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13. Current Knowledge of Methods for Assessing Surface Water Pollution with Microplastics and their Impact on Aquatic Species

TEODOROF Liliana[1*], BURADA Adrian[1], DESPINA Cristina[1], SECELEANU-ODOR Daniela[1], ȚIGĂNUȘ Mihaela[1], IBRAM Orhan[1], TUDOR Iuliana-Mihaela[1], SPIRIDON Cosmin[1,2], SALI Yasemin[1], CIORPAC Mitică[1], TOSIC Katarina[1], TAFLAN Elena[1], HOLOȘTENCO Daniela[1]

[1]Danube Delta National Institute for Research and Development, Tulcea, Babadag Street, 165, Tulcea, RO-820112, România,
[2]"Dunărea de Jos" University of Galați, Faculty of Science and Environment, Domnească Street, 47, RO-800008, Galați, România;

[*]Adress of author responsible for correspondence: Liliana TEODOROF Danube Delta National Institute for research and Development, Tulcea, Babadag Street, 165, Tulcea, RO-820112, România, email: liliana.teodorof@ddni.ro

Abstract: In order to identify and quantify a new class of pollutants, the microplastic, originating from anthropogenic activities, proposed to be included in the list of indicators monitored for deltaic ecosystems quality establishment, it was necessary to carry out a screening of the literature regarding the pollution of continental waters and marine waters with microplastics, sampling, purification and analysis methods, the overall impacts on aquatic fauna, with an emphasis on the toxicity of ingested microplastics, the susceptibility of organisms to the ingestion of microplastics and the physical impact of microplastics, especially on freshwater aquatic species. Analysis of the literature was done by querying the database of ISI quoted articles, Web of Science Core Collection, using specific keywords.
Key words: microplastics, aquatic ecosystems, sampling, purification, methods

INTRODUCTION

Anthropogenic activities have resulted in the accumulation of numerous and diverse types of materials in the sediments of aquatic ecosystems, including synthetic polymers (plastic). The production of these chemical synthesis compounds, along with the development of mass production technologies, has made plastic one of the most commonly used materials. Among the major findings was the elucidation of sulfur vulcanization of natural rubber. At the beginning of the nineteenth century, several attempts were made to create synthetic polymers, including polystyrene (PS) and vinyl polychloride (PVC), but at that time the compounds were either too coarse or were unable to keep form to have commercial significance. The first synthetic polymer that was produced *en masse*, was Bakelite, a substance of resinous origin, developed by the Belgian chemist Leo Baekeland, in 1909.

Modern forms of PVC, polyethylene terephthalate (PET), polyurethane (PUR), were made during the 1930-1940, so that in the early 1950s the method to be developed the method for the production of high-density polyethylene.
In order to meet the various purposes for which plastic is made, the polymer-based materials from which it is composed are processed together with numerous chemical additives such as plasticizers to ensure flexibility, flame retardants, inorganic fillers to provide impact resistance, or pigments, for coloring. It follows that plastic is a complex of chemicals, each having a potential negative impact on the environment. The impact is even greater as it is a material whose use tends to grow. The plastics market is dominated by four main classes: (1) polyethylene - PE - with a world production of 73 million tons in 2010, (2) polyethylene terephthalate - PET - 53 million tos in 2010, (3) polypropylene - PP - 50 million tons in 2010 and (4) polyvinyl chloride - PVC - 35 million tons in 2010 (UNEP 2016).
The term "microplastic" refers generally to plastic particles with a diameter of <5 mm, this being the definition used by most authors. It has also been suggested to redefine the term for plastic objects <1mm (Andrady 2011; Browne et al., 2011). Lambert (2014) describes macroplastics as particle size > 5mm,

mesoplastics with dimensions ≤ 1 mm to> 0.1 μm and nanoplastics ≤ 0.1 μm. However, the upper limit of 5 mm is generally accepted because this dimension includes a wide range of particles that can be easily ingested by aquatic organisms.
Plastic enters into the aquatic environment from various sources and through various ways such as: (1) passing through water treatment plant filters, (2) applying biosolids - nutrient-rich materials resulting from domestic water treatment - in agriculture (Nizzetto et al., 2016), (3) incidental emissions such as, for example, those resulting from the wear of tires, (4) emissions from various industrial processes, and (5) atmospheric transport and fiber deposition.
The microplastics isolation from the environment can be particularly laborious especially if the samples contain a lot of organic substance. Also, the spectroscopic identification of synthetic polymers is limitated by high concentrations of pigments and the wear and disintegration of particles and microplastic fibers over time. As a result, the detection and confirmation of microplastics by analytical methods requires access to sophisticated equipment.
Because they have been investigated for a short time, the long-term effects of exposure to microplastics are unknown. Freshwater species represent a part of a complex trophic state, where there is a wide variety of food types and different feeding strategies. Research on marine organisms has reported the phenomenon of malnutrition associated with intensive feeding with microplastics that replace part of natural food (Cole et al., 2015, Phuong et al., 2016, Welden and Cowie 2016).
The purpose of this study is to identify through analysis of the literature the current state of knowledge regarding methods of evaluation of microplastics in freshwater and their impact on biota.

MATERIAL AND METHODS

The methods used were a screening of the literature on continental and sea water pollution with microplastics, the general impact on aquatic fauna, with an emphasis on ingestion of microplastics, susceptibility of organisms to ingestion of microplastics and the physical impact of microplastics, especially on freshwater aquatic species. This specialized literature screening was conducted by querying the database quoted articles from Web of Science Core Collection (Clarivate Analytics, USA) by using a keyword suite (Table 1).

Table 1 Web of Science Core Collection Database Query Summary and Search Keys Used (source: http://apps.webofknowledge.com/WOS_GeneralSearch_input.do; accessed in 18 March 2018)

Key words used	Data base	The time period that the identified items were published	The number of results identified identificate [1]
microplastic pollution	*Web of Science Core Collection*	2004-2018	625
microplastic pollution in freshwater	*Web of Science Core Collection*	2007-2018	105
marine microplastic pollution	*Web of Science Core Collection*	2004-2018	538
microplastic ingestion	*Web of Science Core Collection*	1976; 2008-2018	285
microplastic ingestion in freshwater	*Web of Science Core Collection*	2014-2018	53

The articles were selected according to relevance (objectively set by the authors of this report) for the topic approached by the degree of novelty and the area of study investigated (Figure 1). Although this analysis is not an exhaustive summary of the literature, we consider that the present study provides a real, correct and objective picture of the current state of knowledge in the field of microplastic pollution impact assessment in aquatic ecosystems.

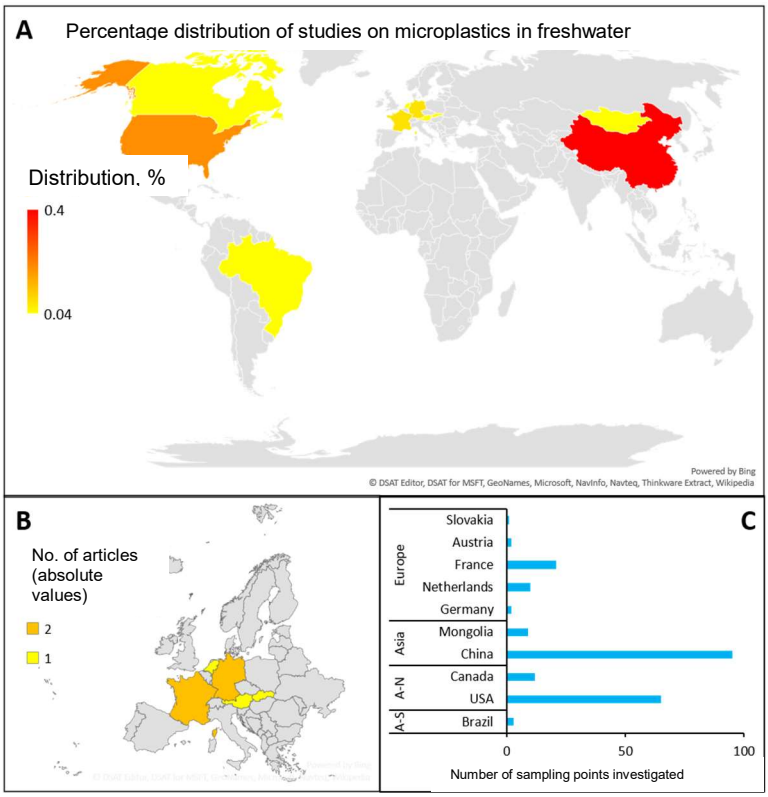

Figure 1. Geographical distribution of studies conducted on microplastics from freshwater. A - Percentage distribution of the number of studies conducted on microplastics from freshwater; B - The (absolute) distribution of the number of studies conducted on microplastics from freshwater at the European level; C - Number of sampling points investigated in studies conducted on microplastics from freshwater (A-S = South America, A-N = North America)

RESULTS ANS DISCUSSION

Sampling for the determination of microplastic concentrations in surface waters, bottom sediments and biota

In order to obtain analytical data on microplastic content in the aquatic environment, it is necessary that the sampling step is properly carried out, taking in account the multitude of sources that may contaminate samples during sampling and transport. Therefore, particular attention should be paid to exogenous contamination (induced by the protective equipment used - clothing) that may compromise the sampling, leading to overestimation of the microplastic concentrations in the sampled material. At the same time, during laboratory analyses, one must keep in mind the ability of microplastics to move into the air, especially fibers with high contamination potential, which can cause problems during the actual determinations (Hidalgo-Ruz et al., 2012, Nuelle et al., 2014, Norén & Naustvoll 2010). As a measure to limit and remove potential sources of contamination from the laboratory, it is advisable to replace plastic devices/equipment or with plastic content, with glass or porcelain devices/equipment.

In the case of microplastics sampled from surface waters, it should be considered that in this type of substrate, the concentrations of this contaminant type are relatively low and the sampling of microplastic particles involves the filtration of large sample volumes (Doyle et al., 2011). In most situations, for sampling microplastics from surface waters, it is enough to trace the sampling net (Figure 2) even below the water's surface, considering the water volume that passes through the sampling device. Depending on the density of the plankton in the area, nets with mesh sizes between 100 and 300 µm will be used.

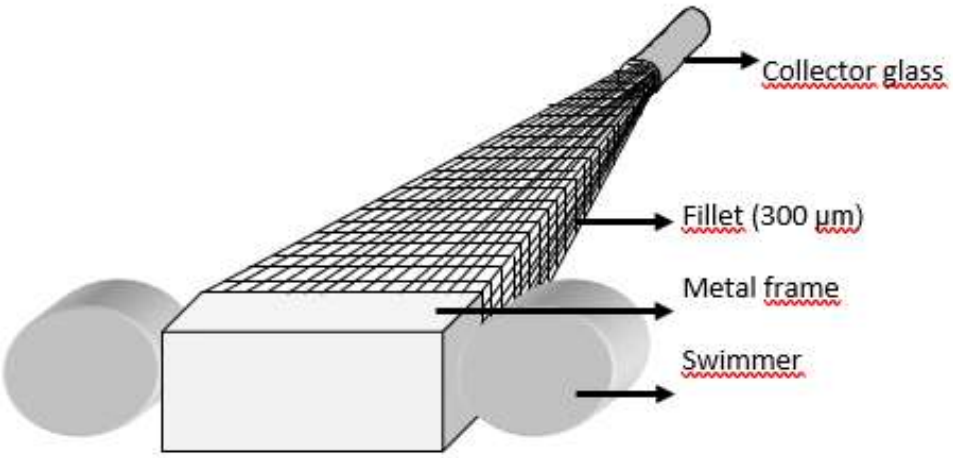

Figure 2. Device for microplastic sampling in surface waters

Determining the volume of filtered water will be the basis for calculating the microplastic concentrations (elements / grams) per volume of water. The volume of filtered water can be indirectly quantified by a flowmeter mounted on the sampling device, based on the flow rate of the water and the traction time. The concentrated sample at the bottom of the sampling device (collector glass) will be transported in a container that does not pose a risk of contamination of the sample and is kept in a dark place under low temperature conditions. If particles of visible plastic material are identified, they will be directly sorted, dried and stored in the dark until further analysis (Hidalgo-Ruz et al., 2012). At the same time, it is important to ensure that no residual sample is left in the sampling device, which would lead to a microplate transmission at the next sample. From the analysis of the literature, the mesh sizes used for the sampling of microplastics varied between 50 and 3000 µm (Hidalgo-Ruz et al., 2012), and the sampling fillet sizes ranged from 2 to 5 m in length. Considering the level of planktonic density in surface waters characteristic of the Danube Delta Biosphere Reserve (Spiridon et al., 2015), the optimal device for the sampling of microplastics is characterized by a length of 2-4 m, an opening of the metallic sampling frame 400 x 700 mm and a mesh size of approximately 300 µm. It is worth mentioning that this device type is most commonly used in order to avoid the risk of mesh clogging. The literature is relatively "poor" in the sampling methods presentation for bottom sediments, characteristic of freshwater aquatic ecosystems, and the presented are briefly detailed. At the European and international level, it was found that sampling of this type of substrate is made from the surface layer, 5 to 10 cm (Van Cauwenberghe et al., 2013), and the sampling quantity varies between 500 g and 10 kg (Hidalgo-Ruz et al., 2012). The sampling of the 5-10 cm layer is a fairly common approach, but there have also been identified cases where the sampling was at a depth of 30 cm, as reported by Claessens et al. (2011).

Regarding sampling site selection methodology, in the studies conducted until the present, there seems to be inadvertencies, because many authors state that the distribution of microplastic particles is as dynamic as deposition sedimentation itself. In many scientific articles, applied sampling methodologies include random sampling in multiple locations, perpendicular or parallel transects with a brief description of the sampling point and, in some cases, the presentation of geographic coordinates. However, it can be observed that in most studies microplastic samples in the sediments are made at at least 5 distinct sampling points, considering that they are representative for two microplastic categories, respectively: microplastics with dimensions between 1-5 mm and 20 µm.

In the case of microplastic particles from aquatic ecosystem biota, the methods have a very large variability and depend on the biological characteristics of the studied species. In marine research, many studies refer to large-scale biota, where direct ingestion of plastics is studied. In these cases, the plastic material in the intestinal content and excretions is relatively easy to recognize and counted (Wright et al., 2013, Ivar do Sul & Costa 2013). In the case of small biota, for example transparent planktonic organisms, it is used the proper specimen, utilizing fluorescent solutions that facilitate the identification

and counting the microplastics. For this category of biotope, Raman or IR spectroscopy is used (Wright et al., 2013. Investigations on the ingestion of microplastes by vertebrates require a significantly higher effort and it seems that in this field, research is at an early stage, and specialist publications are rare. From the vertebrate category, the most frequent studies are made on fish specimens that can be sampled in scientific fishing campaigns and examined for microplastics ingested. For this category, the intestinal contents or the entire digestive tract is taken with metallic instruments and will be conserved by freezing for further transport and analysis in the laboratory. Only low-invasive methods have been identified for the sampling of microplastic specimens in ornithofauna without the specimens being studied sacrificed or sustained consequences with term effects. For this category, the sampling is made of regurgitated material (pelicans and cormorants) and faeces. Small invertebrates such as oligochaeta, bivalves and snails are collected according with the methodology of sampling of benthic organisms (Besseling et al., 2012, Claessens et al., 2013) and are subjected to microplastic extraction and purification operations.

Sample preparation - microplastics extraction and purification

The microplastic extraction methods are based on plastic polymer densities ranging from 0.8 (silicone) and 1.4 g / cm^3 (e.g. polyethylene terephthalate - PET), polyvinyl chloride (PVC), while foam expanded (polystyrene) has only a fraction of the initial polymer densities (<0.05 g cm-3). In the extraction step, the microplastic particles are separated from sediment residues that were taken with the sample by floating in high density supersaturated saline solution. Thus, the sample is mixed in the supersaturated saline solution by agitation or aeration for a determined time period. Upon completion of the agitation / aeration step, the plastic particles float to the surface and / or mass of the supersaturated saline solution, while the high densities particles settle on the bottom of the container. Subsequently, microplastics are recovered by removing the supernatant. In most cases, a saturated NaCl solution is used (Thompson et al., 2004, Browne et al., 2010, Claessens et al., 2011, Browne et al., 2011). The extraction efficiency varies depending on the saline solution used, but also depends on the shape, size and origin of the microplastic particles. Generally, the rate of recovery reaches a percentage of 80-100% (Fries et al., 2013; Imhof et al., 2012).

The purification of microplastic samples is a mandatory step, especially for instrumental analyzes (FTIR / Raman spectroscopy, pyrolysis-GC / MS) to avoid interferences that may cause some errors in their identification. The simplest way to purify microplastic samples, but not the most effective, is stirring and rinsing with distilled / bidistilled water (McDermid and McMullen 2004). Another method for the purification of microplastic samples is the use of ultrasounds (Cooper and Corcoran 2010), which also involves the risk of sample degradation because some aging and brittle plastic particles may break during treatment. Among the most effective purification techniques are the method presented by Andrady (2011), which involves the use of mineral acids for the disintegration of organic impurities from samples taken from surface waters, and for the removal of soft tissues of biological samples, Claessens et al. (2013) recommends the use of hydrogen peroxide (H_2O_2) or nitric acid (HNO_3). In the case of purification of microplastics taken from the sediment, the use of a 30% solution of hydrogen peroxide seems to remove with increased efficiency natural organic residues (Imhof et al., 2012, Nuelle et al., 2014, Dubaish and Liebezeit, 2013).

Methods used for detection and identification of microplastics

The methodologies used to detect and identify microplastics are based on the molecular composition and "fingerprints" of polymers, which allow a clear assignment of studied microplastics to a certain polymeric origin.
The GC-MS techniques (gas chromatography-mass spectrometry) have been applied in various studies of concentration determination and identification of microplastic categories. The polymeric origin of microplastics is achieved by comparing certified reference materials that generate characteristic pirograms (Nuelle et al., 2014, Fries et al., 2013). By using this technique, several types of polymers can be analyzed simultaneously. Although the GC-MS pyrolysis allows a relatively good distribution / identification of the microplasties to the type of polymer, it has the disadvantage that large particles can not pass through the pyrolysis tube and the technique is only suitable for the small microplastics category. Moreover, the technique only allows the analysis of a small part of samples and is not suitable for the analysis of a large number of samples collected in the monitoring programs, since it involves a long time

for proper analysis. However, there are expectations that in the near future this technique will be improved in terms of quantitative analysis.
Raman spectroscopy is a simple technique that has been successfully used to identify microplastic particles in various high-reliability environmental samples (Van Cauwenberghe et al., 2013, Cole et al., 2013, Murray and Cowie 2011, Imhof et al., 2012). Since plastic polymers have characteristic spectra, the technique can be applied based on comparison with the reference spectra (certified reference material). Raman spectroscopy is a "surface technique," and has the ability to identify a wide range of microplastic sizes, to very small particles, less than 1 μm (Cole et al., 2013). Raman spectroscopy may be combined with spectral imaging and gross results are generated in the form of images at a resolution below 1 μm^2, based on the spectra of the analyzed sample. Theoretically, this would facilitate the detection of the smallest microplastic particles in aquatic ecosystems and beyond, but so far, such methodology has not been validated (Cole et al., 2013). One disadvantage of Raman spectroscopy is that the laser-excited fluorescence samples (some residues of biological origin in the samples) can not be measured because of the generation of erroneous spectra, which would lead to a compromised result. For this technique, it is strictly necessary to carry out a sample purification step in order to prevent the fluorescence effect and to ensure a clear identification of the type of polymer.

Infrared (IR) or Fourier Transform Infrared (FTIR) spectroscopy offers the possibility of accurately identifying plastic polymer particles based on comparison with reference spectra (Thompson et al., 2004, Obbard, 2006, Vianello et al. 2013, Harrison et al., 2012, Frias et al., 2010) The plastic polymers have specific IR spectra with distinct band patterns, making IR spectroscopy an optimal technique for identifying the type of microplastics (Fig. Hidalgo-Ruz et al., 2012). At the same time, FTIR spectroscopy can provide additional information on the level of physico-chemical degradation of oxodisable microplastics by detecting the degree of oxidation (Corcoran et al., 2009). FTIR spectroscopy is characterized by two measurable ways of reflection and transmission. The first way (reflection mode) has the disadvantage that measurements on irregular shape microplastics may result in compromised spectra due to refractive error (Harrison et al., 2012). The second mode (transmission mode) is limited by a certain thickness of microplastic problems. It is only suitable for small microplastics. A significant advantage of FTIR spectroscopy is given by the ability to simultaneously record several thousand spectra in a single measurement area and therefore to generate very good resolution images 10 μm to 1000 cm^1).

Assessing the impact of microplastics on aquatic species

The first reports of sea and ocean water pollution with plastics date from the 1970s (Carpenter et al., 1972, Carpenter & Smith, 1972, Colton et al., 1974, Fowler, 1987, Coe & Rogers, 2012) initially unsuccessful in drawing the attention of the scientific community to the potential effect on marine fauna. In the following decades, the interest in plastics pollution increased considerably, with an emphasis on the impact of plastic litter on aquatic species. Initially, the studies on marine waste concerned marine mammals (Laist, 1997), especially cetaceans (Clapham et al., 1999), but other marine species commonly caught in fishing nets were also targeted (Bullimore et al., 2001; Eriksson & Burton, 2003, Tschernij & Larsson, 2003). Also, the ingestion of marine material by sea species (Cadée, 2002, Mallory, 2008) and turtles (Bugoni et al., 2001; Tomás et al., 2002; Mascarenhas et al., 2004) in the world were studied, with an emphasis on their effects. Approximately 44% of the marine bird species have been confirmed (Rios et al., 2007), and moreover, in the case of a species of albatross *Phoebastria nigripes*, the feeding of plastic waste to chicks has also been documented (Andrady, 2011). With the almost exponential increase in the incidence of reports on the pollution with plastic materials in the North Pacific (Moore et al., 2001a, Moore et al., 2001b, Moore et al., 2002, Moore, 2008) plastic has become a priority research field in marine biology (Derraik, 2002, Page et al., 2004, Arthur et al., 2009). Particular concern was given to small pieces of plastic scrap, especially those that are not visible to the naked eye (Andrady, 2011), later called microplastics. Special attention has been paid to the intake of microplastics by marine and ocean fauna. More than 270 ISI articles strictly targeted to microplastics intake (Figure 3) have been published since 2011, with an almost exponential increase.

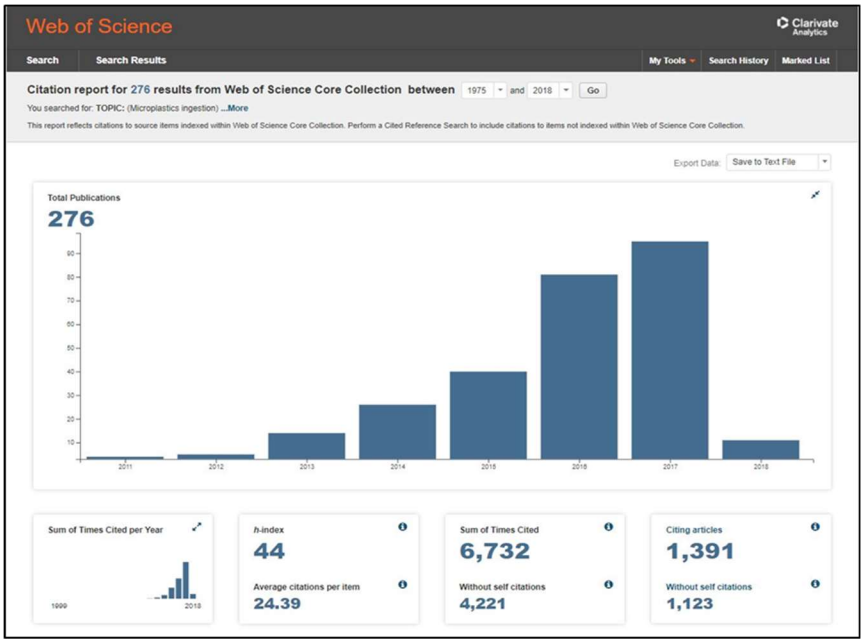

Figure 3. The number of quoted articles from Web of Science Core Collection published since 2011, strictly focused on the ingestion of microplastics and the number of citations in Web of Science Core Collection (Web of Science Core Collection - accessed on 07.03.2018)

Also, the number of studies oriented partially or tangentially to the effect of microplastics ingestion is much higher, being reflected by ~ 6732 citations of articles dedicated to the ingestion of microplastics. Of course, the number of studies that just recall or simply briefly relate to the effects of microplastic pollution on aquatic fauna is much higher.

The ingestion of microplastics by aquatic fauna is a stringent problem worldwide and we therefore propose a screening of the literature on the impact of microplastics on aquatic fauna, with emphasis on the toxicity of ingested microplastics, susceptibility of marine organisms to ingestion of microplastics and respectively the physical impact of microplastics on marine, vertebrate and invertebrate marine species.

Marine waters naturally contain many micro- and nano-particles (~ 106-107 particles per mL, 10 to 500µg / L), most of them smaller than 100 nm (Rosse & Loizeau, 2003). Filtering organisms, from zooplankton to whales, commonly interact with these particles without any apparently unfavorable effect (Andrady, 2011). Since none of these organisms have enzymatic pathways to decompose synthetic polymers, ingested microplastics are not digested or absorbed into the digestive tract and should therefore be bio-inert. On the other hand, microplastics ingestion by microbiota presents a totally different problem. Microbiota generally has an increased potential for high persistence transfer of Persistent Organic Pollutants (POPs) and marine fauna (Bowmer & Kershaw, 2010). In particular, persistent organic pollutants taken from the water are those that give toxic effects.

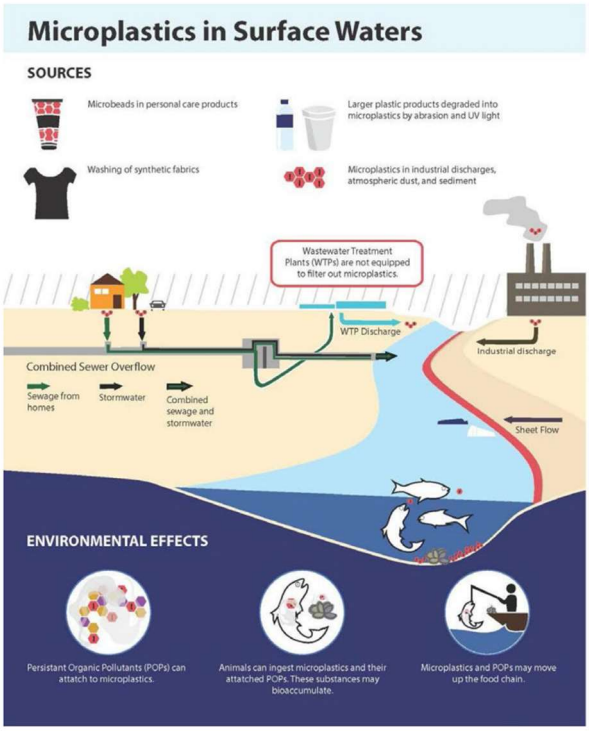

Figure 4. Sources, transport and potential bioaccumulation of persistent organic compounds (POPs) associated with microplastics released into the environment (Ravit et al., 2017)

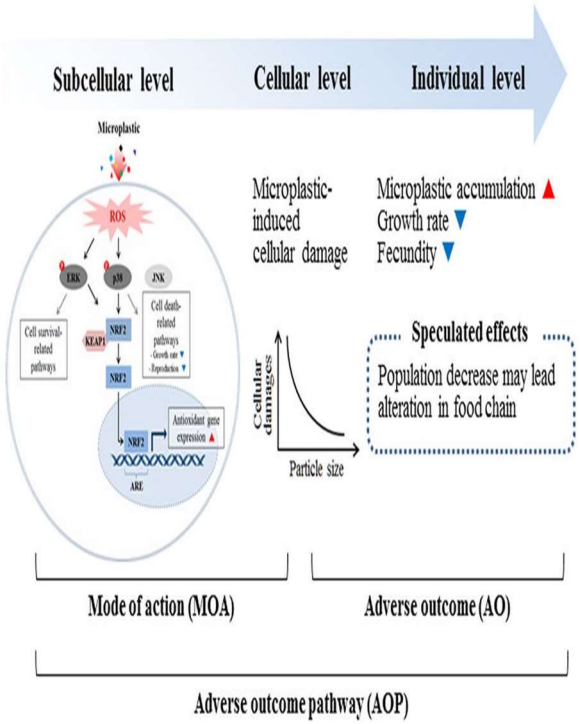

Figure 5. Schematic representation of proposed action pathways for Adverse outcome pathway (AOP) induced microplastic exposure in the case of *Paracyclopina nana* marine copepod. Mode of action (MOA) is present at the subcellular level, being represented by oxidative stress-signaling pathways mediated by microplastic exposure, Adverse outcome (AO) is presented at the cellular and individual level (after Jeong et al., 2017)

Any toxicity associated with plastics in general, also those associated with meso- and microplastics, may be attributed to one or more of the following factors: the release from the ingested plastic of toxic additives used in its manufacture and / or residual monomers from the process production. An example of residual monomer with toxic potential on fauna is bisphenol A (BPA) residual in polycarbonate products (Vandenberg et al., 2007), as well as the toxic potential of phthalates plasticizers used in PVC has been widely discussed in the literature (Latini et al., 2004), the toxicity of some intermediate chemical agents resulting from the partial degradation of plastics. For example, burned polystyrene, partially or totally, can produce styrene and other aromatic substances. Styrene, at low concentrations, may cause hearing loss and ingestion may cause changes in the nasal mucosa and then a considerable impairment of liver functions (William & Roper, 1992), aggregation of persistent organic pollutants presents in water by absorption and slow concentration in microplastic fragments. Plastics waste "cleanses" the water from dissolved chemicals (Figure 4), and by ingestion by fauna, they can become bio-available (Endo et al., 2005).

The literature regarding the ingestion of microplastics by vertebrates is very rich (Laist, 1997, Denuncio et al., 2011, Lazar & Gračan, 2011, Van Franeker et al., 2011; Yamashita et al., 2011), all over the world, including: internal and / or external abrasions and ulcers, respectively; and blockages of the digestive tract, which can lead to apparent satiety and subsequently to starvation and physical deterioration. In turn, this can lead to reduced reproductive capacity, drowning, decreased predator avoidance performance, impaired feeding capacity, potential transfer of harmful seawater toxins and, ultimately, death (Gregory, 2009). Such harmful effects can also be applied to small organisms, including invertebrates, which ingest microplastics, for example, potentially lethal lesions such as digestive tracts or abrasions with sharp objects. Other possible impacts have been suggested by the Marine Strategy

Framework Directive - Working Group 10 (Galgani et al., 2010), these include: blocking the production of enzymes; diminishing feeding stimulus; nutrient dilution; low growth rates; decrease in steroid hormone levels; delayed ovulation and reproductive failure; and absorption / accumulation of toxins (Figure 5). There is a possibility that microplastics may clog up and block feed nets of marine invertebrates or even be embedded in tissues (Derraik, 2002). Fragments of plastic materials, fiber and / or polypropylene monofilaments (PP) were found in two filtration feeding devices tissues, the *Thetys vagina* species, collected from neuston samples in the central Pacific North region (Moore et al., 2001a).

The ability to accumulate microplastics in an organism probably greatly affects the physical impact associated with microplastic ingestion. To date, there is limited literature on the accumulation of microplastics in marine invertebrates and freshwater. However, since the 1970s, microplastics were ingested in a species of aquatic worms, *Parasagitta elegans* (Fam. Sagittidae), which had a microplastic sphere with a diameter of 0.6 mm, as the body had a total length of 20 mm (Carpenter et al., 1972). This example highlights the invertebrate ability to accumulate the particles formed by microplastics or similar particles. Accumulation of microplastic particles in invertebrates could cause blockages of the digestive system, suppressing feeding due to apparent satiety. Alternatively, the predator consumption of invertebrates contaminated with microplastics represent a way of their transfer and accumulation in the food chain (Wright et al., 2013). In addition to internal accumulation, external adsorption of microplastics can also produce adverse effects. It has also been shown that the attachment of microplastic particles to some algae species, the genus *Chlorella sp.* and *Scenedesmus sp.* (Bhattacharya et al., 2010) inhibited photosynthesis (most likely due to physical blocking of light and air) and induced an increase in oxidative stress (by increasing the production of reactive oxygen species). Since algae play an essential role in aquatic feeding networks, the productivity and resistance of ecosystems could be compromised by the adverse effects of plastic particles, producing a domino effect with repercussions on the entire ecosystem.

The potential adverse effects associated with the presence of microplastics may vary depending on the particle form. Benthic organisms have an additional vulnerability to toxicity depending on the form of ingested micro- and nano-plastic particles. If initially it was thought that microplastics could be considered bio-inerts (Andrady, 2011), due to the lack of enzymatic pathways available to decompose plastics in the filtration organisms, being unlikely to be digested or absorbed, it was subsequently shown that they could pass through cell membranes and become incorporated into body tissues after ingestion. Of course, more research is needed to determine the upper and lower limits of particle size capable of translocation in different organisms. In addition, it is necessary to determine the behavior of micro-particles of different polymers types and forms. In the natural environment, organisms can be exposed to microplastics throughout their lifetime, unlike short experimental times. Thus, ingestion continues and the accumulation of such particles can have chronic effects. Moreover, a diverse range of polymers (compared to the laboratory-controlled conditions) may appear in the environment, which may cause a different response to a particular polymer (Wright et al., 2013).

Other than the physical and chemical impact, microplastics also have a potential role in providing a new hard-shell habitat for rafting communities (communities located on drifting elements) that were previously limited to natural elements such as floating wood, pumice stone and shells. Moore et al. (2001a) found submerged mono-filaments colonized with diatoms and other microscopic algae. Recently, microplastics have been identified as an important resource for oviposition in the *Halobates sericeus* pelagic insect (indicating a degree of positive correlation between *H. sericeus* eggs on microplastics and microplastic abundance). The pelagic invertebrate community is a crucial link between primary and non-protozoal species. Thus, changes in the population structure, in the case of *H. sericeus* species, can produce widespread consequences on the ecosystem (Goldstein et al., 2012). The increasing abundance of microplastics may be able to modify the structural parameters of aquatic organisms. In addition, microplastics have a mechanism for long-range transport of species, improving biogeographic connectivity. Shellfish species such as Cnidaria, Crustacea and Ectoprocta (Gibson et al., 2005) can be considered the most vulnerable population at changes associated with microplastics

At trophic chain level, only few studies are available regarding the bioaccumulation of plastics and persistent organic pollutants associated with them. Considering the organisms from the lower trophic level, especially invertebrates, can ingest and accumulate microplastic particles, it is very probably that these microplastics are introduced into the trophic chain. Laboratory studies on microplastic ingestion mainly addressed invertebrates, but microplastic ingestion in several species of vertebrates was reported in situ. The ingestion of microplastic particles in vertebrates has been reported in countless species of fish (Lusher et al., 2013), benthic and pelagic, whales and other filtering organisms, superior organisms in the food chain such as seals or sea lions (Goldsworthy et al. 1997, McMahon et al., 1999, Eriksson & Burton, 2003) and countless species of birds. Currently, there is limited information on the impact of microplastics on trophic chain, as their laboratory experiments are currently unavailable. Therefore, it remains undetermined if plastic of any size can be transferred to higher trophic levels. There are, however, well-documented examples of trophic transfer for many persistent organic pollutants in marine trophic networks, many of which are reported to be associated with plastic waste (Ogata et al., 2009) and the potential of some as bio-magnifiers (Hu et al., 2005). The effect of co-intake of microplastics on the trophic-dynamic behavior of POP additives and plastic additives remains an important subject. Other important factors that need to be considered for the transfer of microplastics and associated POPs are retention times of the intestine (taxon addicts) as well as the fraction of microplastics consumed, able to move on the intestinal epithelium and other tissues or organs.

CONCLUSIONS

The importance of this study started from the need to know the existing methods and instruments at the European and international level for tackling microplastics problems in aquatic ecosystems, with the final goal to lay the foundations of a suitable methodology for monitoring microplastic pollution at the Danube Delta Biosphere Reserve level.

As a result of the research carried out, it was found that in the Danube Delta Biosphere Reserve, for collecting samples of microplastics in the surface waters, it is recomended to use the fillets with 2-4 m length, with openings of the inlet area of 400 x 700 mm and mesh size of about 300 μm. For sampling microplastics in the bottom sediment, it was determined that sampling should be carried out with a Marinescu metal drain from the 0-20 cm substrate of the bottom sediment in the deposition areas. Sample storage and transport should be carried out in low temperature containers and away from sunlight. For biota, the, sampling procedures should be tailored to the characteristics and dimensions of the species to be studied.
For extraction of microplastics, saturated NaCl solutions should be used. This procedure has a recovery rate of 80 to 100%.

From the point of view of the efficiency of purification methods, it has been established that for microplastics in surface water, the use of mineral acids has the highest efficiency in the disintegration of organic impurities. In the case of microplastics originating from the bottom sediment, maximum efficiency was identified in the procedure based on the disintegration of organic impurities in the 30% solution of perhydrochloride.
Although it was initially believed that ingested microplastics should be bio-inert, the toxic potential of the ingested microplastics was reported and characterized, from the molecular level to the individual / population level. The toxicity associated with plastics in general, including those associated with meso- and microplastics, can be attributed to releases of toxic additives used in the manufacture thereof and / or residual monomers, intermediate chemical agents resulting from partial degradation and respectively the aggregation of organic pollutants persistent in water in microplastic fragments by absorption and slow concentration.
The physical impact of microplastics on aquatic organisms has been reported in different vertebrate and invertebrate groups, mainly induced by: internal and / or external abrasions and ulcers, respectively; blockages of the digestive tract, which can lead to apparent satiety and later to starvation and physical deterioration, respectively. In turn, this can lead to a reduction in reproductive capacity, drowning, decreased predator avoidance performance by impairment of feeding capacity and adverse effects. Benthic organisms have an additional vulnerability to toxicity linked with ingested micro- and nano-plastic particles form, which is also accentuated by translocation phenomenon in the tissues adjacent of the digestive tract.

In addition to internal accumulation, external adsorption of microplastics can also produce adverse effects. Also, attaching microplastic particles to some algae species can cause a domino effect with repercussions on the entire ecosystem.
The bioaccumulation of plastic materials and persistent organic pollutants associated with them at the level of tropical and marine trophic chains is currently insufficiently documented, but it is very likely that these microplastics are introduced into the trophic state, given that organisms at lower trophic levels, especially invertebrates, can ingest and accumulate microplastic particles.

In view of all of the above, it is necessary to evaluate the microplastic pollution from aquatic ecosystems in the Danube Delta in order to create the premises of a sustainable management strategy capable of providing a favorable environment for biota and implicitly for human health.

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