

# Characterisation of microplastics in Irish freshwaters

Mwana-Scoe L., Germaine K., Kakouli-Duarte T. And Cleary J.\*

EnviroCore, Department of Science and Health, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland  
\*corresponding author: e-mail: John.Cleary@itcarlow.ie

## Abstract

Microplastics (MPs) in water is an emerging environmental issue worldwide. This study aims to assess the prevalence of microplastics in Irish freshwater and wastewaters and to characterise the interactions of microplastics with organic pollutants and microorganisms. Microplastics recovered from five cosmetic products were used as reference materials in order to develop and assess techniques for recovery and preliminary characterisation of microplastics. Most of the microbeads extracted from various personal care products were polyethylene with particle sizes between 100 and 1000  $\mu\text{m}$ . Polyethylene microplastic was recovered from River Barrow using three steps adapted from National Oceanic and Atmospheric Administration (NOAA) standard method which includes visual extraction, sieving, and flotation, followed by polymer identification using infrared spectrometry. The reference material exhibited gradual changes in its infrared spectrum between three weeks and two months.

**Keywords:** Microplastics, freshwaters, sediment, sampling, detection

## 1. Introduction

In recent decades, microplastics have been recognised as an emerging issue in both marine and freshwater environments. Plastics have been produced on large scale since the 1960s and are now ubiquitous in across numerous consumer and industry sectors. Global plastic production has reached 311 million tonnes in 2014. The first report on the emergence of these materials in marine environments was reported in 1970s by Carpender and Smith. Studies show that most microplastics are not retained by waste water treatment processes, and hence end up in the receiving waters (Horton *et al.*, 2017). Polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate and polyethylene are the main produced polymers as plastics (Rocha-Santos and Duarte, 2015). Microplastics can be classified as primary and secondary microplastics. Primary microplastics are polymeric particles produced with diameters less than 5mm, including cosmetics (exfoliating scrubs, for example microbeads) and glitter, industrial pellets, clothes fibres, air blasting and medical vectors, in contrast secondary microplastics result from the breakdown of larger plastics, for example the breakdown of *in situ* litter (van der Hal, Ariel and Angel, 2017; Horton *et al.*, 2017). Plastics debris accumulation has increased recently with an estimation of 5.25 trillion items with an approximated weight of 269,000 tonnes. (Keswani *et al.*, 2016). To date, there has been less attention on microplastics in freshwater environment (Free *et al.*,

2014), despite their significant impact in marine environment (Rocha-Santos and Duarte, 2015). Microplastics are characterized based on their size, shape and density. Low density plastics have been identified due to their flotation properties, whereas high density plastics such as polyvinyl chloride (PVC) which sink and accumulate in sediment are more difficult to recover and characterise. Microplastics possess an intrinsic toxicity property due to their large surface area and hydrophobicity of organic xenobiotics, which causes the adsorption and concentration of polychlorinated biphenyl, nonylphenol and dichlorobiphenyl trichloroethane (Wagner *et al.*, 2014). These materials are of concern due to their potential for delivery of toxic chemicals by ingestion and subsequent bioaccumulation by many organisms include vertebrates and invertebrates, as well as their potential to leach additives such as stabilisers, flame retardants, pigments and fillers. Studies have highlighted evidence of accumulation of toxic chemical such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), organo-halogenated pesticides and nonylphenols on microplastics (Avio *et al.*, 2015; Bakir *et al.*, 2014). Wastewater treatment plants (WWTPs) have been identified as potential sources of microplastics in marine and freshwater environments (Carr, Liu and Tesoro, 2016). Presently, domestic and industrial products have been considered as the sources of microplastics materials entering marine and freshwater environments leading to unknown outcomes in the future. These particles include fibres released from synthetic clothing and cosmetic products. The majority of these microbeads used in cosmetic products are polyethylene. As long as different types of plastics used in packaging such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyterephthalate (PET) have high likelihood of ending up in marine and freshwater environments, these particles are transported from wastewater treatment facilities, and may have an impact in the environment and on health. Microplastics occurrence has been determined in marine water, sediment or biota samples. Recently, studies have been carried out on the quantification of microplastics in major rivers e.g. the Thames. Most published studies have been relying on density separation using sodium chloride or sodium iodide solutions. Nevertheless, plastics such as polyvinyl chloride (PVC), polyethylene terephthalate (PET) have a greater density than saturated solution (Andrady, 2011; Tagg *et al.*, 2015). This study focuses on the detection, quantification and characterization of microplastics collected from a range of sources such as Irish freshwaters, waste water treatment plants (WWTPs)

and sediments. Physicochemical tests and Fourier transform infra-red (FT-IR) spectroscopy were used to identify and classify different polymers.

## 2. Methods

### 2.1 Sample preparation

Five facial scrubs were selected and labelled 1-5 to isolate microplastics. Products used included Clean And Clear Exfoliating Daily Wash (product 1), Clean And Clear Blackhead Clearing Daily Scrub (product 2), Cien Aqua Rich Daily Exfoliating Facial Wash (product 3), Neutrogena Spot Stress Control (product 4) and Neutrogena Deep Clean (product 5) Each products were diluted to approximately 1L of boiling water to extract microbeads for further analysis. The mixture was then subjected to vacuum filtration using a suction pump, buchner funnel with whatman filter paper N°4. Filter papers containing microbeads were dried in the oven at 60°C for 2 minutes. Once dried, the particles were transferred into labelled petri dishes. Prior to FT-IR spectroscopy, microbeads were analysed under light and dissecting microscopes from 10X to 40X magnification. Fourier transform infrared spectroscopy (ATR-FTIR) was used to analysed these particles to produce reference for freshwater samples. Physicochemical tests were carried out to identify and quantify these microbeads, including gravimetric test involving the flotation in media of various densities such as deionized water, olive oil and isopropyl alcohol. The copper wire, acetone solubility and heating tests were carried out to characterise the product polymers. A specific amount of these microbeads were immersed in a 250ml beaker containing freshwater sample and stir bar. The mixture was spinned using a hot plate. Microbeads polymers were filtered again after 2 months and analysed under FTIR spectrometer using the same process and same materials. The same polymers were analysed furthermore after about 3 months in order to analyse the chemical adsorption of these materials as biofilm was formed around these microbeads from being in the presence of freshwater for months.

### 2.2 Analysis of freshwater and sediment samples

Samples were collected from two rivers: (R. Barrow and R. Burren, Co. Carlow) at 3 different sites (Carlow, St Mullin's, and Milford) by in situ and ex situ methods using appropriate containers. In situ sampling was achieved by immersing the net directly into the river for a short period of time. The collected freshwater and sediment samples were sieved in order to isolate any microplastics present, then the samples were analysed using three different steps including (i) visualisation step using light and dissection microscopes, the samples were analysed visually and sieved to extract microplastic particles, (ii) flotation step was carried out using wet peroxide oxidation which involves the use of various chemicals to facilitate microplastics suspension from the samples and (iii) FTIR spectrometer step to identify the recovered polymer types. The same procedure used to extract the reference samples (microplastics from cosmetics) was applied to freshwater and sediment samples which were collected from four locations at River Barrow and Burren (Carlow, Co. Carlow) by in situ and ex situ methods using appropriate containers.

### 2.3. Wet peroxide oxidation (WPO)

National Oceanic and Atmospheric Administration method (Masura *et al.*, 2015) was adapted to detect and quantify microplastics by processing freshwater and sediment samples via wet peroxide oxidation (WPO) to remove any labile organic substance. In brief, samples were subjected to 30% hydrogen peroxide ( $H_2O_2$ ) solution in the presence of Fe(II) catalyst leading to the oxidation of natural organic matter without affecting the synthetic plastic materials in the mixture. This process was carried out using 0.05M Fe(II) solution which was made up by dissolving 3.75g of iron sulphate ( $Fe_2SO_4$ ) in a 250ml water/sediment beaker. 30ml of concentrated sulfuric acid was added into the beaker for density separation of microplastics via flotation. A clock glass was placed on top of the beaker and after standing for 2 hours the mixture was subjected to vacuum filtration. The recovered materials were rinsed with deionized water and dried in the oven at 60°C for 5 minutes. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was used to analyse the recovered materials from the reference (cosmetic products) and freshwater and sediment samples. Spectra were recorded as % transmission using a Perkin Elmer Spectrum 65 FT-IR Spectrometer. The same process used to analyse microbead polymers from cosmetic products were applied on the actual samples.

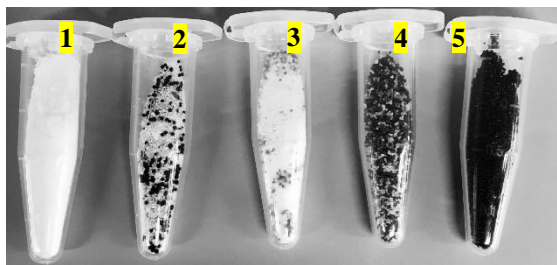
## 3. Results and Discussion

Polymers from product 1 were immersed in freshwater samples for between 2 to 3 months to examine the adsorption properties of microplastics. The identification of microplastic polymers in River Barrow was completed by comparison of the spectra polymers extracted from cosmetic products to procure reference data using ATR-FTIR method, see Fig. 4. Microbeads from the five cosmetic products used consisted of polyethylene.

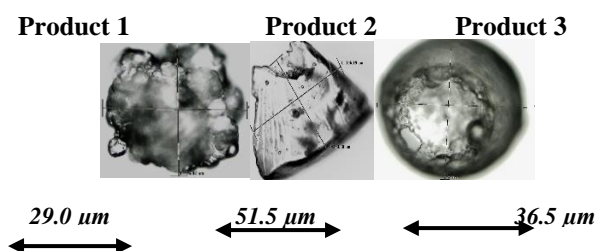


**Figure 1.** Sampling location at River Barrow showing variety of plastic litter. Inset: 4mm white microplastic recovered from River Barrow sample.

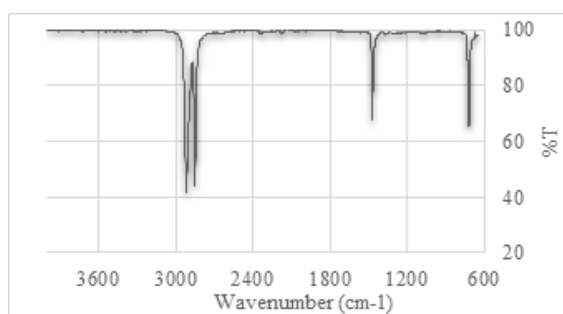
Results from physicochemical tests carried out confirmed that all the microbeads polymers from the reference and the recovered polymer were low density polyethylene. Copper wire and oil tests were effective methods to identify polymer types despite challenges due to the flame visualization during the copper wire test.



**Figure 2.** Color variation among microplastics (MPs) extracted from cosmetic products 1–5.



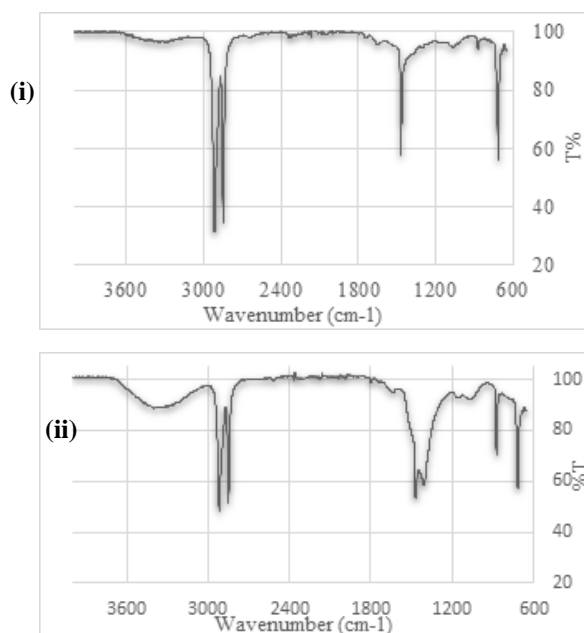
**Figure 3.** Light microscope images of microplastics from products 1, 2, and 3.



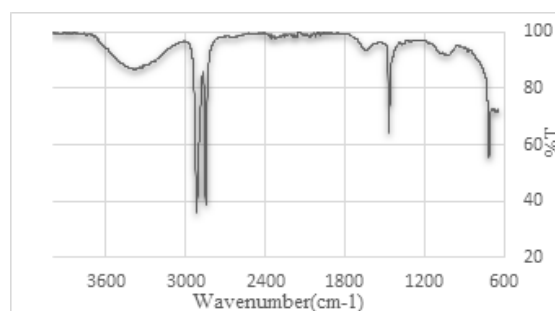
**Figure 4.** ATR-FTIR spectrum of microbead isolated from product 1.

Microplastics extracted from product 1 were analyzed using ATR-FTIR (Fig. 4 and Fig 5). Fig. 4 shows the spectrum obtained for a particle recovered from product 1, which can be identified as polyethylene based on comparison with a reference spectrum for this polymer (see peak assignments in table 1). Fig. 5 shows spectra obtained for particles from product 1 after being exposed to freshwater from River Barrow for periods up to 3 months. These spectra show gradual development of peaks at approximately  $1650\text{ cm}^{-1}$  and in the  $3100\text{--}3600\text{ cm}^{-1}$  region, when polyethylene microbeads were exposed to freshwater samples for prolonged periods. These regions of the spectrum are of interest due to their compatibility with the C=O and N–H stretch respectively of the amide group, a possible indication of microbiological growth on the microplastic surface following exposure to freshwater. However due to the relatively high intensity of the  $3100\text{--}3600\text{ cm}^{-1}$  peak, absorption of water by the polymer is perhaps a more likely explanation, as reflected by peak assignments in Table 2. Fig. 6 shows the ATR-FTIR spectrum of a polyethylene microplastic recovered from the River Barrow after an unknown period of exposure. As summarized in Table 2, this spectrum has similarities to

that shown in Fig. 5 (ii), with the exception of unassigned peak at approximately  $890\text{ cm}^{-1}$  and  $1400\text{ cm}^{-1}$  in Fig. 5 (ii), neither of which appear in Fig. 6.



**Figure 5.** ATR-FTIR spectra of microplastics from product 1 after immersion in freshwater from River Barrow for 2 months (i), and 3 months (ii).



**Figure 6.** ATR-FTIR spectra of microplastic recovered in R. Barrow (Co. Milford).

**Table 1.** ATR-FTIR peak data and assignments for polyethylene microbead isolated from product 1.

Peak number	Wavenumber (cm <sup>-1</sup> )	Assignment
1	2915-2917	C–H asymmetric stretch
2	2849-2896	C–H symmetric stretch
3	1470-1472	C–H bend (CH <sub>2</sub> )
4	1464-1466	C–H bend (CH <sub>3</sub> )
5	716-718	CH <sub>2</sub> rocking

**Table 2.** ATR-FTIR data for additional (non-polyethylene) peaks appearing in freshwater-exposed microplastics, as per Fig. 5 (ii) and Fig 6.

Wavenumber (cm <sup>-1</sup> )		Assignment
Fig 5 (ii)	Fig 6.	
3100-3600	3100-3600	O–H stretch
1650-1662	1652-1691	H–O–H bend
1398-1400	n/a	unassigned
1035-1200	1035-1200	unassigned
870-872	n/a	unassigned

The extraction of microbeads from cosmetic products was challenging due to the buildup of foam during vacuum filtration. To overcome this problem, deionized water was first boiled in a beaker and the products were immersed with a stir bar to facilitate the separation of the polymers from the cream and also to reduce the formation of foam during filtration. A 4 mm microplastics was recovered after sieving freshwater sample. After being analyzed under FTIR spectroscopy, this particle was identified as polyethylene. The remainder of polymers recovered were macroplastics ranging in size from 10.7mm to 25mm. Characterisation by ATR-FTIR showed that these consisted of polyethylene. Most plastics recovered visually and using the three steps were secondary microplastics. All the methods used to isolate and identify microplastics were effective. Vacuum filtration was an effective method for detecting microplastics but recovery of microplastics < 1mm in diameter from Whatman filter paper N°4 proved difficult, which presented a barrier to further analysis of these particles. Wet peroxide oxidation was the most effective method as it enabled the full recovery, identification and quantification of microplastics in freshwaters.

#### 4. Conclusions

Microplastics recovered from freshwater environments (River Barrow) and the ones extracted from 5 different facial scrubs varied in sizes (Fig. 4) and were identified as polyethylene after being analysed under Fourier transform infrared. After subjecting freshwater and sediment samples to vacuum filtration, the results showed the presence of microplastics in both samples. However, these materials could not be removed from the filter paper for further analysis. No microplastics were recovered after subjecting sediment samples to wet peroxide oxidation (WPO) method whereas, this is an effective method. Only one microplastic was recovered from the limited number and volume of freshwater samples analysed to date; this was identified as polyethylene after being analysed using ATR-FTIR. Further sampling will be carried out at different locations including WWTPs to provide a better understanding on the impact and fate of these particles in Irish freshwaters. This study was carried to investigate the fate and behaviour of microplastics in freshwater as there has been less focus on this area despite the obvious importance of rivers as inputs to coastal and marine

environments. Further research is needed to consider the fate and quantification of these particles.

#### References

- Horton, A., Svendsen, C., Williams, R., Spurgeon, D. and Lahive, E. (2017). Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective quantification. *Marine Pollution Bulletin*, 114(1), pp.218-226.
- Rocha-Santos, T. and Duarte, A. (2015). A critical overview of the analytical approaches to the occurrence, the fate and the behaviour of microplastics in the environment. *TrAC Trends in Analytical Chemistry*, 65, pp.47-53.
- van der Hal, N., Ariel, A. and Angel, D. (2017). Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. *Marine Pollution Bulletin*.
- Keswani, A., Oliver, D., Gutierrez, T. and Quilliam, R. (2016). Microbial hitchhikers on marine plastic debris: Human exposure risks at bathing waters and beach environments. *Marine Environmental Research*, 118, pp.10-19.
- Free, C., Jensen, O., Mason, S., Eriksen, M., Williamson, N. and Boldgiv, B. (2014). High-levels of microplastic pollution in a large, remote, mountain lake. *Marine Pollution Bulletin*, 85(1), pp.156-163.
- Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A., Winther-Nielsen, M. and Reifferscheid, G. (2014). Microplastics in freshwater ecosystems: what we know and what we need to know. *Environmental Sciences Europe*, 26(1).
- Avio, C., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L. and Regoli, F. (2015). Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environmental Pollution*, 198, pp.211-222.
- Bakir, A., Rowland, S. and Thompson, R. (2014). Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environmental Pollution*, 185, pp.16-23.
- Carr, S., Liu, J. and Tesoro, A. (2016). Transport and fate of microplastic particles in wastewater treatment plants. *Water Research*, 91, pp.174-182.
- Andrady, A. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62(8), pp.1596-1605.
- Tagg, A., Sapp, M., Harrison, J. and Ojeda, J. (2015). Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. *Analytical Chemistry*, 87(12), pp.6032-6040.
- Masura, J., Baker, J., Foster, G. and Arthur, C. (2015). Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOSOR& R-48. National Oceanic and Atmospheric Administration, Silver Springs, MD