


Determination of large microplastics: wet-sieving of dewatered digested sludge, co-substrates, and compost

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ABSTRACT

Dewatered digested sludge and compost may act as a conduit for microplastics (<5 mm) in terrestrial and subsequently aquatic systems. However, standardized methods for microplastics analyses are lacking. Thus, the aim is to demonstrate the applicability of wet-sieving as a way to quantify large microplastic particles (MPP, 1–5 mm) in dewatered digested sludge and compost. Additionally, we investigated the organic fraction of municipal solid waste, expired drinks and slaughterhouse waste used as co-substrate for anaerobic digestion at wastewater treatment plants (WWTP). Therefore, we collected samples from six WWTP and two biogas plants. These were then wet-sieved and potential MPP analysed via attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). In dewatered digested sludge the amount of microplastics ranged from 0 to 326 MPP/kg TS (total solids) while compost contained 39–102 MPP/kg TS. Our results show that with 0–36 MPP/kg TS co-substrates are not necessarily a source of microplastics in WWTP. Furthermore, we found film to be the most abundant shape in the biogas plant samples, whereas, in WWTP samples film, fragments and fibers were detected the most. ATR-FTIR revealed that polyvinyl chloride, polyester, polypropylene, and polyethylene were the most abundant materials found across all samples.

Key words | ATR-FTIR, compost, co-substrates, dewatered digested sludge, microplastics, wet-sieving

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HIGHLIGHTS

- Standardized methods for MP analysis are still lacking.
- Applicability of wet-sieving as method of detection for MP of 1–5 mm is demonstrated.
- It is proposed that the number of MPP should always be given in reference to the total solids of the respective sample.
- More research is needed to evaluate the effect of co-substrates on the number of MPP in WWTPs.

INTRODUCTION

Plastic products make our everyday lives easier and one has a hard time imagining a world without plastics. The mass production and common use of plastics only occurred after World War II. While initially the production of plastics was 2 million tons per year in the 1950s, it increased to an annual production of 380 million tons in 2015 (Geyer

et al. 2017; PlasticsEurope 2018). In recent years, so called microplastics (MP), particles of <5 mm, became of increasing concern. Microplastic particles (MPP) can be classified as primary and secondary MP. Primary MP are directly produced this size, while secondary MP are formed through disintegration of larger plastics (Arthur *et al.* 2009). The most common polymers are polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC), followed by polyethylene terephthalate (PET), polyurethane (PUR) and polystyrene (PS) (Geyer *et al.* 2017).

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Among the many sources of microplastics in aquatic and terrestrial systems are wastewater treatment plants (WWTP) with effluent and dewatered digested sludge (henceforth referred to as sludge) as well as compost derived from the organic fraction of municipal waste (OFMW) (Talvitie *et al.* 2015; Weithmann *et al.* 2018; Crossman *et al.* 2020). The number of MPP entering the WWTP depends on the size of the facilities, industrial activity, and urbanization in the area (Raju *et al.* 2020).

Microplastics may be added to detergents, cleaning agents or cosmetic products (e.g. peelings). Through their everyday use, MP are introduced into wastewater. Furthermore, washing synthetic clothing emits MP fibers that end up in wastewater. Another source may be the improper disposal of hygienic products (e.g. cotton-swab). Besides these contaminations from household items, MP may also be introduced into wastewater through road runoff or through industrial wastewaters (Carr *et al.* 2016; Lambert & Wagner 2018; Talvitie 2018). The amount of MPP discharged can be linked to the design and operation of WWTP (Magnusson *et al.* 2016). Many studies report a similar rate of retention of MPP, which is >90% in WWTP (Murphy *et al.* 2016; Wick *et al.* 2020b). These retained MP end up in sludge.

An additional source of entry may be via co-substrates in WWTP practicing co-digestion. Co-digestion is a way for WWTP to increase their biogas production. There are different kinds of co-substrates like residues from dairy production, slaughterhouse waste, expired food and drinks, or OFMW (Rauch-Williams *et al.* 2018; Hubert *et al.* 2019a). The latter is made of kitchen and food waste (70%), yard waste (10%), food that is still inside the packaging (18%) and other organics (2%) (Dornbusch *et al.* 2020). To enter anaerobic digestion at WWTP, the OFMW is pretreated to remove packaging and other physical contaminants like metals or glass. Subsequently, it is homogenized and added to a digester.

Similarly, OFMW is also used as substrate in biogas plants. There, it is either used directly for dry fermentation or pretreated in the way stated previously for wet digestion. The digestate can subsequently be used as substrate for composting (Weithmann *et al.* 2018).

While compost is land applied, sludge offers multiple ways of disposal. In 2018, three-quarters of the produced German sludge was incinerated, 16% was used as fertilizer and the rest was composted or disposed of otherwise (DESTATIS 2020). The Netherlands and Japan also incinerate the majority of the sludge produced. However, in the US, Bulgaria, and Spain most of the sludge is land applied (Nizzetto *et al.* 2016; Hubert *et al.* 2019b; Eurostat 2020). Depending on the country, sludge needs to fulfill certain

requirements for land application regarding (among others) nutrient and heavy metal content as well as physical contaminants. In Germany and Austria, the Fertilizer Ordinance (DMVO 2004; DüMV 2019) states that physical contaminants including hard plastics >1 mm in sludge and compost (among other fertilizers) must not exceed 0.4% on a dry weight basis. For deformable plastics >1 mm the limit is <0.1%. In contrast, France's regulation only considers physical contaminants >5 mm (Watteau *et al.* 2018).

One of the major research challenges is the sampling, isolation and quantification of MP in environmental samples (Sun *et al.* 2019; Wick *et al.* 2020a). Currently, there are no standardized methods or uniform reporting of the results, which makes the comparison of studies difficult. Given the restrictions and regulations in Germany, our aim with this study is to document the applicability of wet-sieving as a method to isolate large microplastics in sludge, co-substrates and compost samples. Furthermore, we want to give more insight into MPP in sludge compared to compost.

METHODS

Site and sample description

Figure 1 shows the steps taken during this research project. The first step was to take co-substrates (food waste) and dewatered sludge from six different WWTP (A–F) as well as anaerobically digested OFMW (digestate) and composted digestate (compost) from two biogas plants (G–H) in Bavaria and Hesse, Germany (Table 1).

The size of the WWTP ranged from 50,000 to 2,000,000 population equivalent. All of the sampled WWTP operated their digesters at mesophilic conditions at the time of sampling. The most commonly used device for dewatering sludge was a centrifuge. Subsequently, the sludge is incinerated. The selected plants all treat municipal wastewater. However, the influent of plant B is additionally heavily influenced by dairy production wastewaters. Plant D, E, and F practice co-digestion. The plants accept OFMW and residual products from food production, fat, oil and grease, spoiled milk, slaughterhouse waste, and expired drinks. The ratio of co-substrate to sludge in the digester feedstock ranges from 1:3 to 1:1.5. From all WWTP, sludge was sampled. Homogenized food waste was sampled as co-substrate from plant D, while from plant E and F slaughterhouse waste and expired drinks were sampled, respectively.

Both biogas plants digest OFMW. For plant G, this is 30,500 Mg/a and for plant H 22,500 Mg/a OFMW per year. The difference between the plants is that plant G

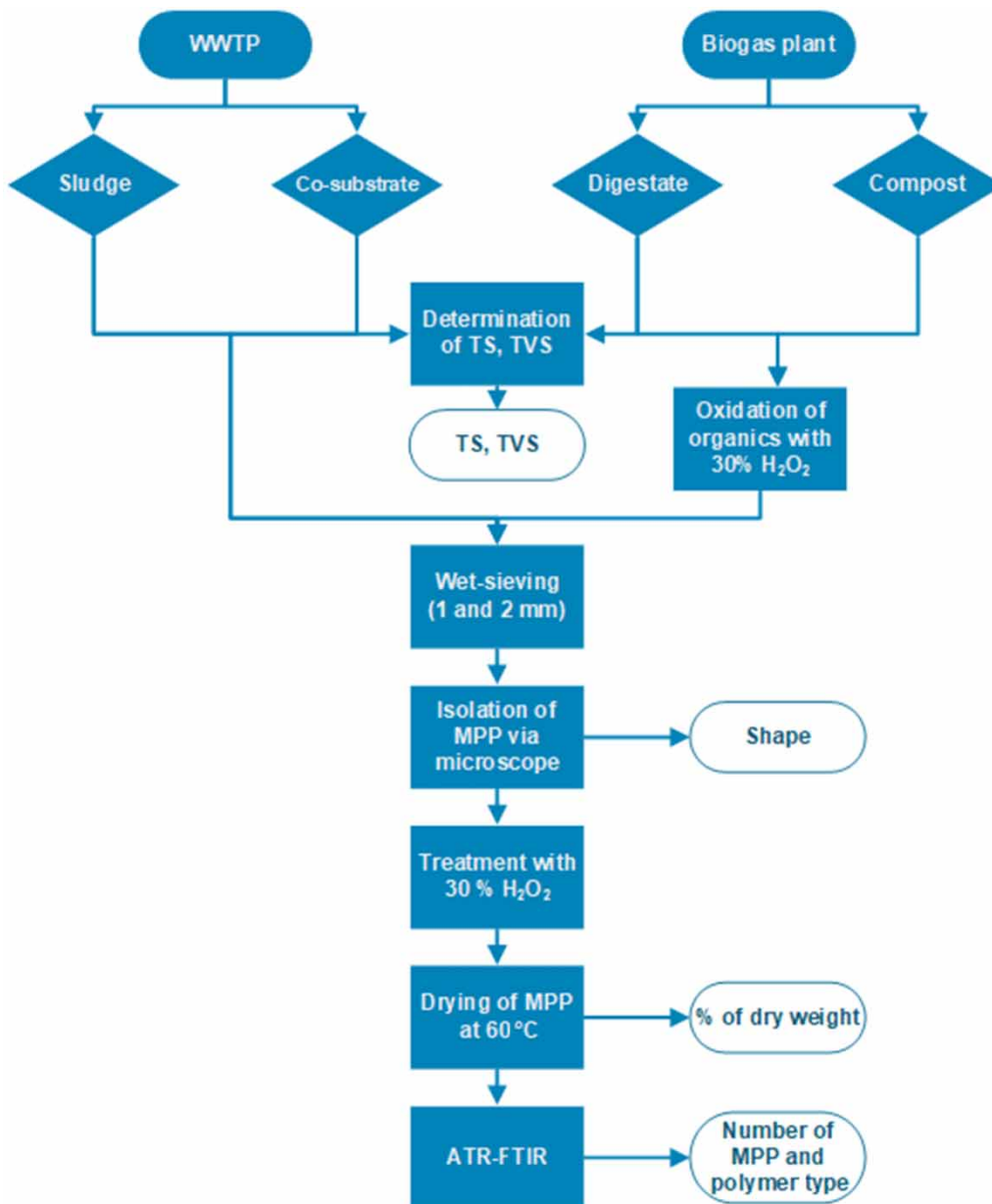


Figure 1 | Flow scheme of the steps taken from sampling to analysis of microplastic particles.

operates a wet digestion (total solids (TS) < 15%) and plant H a dry fermentation (TS > 15%). After the fermentation process, the digestates are composted. In a final step, the plants sieve the compost with a 15 mm star screen. Subsequently, the compost is marketed/used as fertilizer.

Sampling

Sampling occurred between February and August 2020. Grab samples of sludge from WWTP and dewatered digestate from

biogas plants were taken directly after the dewatering device, while co-substrates were sampled directly after the on-site homogenization step or from the storage container. Compost samples were taken from various points of windrows with a soil sampler. During transportation the samples were kept cool and in the dark. In the laboratory, the samples were stored at 4 °C until further processing.

The initial sample volume was approximately 7–10 kg. The volume was reduced in the laboratory by, firstly, making a pile of the sample in one spot. This pile was

Table 1 | Summarized information of the sampled plants (sludge = dewatered digested sludge, digestate = digested OFMW; PE, population equivalent)

Type	Identifier	Size	Dewatering device	Sample(s)
WWTP	A	50,000 PE	Centrifuge	Sludge
	B	400,000 PE	Centrifuge	Sludge
	C	2,000,000 PE	Centrifuge	Sludge
	D	50,000 PE	Centrifuge	Sludge, homogenized OFMW
	E	200,000 PE	Centrifuge	Sludge, slaughterhouse waste
	F	325,000 PE	Chamber press	Sludge, expired drinks
Biogas plant	G	30,500 Mg/a	Centrifuge	Digestate, compost
	H	22,500 Mg/a	–	Digestate, compost

transferred to another spot by trickling the sample from a small shovel. This process was repeated a total of four times to assure proper mixing. After the last round of transferring, the pile was divided in the middle and a new pile was formed with half of the sample, discarding the rest. This step was repeated until the desired volume of 500 g was reached. Physicochemical analysis followed within a day of sampling, whereas wet-sieving occurred within a week.

Determination of dry weight and organic content

TS and total volatile solids (TVS) of all samples were determined according to DIN EN 12880 (DIN 2001) and DIN EN 15935 (DIN 2012).

Isolation and characterization of microplastic particles

Isolation of MPP was done according to Weithmann *et al.* (2018). For wet-sieving, 500 g and 1 kg were taken from the sampled sludge and co-substrates, respectively. In order to validate if 500 g of sample was a sufficient amount of volume, two samples were analyzed in duplicate.

Initially, all samples were treated with an oxidative agent to remove excess organic matter for easier sieving. However, the treatment was only continued with digestate and compost samples. In order to keep foam and heat development to a minimum, 300 mL of 30% hydrogen peroxide (H₂O₂) was slowly added under constant stirring. After the foaming stopped, the samples were left overnight at room temperature in the dark.

Wet sieving was done with stacked sieves of 1 and 2 mm mesh size with an area of approximately 452 cm². The sieves were left to air dry. Afterwards, potential MPP were identified following the guidelines proposed by Norén (2007) under a Leica M165 FC stereomicroscope and photographed with the attached camera (Leica DMC 4500). The particles that did not show organic structures on the surface, fibers that were equally thick and not entirely straight, and particles that were homogeneously colored were counted and selected for further analysis to prove the particles are plastics. Furthermore, the shape was determined. MPPs were divided into four categories: fragments (similar length and width), film (very thin), spheres (round particles), and fibers (longer than wide) (Sun *et al.* 2019).

In order to determine the dry weight of the previously identified MPP, the particles were treated with 30% H₂O₂ for 24 h to remove any excessive organics. Subsequently, the MPP were dried to constant weight at 60 °C to prevent breakdown of some plastics.

All MPP were then analyzed with attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The spectrometer used is the Nicolet iS10 (Thermo Fischer Scientific) with the SMART iTX element. Diamond was used as material for the ATR-FTIR reflection element. Spectra were analyzed in transmission mode with 16 scans per sample with wavenumbers in the range 4,000–400 cm⁻¹ and a resolution of 4 cm⁻¹. The software to obtain the spectra as well as the library search to identify the spectra was OMNIC 9 and OMNIC Spectra 2.0 (Thermo Scientific), respectively.

RESULTS AND DISCUSSION

Sample preparation and characterization

The TS ranged from 19.9 to 36.2% while the TVS was between 45.9 and 67.3% for sludge (Table 2). The co-substrates had a TS from 0.9 to 13.7% and TVS from 80.0 to 96.4% while the digestate and compost samples had 23.3–61.6% TS and 35.8–68.7% TVS. These values show a variability among the samples, which derives from different inputs as well as process differences.

The analyzed duplicate samples showed that 500 g of sample volume was sufficient. In each of the duplicates the same number of MPPs was found. Additionally, a larger sample would have clogged the sieves with plant residues and grit. Compost and digestate samples were treated with H₂O₂ to reduce some of the organics. This step allowed

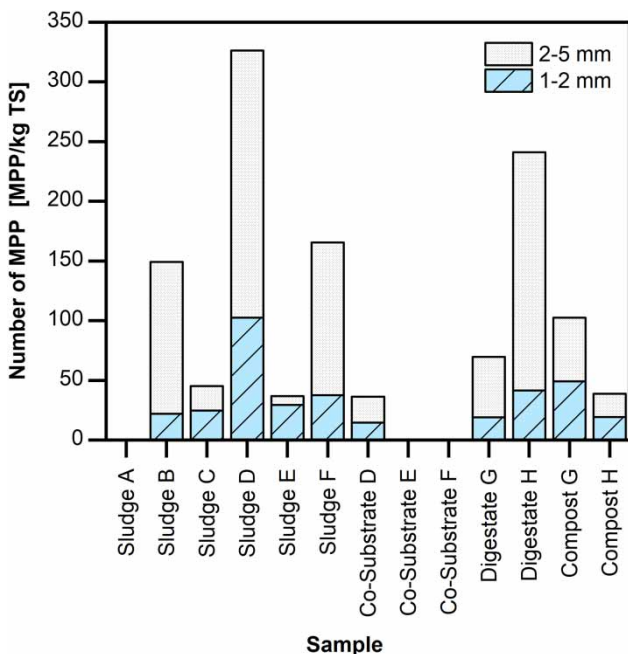
Table 2 | Summary of TS and TVS for all samples

Plant	Substrate	TS [%]	TVS [%]
A	Sludge	19.9	63.8
B	Sludge	36.2	45.9
C	Sludge	24.3	66.0
D	Sludge	21.5	67.3
E	Sludge	27.2	58.4
F	Sludge	26.6	49.7
D	Co-substrate	13.7	86.0
E	Co-substrate	7.0	80.0
F	Co-substrate	0.9	96.4
G	Digestate	31.6	68.7
H	Digestate	23.3	35.8
G	Compost	50.2	46.7
H	Compost	61.6	57.4

for a faster sieving process reducing the time from several hours to approximately 30 minutes. However, we did not find a time advantage when tried on the sludge samples.

Quantity of microplastics

The found number of MPP (Figure 2) in sludge was in the range 0–122/kg TS for the 1–2 mm fraction and 0–224/kg

**Figure 2** | Number of MPP for the fraction 1–2 mm and 2–5 mm.

TS for the 2–5 mm fraction. Overall, up to 326 MPP/kg TS were found in sludge. The sludge sampled from WWTP A and the co-substrates of WWTP E and F did not contain any MP between 1 and 5 mm. For the sludge of plants B, D, F as well as the digestate and compost of plant G, the 2–5 mm fraction contained more MPP than the 1–2 mm fraction. With a total of 326 MPP/kg TS, the sludge of plant D contained the highest number of MPP of all samples. The sludge with the fewest MPP was plant E, closely followed by plant C with 10 and 11 MPP/kg TS, respectively.

There are two reasons why digested dewatered sludge was chosen in this investigation. Firstly, digested dewatered sludge is the final form of sewage sludge in the WWTP. Secondly, the process of dewatering sludge concentrates MPP; thus, a smaller sample volume can be analyzed. However, we are aware that especially centrifuges can separate the low-density MP from sludge (Rosenwinkel 2019). On the other hand, the 165 MPP/kg TS found in sludge from plant F using a chamber filter press as dewatering aggregate is comparable to the 149 MPP/kg TS of plant B, which operated a centrifuge. This result is also interesting because plant F practices co-digestion and plant B does not. The sampled co-substrate of plant F, the expired drinks, did not contain MPP between 1 and 5 mm. Consequently, this will not add MPP to the digester. Furthermore, the plant had not gotten a delivery of OFMW for several weeks before sampling. Hence, the MPP concentration might be higher in sludge when OFMW is added to the digester. This theory is supported by the high amount of MP found in sludge from plant D, whereas sludge from plant A that does not practice co-digestion did not contain any MPP. The sludge of plant E also contained a low number of MPP. The WWTP practices co-digestion; however, no OFMW is used as co-substrate.

Our results lie within or below those of other studies. Though the lack of standard methods and a uniform reporting of results make the comparison challenging. Raju et al. (2020) found 7.91 ± 0.44 MPP/L in waste activated sludge (MPP > 1.5 μm) of which 9.04% were MPP between 1 and 5 mm. Assuming a TS concentration of 6–9 g/L this would be approximately 80–120 MPP/kg TS (1–5 mm), which lies within our results. Dewatered sewage sludge from a municipal WWTP in China contained 240.3 MPP/g TS where 5% of the MPP were of the size of 1–5 mm. This results in 12,000 MPP/kg TS, which is significantly higher than what we found. Another study found 1,200 MPP/kg wet weight in sludge (Murphy et al. 2016). However, the authors did not specify different size fractions, only that the smallest

mesh size used was 65 μm and the average particle size 1.6 ± 0.5 mm. Similarly, Carr *et al.* (2016) analyzed 1,000 MPP/kg wet weight (5,000–45 μm) in sludge, with the fraction of MPP > 400 μm having the least amount of MPP compared to the others. A study from Canada found 8,700–14,000 MPP/kg TS (Crossman *et al.* 2020).

The different numbers of MPP in digestate and compost of plant G may be due to the composting process, where large plastics (>5 mm) further disintegrate to MP. For plant H the digestate shows a higher concentration of MPP than the compost sample, which may be evidence for an effective sieving process of the compost. The MPP concentration we found in compost was 39–102 MPP/kg TS, which lies within the range of 0–146 MPP/kg TS found elsewhere (Weithmann *et al.* 2018). As mentioned previously, the German Fertilizer Ordinance states a weight limit of 0.4% of hard plastics and 0.1% of other plastics > 1 mm. Our results show that both sludge and compost samples can comply with the regulation (Figure 3). How much compost and sludge is land applied depends on the nutrient content and requirement of the soil to be fertilized (DüV 2017). Weithmann *et al.* (2018) estimated that in Germany alone 35 billion to 2.2 trillion MPP > 1 mm are introduced to terrestrial ecosystems through fertilization with compost and sludge. After a 10-year period, researchers analyzed the MP retention in French agricultural soil that was amended with 18.13 Mg wet weight/ha compost every

other year (Watteau *et al.* 2018). The authors of the study analyzed MP contents of the following soil fractions: <50 μm , 50–200 μm , 200–2,000 μm , 2–5 mm, and >5 mm. With pyrolysis coupled to gas chromatography and mass spectrometry they determined the 200–2,000 μm soil fraction to contain, with 464–503 g MPP/kg TS, the most MP. Hence, they concluded compost is a conduit for microplastics in agricultural soil (Watteau *et al.* 2018).

An investigation of MP content of agricultural soils after sludge application showed similar results (Crossman *et al.* 2020). Three fields in Ontario (Canada) were fertilized with sludge. The authors also found a preference for fibers being retained in soil, rather than fragments. However, after the 8-month study period, 99% of MPP across all fields were exported from soil. This may have happened due to soil density and runoff after rain events (Crossman *et al.* 2020). In contrast, Corradini *et al.* (2019) sampled 31 agricultural fields that had records of frequent sludge application for 10 years prior to the study. Their results provide evidence that MP accumulate over time in soil amended with sludge.

Shape of microplastic particles

The relative abundance of the different shapes varied among the samples (Figure 4). Fragments were found in all samples containing microplastics. Film was found in all sludge, digestate and compost samples containing MPP. In contrast to the biogas plant samples, sludge samples contained fibers except for plant D. Plant F contained 32%, plant B 7%, plant E 20%, and plant C 55% fibers.

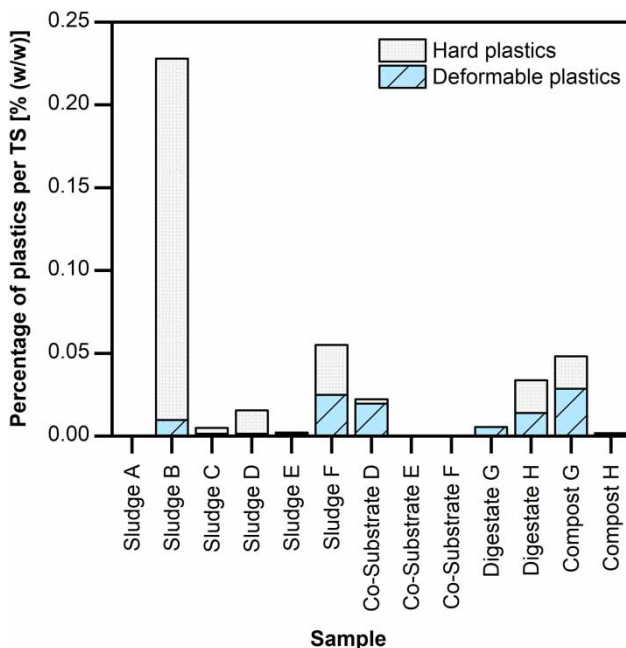


Figure 3 | Percentage of dry weight of hard and deformable MP per kg TS.

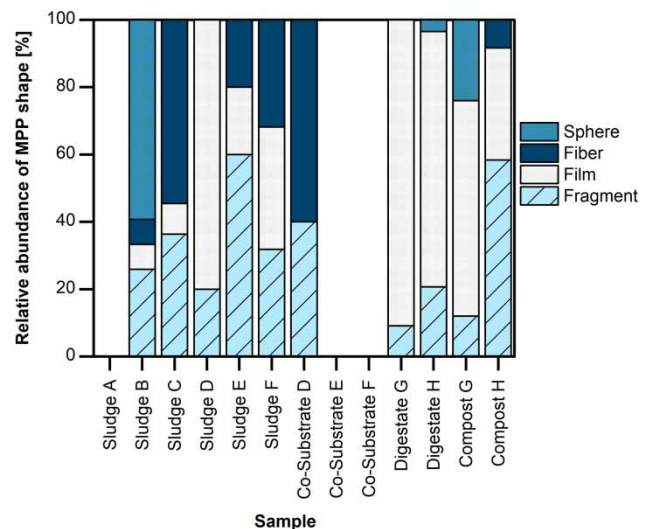


Figure 4 | Relative abundance of the shapes film, fiber, fragment and sphere for each sample.

Raju *et al.* (2020) found fibers to be the most abundant shape of MPP in waste activated sludge. Similar results were reported by Corradini *et al.* (2019) for sludge and soil. On the other hand, Carr *et al.* (2016) and Crossman *et al.* (2020) determined a dominance of fragments over fibers. Considering the absolute number of MPP found across all sludge in this current study, the most abundant shape of the MPP was film followed by fragments and fibers. As fibers have a significantly greater length than width, it is very likely that shorter pieces have passed the 1 mm mesh size sieve; hence, a lower number of fibers was detected. Another reason can be that fibers pass through WWTP, being released into the environment with the effluent (Sun *et al.* 2019). The large percentage of spheres in the sludge of plant C derived from a car wash using the polymer spheres to clean the tires (personal communication).

Similar to the digestate samples of the biogas plants, the sludge sample of plant D contained 80% film. This may be attributed to the co-digestion of OFMW with sewage sludge. However, the OFMW sample of plant D did not contain film particles. The digestate of biogas plant G contained 91% film particles, whereas the compost contained 64% film. Additionally, film was the most abundant shape in the samples from WWTP H. It can be assumed that the film and fragments are secondary microplastics caused by disintegration of packaging material and plastic bags because some of the particles were recognizable as torn garbage bags. The percentage of film decreased in accordance with the feedstock processing. For plant G the dewatered digestate contained more film than the compost sample. The same is true for the digestate and compost samples of plant H. An explanation for this could be that the film is retained during the sieving process, even though the sieve is 15 mm. Moreover, the particles could be further disintegrated during composting, resulting in particles of <1 mm.

Polymer type of microplastic particles

The various polymers are distributed unevenly within the samples (Figure 5). For example, plant B has 87% PP and the sludge of plant D has 71%. Considering every isolated MPP from all samples, PVC is the most common polymer (29%). With 20%, PP is the second most abundant polymer of all samples. Moreover, the overall abundance of PE and PET is 10% each. This is also in line with previous studies (Murphy *et al.* 2016; Weithmann *et al.* 2018; Sun *et al.* 2019), although Raju *et al.* (2020) detected predominately PET and nylon in waste activated sludge samples. The mentioned plastics are the most produced plastics: PE (36%), PP

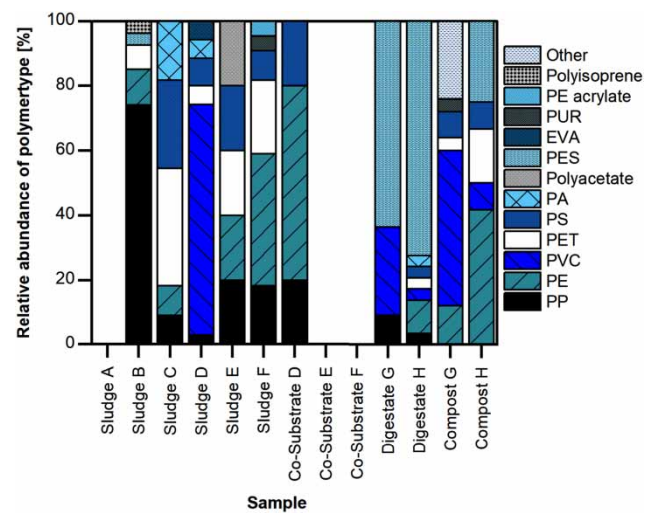


Figure 5 | Relative abundance of polymer type for each sample. PE acrylate: polyethylene acrylate; PUR: polyurethane; EVA: ethylene vinylacetate; PES: polyester; PA: polyamide; PS: polystyrene; PET: polyethylene terephthalate; PVC: polyvinyl chloride; PE: polyethylene; PP: polypropylene.

(21%), PVC (12%) followed by PET, PUR and PES (polyester) (<10% each) (Geyer *et al.* 2017).

CONCLUSIONS

In recent years, scientists and other people have become increasingly concerned by microplastics polluting the environment. For the most part, researchers put their focus on the effects and sources of microplastics in aquatic systems. As a result, WWTP were hypothesized to be a major conduit for microplastics in the environment. Due to the lack of standardized methods, the comparison of the various studies is difficult. During this research we demonstrated the applicability of wet-sieving as a method to isolate plastic particles from sludge, co-substrates, and compost taking a step towards standardized methods for MP from 1–5 mm, while contributing information about the MP contamination of the analyzed samples. We also propose that the number of MPP should always be given in reference to the total solids of the respective sample. Our results suggest that digested dewatered sludge and compost are comparable regarding the amount of large MPP they contain. Additionally, we hypothesized that the OFMW used as co-substrate during anaerobic digestion of sewage sludge could be an additional source of MP in WWTP. However, our data could neither confirm nor deny that co-substrates are a relevant source of large MPP in WWTP. Thus, further investigations are needed.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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