.

There is a very wide range of colours in which microplastics can be found. The colours can be used to perform a preliminary identification of the chemical composition of the most common particles, although additional analysis is necessary for the conclusive identification of the composition. The lighter plastic granules are associated with polypropylene, while the white plastic granules are attributed to polyethylene, on the other hand, the opaque granules are associated with low-density polyethylene, and the transparent plastic granules are attributed to the ethyl vinyl acetate (24).

It should be noted, in relation to the sources of the microplastics, that the sources of these plastic pellets are mainly associated with processing plants near the study sites. However, plastic pellets are also found on urban beaches located long distances from these potential sources, which implies long-distance maritime transport (24). This fact causes that some of the properties of plastic pellets can change during their residence at sea.

4.4. IDENTIFICATION OF THE CHEMICAL COMPOSITION OF MICROPLASTICS

As already described above, plastics are synthetic polymers manufactured from a wide range of chemical compounds that give rise to many different plastics with different characteristics.

There are several methods for the identification of the chemical composition of microplastics.

Identification is possible with infrared spectroscopy. This method is based on the comparison of the infrared spectrum of a sample of an unknown microplastic, with spectra of known polymers. There are different types of spectroscopy applied for the identification of microplastics, the infrared spectrophotometer, Fourier transform infrared spectroscopy, and the near-infrared spectrometer. From these techniques it is possible to identify a range of polymer such as polypropylene, polyethylene and polyester.

Infrared (IR) or Fourier transform infrared (FTIR) spectroscopy are techniques from which particles of plastic polymers are precisely identified (32,33). A sample is placed in an infrared cell, and is subjected to a source of infrared light with different wavelengths. This infrared radiation excites the molecular vibrations (which depend on the composition and molecular structure of the substance), which are specific for each wavelength. The amount of energy absorbed by the sample will be measured (which will be the difference between the intensity of light that enters and the one that passes through it), thus obtaining the characteristic IR spectra (28). These IR spectra are very characteristic, with different band patterns, for each plastic polymer.

Another chemical analysis, which provides information about the structure of the polymer, is Raman spectroscopy. The Raman spectroscopy is an analysis technique that consists of irradiating a sample with a monochromatic laser source (with a wavelength that depends on the available lasers, normally between 500 and 800 nm) (28), this light interacts with the molecules and atoms of the sample producing a backscattered light with a frequency different from that of the irradiating laser. This difference is due to possible vibrations, rotations and other low-frequency interactions. This is called Raman shift,

which can be detected. Plastic polymers have characteristic Raman spectra, that is why the polymer can be detected by comparison with reference spectra. This technique is simple and has been successfully performed for the identification of microplastics (29,30,31).

From this technique it is possible to analyse both large particles that have been previously classified visually, and microscopic particles if combined with microscopy, being able to characterize microplastics of sizes smaller than 1 μm (30).

One disadvantage of this technique, Raman spectroscopy, is that when the laser irradiates a fluorescent sample, the fluorescence of this sample prevents the generation of an interpretable Raman spectrum. That is why a purification stage is adequate before the Raman spectroscopy technique, to prevent fluorescence.

In addition, it can be used to determine the polymers from which the microplastics are made, the smoke characteristic of combustion and testing with solvents. From the characteristic pyrograms it is possible to identify the type of polymer. These characteristic pyrograms are obtained as a result of the pyrolysis of plastic polymers. From the comparison of the pyrograms of the combustion products obtained, with reference pyrograms of known polymers, the origin of the polymer is obtained (27). This technique is known as pyrolysis-gas chromatography (GC). This technique in combination with mass spectrometry (MS) is used to evaluate the chemical composition. This spectrometry serves as a detector of a gas chromatograph, and it is an analysis technique that allows us to determine the distribution of the molecules of a substance as a function of its mass. The results obtained by this method of pyrolysis GC - MS are quite good at identifying polymers, however it has some disadvantages, one of them is that you have to place the particles manually, which implies that the particles have to be large enough to be manipulated manually, and another disadvantage is that it will not be possible to analyse

large amounts of samples, since this technique only allows analysing one particle per cycle.

In 2010 (26) the specific densities were used to identify the origin of the polymer of the microplastics. For this purpose, the sample was first introduced in distilled water, adding ethanol or concentrated solutions in calcium or strontium chloride depending on the density of the sample, finally getting the sample to float neutrally. The precise determination of the density is done by evaluating the weight of a certain volume of the solution. From a subsequent analysis C-H-N the plastic origin of the particle is identified, since the characteristic elemental composition is different for the different groups of polymers. This technique involves a approximate identification of particles, but it is not a rigorous chemical analysis.