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Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK

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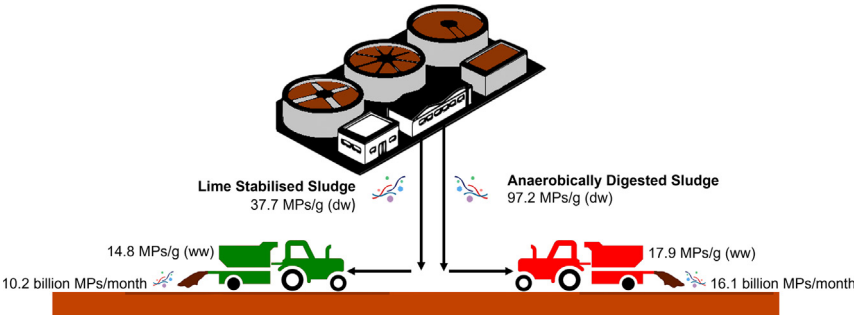
HIGHLIGHTS

- Microplastic identification and quantification in sludge and biosolids down to 50 μm
- Presumed microplastics confirmed with μ-Fourier Transform infra-red spectroscopy
- Microplastics concentrations range from 37.7–286.5 microplastics/gram of sludge (dw).
- 1.02×10^{10}–1.61×10^{10} microplastics retained in biosolids every month.
- Equivalent of >20,000 bank cards potentially recycled to land every month

GRAPHICAL ABSTRACT



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ABSTRACT

There is an increasing concern about the impacts of microplastic pollution in the terrestrial environment. Identifying sources, pathways and sinks of terrestrial microplastics is crucial to determining environmental exposure and applying efficient intervention measures. In the UK alone, 3.5 million tonnes (wet weight) of biosolids from the wastewater industry are recycled each year to agricultural land, raising the possibility that recycling of biosolids could be a significant source of microplastic pollution to the terrestrial environment. To address this issue, the present study determined the presence of microplastics from across the whole sludge treatment stream from one exemplar wastewater treatment works in the UK. Both sewage sludge (a liquid by-product produced from the wastewater treatment processes) and biosolids (sewage sludge that has undergone a treatment process) were examined as a source of microplastics to the terrestrial environment. Microplastics were detected in all samples taken from across the treatment process with concentrations ranging from 37.7–286.5 number of microplastics/g of sludge (dry weight). The microplastic load in the final biosolid products produced at the site ranged from 37.7–97.2 number of microplastics/g of sludge (dry weight). The wastewater treatment works in this study produces 900 tonnes of anaerobically digested sludge cake and 690 tonnes of lime stabilised cake per month. Based on the results from this study, the application of these biosolids to agricultural land as fertilisers can potentially release 1.61×10^{10} and 1.02×10^{10} microplastics in anaerobically digested and lime stabilised sludge respectively, every month (equivalent to the same volume as >20,000 plastic bank cards). The results illustrate the extent to which microplastics may enter the terrestrial environment through this route.

Abbreviations: MP/s, microplastic/s; WwTWs, wastewater treatment works; Dw, dry weight; Ww, wet weight; MPs/g, microplastics per gram; CSO, combined sewer overflow; Avg, average; MBR, membrane bioreactor; μFT-IR, micro-Fourier Transform-Infrared Spectroscopy; μm, micrometre; PVC, polyvinyl chloride; PA, polyamide; PVA, polyvinyl acetate; PET, polyethylene terephthalate; PAN, polyacrylonitrile; PS, polystyrene; SAN, styrene-acrylonitrile resin; ABS, acrylonitrile butadiene styrene; ASA, acrylonitrile styrene acrylate; PCT, polycyclohexylenedimethylene terephthalate; EPM, ethylene propylene rubber.

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1. Introduction

1.1. Microplastics; contaminants of emerging concern

The general term 'microplastics' is used to describe particles and fibres of a diverse range of polymers less than 5000 μm in size (with the minimum particle size subject to methodological limitations (Horton et al., 2017)). These can be intentionally manufactured within this size range (primary microplastics) or formed by the fragmentation or break-up of larger plastics or plastic-containing items, known as secondary microplastics (Hartmann et al., 2019). Microplastics have been identified as a contaminant of emerging concern (Lambert and Wagner, 2018) and their presence in the environment raises questions about the risk they pose to human and environmental health. This has led to microplastics receiving increased interest from the public, water companies and regulators. Wastewater treatment works (WwTWs) have been identified as an important pathway for microplastic pollutants to enter the terrestrial environment (Sun et al., 2019; Karapanagioti and Kalavrouziotis, 2019). Microplastics are released to the environment either through the discharge of final effluent, from the release of untreated sewage via combined sewer overflows (Woodward et al., 2021), or through the recycling of sewage sludge (a by-product generated from the treatment of wastewater) to agricultural land. Due to the importance of WwTWs as a pathway for the release of microplastics to the terrestrial environment it is important to have a thorough understanding of the fate and behaviour of microplastics in wastewater and sludge, and to identify the potential environmental exposure and associated risks.

1.2. Microplastics in wastewater treatment works

Microplastics have been reported in wastewater influents, with values ranging from 0.631 particles/Litre - a value which included microplastics and non-synthetic anthropogenic fibres - (Magnusson et al., 2016) to 130,000 number of microplastics/Litre (MPs/Litre) (Vollertsen and Hansen, 2017) (unless stated otherwise, microplastic concentrations are reported as number of microplastics per weight or volume). Despite the high concentration identified in the influent, WwTWs have been found to be effective at removing microplastics from the wastewater stream, with removal rates of 57%–99% following secondary treatment processes (Carr et al., 2016; Gies et al., 2018; Liu et al., 2019). Captured microplastics end up in the sewage sludge with concentrations varying considerably between studies, from 1.00 microplastics/gram (MPs/g) (Carr et al., 2016) to 169,000 MPs/g (Vollertsen and Hansen, 2017) (Table 1). Table 1 illustrates the range of microplastic loads previously determined in different sludge sample types and across different locations

1.3. Effects of sludge treatment on microplastic concentration and characteristics

Sludge is treated before it is recycled to land (unless injected or worked straight into soils under certain conditions) to minimise the risk to human health, (Department for Environment, Food and Rural Affairs, 2018; European Commission, 2021; The Council of the European Communities, 1986). These treatment processes can consist of biological, chemical and heat-treatment. For example, in the UK, 73% of sludge is treated via anaerobic digestion (AD) followed by lime-stabilisation (22%) (Assured Biosolids Ltd, 2019). This is also the case in Europe where anaerobic digestion is the most common sludge stabilisation process employed in twenty-four European Union (EU) countries (Gianico et al., 2021).

A limited number of studies have investigated the effects of sludge treatment on microplastic concentration and characterisation. Some studies have found sludge treatment processes are inefficient at removing microplastics from sewage sludge but effects on the characteristics of microplastics have been identified. Synthetic fibres in alkaline-stabilised sludge have been found to be more brittle, shorter and exhibit erosion (compared to dewatered, pelletized and composted sludge) (Zubris and Richards, 2005). It is thought this is due to an elevated pH and temperature, and mechanical mixing, within the lime stabilisation process which also likely produces an elevated number of smaller particles (Mahon et al., 2017a). A significantly lower number of microplastics were found in sludge that had undergone anaerobic digestion (2,743 MPs/kg (dw)) than thermal drying (10,012 MPs/kg (dw)) at the same site in a study by Mahon et al. (2017a). In another study, fewer nylon (polyamide) microplastics were found in anaerobically treated sludge samples compared to the wastewater samples from the same treatment works (Vollertsen and Hansen, 2017). It is suggested that anaerobic digestion could affect some plastics, such as nylon, by either causing the particles to break-up into smaller pieces which are not detected by the methodologies employed, or from biological digestion (Vollertsen and Hansen, 2017). This could possibly explain the lower concentration of microplastics (specifically nylon) found in sludge compared to wastewater samples from the same site (Vollertsen and Hansen, 2017), suggesting that further research is required to understand if and to what extent this occurs. The breakup of microplastics is of a concern, as weathering and erosion of polymers increases the surface area through fragmentation which could in turn affect sorption characteristics (Teuten et al., 2007). Changes in microplastic characteristics during wastewater treatment and sludge treatment processes could cause variations in adsorption potential of microplastics for metal pollutants and potentially enhance their adsorption capacity (Li et al., 2019). Fragmentation of particles increases the surface area to volume ratio, thus increasing adsorption rates. Uneven and rough surfaces, enhanced by sludge treatment processes such as lime stabilisation, can allow microorganisms, chemicals and heavy-metal contaminants to concentrate on the surface while smaller sizes may increase bioavailability via ingestion (Li et al., 2018; Mahon et al., 2017b). Although initial research indicates sludge treatment may have an effect on microplastic morphology, there is contradicting evidence on whether sludge treatment influences microplastic concentration, leading to the conclusion that sludge treatment processes are not the dominant control on microplastic abundance in sludge (Hurley et al., 2018a). It has been suggested that concentration of microplastics in sludge could be significantly influenced by factors in addition to sludge treatment, such as temporal differences based on seasonal and weather conditions (Horton et al., 2021).

Despite the large range of microplastic concentrations reported in sewage sludge, a recent study suggests that only a small fraction of the microplastics removed from the wastewater stream are accounted for in biosolid samples, while up to 96% of microplastics entering WwTWs may remain undetected in sludge samples with current methodologies (Koutnik et al., 2021). Given the large variability inherent in current methodologies employed in identification and quantification of microplastics in sludge and biosolid samples (Koutnik et al., 2021), uncertainty remains over the extent to which microplastics enter the terrestrial environment through the application of biosolids to land.

1.4. Biosolids as a source of microplastics to the terrestrial environment

Soils are likely a significant environmental reservoir of microplastics (Hurley et al., 2018a) and the recycling of biosolids to land can create a pathway for microplastic contamination of agricultural soils. It is estimated sludge application is responsible for 63,000–430,000 tons of microplastics deposited annually on European agricultural soils (Nizzetto et al., 2016) while 446 billion microplastics are released annually to agricultural soils via the application of biosolids to land in Norway, and 1,000–3,100 tons of microplastics per year are reportedly retained in sludge across Denmark (Vollertsen and Hansen, 2017). It was estimated that 3.7 billion microplastics were introduced to a single field during one biosolid application in Canada (Crossman et al., 2020). These values indicate the recycling of biosolids could be a significant source of microplastics to terrestrial environments (Hurley et al., 2018a). Microplastic fibres have been identified in soils more than five years after biosolid application in soil column array tests (Zubris and Richards, 2005). Soils in agricultural fields with a history of biosolid application contain significantly higher concentrations of microplastics compared to fields which have not received biosolids (van den Berg et al., 2020). Increasing applications of sludge additionally leads to an increasing microplastic abundance in soil samples taken from agricultural fields,

Table 1
Reported MP concentration in sludge from various studies, and removal rate of MP from wastewater stream if stated.

Study	Location	microplastic size range	Microplastic (MP) load in sludge (number of MP pieces/g or mass mg/g)	% Removal of MPs from wastewater stream	Sample location
Carr et al. (2016)	America	45–400 μm	5 MPs/g primary skimming 1 MPs/g[a] biosolids	99.9%	Skimming's, sewage sludge (before sludge treatment), and biosolids
Gies et al. (2018)	Canada	1–65 μm	Primary sludge: 14.9 (±6.3) MPs/g. Secondary sludge: 4.4 (±2.9) MPs/g	97.1–99.1%	Primary and secondary sludge
Hansen and Vollertsen, (2017)	Denmark	20–500 μm	Avg[b]. 4.5 mg/g. Avg. 169,000 MPs/g [c]dw sludge	99%	Digested sludge.
Hurley et al. (2018a)	Norway	≥50 μm	Avg. 6 MPs/g (dw)	–	Dewatered raw sludge (before lime stabilisation treatment) or stabilised and dried sludge
Lares et al. (2018)	Finland	≥250 μm	Avg. 170.9 (±28.7) MPs/g dw sludge. (Range 8.2–301.4 MPs/g dw sludge). Digested sludge 170.9 (±28.7) MPs/g dw. MBR sludge 27.3 (±4.7) MPs/g dw	98.3–99%	Activated sludge after aeration, [d]MBR and digested sludge.
Li et al. (2018)	China	37–5000 μm	1.60–56.4 MPs/g dw sludge. Avg. of 22.7 (±12.1) MPs/g dw sludge	–	Dewatered sludge samples collected from twenty-eight WWTWs-receiving varying secondary and dewatering treatment processes.
Liu et al. (2019)	China	20–5000 μm	240.3 (±31.4) MPs/g dw sludge	64.4%	Activated sludge samples; combines primary and secondary sludge
Magnusson and Norén, (2014)	Sweden	≥300 μm	16.7 MPs/g dw sludge	99%	Sewage sludge collected after mechanical, chemical and biological WW treatment processes
Mahon et al. (2017a)	Ireland	250–4000 μm	2.7–15.4 MPs/g	–	Sludge after lime stabilisation, anaerobic digestion, and thermal drying
Magni et al., (2019)	Italy	63–5000 μm	113 (±57) MPs/g dw sludge	84%	Recycled activated sludge
Mintenig et al. (2017)	Germany	20–500 μm (no microplastics above 500 μm found in sludge samples)	1–24 MPs/g dw sludge	93–98%	Six WWTWs sampled. Samples taken from plants which use skimming primary treatment.
Murphy et al. (2016)	Scotland	≥65 μm	7.8 MPs/g in grease fraction ~1.6 MPs/g grit fraction. ~0.8 MPs/g sludge cake.	98.41%	Grit and grease solid fraction and sludge cake after centrifuge treatment.
Horton et al. (2021)	United Kingdom	25–178 μm	Individual microplastic concentrations ranged from 301 to 10,380 microplastics/g (dw). Avg. concentration at each site ranged from 499.67–7651.60 MPs/g	99.8%	Sludge cake (anaerobic digestion and limed sludge) was collected from five sites on five different occasions.
Hongprasith et al. (2020)	Thailand	300–4750 μm	103.4 pieces/L	–	Secondary sludge collected after secondary sedimentation
Zhang et al. (2020)	China	200–5000 μm	Avg. 2.35 items/g (dewatered sludge), 0.353 items/g (raw sludge compost), 0.708 items/g (after eight days of composting) and 0.246 items/g after twelve days of composting		Samples collected after dewatering, raw sludge compost, before composting, and after eight and twelve days of composting.

[a] MPs/g (microplastics per gram).
[b] Avg (average).
[c] dw (dry weight), WW (wastewater).
[d] MBR (membrane bioreactor).

suggesting that microplastics accumulated over time with repeated applications (Corradini et al., 2019; van den Berg et al., 2020).

1.5. Aim of current study

Although it is clear microplastics are present in sewage sludge, their fate and behaviour through the sludge treatment process is less clear and there is a lack of data on how they accumulate and alter through the sludge treatment process. There is currently insufficient evidence to suggest the recycling of biosolids to land poses a significant risk to the environment or human health (Assured Biosolids Limited, 2018; Nicholson et al., 2018) and in the UK, industry bodies have called for research to better understand the source, fate, and impact of microplastic pollution in sludge. This includes increasing understanding of the total load of plastics introduced to soil through biosolid application and identifying a threshold of harm for sludge applied to land while maximising the value of sludge to support a circular economy (UK Water Industry Research, 2019). Further understanding is required on the source and environmental impacts of microplastics in sewage sludge and biosolids, as well as the impacts that sludge treatment processes have on the characteristics of the microplastics.

In response to these issues, the aim of this study was to make a detailed investigation of the occurrence and characteristics of microplastics across the sludge treatment process of a typical WwTWs in the UK, and to assess what this may mean in terms of soil contamination. To achieve this, we investigated microplastic concentration and characteristics from samples collected at every stage of the sludge treatment stream at one wastewater treatment works, including from two different biosolid products. The study sought to identify whether microplastics in sewage sludge are a source of concern regarding microplastic pollution of the terrestrial environment. The results provide a comprehensive investigation of the presence of microplastics and characteristics in sludge samples throughout the whole sludge treatment stream and from two different sludge treatment processes at one wastewater treatment works.

2. Methodology

2.1. Site description

The WwTW investigated in this study is located in Devon in the Southwest of England. It has a resident only Population Equivalent of 157,946 and a maximum wastewater treatment capacity of 1,000 L/s. Both wastewater and stormwater are delivered to the site through the same system, this is known as a combined sewerage network (Butler and Davis, 2011). As well as treating sewage sludge produced on site, the site also receives sludge from external WwTWs which has received minimal treatment (dewatering via gravity). The site in this study has two sludge

treatment streams; anaerobic digestion and lime stabilisation, Fig. 1, which produces two levels of treated sludge. Anaerobic digestion produces digested sludge cake, known as conventionally treated sludge. This stabilisation process ensures at least 99% of pathogens are destroyed and the *Escherichia coli* pathogen concentration is $<10^5$ colony forming units/g dry solid. The conventionally treated sludge undergoes a two-stage digestion processes which involves fifteen days in the anaerobic digester followed by fifteen days in the open lagoons before being centrifuged (dewatered) and forming a more solid sludge cake (total solid content of ~18%). The anaerobic digester operates in the mesophilic temperature range (32–40 °C) and as there is no pasteurisation processes, the digested sludge must be held as batches (secondary digestion in the lagoons) to reduce the pathogen content further (Assured Biosolids Limited, 2017). Before being stored prior to anaerobic digestion, after the reception tank, the sludge is thickened to about 6% in a drum thickener with the addition of a copolymer known as polyacrylamide, this is used to consolidate and dewater the sludge. The limed cake, known as enhanced treated sludge, requires the addition of lime which raises the pH and temperature, and ensures 99.9999% of pathogens are destroyed, with an *E. coli* concentration of $<10^3$ in 2 g of dry solid, and free from *Salmonella* spp. The sludge is transferred directly from the reception to the centrifuge where it is de-watered, following this, lime is added, and the sludge then passes through a rotating drum which mixes the sludge and lime (to form total solid content of ~39%) before entering the storage barn.

2.2. Sample collection

All samples were collected on the 18th of October 2019 between the times of 11:30 and 14:30 from seven different locations along the sludge treatment stream, and two samples (duplicates) were collected at each location. Included in the sampling locations are two different end products (biosolids). Details about the location and sample names are as follows:

1. **Reception Tank:** The reception tank receives all primary and secondary sludge produced on site as well as receiving sludge from external WwTWs (transported to the site by tankers). The samples were collected directly from the reception tank.
2. **Thickened:** From the reception tank, prior to anaerobic digestion, the sludge is thickened to have a solids content of ~6% with the addition of the chemical coagulant polyacrylamide, and then passed through a rotating drum. The samples were collected directly after this process.
3. **Digestate:** The sludge is then stored before being fed to the anaerobic digestors where it undergoes mesophilic digestion for fifteen days. The anaerobically digested sludge (digestate) samples were collected from a discharge pipe after the anaerobic digestors, before it enters the secondary digestion process (open lagoons).
4. **Centrifuge Feed Tank:** Following anaerobic digestion, the sludge is held in open lagoons for fifteen days (this is the secondary digestion process). The sludge samples were collected from the centrifuge feed tank, after the secondary digestion, where it is held before being centrifuged. Centrate from the centrifuge process is recycled back into the treatment works.
5. **Digested Sludge Cake**: A sludge cake is produced after centrifugation; the sample was collected before it dropped onto the conveyor belt prior to entering the barn where the sludge cake is stored.
6. **Pre-limed**: The sludge in this stream is centrifuged directly from reception tank to reduce the water content and thicken the sludge. Samples were taken following this process before the addition of lime.

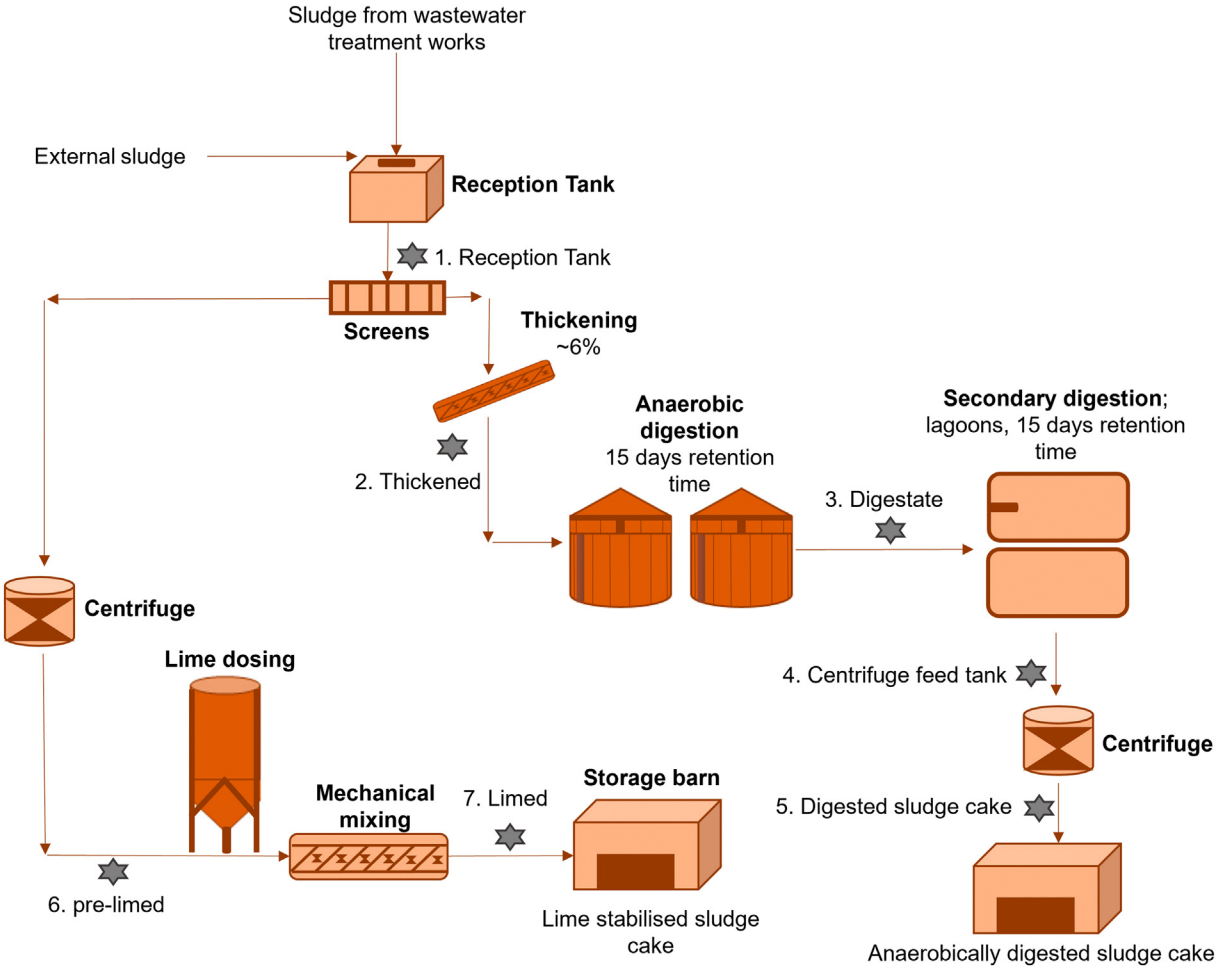

Fig. 1. Schematic of the two sludge treatment streams at the wastewater treatment works, plus the seven different sampling locations.

7. **Limed Stabilised Sludge Cake**: After dewatering via the centrifuge, lime is added to the sludge and mixed via a corkscrew mixing drum. Samples were collected following the addition of lime process from the conveyer belt before the limed sludge cake enters the storage barn.

A sampling pole with 1 L stainless steel collecting bucket attachment was used to collect samples from the reception tank, anaerobic digestion discharge pipe and centrifuge feed tank, this was deemed the best equipment and procedure for the locations and sludge type following an initial site visit. The anaerobically digested and limed sludge were collected with a metal scoop/metal jug from conveyer belt leading into the storage barn. The collected samples were transferred into either a 212 mL or 230 mL glass sampling containers. In the case of the thickened and pre-limed samples, the collecting container was held under the 'flow' and removed once full. The samples were stored in a cold store at 3–5 °C prior to the pre-treatment and extraction processes (Lares et al., 2018).

2.3. Sample pre-treatment and microplastic extraction

2.3.1. Method overview

The pre-treatment and extraction processes were adapted from the method developed by Hurley et al. (2018a). Sludge and biosolids contain organic and inorganic material (such as clay particles), which need to be removed to allow for target analysis of microplastics presence (Hurley et al., 2018a). The methodology used for pre-treatment of the samples (also known as microplastics extraction) intends to separate microplastics from the organic and inorganic material presence in sewage sludge. The organic content was removed via an advanced oxidation process using Fenton's reagent (also known as a wet peroxide oxidation process). During this process ferrous sulphate ($FeSO_4{\cdot}7H_2O$) (Fe^{2+}) was used as a catalyst with the oxidising reagent hydrogen peroxide (H_2O_2). Fenton's reagent is effective at destroying organic material with validation testing by Hurley et al. (2018a) and Hurley et al. (2018b), no evidence of changes or degradation to polymers were found, with extraction efficiencies of 79–100% for spiked samples. The density separation method was used to separate the inorganic matter, not digested in the first stage, from the microplastics, by encouraging the flotation of microplastics in the supernatant and the settling of the inorganic material. The supernatant containing the suspended microplastics was then filtered.

The addition of ferrous sulphate to hydrogen peroxide can be highly reactive (Masura et al., 2015) and the reaction can produce heat and, in some cases, cause the sample to heat up rapidly. Due to the highly reactive nature of the samples, it was not necessary to heat them. It was also noted, that the nature of the wet peroxide reaction, and how vigorous it became, varied between samples. To avoid degradation or damage of synthetic polymers (especially during the oxidation process), the temperature was monitored using a glass thermometer and kept below 50 °C, this is recognised in a report by Koelmans et al. (2019) as the safest upper temperature limit to avoid effects on polymers (if the temperature was to become elevated above 50 °C, the sample containers were placed in trays containing ice).

2.3.2. Preparation of ferrous sulphate solution

0.05 M of Fe(II) sulphate solution was used for the digestion processes and was prepared by mixing 15 g of ferrous sulphate heptahydrate (FeSO4.7H2O) with 1 L RO water. To reduce the formation of iron deposits, 3 mL concentrate sulphuric acid (95%) was added (Masura et al., 2015). The solution was thoroughly mixed using a stir-plate.

2.3.3. Catalytic wet peroxide oxidation

The different sludge samples had varying amounts of water content. Processing these 'wet' samples would be complicated, especially during the digestion process because different volumes of reagent would be required for the varying samples (based on their organic content). To simplify this, sludge samples were dried prior to extraction to make the digestion process more straightforward by allowing consistent volumes of reagents to be used (Ziajahromi et al., 2021). The wet sludge samples were thoroughly mixed before a subsample of 5–15 g was placed in a petri dish and then placed in an oven (along with a blank filter paper to monitor aerial microplastic contamination) and dried at a temperature of 50 °C for about twenty-four hours or until a steady mass was reached. The dry weight of each sample was calculated.

Roughly 0.2 g of dried sample was transferred into a glass conical flask and 30 mL of 30% H_2O_2 was added to each sample. Following this 30 mL 0.05 M $FeSO_4$ solution was added to the conical flask and mixed gently. A metal/glass rod was used to break-up the dried sample if required. The reaction and temperature were monitored for an hour to ensure the temperature stayed below 50 °C and that the reaction did not become too vigorous (which would lead to a rapid increase in the temperature of sample). Once the reaction became less vigorous, the flasks were placed in an oven at 40 °C and left overnight for completion of the digestion process. Generally, all samples required a second digestion, therefore the whole digestion process was repeated the following day for all samples. During the digestion process all samples were covered with foil to avoid airborne contamination. Following this digestion process, not all the material was digested, and sediment was still visible at the base of the flask. The supernatant was vacuumed filtered through 1.2 μm Whatman GF/C filter papers.

2.3.4. Density separation

Following digestion of samples, 1.5 g cm^{-3} zinc chloride ($ZnCl_2$) was added to conical flasks and remaining sediment, and the solution was mixed before being left to settle overnight. The supernatant was vacuumed filtered onto a second 1.2 μm Whatman GF/C filter. Two filters were obtained for each sample for subsequent analysis (one from the filtration of supernatant after digestion and one from the filtration of supernatant following the density separation).

2.4. Microplastic identification

The identification and quantification process involves the visual sorting and physical analysis of all presumed microplastics. Each filter was analysed using an Olympus SZX16 dissection microscope in conjunction with a camera attached to the microscope and CellSens Standard computer software to determine the physical characteristics of the microplastics. For each sample a presumptive count was determined initially and microplastic number, microplastic type (particle or fibre), size (length of longest axis in μm) (Hurley et al., 2018a) and colour were recorded for every presumed microplastic observed. A subsample of recorded presumptive microplastics was 'picked' using very fine tweezers (Dumont #5) and put onto a new filter for chemical analysis via micro-Fourier Transform-Infrared Spectroscopy (μFT-IR), (Koelmans et al., 2019). For six out of seven locations a subsample of over 50% was chemically analysed (as many particles/fibres as possible were picked for chemical analysis), as is recommended by Koelmans et al. (2019) (for one location it was not possible to chemically analyse over 50% of presumptive microplastics because of loss during 'picking', instead 40% of the presumed microplastics were chemically analysed). For example, for the reception tank, an average count of 33 presumed microplastics was identified (down to 50 um), of that, 19 were subjected to chemical analysis, and 13 of these were confirmed as microplastics (68.8% of subsamples was confirmed) (Table 3). Kedzierski et al. (2019) used statistical approaches from survey studies to conduct a theoretical evaluation of the accuracy of subsampling as a function of the size of the population sampled. In this case, their samples contained potentially tens of thousands of particles. They found that accuracy did not increase in a linear fashion with subsample size, but reached a plateau when the subsample size reached the hundreds to thousands, In this case, a 3% subsample size was found to provide sufficient accuracy in determination for such large scale samples.

Following the physical analysis, the subsamples of presumptive microplastics were chemically analysed using a Perkin Elmer, Spotlight 200, μFT-IR microscope with electronic stage controller (www.perkinelmer.com) (manufactured in the UK). The Spectrum Software (with spectrum libraries for comparison) was used alongside the μFT-IR microscope and the set-up details of the microscope are as follows (Murphy et al., 2016).

- Wavelength range: 4000–600 cm^{-1}
- Wavelength resolution: 4 cm^{-1}
- Accumulation: 16 (scans)
- Microscope set to transmission (for use with diamond compression cell).
- Apertures: 100 × 100 μm

Presumed microplastics were transferred to a diamond compression cell which was placed on the μFT-IR microscope stage and scanned under transmission, the spectrums generated were compared to a library bank. Microplastic spectrums with a match to the library generated spectrum of 70% and over were accepted (Edo et al., 2020). The study '*limit of detection*', or smallest microplastic size specified, was 50 μm (Xu et al., 2020), therefore microplastics in this study are defined as between 5000 μm and 50 μm. Although it is possible for the μFT-IR to work down to ~10 μm, it was not feasible to test microplastics below this using the μFT-IR due to the difficulty in 'picking' smaller particles and transferring them to the compression cell for chemical analysis, therefore we cannot be sure we are getting quantitative data because of this protocol limitation. Obtaining a high-quality spectrum for particles lower than 50 μm, proved difficult as it was getting close to the μFT-IR's absolute resolution of 10 μm. The detection methods applied to sludge samples are not generally suitable for identifying microplastics below 50 μm (Koutnik et al., 2021) and therefore any particles below 50 μm were excluded from the analysis.

2.5. Contamination control and quality assurance

During the sampling process, the contamination of samples by external microplastics during collection where considered, and the risk of external contamination (from equipment/materials used, cloths and aerial deposition (GESAMP, 2019)) was minimised. To reduce the risk of contamination, glass and metal equipment was used were possible, and all sampling equipment was rinsed with Milli-Q water before use and covered with foil until needed.

2.5.1. Blank controls and procedural blanks

Blank filters were left exposed to the air to monitor airborne contamination in the different laboratory environments. Blank filters were left exposed in the oven during the drying process, in the fume hoods during the digestion and filtration processes, and in the laboratory during analysis. To monitor contamination of reagents, the reagents were also processed along with field samples.

To account for possible external contamination, procedural blanks were carried out using Milli-Q water instead of sample material. Two sampling containers (212 mL and 230 mL glass jars) were filled with Milli-Q water then taken out onto the field and back to the lab, the blank samples (212 mL and 230 mL Milli-Q water) were then processed in the lab along with the sludge samples. These procedural blanks monitor possible contamination from sample containers and from the extraction process (including the digestion and density separation steps). A third blank sample was filtered with $ZnCl_2$ only to monitor contamination incurred during the density separation process and from the $ZnCl_2$ solution.

2.5.2. Positive controls

The method validation process was performed on additional sludge samples with four different polymers, each sample was carried out in duplicate. The polymers and sizes used are as follows; polyvinyl chloride fluorescent particles between 50 μm and 150 μm, polyamide particles (dyed with Nile red) between 150 μm and 300 μm (sourced from www.Goodfellow.com), polyethylene terephthalate 381 μm glitter and polyvinyl chloride 1000 μm beads (sourced from www.theglittershop.co.uk). A total of fifty individual 1000 μm beads and fifty individual polyethylene terephthalate glitter particles were added to separate samples, 20 μL of 50 μm–150 μm polyvinyl chloride particles (containing an average of 49.4 particles) and 20 μL of 150 μm–300 μm polyamide particles (containing different dilutions of averaging 79 particles and 13.25 particles) were added to separate samples. A fluorescent microscope was used for the fluorescent polyvinyl chloride particles and polyamide particles dyed with Nile red. The glitter and beads were observed under standard light conditions. Background microplastics were disregarded.

2.6. Data synthesis

Two samples (duplicates) were collected for each of the seven locations. Firstly, the mean presumed microplastic load for the two samples was calculated. This is known as the presumptive count and was obtained during the initial visual sorting process. A subsample of the presumed microplastics was subjected to chemical analysis for microplastic confirmation and polymer identification, and of this, the percentage of microplastics confirmed as plastic was calculated. The percentage of sub-sample confirmed as plastic was factored into the results (Edo et al., 2020; Xu et al., 2020), this is known as the correction ratio and deemed the 'total confirmed microplastic count' in this study. The results for the presumed microplastic count and total confirmed microplastic count (correction ratio applied) were reported as 'mean ± standard error' (Table 3).

The correction ratio and percentage of microplastics were calculated as follows:

Correction Ratio

$$N = (a \times d)/100 \quad (1)$$

N = Total confirmed microplastic count
a = Average number of presumed microplastics
d = Percentage of microplastics in subsample confirmed as plastic by μFT-IR analysis

Percentage of Microplastics

$$d = (c/b) * 100 \quad (2)$$

d = Percentage of microplastics in subsample confirmed as plastic
b = Average number of presumed microplastics in subsample
c = Average number of presumed microplastics confirmed as plastic (confirmed count).[1]

All[2] graphs and descriptive statistics were carried out using GraphPad Prism version 9.2.0 (332) for Windows, (GraphPad Software, San Diego, California USA, www.graphpad.com). Due to the limited number of data points, statistics analytical tests were not performed.

3. Results and discussion

3.1. Blank control results

Only fibres were found on the blank samples, and when analysed under the μFT-IR, only two were confirmed as plastic (polyester and Nylon). Ten fibres were identified as cellulose.

The results from the blank controls indicated that external contamination from microplastics, either from the extraction process or from airborne contamination, was insignificant compared to the number of microplastics identified in the sludge samples. Due to the low contamination observed (and the high number of microplastics identified in real samples), it was decided not to adjust final calculations to include results from contamination analysis in line with other research studies (Mahon et al., 2017a; Mahon et al., 2017b; Chen et al., 2019; Corradini et al., 2019).

3.2. Positive control results

The polymers used for the positive controls, and recovery rates established, are presented in Table 2. The results from the positive controls

[1] Equation variables associate with data in Table 3.
[2] Equation variables associate with data in Table 3.

Table 2
The polymers and their sizes, used in the method validation process, and the average recovery rates for each polymer.

Polymer	Size μm	Average recovery rate %
PA particles	>150 and <300	102
PVC particles	>50 and <150	97
PET glitter	381	60
PVC beads	1000	74

were not factored into the final results but are an indication of the accuracy of the method employed.

The variation in recovery rates from these polymers may be due to several reasons. Firstly, the polyamide and polyvinyl chloride were ground down in the laboratory (with a 6875 Freezer/Mill® High-Capacity Cryogenic Grinder), and the sizes may vary beyond expected size range, additionally the particles may not be evenly spread in solution and each sample pipetted may vary slightly in the particle concentration (this may indicate why the recovery rate for polyamide particles was higher than expected). Although measures were taken to minimise loss of microplastics during the extraction process, there still may be a chance microplastics were lost when supernatant was filtered, this may explain the lower recover rates for polyethylene terephthalate glitter. The recovery rates reported in this study are in line with rates reported across literature, with rates reported for various polymers and sizes of 30%–44% (Van Echelpoel, 2014), 49%–78% (Corradini et al., 2019), 67%–98% (Li et al., 2018) and 61%–120% (Vollertsen and Hansen, 2017).

3.3. Microplastic quantification

A subsample of 39.5%–61.1% of presumptive microplastics were picked for chemical analysis via μFT-IR (it was not practically possible to pick 100% of presumed microplastics for chemical analysis). The percentage of the subsample confirmed as plastic, recorded in Table 3, varies from 41.2% to 81.6% with a mean percentage of 67.1% of the subsample of presumed microplastics confirmed as plastic during chemical analysis.

A total of 103 ± 8.63 microplastics were confirmed via the μFT-IR (the sum of the averaged confirmed count at each location) and after recalculation the total confirmed microplastic count was 186.8 ± 16.8 (the sum of the averaged total confirmed microplastic count).

The results for the number of presumptive microplastics counted, the number in the subsample subjected to chemical analysis, the number confirmed as plastic and the recalculated total confirmed microplastic count are recorded in Table 3, as well as MPs/g of dry weight and wet weight of each sample.

Across each location, the microplastic count per gram of dry weight of sludge ranged from 37.7 to 286.5 MPs/g (dw), samples collected from the centrifuge feed tank contained the highest number of microplastics with 286.5 MPs/g (dw). Prior to the centrifuge feed tank, the sludge was held in open lagoons for fifteen days. This could possibly allow aerial contamination of the sludge as past studies have shown atmospheric microplastic contamination with microplastic fallout values ranging from 136.5–512.0 microplastics/m^2/day across rural and urban locations (Klein and Fischer, 2019). The concentration of the sludge cake is much lower than the centrifuge feed tank at 97.2 MPs/g (dw), possibly indicating loss of microplastics during centrifugation of the sludge. The next highest number of microplastics were recorded in the digestate samples with 180.7 MPs/g (dw), this is three times higher than the concentration of microplastics from the thickened samples (50.2 MPs/g (dw), although these samples should be treated independently, being fifteen days older.

The limed cake contained the lowest number of microplastics with 37.7 MPs/g (dw). This is a lower number of microplastics compared to the sludge cake which had a microplastic concentration of 97.2 MPs/g (dw). The biosolids (limed cake and digested sludge cake) are applied to land in their 'wet' form, therefore knowing the microplastic concentration per wet weight is important for determining the potential microplastic load introduced to agricultural soils. The wet weight takes into account the total water content of each sample (before drying), and in this study microplastic loads for the wet weight of biosolids are similar with the sludge cake containing a slightly higher number of microplastics 17.9 MPs/g (ww) compared to the limed caked (14.8 MPs/g ww).

The microplastic load per gram of sludge (dry weight) in this study is inline with other findings across literature, presented in Section 1 (excluding the elevated results from Hansen and Vollertsen (2017) which are unusually high compared to results reported in other studies). However, microplastic concentrations varied widely between locations in this study (with the highest recorded microplastic load/g of sludge (dw) being 7.6× larger than the lowest concentration) and further investigation would be needed to determine if this variation is due to random variability. The number of microplastics in the biosolids (the treated sludge) ranging from 97.2 MPs/g dw for digested sludge cake and 37.7 MPs/g dw for lime cake, is higher than the number of microplastics found in biosolids from other studies (Carr et al., 2016; Mahon et al., 2017b; Murphy et al., 2016; Zubris and Richards, 2005) (Table 1). Mahon et al. (2017b) observed a lower microplastic concentration in sludge samples with an average of 3.9 MPs/g for anaerobically digested sludge and 12 MPs/g for lime stabilised sludge. However, the study by Mahon et al. (2017b) had a minimum detection limit of 250 μm, whereas the minimum limit of detection for this current study is 50 μm, allowing smaller microplastics to be identified. The digestate in this study had an average microplastic concentration of 180.7 MPs/g (dw) this is similar to findings by Lares et al. (2018) which found an average concentration of 170.9 MPs/g dw of digested sludge.

The highest microplastic concentration was found in samples taken from the digestate and centrifuge feed tank (180.7 and 286.5 MPs/g dw respectively). In the sludge treatment stream, the number of microplastics per gram dry weight of sludge increased after anaerobic digestion and after secondary digestion (open air lagoons) before decreasing following centrifugation. However, we do have to take caution when comparing the different

Table 3
The total confirmed microplastic count (which includes correction ratio), and microplastic load per dry weight and wet weight of sludge are displayed. The values for the average presumptive count, confirmed count and total confirmed count were obtained from ~0.2 g dried sludge, the results for 'MPs/g sludge' (dry weight and wet weight) were calculated using 'total confirmed count' (correction ratio) and exact weight of dried sludge sample used.

Location	Presumptive count[a]	Subsample count[b]	Confirmed count[c]	Percentage of subsample confirmed as plastic (%)[d]	Total confirmed MP count [N]	MPs/g sludge (dw)	MPs/g sludge (ww)
Reception tank	33.0 ± 16.0	19.0 ± 5.0	13.0 ± 4.0	68.8	22.6	107.5	2.4
Thickened	16.5 ± 7.5	10.0 ± 2.0	7.0 ± 1.0	70.0	11.6	50.2	2.4
Digestate	56.5 ± 8.5	33.5 ± 8.5	22.5 ± 6.5	67.2	37.9	180.7	3.8
Centrifuge feed tank	75.5 ± 0.5	38.0 ± 3.0	31.0 ± 1.0	81.6	61.6	286.5	4.6
Sludge cake	37.0 ± 7.0	20.5 ± 2.5	14.0 ± 3.0	68.3	25.3	97.2	17.9
Pre-limed	27.0 ± 8.0	16.5 ± 3.5	12.0 ± 2.0	72.7	19.1	74.7	21.2
Limed cake	21.5 ± 9.5	8.5 ± 1.5	3.5 ± 0.5	41.2	8.9	37.7	14.8
Total	267.0 ± 19.4	146.0 ± 10.3	103.0 ± 8.63		186.8 ± 16.8		

a = average number of presumed microplastics, b = average number of presumed microplastics in subsample, c = average number of presumed microplastics confirmed as plastic d = percentage of microplastics in subsamples confirmed as plastics by μFT-IR analysis. Total confirmed microplastic count is presented as dry weight (dw). MPs/g is presented as dry weight (dw) and wet weight (ww).

locations due to all samples being collected on one day. This does not consider the retention time of the sludge treatment processes (15 days for primary digestion in the anaerobic digester and another 15 days secondary digestion in the lagoons), therefore the samples may not correlate. The digestate, centrifuge feed tank and sludge cakes samples were much older (up to 30 days) than the samples collected from the reception tank, thickened, pre-limed and limed. There is no time-lag occurring in the lime treatment stream, sludge from the reception is treated on the same day, therefore samples are more comparable in terms of their age. The microplastic concentration decreased along the lime treatment stream from the reception tank, which had the highest count (107.5 MPs/g dw), to the limed biosolid (37.7 MPs/g dw). Mahon et al. (2017b) found a lower number of microplastics in anaerobically digested sludge compared to lime stabilised (this is in contrast to what was observed in this study), however Mahon et al. (2017b) collected samples from the final sludge product only and not before treatment, whereas samples were collected across the whole treatment stream in our study. In addition, the analytical methods between the studies differ, Mahon et al. (2017b) adopted a limit of detection of 250 μm, which is higher than the adopted limit of detection of 50 μm adopted in this study. Additionally, the extraction process was different with Mahon et al. (2017b) adopting a density separation process only. Further research is required and additional digestate samples collected will provide a clearer picture as to whether microplastic concentrations are indeed elevated.

Horton et al. (2021) quantified between 301 and 10,380 microplastics/g (dw) in anaerobically digested and lime stabilised sludge (Table 1). The mean concentration at each of the five sites ranged from 499.67 to 7,651.60 microplastics/g (Table 1). These results are higher in comparison to the results reported in this study, and generally higher than results reported across the literature (Table 1) despite adopting a limited microplastic size range of 25 μm–178 μm and with a focus on identifying nine polymers only. Our study relied on visual identification of microplastics before confirmation via chemical analysis, while Horton et al. (2021) adopted an automated analytical method without an initial visual identification step. In the study by Horton et al. (2021) all microplastics were identified and quantified within a selected filter area with an imaging μFTIR spectrometer (the software was restricted in its ability to identify fibre-like particles) (Ball et al., 2019; Horton et al., 2021). It has been suggested that visual sorting may be less accurate for smaller microplastics (below 100–500 μm), and may miss microplastics below 50 μm, due to a tendency for smaller particles to be overlooked. This could result in false negative results and an increased probability of misidentification (Chand et al., 2021; Xu et al., 2019). When chemical analysis is applied after visual sorting, the issue of false negatives remains, as microplastics not detected by visual sorting will remain uncharacterised, unless it is possible to measure each particle in a sample (Xu et al., 2019). Full FTIR analysis, or the adoption of an automated chemical analysis (either with Raman spectroscopy or FTIR) leads to a larger estimate of microplastic abundance, which is especially pronounced for smaller microplastics (below 50 μm), and the measurement of all particles through an automated methods address the issues of false negatives (Xu et al., 2019). This may explain the difference between the elevated results reported by Horton et al. (2021), and the lower values reported in this study (and across the literature). Automated imaging and chemical analysis methods may eliminate human bias and sensitivity limitations associated with visual sorting and chemical analysis of individual particles. But automated imaging methods also possess limitations, such as the prolonged time taken to analyse each filter, the inability to identify microplastic morphological characteristics, and in their lack of widespread availability. Automated sampling was not available for use in the current study; hence a visual sorting methodology was adopted prior to chemical analysis.

3.4. Microplastic type

The microplastics were recorded either as particle or fibre, and overall, more particles than fibres were reported. Examples of the different confirmed microplastic types are shown in Fig. 2. All microplastic particles confirmed via chemical analysis were secondary microplastics apart from one red bead (a primary microplastic confirmed as polymethyl acrylate). Of the total confirmed microplastic count, 42.5% of microplastics were fibre and 57.5% were particle. Fig. 3 presents the percentage of the two different microplastic types at each location, and at all locations apart from the reception tank, more particles were recorded over fibres. The limed cake had the highest ratio of particles followed by the pre-limed and sludge cake whereas the reception had a higher number of fibres to particles. The thickened, digestate and centrifuge feed tank samples had a similar ratio of particles to fibres, while the ratio was larger in the sludge, pre-limed and limed samples. The limed samples had the biggest ratio, 71.4% particles and 28.6% fibres. This is different to other studies (Lares et al., 2018; Mahon et al., 2017a; Mahon et al., 2017b) where fibres made up the majority of microplastics found in sludge samples. Lares et al. (2018) found synthetic fibres to be the most abundant microplastics type in sludge samples collected with an abundance of 94% in the activated sludge samples and >90% in the digested sludge. However, Liu et al. (2019) and Hurley et al. (2018a) found fibres did not make up the majority of the microplastic type. Hurley et al. (2018a) found beads to be the most common microplastic type. Only one microplastic bead was identified in this study, from the digested sludge samples, this may be due to the sampling procedure and use of 'grab samples'; the absence of microbeads in samples collected does not mean absence in all sewage sludge produced at the plant. However, in the UK, the government put a ban on the sale of products that contain microbeads on the 19th of June 2018 (this followed a ban on the manufacture in products containing microbeads on the 9th of January 2018) (Department for Environment, Food, and Rural Affairs, 2018). Fibres made up 28.9% of total microplastics observed by Hurley et al. (2018a) (compared to 43.5% in this study). Liu et al. (2019) found fragments to make up the largest proportion of microplastics identified in their sludge samples followed by fibres, again microbeads were also identified and made up 17.1% of microplastics type in their study. Magni et al. (2019) found the proportion of microplastics particles to fibres was similar wo evidence of changes or degradation to polymers with 59.5 microplastic particles/g dw and 53.3 microplastic fibres/g dw.

The high number of synthetic fibres present in wastewater (and hence sludge samples) is not a new revelation (Lares et al., 2018; Magnusson and Norén, 2014; Magni et al., 2019) with research finding 496,030 fibres are potentially lost in a 6 kg wash (Napper and Thompson, 2016). It is clear the microplastic type varies between studies and the proportion of the different types of microplastic identified varies. This could be down to several factors such as the variability of wastewater and hence sludge, or it could be down to the sampling and analytical techniques and reporting style employed by the researchers. Of all the particles found in this study, only one particle was identified and confirmed as a primary plastic and confirmed by chemical analysis, this was found in the digestate samples. A red bead (50.76 μm) confirmed as polymethyl acrylate. All other plastic particles appeared to be secondary and had experienced weathering; this indicates the particles found were fragments from larger plastic items, or from paints or resins.

3.5. Microplastic size

The sizes of all microplastics were recorded and grouped into the following size classifications: ≥1000 μm, ≥500 μm and <1000 μm, ≥100 μm and <500 μm, <100 μm. The majority of the microplastics confirmed at most of the locations (except the limed and thickened samples) were between 100 μm and 500 μm with ranges of 33.3% to 46.7%, Fig. 4. However, the majority of microplastics on the limed samples where <100 μm (the minimum size limit is 50 μm). At all locations, most microplastics were <500 μm with values ranging from 57.1% to 82.1%.

The size range varied across the treatment streams with the end products (biosolids) containing the lowest average size range; the digested sludge cake and limed cake contained the lowest average microplastics size of 246.8 and 212.5 μm respectively. The average size of microplastics in the limed cake is smaller than those in the pre-limed, which had an

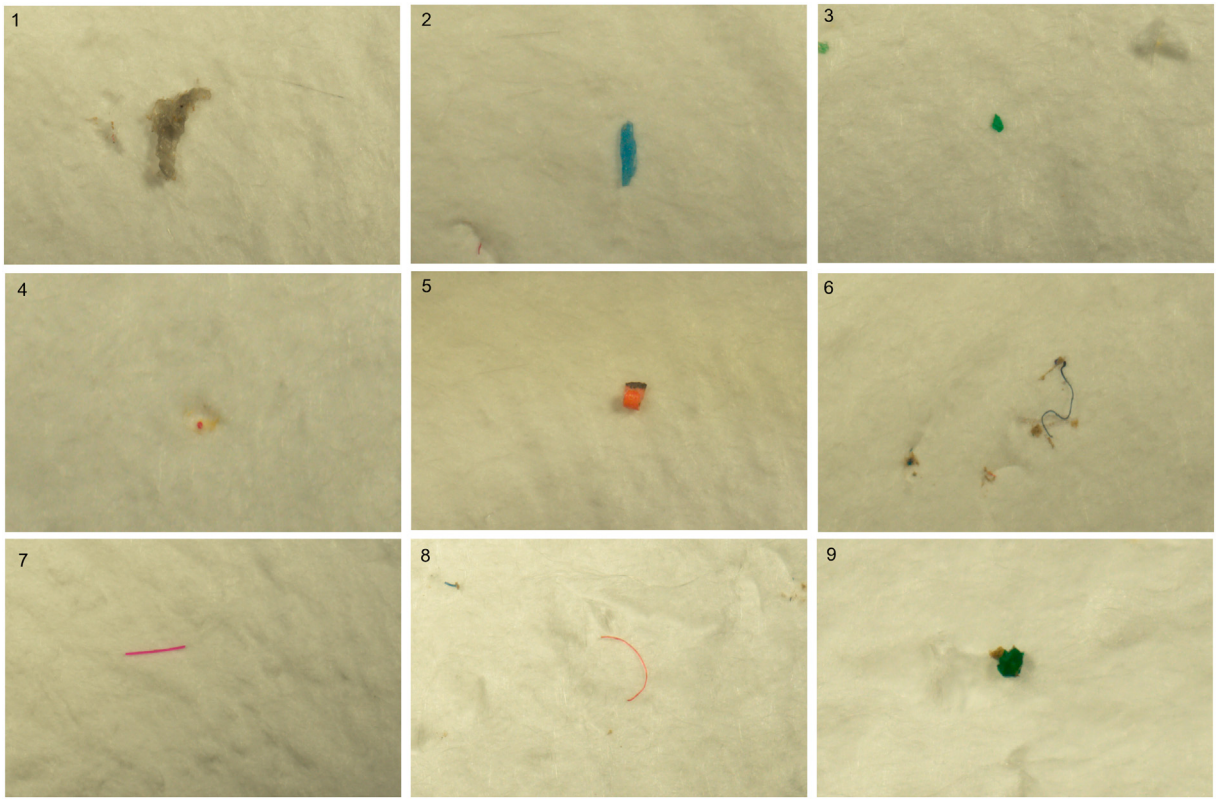

Fig. 2. Confirmed microplastics found in sludge samples; 1. Grey particle (1230.20 μm) confirmed as polyethylene (pre-limed). 2. Blue particle (609.68 μm) confirmed as polypropylene (pre-limed). 3. Green particle (227.92 μm) confirmed as acrylonitrile styrene (digestate). 4. Red bead (50.76 μm) confirmed as polymethyl acrylate (digestate). 5. Red particle flake (316.72 μm) confirmed as alkyd aliphatic (CFT). 6. Blue fibre (1237.37 μm) confirmed as polyester (CFT). 7. Red fibre (1072.86 μm) confirmed as Nylon (pre-limed). 8. Red fibre (943.05 μm) confirmed as polyester (digested sludge cake). 9. Green particle confirmed as polyvinyl acetate (CFT).

average size of 693.9 μm, which supports the theory that the elevated pH and temperature within the process produces smaller microplastics (Mahon et al., 2017a).

At every location most microplastics were <500 μm, indicating the importance of quantification microplastics of a lower size range. The percentage of microplastics within the size range 50–250 μm (lower than the minimum detectable limit of other studies (Mahon et al., 2017b)) varied form 42.6%–78.6% of the total number of microplastics identified at each location. This would result in many microplastics missed if the limit of detection in this study was 250 μm instead of 50 μm, and this may be one reason why a higher microplastic concentration was identified in this study compared to others (Mahon et al., 2017a; Mahon et al., 2017b) where microplastic load may be underestimated because smaller microplastics were not accounted for. However, a microplastic size limit of 50 μm adopted in this study may still provide an underestimated total microplastic count. Horton et al. (2021) observed that the vast majority of the microplastics identified in sludge samples were in the lower particle size range, between 25 μm to 100 μm, and it was observed that smaller particles appeared to be present

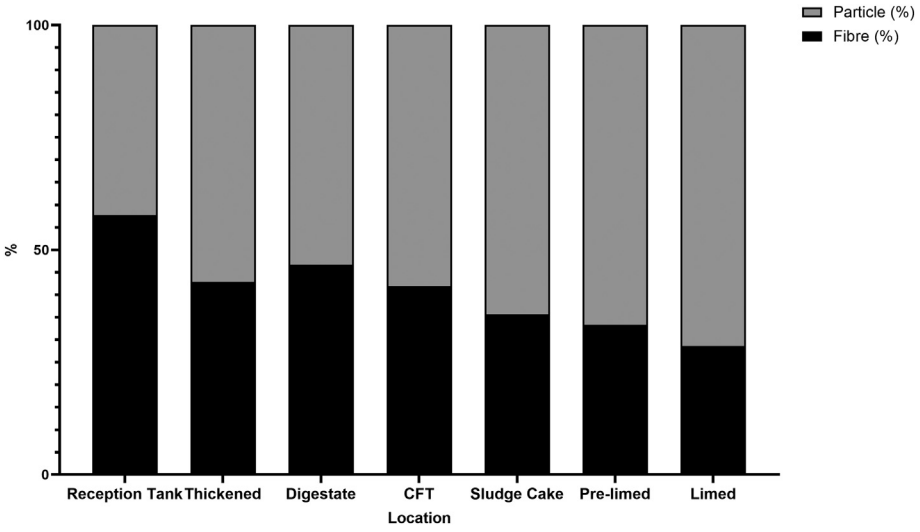

Fig. 3. Percentage of microplastic type (fibre/particle) at each location.

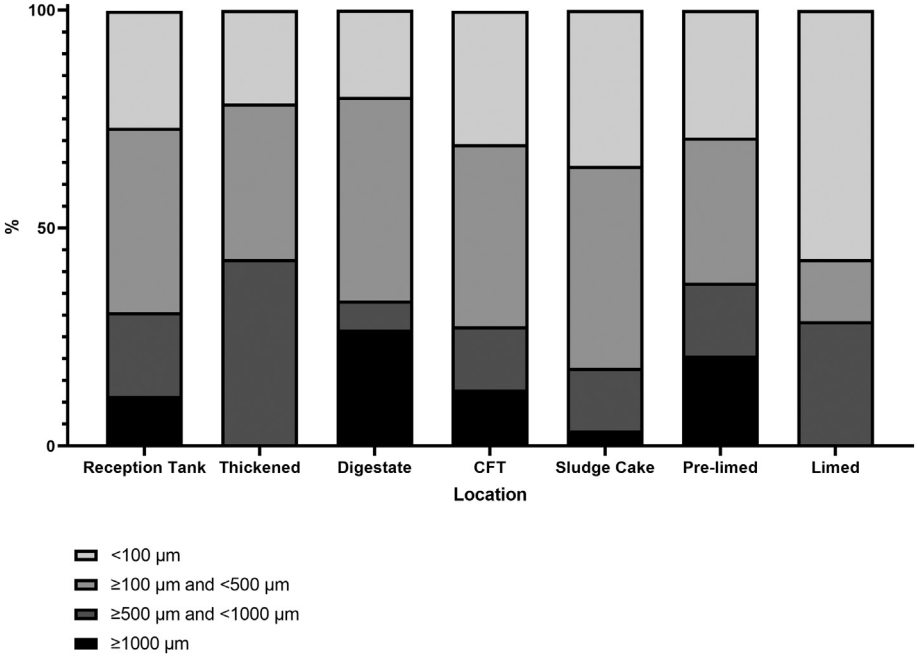

Fig. 4. Size distribution of microplastics (%) at the different locations along the sludge treatment stream.

in greater number (Horton et al., 2021). Adopting a limit of 50 μm may mean smaller particles (which may be present in greater number) will be missed.

Both the sludge and limed cake had a high proportion of particles to fibres, and the lowest average microplastics size, possibly indicating a break-up of particles during sludge treatment (although both locations had the lowest concentration of microplastics per dry weight). The majority of microplastics, 57.1%, were <100 μm in the limed samples (compared to all other samples where the majority of microplastics were between ≥100 μm and <500 μm or ≥500 μm and <1000 for the thickened sludge). The smaller microplastic particles identified in the lime stabilised sludge in this study is similar to what Mahon et al. (2017b) observed where it was suggested elevated pH and mechanical mixing lead to elevated number of smaller particles.

Fibres tend to have a longer length than particles and were on average 5 times larger than particle sizes. At every location in this study the average fibre size is larger than the average particle size (the longest side). Overall fibres had an average length of 969.9 μm compared the overall average particle size of 170.6 μm. Other studies found smaller microplastics were more likely to be transferred to sludge (Liu et al., 2019) (only sludge samples were collected in this study therefore wastewater samples are not available for comparison). Liu et al. (2019) found the mean microplastic size in the sludge samples of 203.8 μm, this is smaller than the average size in this study (464.3 μm), but they also found larger microplastics were dominated by fibres and smaller ones by particles (Liu et al., 2019). Lares et al. (2018) found that 64% of all microplastics (in wastewater and sludge samples) were less than 1000 μm, where our study found 86% of all microplastics identified were below 1000 μm.

3.6. Microplastic colour

Eleven different colours were recorded for all microplastics confirmed, with green, blue and red the most common colours identified accounting for the top three highest percentages at most locations. The top three colours in the thickened sample were green, blue and black, and in the limed samples only green, red and grey were confirmed. It is likely that there was a biasness towards microplastics of brighter colours and white or clear microplastics were not easily identified due to the nature of the filters. This could have resulted in clear and white particles and fibres being potentially underestimated. It was difficult to distinguish white and clear fibres from the cellulose matted on the filters. The undigested material present on the filters may have acted as an inhibitor and have hindered observation of microplastics of a less bright colour and smaller microplastics. Of all confirmed micropalstics, green, blue, and red respectively accounted for 33.5%, 31.5% and 23.0% of all colours identified. Clear and white microplastics only accounted for 2.5% and 0.5% of the total microplastic count respectively. It becomes more apparent that clear and white microplastics are underrepresented in this study when comparing to results from literature, where transparent/clear microplastics account for between 38.3% and 41% (Hurley et al., 2018a; Liu et al., 2019). It has been suggested this is linked to the high number if microbeads identified which are more likely to be clear with 65.8%–93% of microbeads recorded as transparent (Lares et al., 2018). These results could imply that the lack of clear microplastics may be due to the lack of microbeads (now banned in the UK), however it could also be said that the lack of microbeads identified could be due to the biasness towards brightly coloured microplastics and clear and white microplastics being missed.

3.7. Polymer type

An average of 66.7% of the subsamples were confirmed as plastic during chemical analysis (with the values ranging from 41.2% to 70.6% of the subsamples were confirmed as plastic). This is in-line with other research where 39% to 100% (Blair et al., 2019; Li et al., 2018) of presumptive microplastics were confirmed as plastic after chemical analysis.

Twenty-one different polymers/polymer groups were identified (Fig. 5) during the chemical analysis process. The most abundant polymer identified was polyester, accounting for 39.8% of all confirmed microplastics, followed by polyvinyl acetate (13.6%) and polyethylene (13.1%). Together these three polymers accounted for 66.5% of all polymers identified, while the remaining thirteen accounted for only 33.5%. Polyester and polyethylene are present at all locations while polyvinyl acetate is found at all locations apart from the limed sludge. At all locations, apart from the limed cake, polyester was the single most abundant polymer ranging from 28.6% to 53.8% of confirmed microplastics. Polyacrylate was the most abundant polymer in the limed cake, accounting for 42% of polymers found at this location (although the total number of polymers identified was low with a mean of 3.5 ± 0.5). In the digestate samples, acrylonitrile butadiene styrene (ABS) was the third most abundant polymer with

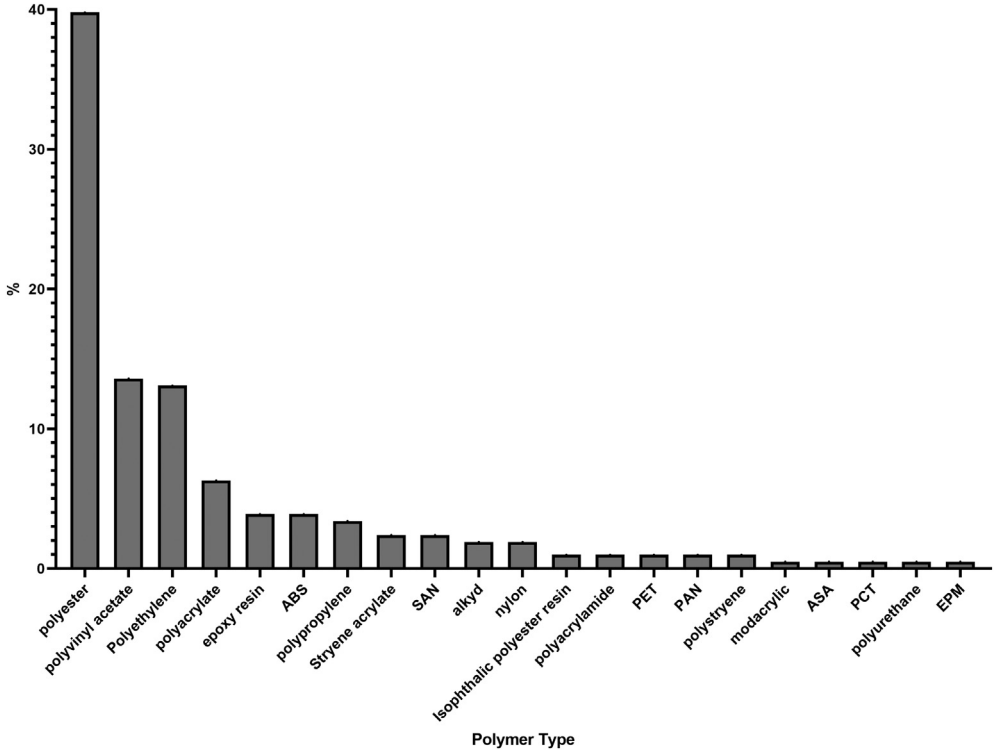

Fig. 5. Polymer type as a percentage of total confirmed microplastics. ABS = acrylonitrile butadiene styrene, SAN = styrene-acrylonitrile resin, PAN = polyacrylonitrile, ASA = acrylonitrile styrene acrylate, PCT = polycyclohexylenedimethylene terephthalate, EPM = ethylene propylene rubber.

polyacrylate (after polyester (48.6%) and polyethylene (13.5%)) accounting for 8.9% of total polymers found at this location. ABS was also present in the centrifuge feed tank (4.8%) and sludge samples (3.6%). Interestingly, no polyvinyl chloride was recorded at any of the locations despite being widely reported in aquatic and marine samples. The absence of polyvinyl chloride in the samples might not necessarily indicate absence of polymer, just that it was detected in samples collected. Polyvinyl chloride has been reported as the most commonly found polymer in sludge (Wei et al., 2019).

The majority of confirmed synthetic fibres (which make up 42.7% of the total microplastics) were polyester which was the single most abundant polymer identified. Chemical analysis can give an indication of possible sources (although exact sources cannot be easily proven). It is not surprising to see the presence of polymers with possible sources linked to automotive industry, (such as ABS and propylene etc.), considering the WwTW in this study is supplied by a combined sewerage system as stormwater can wash the pollutants from the catchment surface to the sewerage system (including pollution from vehicles on road surfaces) (Butler and Davis, 2011). Similar polymers have been recorded in influent samples, including copolymers acrylonitrile butadiene and ethylene propylene, as well as polyester and polyethylene being the most abundant polymer identified (Magni et al., 2019). In this present study, Polyester and polyethylene are the topmost abundant polymers with a total of 41 and 13 microplastics identified respectively. Polyolefins (polypropylenes and polyethylene) are the leading polymers in plastic demand in Europe, with polyethylene (LD-PE and HD-PE) being the leading polymer type (PlasticsEurope, 2018). Polyethylene and polypropylene are polymers used in packaging industry which represent the largest end users in Europe, accounting for 39.9% of the total European plastic demand (PlasticsEurope, 2018). The third most abundant polymer found in this study is polyvinyl acetate (12.4%) this is used as an adhesive, coatings or in co-polymers (Ravve, 2012).

3.8. Biosolids as a pathway for microplastic presence in the terrestrial environment

Both anaerobically digested sludge cake and limed cake are end products in this current study and all the sludge produced at the WwTWs is recycled to agricultural land as a fertiliser and soil conditioner. At the WwTWs in this study, 900 tonnes of anaerobically sludge cake and 690 tonnes of limed cake are produced per month. Based on the results from this study, 1.61×10^{10} and 1.02×10^{10} of microplastics end up in the biosolids (ww) every month from one WwTWs in England from anaerobically digested sludge cake and limed cake respectively. The microplastics then enter the terrestrial environment when the biosolids are recycled to agricultural land. There is a strong case for further exploration of the potential implications and long-term prevalence of microplastics in agricultural soils subject to biosolid applications. For a representative example, the microplastic load reported in this study is equivalent to 20,579 bank cards or 11.4 footballs ending up in the combined biosolids every month. To obtain these figures, the length for each microplastic was measured during physical analysis, from this, the average length of particles and fibres was calculated for each location. From these figures the over-all average length for particle (170.6 μm) and fibre (969.9 μm) were calculated. Textile fibres generally have a width of 10–30 μm (Lusher et al., 2020), hence the smallest width of 10 μm was taken as an average fibre width for this study. All fibres were assumed to be cylindrical, and particles were assumed to have an equal width to length. The average thickness of the particles was deemed to be 60% of the shortest axis (Vollertsen and Hansen, 2017) therefore an estimated particle thickness of 102.3 μm was calculated. From these estimated average dimensions, a volume for fibres (7.62×10^{-5} mm^3) and particles (2.98×10^{-3} mm^3) was calculated and from this, the equivalent number of bank cards and footballs were calculated based on the ISO/IEC 7810:2003 dimensions for a standard bank card, and the standard diameter for a size 5 football according to FIFA.

3.9. Limitations of study

Due to the complex matrix of sludge, not all material (especially cellulose) was digested during the extraction process. This resulted in the presence of undigested material and sediment on the filters following digestion and density separation, potentially hindering the observation of microplastics especially smaller plastic particles which may be obscured or covered by this

material. The nature of the filters (presence of white cellulose material and sediment on filters) hindered the observations of white or clear microplastics potentially producing an underestimated count. The significant variability between the sludge samples (Karapanagioti and Kalavrouziotis, 2019) resulted in the samples behaving differently during the digestion process (despite samples being dried) and the resulting filters looking very different. All samples were collected in one day, this does not consider the retention time of the sludge treatment processes (fifteen days for primary digestion in the anaerobic digester and fifteen days secondary digestion in the lagoons). Therefore, the samples may not be comparable and because of the difference in ages between some samples we cannot produce mass flow data across the whole treatment stream, the data for each sample should be considered individually, and as a preliminary indication for microplastic load at each location rather than evidence of spatial changes across the treatment streams. Samples were taken only once and provide a snapshot of microplastic concentration at the time and place during which the samples were taken. Data from previous research (Table 1), demonstrates how microplastic load varies greatly over time and location. Results for microplastic analysis are always retrospective and cannot be used to determine present or real-time microplastic loads in wastewater treatment works of the environment. Any variation or differences in results between locations may also be down to random variability; only a small amount (roughly 0.2 g dw) of samples were analysed and extrapolation techniques were employed to apply microplastic load to larger volumes, therefore samples may not be always representative of larger volumes.

4. Conclusion

In this study we quantified and characterised microplastics after different treatment processes along two treatment streams at one WwTWs. All samples were collected on the same day to provide a snapshot in time of the levels of contamination in the sludge treatment stream, to give an indication of the potential microplastic load leaving the treatment works in biosolids and to provide an indication on where the focus of future research is required.

There were microplastics present in all sludge samples from across the sludge treatment stream, with elevated microplastic loads in samples after anaerobic digestion and secondary digestion. Further research is required to determine how this relationship varies with time and whether it is repeated with the collection of successive samples. Data from this study shows that anaerobically digested sludge cake (17.9 MPs/g ww) has a slightly higher concentration of microplastics compared to the limed cake (14.8 MPs/g ww). The presence of microplastics in the biosolids may have implications on future use of the biosolids as a source of fertiliser for agricultural land. From this treatment works, 16.1 billion and 10.2 billion microplastics are present in anaerobically digested and lime stabilised sludge respectively, every month, and can subsequently enter the terrestrial environment when the biosolids are used as a fertiliser on agricultural land. This is equivalent to 20,579 bank cards potentially ending up in the combined biosolids every month. Further detailed research is required to quantify the extent of microplastic load introduced through the recycling of biosolids to land in comparison to other sources of microplastic pollution. There are currently no regulations or guidance concerning the presence of microplastics in sludge and biosolids and microplastic concentration in WwTWs are not monitored. The presence of microplastics in biosolids could have implications for the biosolids market and may put at risk a valuable source of nutrients for the agriculture sector. For a full understanding of the risks to human and environmental health posed by microplastics in sludge and biosolids, further scientific research and evidenced based data is required.

The work carried out in this study was a preliminary investigation and paves the way for further research into the presence of microplastics in the whole sludge treatment stream. It will be of great interest to see whether differences observed between microplastic count between digested biosolids compared to limed sludge are confirmed with future research, and whether the elevated number of microplastics observed after secondary digestion is replicated across other sites. Such studies may help to illuminate the variables which may contribute towards differences in microplastic count.

CRediT authorship contribution statement

Daisy Harley- Nyang: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization, Project administration. **Fayyaz Memon:** Writing - Review & Editing, Supervision. **Nina Jones:** Writing - Review & Editing. **Tamara Galloway:** Writing - Review & Editing, Supervision.

Declaration of competing interest

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