Molluscs as Indicators of Microplastic Pollution in the Gulf of Mexico

Moluscos como Indicadores de Contaminación Microplástica en el Golfo de México

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ABSTRACT

The invention of plastic based on synthetic polymers changed our lives forever; being one of the most versatile materials ever produced. However, its wide use has resulted in 9.5 million tons of discarded plastic waste entering the ocean each year, making plastics the most prevalent form of marine pollution today. Over time, plastics break down into microplastics, or are released directly from other products in which they are important components, such as personal care products (e.g. facial cleansers and toothpaste). These microplastic particles of 300-5000 μm in size are now present in marine sediments and the water column where they can be ingested by marine organisms, and passed up the food chain. This trophic transfer is an indirect, yet potentially major, route of microplastic ingestion by top predators, including humans.

This study was carried out on four species of marine mollusc exhibiting four different feeding strategies: (1) a carnivore (*Melongena corona bispinosa*); (2) a herbivore (*Pseudosuccinea columella*); (3) a filter feeder (*Ischadium recouvum*); and (4) two species considered to be both detritivorous and herbivorous (*Nassarius vibex*). Microplastics were analyzed using a non-destructive method that involved sampling the individuals' feces. Sampling took place primarily in the Yucatan Peninsula, Mexico, ten individuals of each species were sampled. Microplastics were extracted from the feces samples by degradation of the organic matter, followed by re-suspension of the sample and examination under a stereomicroscope and using a scanning electron microscope.

Microplastics were present in all mollusc feces samples and included fragments, fibres and sheets. The filter feeders and carnivorous molluscs were found to have significantly higher abundance of microplastics in their feces than the herbivores and detritivores.

KEYWORDS: Microplastics, pollution, marine reserve

INTRODUCTION

The invention of plastic based on synthetic polymers changed our lives forever; it is one of the most versatile materials ever produced. Over the past century, petroleum-derived synthetic plastic polymers have been widely used, and due to the characteristics of plastic, such as being lightweight and having high durability, these polymers from numerous sources are finding their way into fresh and marine water bodies. Every day about one megaton of plastic is produced, enough to make almost 22 trillion water bottles and more than 90 percent of that will never be recycled. Plastic pollution in our marine environment has reached a critical point, with 9.5 million tons of new plastic waste flowing into the ocean each year. Today, plastic is the most prevalent type of marine debris, representing more than 80% of the debris swirling through the oceans. Plastics are not all the same, they have different densities. Polyethylene present in plastic bags has a density of 0.88 - 0.96 g/cm3 less than 1.02 - 1.03 g/cm^3 of the sea water density. It is lighter than seawater, consequently those plastics float. Other plastics such as PVC have a density greater 1.4 g/cm^3 than seawater, they are found at the bottom of the sea. Over time, as they break down, they turn into microplastics (particles smaller than 5 mm) and these belong to a group considered as emerging pollutants (Andrady 2011, Baini et al. 2018, Bosker et al. 2018). Some plastics are intentionally designed to be small. Other types of microplastics called microbeads and are used in many health and beauty products, such as some cleansers and toothpastes.

Microplastics can be ingested by marine fauna both on the water surface, through the zooplankton and on the seabed, by detritivore and filter organisms and. Besides, microplastics can be ingested by marine animals and pass up the food chain (Isensee and Valdes 2015, Barrows et al. 2018, Wang et al. 2018). It has been shown that indirect trophic transfer of microplastics represents yet potentially major, route of microplastic ingestion for the top predators. Fish were the most commonly studied group of organisms (44%), followed by crustacea (21% for large and small crustacea combined), mollusks (14%) and annelid worms (6%). There were relatively few studies of other organism groups (de Sá et al. 2018).

These microplastics can have negative effects on organisms, such as a decrease in reproduction, survival and respiration rates. They can also affect the immune system of organisms. Perhaps the most serious, yet least documented, is the effect of microplastics as bio-magnifiers of persistent organic pollutants such as agrochemical and insecticides substances. Also, microplastics accumulate aromatic hydrocarbons and heavy metals (Andersson 2014, Auta et al. 2017, Hurley et al. 2018). By ingesting microplastics, animals are exposed to these contaminants and they can accumulate in their tissues (Andersson 2014). The ingestion of microplastics by bivalve mollusks could lead to a significant decrease in lysosomal membrane stability and a significant increase in the formation of tight ball-like collections of immune cells referred to as granulocytomas (von Moos et al. 2012). Further, microplastic ingestion could negatively impact the fecundity and energy allocation of some bivalves, such that energy is allocated to the maintenance and structural growth of the organism at the expense of reproduction, thereby impairing gametogenesis and the gamete quality of the animal (Sussarellu et al. 2016). These chemical contaminants, POPs and additives, have well-known toxic effects. POPs such as PCBs, PAHs, or DDT are endocrine disruptors, ie they mimic, compete or disrupt hormone synthesis (Talsness et al. 2009). The risk of microplastics to human populations is still not known, but they have a potential risk due to their pervasive and persistent nature. The presence of microplastics in marine foods consumed by humans, such as oysters and mussels has already been documented and considered to have a negative effect on human health (Van Cauwenberghe and Janssen 2014, Waite et al. 2018).

In the Caribbean region, few studies have been carried out to quantify microplastics (Bosker et al. 2018), but information on the concentration of these particles and their effects is important. For endangered species or for bioethical issues sacrifice of organisms is not optimal. Studies of microplastics in marine organisms usually involve this method. An alternative, is to use a non-invasive techniques analyzing external secretions (Busso and Ruiz 2011). The analysis of feces allows resolution of this issue since it does not involve harming the organism being investigated (Nelms et al. 2018). The use of feces has increased in the study of aquatic organisms; e.g., for determining stress levels in fish (Turner et al. 2003, Lupica and Turner 2009) and assessing reproductive function in whales and dolphins (Rolland et al. 2005) and Molluks (Chong et al. 2019).

Organic matter is considered the main route of transport from microplastics to sediments, thus constituting an important reservoir for microplastics (Cozar et al. 2014). Many species having varied feeding strategies and occupying different trophic levels have been identified as ingesting particles plastics (Laist 1997). Polystyrene particles are more easily ingested by filter organisms when they are associated with phytoplankton, increasing the bioavailability of microplastics for filter organisms (Long et al. 2015).

The present study was carried out using feces samples to determine the relative levels of pollution by microplastics across the wider Caribbean using the mollusk, queen conch (*Strombus gigas*) as an indicator. This study also included the use of mollusks of different trophic levels. Four types of mollusks are considered: carnivorous (*Melongena corona bispinosa*), herbivores (*Pseudosuccinea columella*), filter feeders *(Ischadium recouvum*) and detritivores/herbivores (*Nassarius vibex* and *Strombus gigas*).

METHODS

Sampling Sites

Mollusks having various types of food were sampled in the Celestun lagoon, Yucatan Peninsule Mexico (Figure 1). The mollusks sampling was carnivorous (*Melongena corona bispinosa*), herbivores (*Pseudosuccinea columella*), filter feeders (*Ischadium recouvum*) and detritivores/herbivores (*Nassarius vibex*).

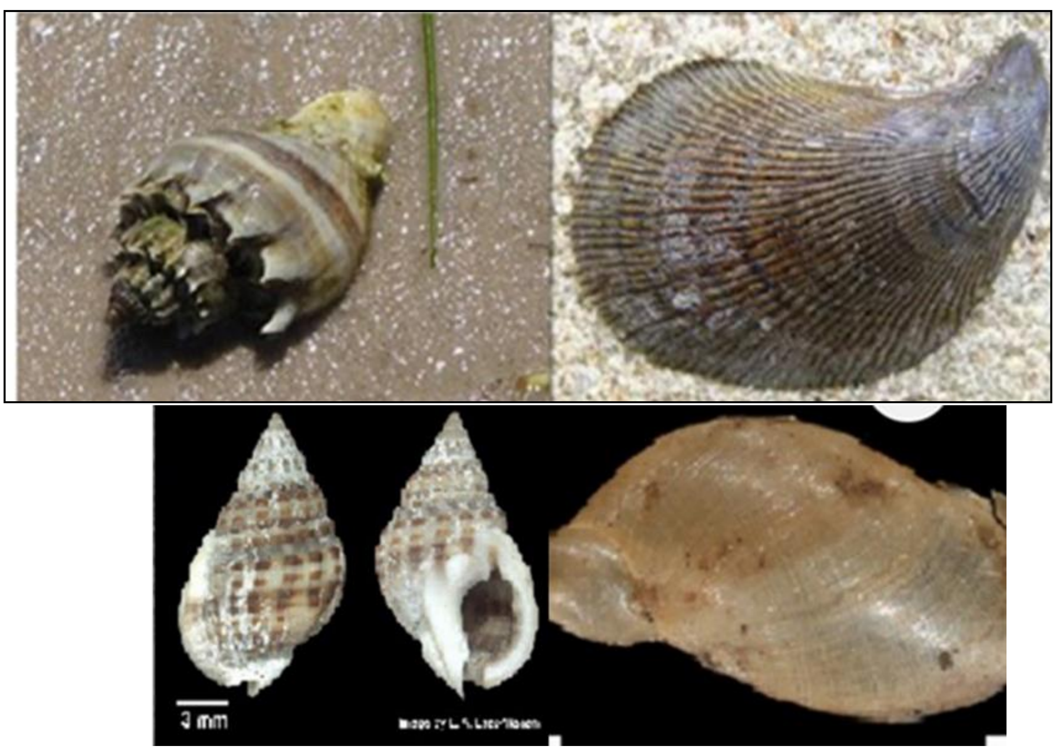

Figure 1. Mollusks sampled from Yucatan Peninsula, Gulf of Mexico with various A) *Melongena corona*, B) *Ischadium recurvum*, C) *Nassarius vibex* D) *Pseudosuccinea columella*

The feces samples were then sent to the Center for Research and Advanced Studies (CINVESTAV), Merida, Yucatan, Mexico, where they were frozen at -5 °C until processing. After sample collection, all conchs were returned to their habitat. The same procedure was carried out for the other mollusks. From them 10 organisms of each species were collected.

Plastic Extraction

Frozen samples were thawed at room temperature. In the laboratory, the methods recommended by Hidalgo-Ruz et al. (2013) and Masura et al. (2015) were followed. To avoid contamination of the samples, all the equipment and glassware was rinsed three times with 96 percent alcohol, and only glassware and cotton coveralls were used. One gram of feces from each conch sample was subsampled and mixed with peroxide (30%) and then left for 48 h to eliminate organic matter. This mixture was then stirred manually and allowed to settle. Subsequently, with the use of a glass Pasteur pipette, the peroxide was removed and replaced. Particles were separated from the sediment via floatation, using a solution of sodium chloride at a concentration of 1.12 g/cm^3 to re-suspend the microplastics (Hidalgo-Ruz et al. 2013, Masura et al. 2015). Five milliliters of the solution were then taken and filtered through a 0.22 µm membrane (Millipore durapore). To remove excess NaCl from the filter, 5 ml of distilled water was then passed through the filter. After filtration, the filters were checked to quantify the abundance and diversity of the microplastics using a Leica Zoom stereoscopic microscope at 40 X magnification. At this magnification it is possible to detect pieces up to 0.001 mm (Waite 2018). The microplastics found were classified into fragments, fibers, sheets and spheres particles following the identification criteria of Hidalgo-Ruz et al. (2013).

In addition, RAMAN Microscopy (WITEC ALPHA 300 Microscope, 488nm Laser) was used, using the Raman distances (cm^{-1}) proposed by Nehrke, Poigner, Wilhelms-Dick, Brey, and Abele (2012) for the identification and quantification of various types of plastics with a laser of 633 nm and observations at 50x. Raman intensity was calculated with OriginPro 8 software, and reported in arbitrary units (a.u.).

RESULTS

In the samples, after digestion of the organic matter it is possible to observe microplastic particles as well as other particles like the calcareous fragments of mollusks, corals or silicon in the diatoms. Thus, it is important to use scanning electron microscopy to discriminate between these particles correctly. These microplastic particles were different shapes and sizes. However, the most frequent microplastic particle types were the same across all sites, with fragments being the most abundant and sheets being the least abundant. The size of the fibers varied between 300 and 4500 µm and fragments between 100 and 700 µm.

About microplastics abundance in mollusks of different trophic level; filterer organisms had an average of 77 ± 31 microplastics per organism being the species most affected by the pollution. Then carnivores, represented by *M. corona* with 73 microplastics per individuals. *Nassarius vibex* (detritivore) and *Pseudosuccinea columella* (herbivorous) exhibited the least amount of microplastics (Figure 2). Figure 3 showed a fiber of microplastic observed in scanning electron microscopy and its analysis EDX microscopy.

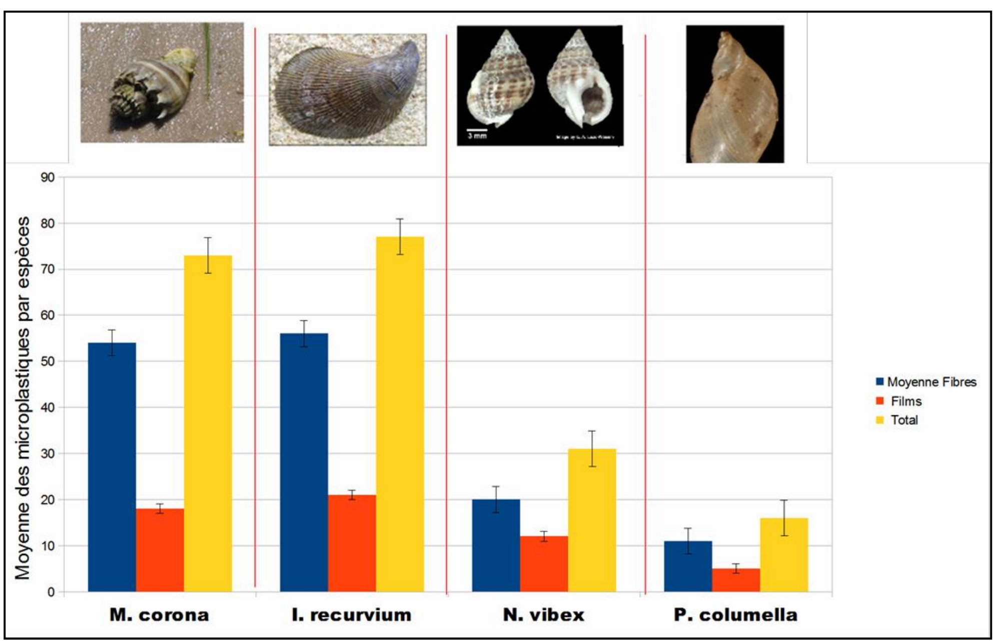

Figure 2. Abundance of microplastics found in feces of various mollusks in Gulf of Mexico (Yucatan Peninsula) with different trophic levels. Carnivorous (*Melongena corona bispinosa*), herbivores (*Pseudosuccinea columella*), filter feeders *(Ischadium recouvum*) and detritivores (*Nassarius vibex*).

DISCUSSION AND CONCLUSION

Feces have been used to study hormone levels in aquatic organisms as well as to monitor the health and stress of captive marine mammals (Amaral 2010) and mollusks (Chong et. al. 2019). Feces have also been used in conch feeding studies (Serviere et al. 2009). In the current study, feces were found to be highly suitable for measuring comparative levels of contamination by microplastics, without sacrifice of organisms. Thus, this method is a very useful alternative to study reproduction, feeding or pollution in marine species, especially when we are working with protected species

Limited research is available regarding the trophic transfer of microplastics, however, the discovery of microplastics in the tissues of various coastal higher trophic level organisms representing a diverse array of habitats (i.e., mammals, birds, fishes and zooplankton) strongly suggests the potential for trophic transfer occurring concurrently with the direct ingestion of microplastics (Eriksson and Burton 2003). Fish taken from the wild had a greater amount of microplastics in examined gastrointestinal tissue than macroplastics (> 0.5 mm), suggesting that the increasing presence of smaller microplastics may pose a greater threat than previously thought, especially when considering the higher surface area to volume ratio that smaller particles have, and the possible contribution of a higher amount of adsorbed contaminants to exposed organisms (Ogonowski et al. 2016, Rummel et al. 2016). Similar results are presented by Beckwith et al. (2018).

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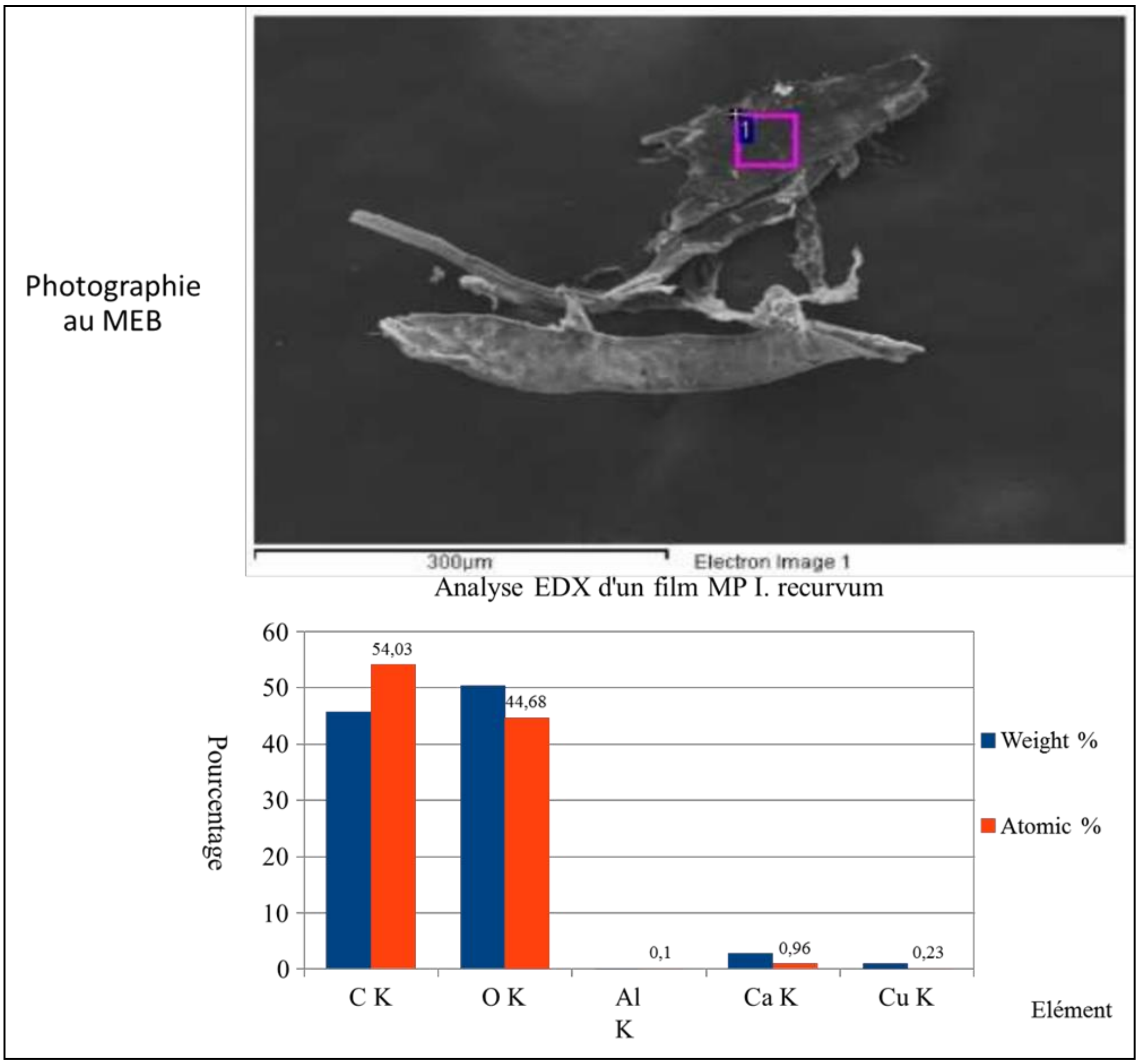

Figure 3. Micro fiber of plastic observed in in scanning electron microscopy and its analysis EDX microscopy of microfiber of plastic.

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