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Microplastic abundance in sludge-treated fields: Variance and estimated half-life

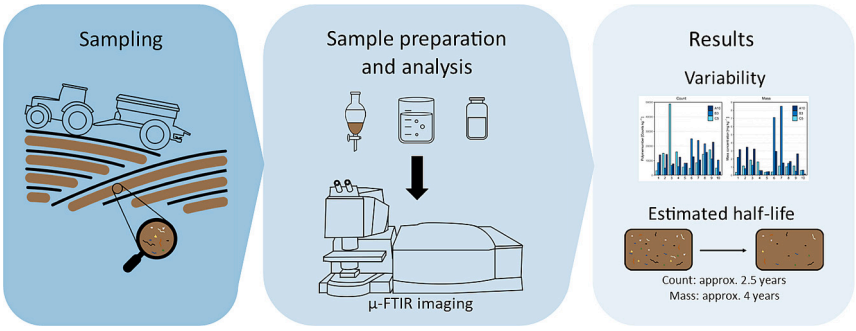
Nanna D.R. Klemmensen *, Rupa Chand, María S. Blanco, Jes Vollertsen

Department of the Built Environment, Aalborg University, Thomas Manns Vej 23, 9220 Aalborg Øst, Denmark

HIGHLIGHTS

- Soil treated long-term with sludge was analysed for MP using μ-FTIR imaging.
- The MP count varied by a magnitude of 20 among the 30 sampling points.
- The most detected polymer types were polyester and acrylic, comprising over 50 %.
- The importance of sampling size was demonstrated with a Monto Carlo simulation.
- It was estimated that MP degrade and/or migrate from the fields over time.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the abundance of microplastic (MP) in agricultural soil fertilised with sludge, assessing the variation in MP count and estimated mass in three long-term field trials treated excessively with sludge in 2003–2012. Ten samples were taken from each of the three fields with concentrations ranging from 2392 to 48,791 counts kg^{-1}, where over 50 % of the MPs were polyester and acrylic. Due to the considerable variation in concentration, the impact of the number of sub-samples on the predicted measured concentration was estimated applying a Monto Carlo simulation approach. Choosing the number of sampling points is a compromise between acceptable sampling error and available resources. The simulations showed an increasingly high risk of obtaining an outlier when taking less than approx. ten subsamples. When ending fertilisation with sludge, the estimated half-life for the MPs measured by counts was approx. 2.5 years, whereas the half-life for the MP estimated mass was approx. 4 years. Hence, smaller particles seemed to degrade and/or migrate elsewhere the fastest.

1. Introduction

Microplastic (MP) pollution has received increasing attention in recent years. Especially as plastic has become an unavoidable part of our day-to-day life – leading to environmental accumulation due to insufficient recycling and disposal (Syberg et al., 2020; Rillig, 2012). MP is found in almost all imaginable matrices, including marine ecosystems (Andrady, 2011), stormwater retention ponds (Liu et al., 2019a; Liu et al., 2019b; Rasmussen et al., 2024), air (Dris et al., 2015), road dust (Rasmussen et al., 2023), agricultural soils (Zubris and Richards, 2005),

* Corrresponding author.
E-mail address: ndrk@build.aau.dk (N.D.R. Klemmensen).

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and remote arctic regions (Kanhai et al., 2020; Kelly et al., 2020). Even though there is a high diversity in the investigated areas, the most well-researched area is the aquatic environment, leaving the knowledge about MP in soil limited (Rillig and Bonkowski, 2018; Zhou et al., 2020; Nizzetto et al., 2016b).

Many countries utilise sludge as fertiliser on agricultural fields (Bläsing and Amelung, 2018; Peccia and Westerhoff, 2015; Nizzetto et al., 2016b). Sludge is used because it is a by-product from wastewater treatment (Peccia and Westerhoff, 2015) and is rich in organic matter, phosphorus, and nitrogen (Singh and Agrawal, 2008). Besides the desired recovery of nutrients when using sludge as fertiliser, the accumulation of hazardous pollutants in the soil raises concerns due to high concentrations herein (Fijalkowski et al., 2017). Therefore, to protect the environment and human health, several EU and U.S. regulations have been established to minimise contamination with pollutants (Bläsing and Amelung, 2018).

Despite the increasing number of publications on MP in wastewater treatment plant sludge, revealing concentrations ranging from 1000 counts kg^{-1} (Mintenig et al., 2017) to 6.36 ($\pm$0.08) $\times$ 10^6 counts kg^{-1} (Chand et al., 2021), the regulation of MP in sludge is yet to be addressed by both the EU and the U.S. While awareness of MP contamination from sludge is growing (Nizzetto et al., 2016b), regulatory frameworks are still lacking (Bläsing and Amelung, 2018).

While it is hypnotised that fertilisation with sludge is one of the major input paths of MP to soil (Bläsing and Amelung, 2018), the research regarding the abundance and fate of MP in sludge-treated fields is currently limited, with only few publications available (van den Berg et al., 2020; Colombini et al., 2022; Corradini et al., 2019; Crossman et al., 2020; Zubris and Richards, 2005; Tagg et al., 2022). These studies all investigated the abundance of MP in sludge-treated fields, yielding varying results. However, an essential aspect overlooked in these studies is the determination of the necessary sample size to obtain a representative sample, a crucial consideration for accurately assessing MP concentrations in sludge-treated fields. Additionally, it is also important to investigate whether the MP stays in the soil after sludge treatment or degraded and/or migrates elsewhere, posing potential risks to other ecosystems.

The primary objective of this study was to investigate the abundance and distribution of MP within three long-term field trials subjected to long-term sludge treatment. The study aimed at determining the required sampling size for adequately representing natural variability within each field, as it is hypothesised that such variability is large. It is, therefore, important to address the problem by finding a suitable sampling size. Furthermore, this study aimed to enhance the understanding of the fate of the MP subsequent to their introduction into these fields, as the knowledge is limited, and it is suspected that the MP disappear from the fields over time. This is done by estimating the half-lives of the MP and thereby assessing the possible risk that the sludge application poses to this and other ecosystems.

2. Material and methods

2.1. Sampling area

The samples were taken from three fields at the long-term field trial 'CRUCIAL', located in Taastrup, approximately 20 km west of Copenhagen, Denmark (Fig. 1a) (López-Rayo et al., 2016). The fields were fertilised with an accelerated sludge level from 2003 to 2012 (Johansen et al., 2023), corresponding to 2295 kg phosphorus ha^{-1} (López-Rayo et al., 2016). The sludge had been anaerobically digested and provided by a municipal treatment plant, receiving wastewater from industry and private households (Poulsen et al., 2013). The specific sludge was not tested as it was applied long before this study, nor was the soil analysed prior to treatment in 2003. The fields were treated yearly in the spring and under sown with clover (Poulsen et al., 2013). The fields were fertilised with small amounts of mineral fertiliser from 2013 to 2021

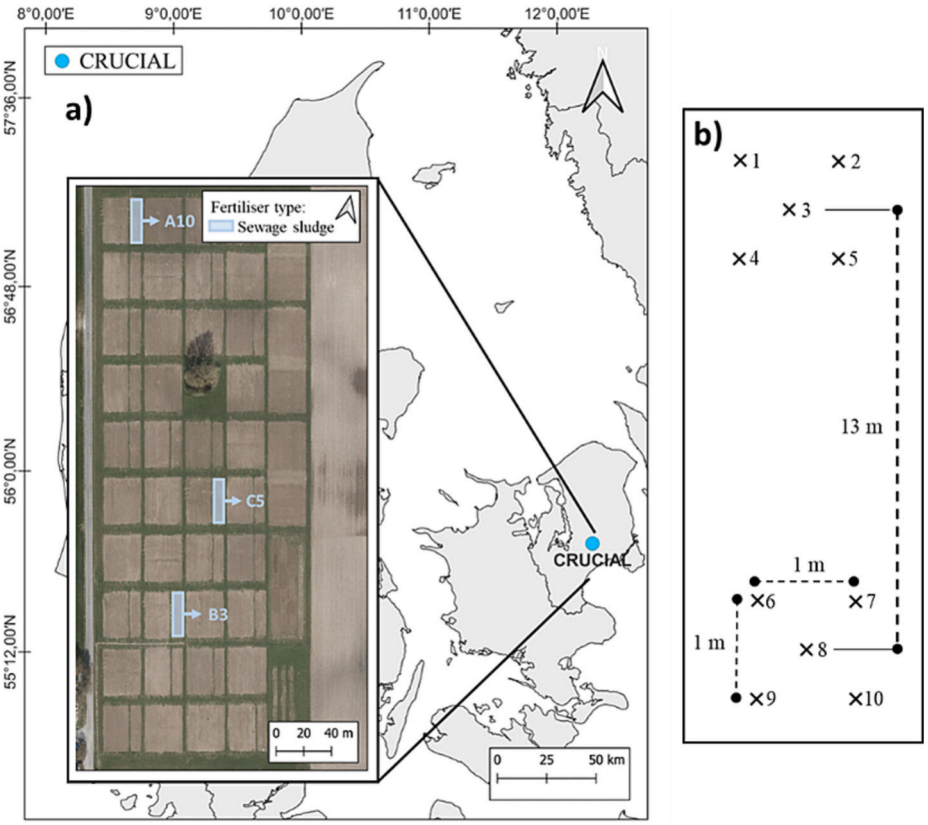

Fig. 1. Map of Denmark (a), with corrosponding zoom of the sampled fields at the CRUCIAL field trial. The sampling strategy per field is likewise illustrated (b).

(Johansen et al., 2023). Agricultural plastic such as mulch film was never used on the fields nor the land surrounding it. There was furthermore no visible plastic or other waste present at or in the vicinity of the fields. MP in the fields should hence arise from the applied sludge, from the machines working the fields, and from atmospheric deposition.

2.2. Sampling

Thirty samples were collected from three CRUCIAL fields in the summer of 2021. Ten samples were collected from each sludge-treated field (A10, B3, C5), meaning 30 samples in total. The plots were flat and the topography featureless, due to their small size (approximately 7 × 33 m). All samples were taken with a metal shovel, transferred to a metal container, and the lid was put on. To avoid contamination, the samples were sampled with headwind, stepping on the sampling location was avoided, and the metal containers were opened as briefly as possible. The samples from the sludge-treated fields were taken from two 1 × 1 m squares with 13 m in between. From each corner and the centre, a sample of approx. 1 L was taken to a depth of approx. 20 cm, creating ten samples per field, as illustrated in Fig. 1b. A sampling depth of 20 cm was chosen as this corresponds to the ploughing depth of the fields. Therefore, the MP that might be added with the sludge should be dispersed within these 20 cm if they stay in the soil. The samples were stored in a fridge until further processing.

2.3. Sample preparation

The sample processing followed the procedure used by Molazadeh et al. (2023) for freshwater sediments, with minor alterations. The procedure is derived from previous methods used by Liu et al. (2019b) for stormwater pond sediments and follows the concept proposed by Löder et al. (2017) and Hurley et al. (2018).

In short, the whole sample was homogenised, and the water content was measured (METTLER TOLEDO HE73 moisture analyser). A subsample of approximately 285 g of soil in dry weight (Table S1) was weighed and transferred to a 5 L beaker containing a coil for aeration and gentle mixing. To break aggregates and start the degradation of organic matter, a pre-oxidation was started by adding water and then gradually adding 50 % H_2O_2 over multiple days until the pre-oxidation was completed, but for no more than 7 days. The concentration of H_2O_2 never exceeded 10 %.

When the pre-oxidation was done, the sample was wet sieved using a 1 mm sieve and milli-Q water to remove sand and gravel larger than 1 mm. The samples were left to settle for at least 48 h to make the filtration easier, whereafter the supernatant was filtered off using a 10 μm stainless steel filter. The filter was cleaned using filtered demineralised water (Glass fibre, 0.7 μm), and the particles transferred to the remaining soil.

The soil was dried in an oven at 50 °C until it was almost dry. It was then transferred to a 2 L separation funnel, and $ZnCl_2$ ($\rho = 1.8$ g cm^{-3}) was added. The density separation was done to remove most of the inorganic material in the samples. The samples were aerated with filtered and dry compressed air for 30 min and left to settle overnight. The next day, the sample was discharged, the top three centimetres were kept for further sample preparation, and the funnel was flushed to wash out particles stuck to its inside. The discharge was poured back into the funnel, and the sample aerated for another 30 min. The top three centimetres were again kept, and the funnel was flushed with filtered demineralised water. The collected top parts were filtered using the same 10 μm stainless steel filter as earlier.

The filter was cleaned using 5 % SDS, and the particles were transferred to approximately 300 mL of 5 % SDS; this treatment was done to break up the remaining aggregates. The same process as for the SDS was repeated. The particles were, however, first transferred to 250 mL TRIS buffer (pH 8.2) with 0.5 mL protease to remove proteins and then 250 mL acetate buffer (pH 4.8) with 0.5 mL cellulase and 0.5 mL viscozyme to remove plant material. To remove the remaining organic matter, the particles were transferred to 200 mL of filtered demineralised water, and a Fenton oxidation was started by adding 145 mL H_2O_2 (50 %), 65 mL NaOH (0.1 M), and 62 mL $FeSO_4$ (0.1 M).

After the Fenton oxidation, the samples were again sieved using a 500 μm sieve. The samples were filtered, the particles were transferred to $ZnCl_2$ ($\rho = 1.8$ g cm^{-3}), and yet another density separation was done to remove the remaining inorganic matter. The top part was kept, filtered, and transferred to 50 % HPLC-grade ethanol. To concentrate the samples, they were evaporated into 10 mL vials in an evaporation bath (TurboVap® LV, Biotage) at 50 °C until dry. The volume of the samples was fixed with 50 % HPLC grade ethanol to 5 mL (Table S1).

2.4. Identification and quantification of MP

No items resembling MP were found in the fraction retained on both the 1 mm and the 500 μm sieve during the sample preparation. Therefore, the only fraction analysed was the fraction below 500 μm, which was analysed using μ-FTIR-imaging combined with the freeware siMPle for data processing. The MP in this fraction was analysed as described by Liu et al. (2019a), Rasmussen et al. (2021), and Liu et al. (2019b). In short, the final sample was vortexed, and a subsample was deposited onto a zinc selenide (ZnSe) window in a compression cell, which restricted the active area to Ø10 (Pike Technologies, USA). Several 100 μL subsamples were deposited over multiple depositions using a 100 μL glass capillary until an appropriate number of particles were on the window. The subsample was dried at 55 °C on a heating plate between each deposition. An appropriate subsample could not be deposited on only one window; therefore, each sample was deposited onto three different ZnSe windows and scanned.

The whole window was scanned in transmission mode using μ-FTIR imaging applying a Cary 670 FTIR spectrometer (Agilent Technologies). The spectrometer was combined with a Cary 620 FTIR microscope, using a 15× Cassegrain objective and a 128 × 128 Focal plane array (FPA) Mercury-Cadmium-Telluride detector, creating a 5.5 μm pixel resolution. The wavenumber range was 850–3750 cm^{-1} with a spectral resolution of 8 cm^{-1} and 30 co-added scans. For the background, 120 co-added scans were made.

The μ-FTIR imaging data were processed using the freeware siMPle (Primpke et al., 2020). For the siMPle analysis, a library consisting of 512 reference spectra within 79 material groups, including MPs and natural materials, was used. All the MPs found by siMPle were manually checked to exclude false-positive particles. siMPle yielded data regarding size, shape, type, and estimated mass. The latter was estimated by siMPle based on calculations described in Simon et al. (2018). The software estimates volume from the 2-dimensional projection of the particle as descried in Liu et al. (2019a): The equivalent ellipse of its 2-dimensional projection is determined, where the first dimension of the ellipse equals the length of the particle. The volume is estimated by defining the particle shape as an ellipsoid with the first and second dimension of the ellipsoid equalling the first and second dimension of the equivalent ellipse, and its third dimension being 0.6 times its second dimension. The particle mass is estimated by multiplying the estimated volume with the density of the polymer identified by the μ-FTIR.

2.5. Contamination and recovery

Blanks and recovery tests were processed alongside the samples. Three blanks and three recovery tests were made. Muffled sand was used as the matrix for the blanks, which then followed the sample processing procedure described for the soil samples. For data analysis, the blanks were not subtracted from the samples due to the challenge of determining which specific MP particles should be excluded from the dataset (Dawson et al., 2023; Shruti and Kutralam-Muniasamy, 2023).

To avoid contamination during the sample preparation, synthetic clothes were avoided, along with always using a cotton lab coat. In addition, only non-plastics such as glass and metal was used during

sampling and analysis. Utensils were always flushed three times with filtered milli-Q water (GF, 0.7 μm) before use. Everything was kept covered with aluminium foil as much as possible.

Muffled sand was also used as the matrix for the recovery tests. Polystyrene (PS) beads with sizes of 52 μm (Blue-colored PS-beads, ρ = 1.05 g cm^{-3}, microParticles GmbH, Germany) and 106 μm (Red-colored PS-beads, ρ = 1.05 g cm^{-3}, microParticles GmbH, Germany) were counted with a Flowcam (FlowCam 8400, colour camera, 488 nm laser). The counted beads were added to the muffled sand, and the sand was treated by the procedure described for the soil samples (excluding the evaporation step). The beads in the extract were then counted again by the Flowcam, yielding a recovery rate for the added beads.

2.6. Mass balance estimation

The accumulation of MP in the soil was calculated based on an estimated MP mass in the sludge, on the soil properties, and the time since the sludge was applied to the field. The MP concentration in sludge was assumed based on the mean MP count and estimated mass reported by Chand et al. (2021) (namely 5.22 × 106 counts kg^{-1} and 300 mg kg^{-1}). Chand et al. (2021) was selected as a reference due to similarities in the sample preparation and analytical method, which is important as even minor alterations may change the output. Additionally, both studies measured MP per dry matter, aligning with the requirements for reporting Danish sludge.

Based on the amount of sludge added to the fields (Table S6) and measured mean MP concentration in sludge, it was estimated that 9.68 × 109 counts corresponding to 556 g of MP should have been introduced to each field from the sludge. The soil was reported as a sandy loam (López-Rayo et al., 2016) with a ploughing depth of 20 cm (Poulsen et al., 2013). The bulk density was assumed to be 1490 kg m^{-3} (Nielsen et al., 2018), and homogeneous mixing of MP in the ploughing layer was likewise assumed. Under these assumptions, the amount of MP from sewage sludge alone was estimated to each field having received 1.41 × 105 counts kg^{-1}, corresponding to 8.1 mg kg^{-1}. This estimation assumes that no additional MP entered the fields through atmospheric deposition or mineral fertilisation since the termination of sludge treatment. The removal was then calculated from difference in actual concentrations and the predicted ones.

2.7. Statistical analysis

The statistical analysis was carried out using R (version 4.2.1). For small datasets, Shapiro-Wilk's test was used to test normality. The normality for larger datasets was determined with Q-Q plots (Fig. S1). Two-sample *t*-test and Wilcoxon rank sum test were used to determine significant differences for normally distributed and non-normal distributed data, respectively. All statistical analysis performed in this study was done at a confidence level of 95 %. The corresponding *p*-values are shown in supplementary data.

A Monto Carlo simulation was performed to assess the relation between the number of soil subsamples and the probability of obtaining a representative sample. First, all datasets from the sludge-treated fields were combined and treated as the ground truth of the MP distribution in the fields, i.e., a distribution containing 30 distinct data points. Then, random values were drawn from that distribution. Initial tests showed that 2500 random draws gave an appropriate prediction accuracy (Fig. S2).

3. Results and discussion

3.1. Contamination and recovery

In the scanned part of the samples, an average MP count of 50 with a corresponding average estimated mass of 4.2 μg was observed per blank. This scanned portion represented approximately 48 (±21) % of the total sample volume (Table S1). When this was extrapolated to the whole sample volume, an average contamination of 107 (±12) counts corresponding to 8.3 (±4.0) μg was determined (Table S5). To provide context, when processing 287 g of soil (dry weight), which was this study's average, the samples were contaminated with 373 (±42) counts kg^{-1} (dry weight), equalling an estimated mass of 28.9 (±13.9) μg kg^{-1} (dry weight). Notably, a significant portion of this contamination was identified as polyester (Table S5), constituting 78 (±5) % of the total MP counts. These polyester particles probably originate from clothing fibres released into the environment (De Falco et al., 2020; Vianello et al., 2019).

In the recovery test, a recovery rate of 48 (±8) % was achieved for PS beads with diameters of 52 μm and 53 (±4) % for the PS beads with diameters of 106 μm. No significant difference was observed between the recovery rates for the two bead sizes (Table S2), suggesting that the sample extraction method was equally effective for both sizes. However, it is crucial to bear in mind that the recovery rate may differ depending on factors such as polymer type, shape, size, and the characteristics of the sample matrix.

3.2. Abundance of MP

When considering all 30 sampling points distributed in the three sludge-treated fields (A10, B3, and C5), it was evident from Fig. 2 that MP was present at all sampling locations at concentrations substantially above the measured contamination, here, each point in the boxplots represents an individual sample. However, both the MP count and the estimated mass displayed considerable variability across the samples. Among the 30 samples, the lowest count was 2392 counts kg^{-1} (dry weight), approx. 20 times lower than the highest count of 48,791 counts kg^{-1} (dry weight). An even more substantial difference was observed when comparing estimated masses, with a factor of approx. 50 separating the highest value of 8.51 mg kg^{-1} (dry weight) and the lowest value of 0.17 mg kg^{-1} (dry weight). Detailed concentration data for both the count and estimated mass at each sampling point can be found in the supplementary material (Fig. S3).

Although great variation was observed in both the count and estimated mass among the different fields, when combing data from all ten sampling points within each field, as illustrated in Fig. 2, no statistically significant difference was observed between the MP counts and estimated masses when comparing the three fields treated with sludge.

The substantial variance observed in the boxplots in Fig. 2 was attributed to natural variability, resulting in wide and fluctuating quartile ranges. This highlights the importance of the number of sampling points to obtain a representative sample. To investigate the optimal number of sampling points for representativeness, a Monto Carlo simulation was conducted, the results of which are shown in Fig. 3. Here, Fig. 3a shows that when increasing the number of sampling points, the variance decreases. Meanwhile, the mean of all the replications remains constant, regardless of the number of sampling points. For instance, when taking only one sampling point, the mean count varied, ranging from 6775 counts kg^{-1} (dry weight) to 15,743 counts kg^{-1} (dry weight), taking the first and third quartiles, respectively. In contrast, with ten sampling points, the range narrowed, falling between 11,083 counts kg^{-1} (dry weight) to 14,528 counts kg^{-1} (dry weight) for the first and third quartiles, respectively (Fig. 3a).

Furthermore, Fig. 3b reveals that the first quartile varied more compared to the third quartile. With just one sampling point, the first quartile varied by 48 % from the mean, while the third quartile only varied by 20 % from the mean. However, with ten sampling points, both the first and third quartile varied approximately 13 % from the mean. This variation was ascribed to the non-normality of the data (i.e., skewness), making it essential to address potential outliers as they can significantly impact the mean when only a few samples are taken.

In addition, before determining the number of sampling points, it is crucial to establish the expected and acceptable error. Choosing only one

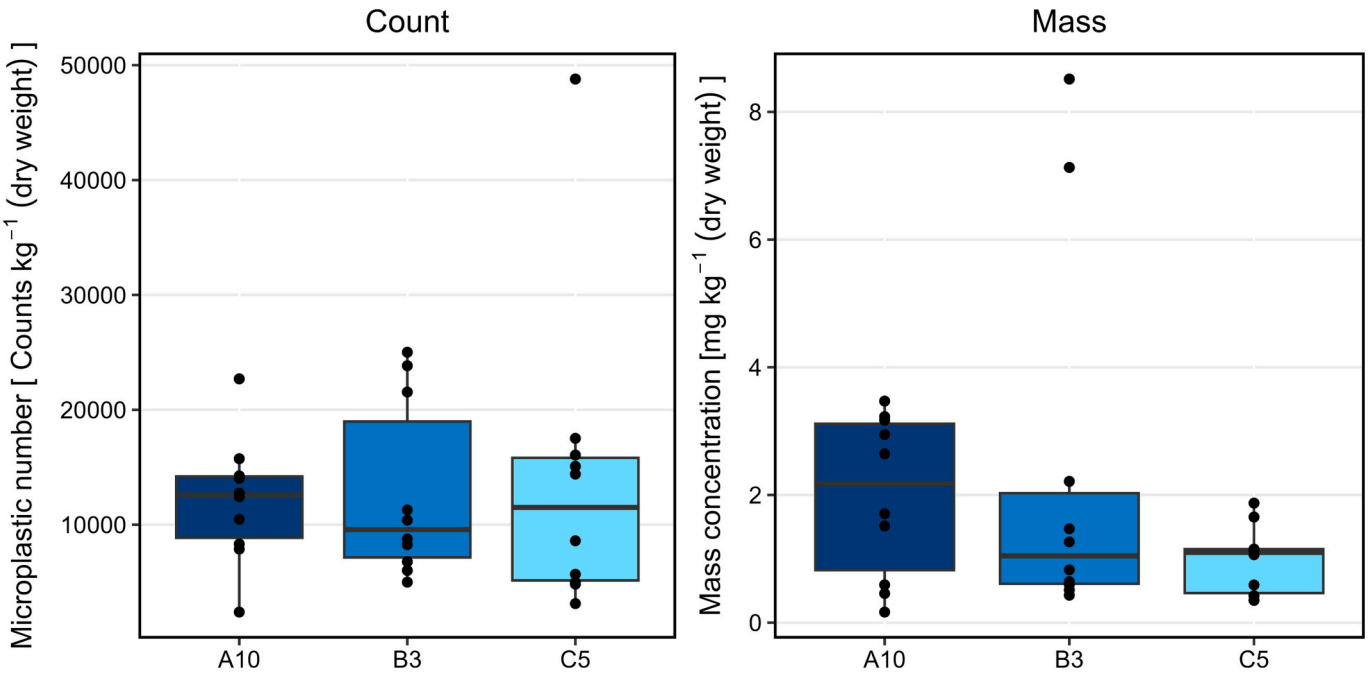

Fig. 2. Boxplots displaying the abundance in terms of both count (left) and estimated mass (right) on the three sludge-treated fields (A10, B3, and C5), each including ten sampling points. Each data point on the boxplots represents a specific sampling point within the respective field.

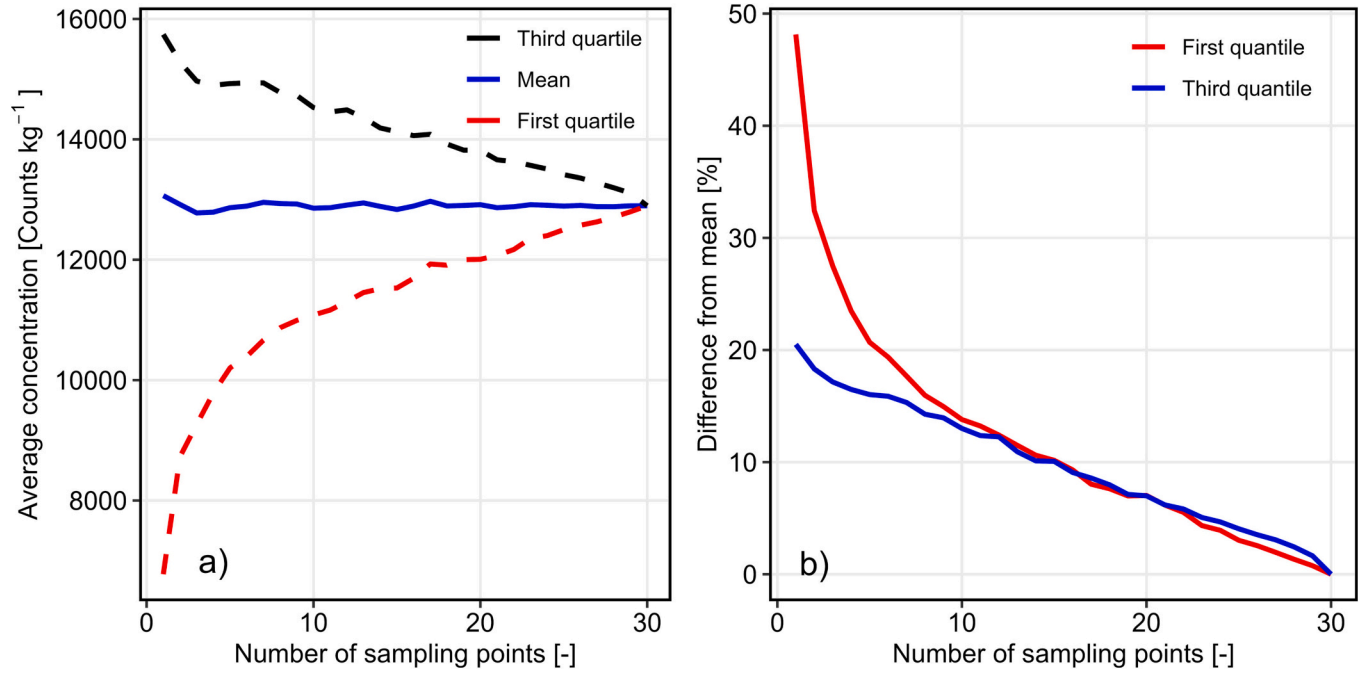

Fig. 3. Monto-Carlo simulation results on numbers of samples versus uncertainty compared to a ground truth. The mean concentration (a) is shown for the mean, the first and third quantile, for a continuum of sampling points. In addition, the difference from the mean is shown for the first and third quartile (b).

sampling point resulted in a situation where 50 % of the obtained mean counts deviated by approx. 70 % from the true mean. However, with ten sampling points, the deviation reduced to approx. 26 % and further decreased to approx. 14 % when taking 20 sampling points. Therefore, when choosing the number of sampling points for a study, it should involve a consideration of the acceptable level of variance within the study relative to the available resources.

Not only was substantial variation observed within the sludge-treated fields investigated in this study, but there were also differences when comparing this study's results to existing literature. Comparing the concentrations observed in sludge-treated fields in this study, lower concentrations were generally noted. In contrast to the average concentration found (12,896 ± 923 counts kg^{-1} (dry weight), Table S6), van den Berg et al. (2020) reported counts 2.5 times smaller. Zubris and Richards (2005) detected only a few fibres per g^{-1} of soil, while Corradini et al. (2019) reported a median ranging from 1100 to 3500 counts kg^{-1}, depending on the number of sludge applications. Crossman et al. (2020) reported concentrations varying from 25 (±20.8)% counts kg^{-1}

to 298 (±39.1)% counts kg^{-1} after the application of biosolids. Tagg et al. (2022), on the other hand, reported concentrations as high as 14,610 counts kg^{-1} in the top layer (0–30 cm) and 210 counts kg^{-1} in deeper soils (60–90 cm). Additionally, Colombini et al. (2022) reported a concentration of 417.4 (±220.5) kg ha^{-1} equivalent to 1.2 × 10^8 (±1.6 × 10^7) counts ha^{-1} after 22 years of fertilising with residual municipal solid waste.

The difference in reported concentrations might originate from several factors. Firstly, the lack of a standardised protocol for processing and analysing samples (Hidalgo-Ruz et al., 2012; Rocha-Santos and Duarte, 2015) leads to inconsistencies in how samples were processed and analysed. Visual and manual detection of MP is common, as it is a cheap and straightforward method, but might introduce uncertainty with smaller MP and tends to be biased by the analyst (van den Berg et al., 2020). Additionally, if organic matter was insufficiently removed from the samples, the identification process becomes challenging (Corradini et al., 2019; Hidalgo-Ruz et al., 2012), leading to potential underestimation or overestimation.

The extraction procedure was also essential. Variations in sieving limits or filter sizes can impact the size range investigated. Colombini et al. (2022) employed sieves with a pore size of 2 mm, investigating only the course MP, potentially causing underestimation due to the lack of smaller MP. Another aspect was the solution used for density separation. Tagg et al. (2022) and this study used solutions with a density of 1.8 g cm^{-1}, whereas Corradini et al. (2019) used 1.55 g cm^{-1}, which means that the heavier MP might not have been included.

Another factor contributing to differences was the variability of MP concentration in sludge and the fertilisation rates. The literature reports a wide range of MP concentrations in sludge, from as low as 1000 counts kg^{-1} dry sludge (Mintenig et al., 2017) and as high as 6.36 (±0.08) × 10^6 counts kg^{-1} (Chand et al., 2021), resulting in different levels of MP input into the fields.

These variations underscore the importance of not only ensuring representative sampling by collecting more than ten samples from an area and analysing them individually or combining them but also standardising the methods to facilitate meaningful comparisons.

3.3. Size and type of MP

Fig. 2 illustrates that fields A10 and B3 exhibited the highest estimated masses, while field C5 had the lowest. The estimated mass was influenced by both the size and type of detected polymer. Consequently, the presence of a single large or heavy MP significantly impacted the total estimated mass. This effect may be partially attributed to the mass estimation algorithm by siMPle. This was amplified in Fig. 4, where the percentage of MP within various bins is presented. This was calculated based on both the count and estimated mass of the MP. Notably, there were only a few MP counts with major dimensions above 300 μm, but those few particles contributed significantly to the total estimated mass.

Analysing the count distribution of sizes (Fig. 4), a similar pattern was observed for the sludge-treated fields, with 59 (±2)% of the total MP falling within the 10–75 μm range. However, significant differences were noted when comparing major dimensions between fields A10 and B3 compared to field C5 (Table S4). Field C5 had a lower mean and third quartile due to a smaller count of large particles. A clear distinction is also visible when examining the size distribution based on the estimated masses in Fig. 4. In fields A10 and B3, over 60 % of the estimated mass originated from MP particles larger than 300 μm, whereas in field C5, only approx. 30 % of the estimated mass came from particles above 300 μm. This variation is due to the presence of a few large polypropylene (PP) particles in fields A10 and B3, accounting for 40 % and 44 % (Fig. 5) of the total estimated mass, respectively, and numerous small polyester

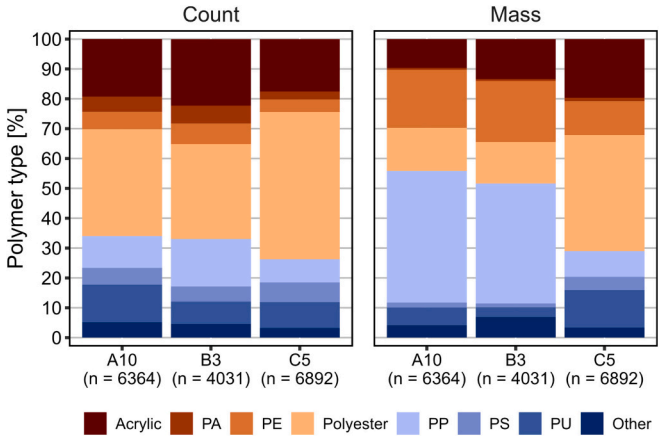

Fig. 5. Type distribution for the three sludge-treated fields (A10, B3, and C5), based on count and estimated mass.

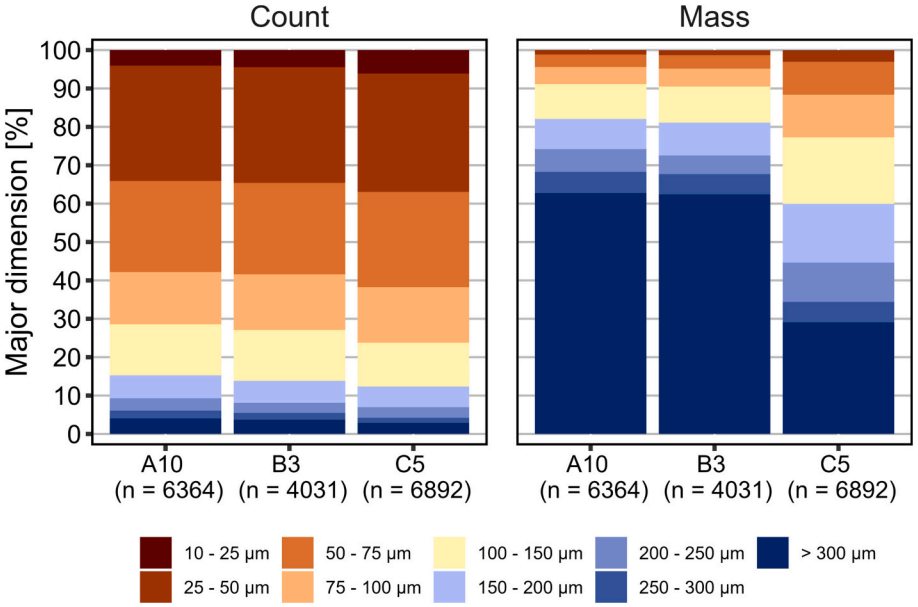

Fig. 4. Size distribution for the three sludge-treated fields (A10, B3, and C5), based on both count and estimated mass.

particles in field C5 (Fig. S4).

Comparing the major dimension of the MP found in this study to the major dimension of those found in sludge, Chand et al. (2021) found that the MP were approx. 10 μm smaller than those observed in the sludge-treated fields, with a median size of 56.9 for sewage sludge and 55.7 μm for digested sludge. Chand et al. (2021) applied a similar sample preparation protocol, utillised the same instrument, and employed identical MP identification software (siMPle) as the present study. Hence, comparisons between these datasets and the current finding are less biased than those between studies employing entirely different analytical protocols. This suggests that larger MP particles may persist in the soil for longer periods compared to smaller ones.

Looking at the count (Fig. 5), the most common polymer found in the samples was polyester, accounting for 39 (±9)%, followed by acrylic with 20 (±2)%. The high concentration count wise of these two polymers is likely stemming from polyester and acrylic fibres entering the wastewater treatment plants when draining washing machines (Browne et al., 2011; Mahbub and Shams, 2022; Napper and Thompson, 2016).

3.4. Estimation of MP accumulation in sludge-treated soil

The investigation of MP in soil remains limited, with sparse information on their migration and degradation, particularly in the context of sludge-treated soils. Previous studies have reported a high concentration of MP in sewage sludge (Chand et al., 2021, 2022), suggesting a considerable input of MP to agricultural fields during sludge treatment.

The fact that sewage sludge-treated fields received a decade of sludge application from 2003 to 2012, whereafter the sludge treatment ended, provided an ideal opportunity to estimate whether MP disappeared after the termination of sludge application. Based on the measured MP content of the soil and the amount added from sludge, the removal in terms of counts was about 90 % while it was about 78 % in terms of mass. Notably, the percentage reduction in counts was approximately 1.2 times higher than the reduction in estimated mass, suggesting that smaller and lighter particles may have degraded and/or migrated more rapidly than larger, heavier ones. This is further emphasized when calculating the estimated half-life based on the calculation described in methods and the observed count and estimated mass in this study. The calculated half-life for MP counts was approx. 2.5 years, while the estimated mass half-life was approx. 4 years. The faster halving of counts compared to estimated mass, further supports the notion that smaller and lighter MP tend to degrade and/or migrate more rapidly.

However, it is important to recognize that the estimation is an approximation. The concentration of MP in sludge can vary depending on factors such as climate, soil type, agriculture practice, and sludge type, changing the input of MP to the field. Chand et al. (2021) demonstrated variation in MP concentration in sludge, even when using the same extraction and analytical methods, with concentrations ranging from 4.08 (±0.25) × 10^6 to 6.36 (±0.80) × 10^6 counts kg^{-1} for sewage sludge and digested sludge, respectively. Variability also exists within the same sludge type. Therefore, the concentration of MP in the sludge applied to the fields investigated in this study may have differed, introducing uncertainties to the estimation.

Nonetheless, the difference in concentration suggests that MP can degrade and/or migrate over time. Several mechanisms may contribute to this phenomenon, including bioturbation creating macropores by earthworms (Rillig et al., 2017b: Huerta Lwanga et al., 2017, Heinze et al., 2021) or plant roots (Rillig et al., 2017a), which facilitate the migration of MP to deeper soil layers. Infiltrating rainwater can transport MP toward groundwater (Kim et al., 2023), and MP can degrade into smaller sizes, potentially forming nanoplastics (NP) over time (Wahl et al., 2021). This study only focused on MP, so the NP would have been overlooked. Lastly, horizontal removal of MP may occur through wind erosion (Borthakur et al., 2022; Rezaei et al., 2019) and surface runoff (Nizzetto et al., 2016a). Further research is necessary to fully understand how the MP is removed from the top layer.

4. Conclusion

MP was found in all analysed samples, with the primary polymer types being polyester and acrylic. Considerable variation was, however, observed in the sludge-treated fields, even within small distances. This means that the number of sampling points taken from a field is crucial when determining the representativeness of the samples. This study identified how many sampling points must be taken from a given field to get within a certain accuracy of the chosen ground truth. It was found that choosing the number of sampling points is a matter of choosing an acceptable margin of error compared to the resources available. However, it is essential to note that the chance of the data being dominated by an outlier increased substantially when fewer than ten sampling points were taken from a field.

When estimating the expected load of MP on the fields, it was assessed that the MP degraded and/or migrated elsewhere. Here, it was found that the MP count decreased more percentage-wise compared to the mass, which means that the larger or heavier particles were better retained. However, more research is necessary to understand the mechanisms that degrade and/or move the MP from the fields.

CRediT authorship contribution statement

Nanna D.R. Klemmensen: Writing – original draft, Visualization, Investigation, Formal analysis. **Rupa Chand:** Writing – review & editing. **María S. Blanco:** Writing – review & editing. **Jes Vollertsen:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171394.

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