X-Ray Computed Tomography: A Novel Non-Invasive Approach for the Detection of Microplastics in Sediments?

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Highlights

- A new application of Computed Tomography (CT) in microplastics research is proposed
- CT scans reveal non-invasive study of microplastics in sediments could be possible
- Larger microplastics in artificial cores were all digitally recovered and isolated
- Scans of natural River Thames cores show data on sediment structure lost by other methods

Abstract

As a non-invasive imaging technique, this study explores the application of Computed Tomography (CT) in microplastics research, assessing its potential to distinguish different types and sizes of microplastics (polypropylene, polyethylene terephthalate, polyethylene, and polyvinyl chloride) from homogenised river-estuarine sediment. When layered in artificial sediment cores, all microplastic types could be observed by CT imagery with good contrast. Large microplastics (4 mm diameter) were detectable when distributed randomly amongst the sediment (spiked cores with sufficient difference in X-ray attenuation (based on image gray level intensity) to allow segmentation between type. Due to limitations on scan resolution, smaller microplastics (≤125 μm diameter) could not be detected. Scans of two sediment cores from an urban tributary of the river Thames (UK) revealed two distinctive sediment structures which could influence microplastic accumulation. This information would be lost using conventional density-based separation procedures. Although more work is needed, this study presents a novel application of CT that could enhance microplastics research.

Keywords

X-ray Computed Tomography, polymer, sediment core, microplastic pollution, image analysis, River Thames

1 Introduction

Microplastics have repeatedly been found within aquatic sediments around the globe over the past twenty years, such that it is now generally agreed that microplastics are ubiquitous within these environments (Thompson 2004, Barnes *et al.* 2009, Andrady 2017, Rochman 2018). Frequent interaction with microplastics by aquatic organisms has been found to cause possible biological effects, ranging from physical abrasions and internal blockages to toxicity from xenobiotics (including plastics acting as vectors for persistent organic pollutants- or POPs, when ingested) (Teuten *et al.* 2009, Wright *et al.* 2013). However, the consequences of microplastic pollutants remain poorly understood, and often contested (Jovanović 2017; Triebskorn *et al.* 2018; Windsor *et al.* 2019).

To tackle the microplastics problem effectively, significantly more data is required to elucidate the behaviour of these pollutants. A key component of this is to understand when and how microplastics are transported or stored in aquatic bed sediments between microplastic sources and their sinks. Analysis of sediment cores has previously revealed the existence of historical microplastic accumulation records as well as the effects of urbanisation on vertical abundance of microplastic particles (Carson *et al.* 2011; Li *et al.* 2020, Lloret *et al.* 2021).

At present, the majority of studies utilise an invasive density separation stage to recover microplastic particles. This involves the agitation of the sample with a heavy liquid (often NaCl or $ZnCl_2$) of a density that causes microplastic particles to float to the surface while sediments settle out (Mohamed Nor & Obbard 2014, Nuelle *et al.*

2014, Coppock *et al.* 2017, Hurley *et al.* 2018). Further stages to improve abundance estimations are often used, including the use of additional density separations and the digestion of organic matter in the samples, yielding microplastic recovery rates of between 55% and 99% depending on the exact methodology followed (Hidalgo-Ruz *et al.* 2012, Nuelle *et al.* 2014, Miller *et al.* 2017, Hurley *et al.* 2018). Suspected microplastic particles can also be chemically characterised using analytical techniques such as Fourier-transform infrared spectroscopy (FT-IR) and Ramen spectroscopy to improve the accuracy of estimations (Hidalgo-Ruz *et al.* 2012, Lusher *et al.* 2016). Density separation steps rely upon the destruction of the sediment structure to extract microplastic particles and yield a low vertical resolution of microplastic abundance, making it difficult to develop a precise understanding of microplastic storage and transfer in sediments.

X-ray Computed Tomography (CT) is a three-dimensional non-destructive imaging technique, which could in theory allow for the non-invasive identification of microplastics in situ from sediment samples (Mooney 2006, Taina *et al.* 2008). Using X-ray imaging, grayscale images of samples can be produced based upon the the X-ray attenuation of different materials (as determined primarily by bulk density and electron density) (Heeraman *et al.* 1997, Wildenschild *et al.* 2002, Helliwell *et al.* 2013). In CT, a series of 2D X-ray projection images are collected of the sample as it undergoes a 360° rotation. These images are then computationally reconstructed to yield a 3D volumetric data output, with different materials represented by different image gray level values. In general, low-density materials appear dark (low gray level) and high density materials appear lighter (high gray level). More comprehensive descriptions of the fundamental principles of CT operation can be found within Wildenschild *et al.* (2002) and Mooney *et al.* (2012).

Over the past two decades, CT imaging has been increasingly used in soil and plant sciences to investigate soil characteristics and biophysical interactions at sub-micrometre scale (Heeraman *et al.* 1997, Mooney 2006, Schrader *et al.* 2007, Taina *et al.* 2008, Helliwell *et al.* 2013). Despite significant technological advances, few studies have applied the technique to the investigation of microplastic particles and their environmental interactions. CT images have been used to investigate the shape and structure of microplastic particles in sediments of Hiroshima Bay (Sagawa *et al.* 2018), and the technique has been paired with neutron tomography to investigate microplastics in an artificial sand core (Tötzke *et al.* 2021). In this current study, the utility of CT was explored as a novel technique for the non-destructive analysis of microplastics in river sediments.

The aims of this investigation were threefold:

1) Identify whether CT imaging could distinguish between layers of plastics and layers of river sediments in artificial cores (known as layered cores).
2) Assess microplastic recovery rates using artificial river sediment cores that were spiked with a known quantity of microplastic particles (known as spiked cores).
3) Establish whether the technique could be applied to real-world samples, using two sediment cores that were obtained from a tributary of the River Thames, UK (cores UT3C and UT3F).

2 Materials and Methods

2.1 Sample Preparation

A total of four artificial cores for each core type (layered cores and spiked cores) were created in PVC-U tubes by packing with a stock of homogenised intertidal foreshore sediment (clay 20%, silt 44% and fine sand 34%) collected from a site adjacent to Purfleet (51°29′04.98″N, 000°13′37.96″E) on the river Thames estuary, UK (Vane *et al.*, 2015). Each of the four cores for both types were spiked with a different plastic polymer to test the influence of plastic type on recoverability. Four types of primary microplastic were purchased from Sigma Aldrich in February 2022 to develop both types of experimental core. These were polypropylene, polyethylene terephthalate (PET), polyethylene, and polyvinyl chloride (PVC). These polymers were chosen because they were the four most in demand polymers in Europe in 2021, and were therefore likely to be abundant types of microplastic in the environment (Plastics Europe 2021). The polymers also covered a range of bulk and electron densities (Table 1) which were hypothesised to have an impact on x-ray attenuation.

To simultaneously investigate the effect of microplastic size on recoverability, the microplastics were selected so that two size categories could be compared: ‘large’ microplastics (~ 4 mm in diameter), and ‘small’ microplastics (~125 μm in diameter). Polypropylene and PET represented large-sized microplastics, while polyethylene and PVC represented small-sized microplastics.

The first experimental cores (layered cores) were designed to assess the ability of the CT scanner to detect plastic particles compared to sediment particles. It was also envisaged this would ascertain whether it was possible to distinguish between individual microplastic particles contained in a layer. Sediment layers were alternated with three microplastic layers, aiming to have the thickest layer of microplastics at the bottom of the core, getting progressively thinner up the core (bottom layer 1-2 cm thick, middle layer ~0.5 cm thick, and top layer ~1-2 particles thick), although this was more difficult to attain for the smaller microplastics (Fig. 1). These layered cores were compiled in small cores of 2.5 cm diameter and 7.5 cm height.

The second suite of experimental cores were designed to mimic real-world urban river samples by spiking 200 g wet mass of sediment with known quantities of microplastic, which were then randomly mixed to make spiked sediment cores of 5.5 cm diameter and 6 cm height (Fig. 1). For cores containing granular microplastics, a total of 70 particles were added, similar concentrations to those found by Horton *et al.* (2017) across several River Thames tributaries (UK), and Willis *et al.* (2017) in the Derwent estuary (Tasmania, Australia). For cores containing microplastic powders, 0.5 g of microplastics were added since particles could not easily be counted.

Two sediment gravity cores were obtained at low tide in February 2022 along a transect of Barking Creek, a tributary of the River Thames (London, UK). Core UT3C was directly extracted from Barking Creek adjacent to Beckton Creekside salt marsh, spanning a depth of 0-30 cm (51° 31' 27.5" N, 00° 05' 33.2" E). Core UT3F was taken from the edge of the salt marsh, 35 m landward of UT3C and spanning a depth of 30-60 cm (51° 31' 27.0" N, 00° 05' 31.5" E). At each site, a polycarbonate tube fitted with a stainless-steel basket catcher was driven into the exposed sediment to recover core material (Vane *et al.* 2007). Cores were contained in the tubes and sealed for transportation to the laboratory, where they were refrigerated until analysis. These cores were collected as part of a wider evaluation of microplastics in Thames sediments and are included here to represent two urban environments namely, urban tributary and salt marsh, where microplastics are thought to accumulate.

2.2 Scanning

Layered cores were scanned using a Phoenix Nanotom 180NF (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) fitted with a 5-megapixel (2316 × 2316 pixels) flat panel detector (Hamamatsu Photonics KK, Shizuoka, Japan) at 13.1 µm resolution. The scans consisted of collecting 2160 projection images over a 360 rotation of the sample using an X-ray tube energy, current and detector exposure time of 85 kV,160 µA and 750 ms, respectively. Scans were made in FAST mode where the sample rotates continuously without the use of any projection image integration (i.e. single image per projection).

All other cores were scanned using a v|tome|x M 240 kV scanner (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany), fitted with a DXR-250 flat panel detector (2014x2014) at 35 µm resolution. The scans consisted of collecting 2400 projection images over a 360° rotation of the sample using an X-ray tube energy, current and detector exposure time of 140 kV, 200 µA and 250 ms, respectively. Each projection image was the integration of two images. The scanner detector shift option was used to supress to occurrence of ring artefacts in the reconstructed data.

3 Results

3.1 Layered Cores

Reconstructed 3D images of layered cores revealed that it was possible to identify all layers of microplastic particles within the sediment (see Fig. 2 for example 2D image slices). The microplastics had average gray level values that were consistent for different particles of the same polymer type, reliably representing the true sizes and shapes of each microplastic type. Most microplastics had a uniform colour throughout, although polypropylene particles were found to have dark void-like regions in the centre of the particles. These voids are likely a result of the manufacturing process, as a cloudy region could be seen in the centre of each (otherwise colourless) polypropylene particle upon visual inspection.

The average gray level value varied between plastic types, indicating that both the bulk density and atomic number of the plastic material are important properties to consider when using CT for microplastics analysis (Fig. 3). The average gray level value of polyethylene and polypropylene were most similar, and the two plastics were also most similar in terms of bulk density and atomic mass. However, there was sufficient and consistent contrast between all plastic types and other materials in the cores, indicating that it may be possible to use gray level values to uniquely identify each polymer type within a sediment core.

3.2 Spiked cores

Larger microplastic particles of 4 mm diameter (PET and polypropylene) were easily recognised in scan images of spiked cores with a clear contrast between microplastic particles and background sediments (Fig 4).

To quantify the number of particles in each core and their approximate volumes, the 3D CT images were further processed using VG StudioMAX© v2.2.5 (Volume Graphics GmbH, Heidelberg, Germany). The gray level values for each scan were first normalised by remapping the background ‘air’ and the column wall to 10000 and 20000, respectively. Microplastic particles were then segmented in 3D to a ‘region-of-interest’ from the sediment based on their average gray level. The defect analysis tool was then used to quantify the volume of the particles using the region-of-interest as a mask for each polymer type (Fig 5). Using these detailed reconstructed 3D images, all 70 microplastic particles could be isolated from other particle types in the cores, based first on the gray level colour of the particles, and then by individual particle shape and volume. Individual polypropylene particles had a mapped volume of between 23 mm^3 and 26 mm^3, while PET particles had a mapped volume of between 15.5 mm^3 and 16.5 mm^3. *Craig is working on obtaining actual values (these are my estimates from the figures, he is on leave over Easter)*

Plastic particles could not be confidently identified in the spiked cores containing the small 125 µm microplastics (polyethylene and PVC), despite their detectability in the layered cores (seen in Fig. 2). Scan resolution of these images (35 µm) meant that individual microplastics were represented in images by 3.6 pixels, making it challenging to unequivocally identify microplastic particles as discrete objects amongst the background sediments in these cores.

3.3 Environmental Samples

Imaging of environmental samples revealed two distinct cores in terms of sediment structure (Fig. 6). Core UT3C from Beckton Creek contained few large particles except for the base of the core, and the sediment structure became increasingly fractured towards the surface. In contrast, core UT3F from the edge of the salt marsh had a clear layering of sediments and contained multiple large particles and root networks. A range of particle types were visible in the imagery, but it was not yet possible to confidently differentiate microplastic particles from others.

4 Discussion

All four microplastic materials were distinguished from background sediments as well as other plastic types in experimentally layered cores, and the same was true for the larger microplastic particles in spiked cores. This suggests that it may be possible to uniquely identify different plastic polymer types and isolate them from other particle types using CT imaging, and therefore use the technique to detect plastic pollution in natural sediment cores. This technique would offer several advantages over other more invasive microplastics analysis techniques currently in use.

Firstly, samples do not require complex preparation or involve the use of (often) expensive and harmful laboratory chemicals over several stages to extract the microplastics (Claessens *et al.* 2013, Coppock *et al.* 2017), although it is noted that the CT instrumentation itself is costly. The microplastics would also not be at risk of degradation from floatation or digestion medias (Lusher *et al.* 2016), and quantification was not significantly affected by the presence of fine sediments (Constant *et al.* 2021, Nava & Leoni 2021). Additionally, the use of CT imaging significantly reduces the occurrence of background/user microplastic contamination compared to invasive techniques, as the samples remain sealed following their collection (Hidalgo-Ruz *et al.* 2012, Fries *et al.* 2013).

More research is, however, required to refine the technique before it can be considered a viable practice for identifying microplastics in environmental cores such as UT3C and UT3F. For example, smaller microplastic particles could not be identified when they were randomly distributed in the experimentally spikes cores due to the scan resolution and the increased vulnerability to partial volume effects as a result. CT imaging could therefore be optimal for investigating larger microplastics and mesoplastics, although exact boundaries need to be established. Scale will be particularly important to consider if CT imaging is to be used for the quantification of microplastic fibres- a microplastic shape that is notoriously difficult to accurately identify using existing methods, and often overestimated in studies relying solely on visual identification (Fischer *et al.* 2016, Lusher *et al.* 2016).

It could also be argued that the larger microplastic particles were only identifiable in the spiked cores because the particles were homogenous in shape and colour which made them easy to isolate from the background sediment matrix. However, identification of microplastic particles was not only possible through visual examination, but they could also be reliably digitally isolated from other particle types using a threshold volume software by specifying the gray level colour range of the desired plastic. Since the microplastics were also at environmentally relevant concentrations, this means that the spiked cores were able to demonstrate a promising proof of concept for the use of CT imaging in sediment microplastic analysis. Further work could seek to isolate a range of microplastic types, shapes, and sizes within the same core to minimise the effect of particle homogeneity.

If successful, it may be possible to develop a reference library database of polymer gray values that can be calibrated between instruments using reference materials, enabling the user to digitally quantify different types of microplastic particles. Polymer matching libraries have been established in the past for vibrational spectroscopic techniques, so could likely be produced for CT imaging (Primpke *et al.* 2018, Cowger *et al.* 2021, De Frond *et al.* 2021). It could also be possible to apply deep-machine learning approaches to differentiate between plastics and other material types in cores; there already exist AI segmentation tools to map root networks in soil cores (e.g. Soltaninejad *et al.* 2020, Tötzke *et al.* 2021, Griffiths *et al.* 2022). The retraining of such algorithms to map microplastics would be a feasible first approach at applying CT deep learning to the study of microplastics.

Of course, as with existing floatation and spectroscopic techniques, it may be possible that other naturally occurring particles have similar attenuation properties to microplastics, or that significantly degraded plastics no longer have the same properties as a virgin polymer, leading to misidentification (Morét-Ferguson *et al.* 2010, Imhof *et al.* 2012, Fischer *et al.* 2016, Lusher *et al.* 2016). Repeated imaging of a wide range of polymers at variable degrees of degradation could aim to resolve this. Neutron tomography has also been suggested as a complementary technique that may improve identification accuracy, since neutrons are strongly attenuated by plastics and not by sediments and therefore have high visibility in the imagery (Tötzke *et al.* 2021).

CT imaging could also prove a valuable complementary analysis tool to be used alongside established techniques. The examination of the environmental cores using CT in this study revealed a detailed sediment structure which would not have been preserved using current microplastic analysis techniques. These structures could prove to be a key factor influencing the storage of microplastics in different sediments, and therefore their analysis is important. In addition, CT could prove useful for investigating interactions between large microplastics and their environment. For example, artificial sediment cores spiked with a known type and quantity of large microplastic could be used to simulate relationships between microplastics, sediment microstructure, vegetation, and other interactions which could then be deciphered through CT imaging.

5 Concluding Remarks

This study confirms that CT imaging can distinguish between microplastic particles and sediments. While further work is needed to make it a robust reproducible screening technique for the investigation of microplastics in environmental samples, this is an important early step in the use of CT within the field. It presents an opportunity for the non-invasive detection of microplastics in sediments and has several other advantages over existing techniques.

It was found that the technique could be used for investigating the relationships between larger microplastics, sediment microstructure and other relationships, by simulating natural sediment cores that are spiked with microplastics of a known polymer material and morphology. Further research could enable the technique to be applied to environmental samples, quantifying the number and types of microplastics without needing to destroy the sediment structure, spend consumables in the lab, or risk modifying the particle chemistry. Deep machine learning could also be developed from existing algorithms to map microplastics in cores, significantly improving the speed at which samples can be processed. Alternatively, it could also be used as a complementary technique to existing microplastic quantification methods where details of the sediment structure would provide a more holistic view of the data. In each case, CT imaging offers a promising opportunity to improve understanding of microplastic storage, interaction and transfers within sediments.

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