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Optimised reduction of total solids and organic matter of sewage sludge matrix for an improved extraction of microplastics

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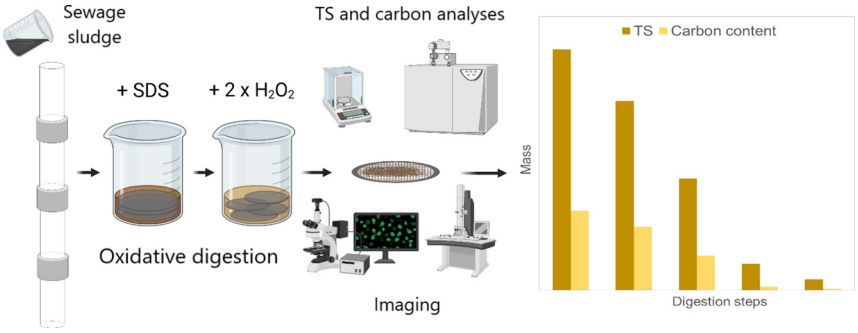
HIGHLIGHTS

- Accurate microplastic analysis from sludge relies on a digestion of organic matter.
- Digestion efficiency was increased by optimising the oxidative digestion of sludge.
- Use of sodium dodecyl sulphate as a pretreatment improved the digestion efficiency.
- Total solids and carbon content were reduced by 95.6% and 98.1%, respectively.
- Incubation at 40 °C with H_2O_2 allowed maintaining the integrity of microplastics.

GRAPHICAL ABSTRACT



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ABSTRACT

The extraction of microplastics from complex environmental matrices, such as sewage sludge, has proven challenging because of their high organic content. A common procedure for the extraction of microplastics from sludge involves conducting a chemical digestion to reduce the amount of organic matter in the sample, followed by a density separation of microplastics. In order to increase the reliability of the density-based separation, an optimisation of the chemical digestion is needed. The aim of this study was to maximise the total solids and carbon content reduction of sludge by optimising the sodium dodecyl sulphate (SDS) pretreatment and the duration of H_2O_2 digestion. A reduction in total solids by 95.6% and in carbon content by 98.1% were achieved with the optimised digestion method, which involved an application of 1% SDS and a 2-day H_2O_2 treatment in the first digestion step. The inclusion of the SDS pretreatment significantly increased the reduction of total solids and carbon content. The optimised digestion process had no significant visible effects on tested reference microplastics and provided an extraction efficiency of 84% for 150 μm reference microspheres and 72% for 650 μm long microfibres. To enable the application of the optimised digestion process to other types of sludges, the consumption of SDS and H_2O_2 were also presented as per grams of organic matter in the untreated sludge.

1. Introduction

Tiny pieces of plastics, referred to as microplastics when smaller than 5 mm, are released during the production and use of plastics, as well as after their disposal. As a consequence of both the continuous release and the persistent nature of plastic, microplastic pollution is currently encountered in environments ranging from urban surroundings (McCormick et al., 2014; Patchaiyappan et al., 2020) to the most distant natural areas (Kelly et al., 2020; Peng et al., 2018).

Some of the most recognised point sources of microplastic pollution include wastewater treatment plants (WWTPs) (Okoffo et al., 2019). From WWTPs the microplastics are released to the environment both via the effluent discharge and, when allowed by national legislation (Hudcová et al., 2019), via a land application of treated sewage sludge (Corradini et al., 2019). The role of land application of treated sludge has gained relatively little attention in the discussion of microplastic pollution related to WWTPs even though the majority of microplastics leaving WWTPs is

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captured in the sewage sludge (Carr et al., 2016; Raju et al., 2020; Salmi et al., 2021).

The land application of sludge enables the circulation of nutrients and carbon back to the agricultural land (Collivignarelli et al., 2019). However, the land application has been both estimated (Edo et al., 2020; Li et al., 2018) and demonstrated (Corradini et al., 2019; Zubris and Richards, 2005) to result in an accumulation of microplastics in agricultural soils. The increasing awareness of the potential effects of microplastics on soil properties, for example on pH and soil aggregation, as well as on root and tissue characteristics of plants (Stubenrauch and Ekardt, 2020; Zhou et al., 2021), has raised a demand to regulate the composition of land-applied sewage sludge in terms of microplastic pollution (Edo et al., 2020). Currently in the EU, for example, the composition of sludge is principally regulated only in terms of heavy metal content, through the Sewage Sludge Directive 86/278/EEC (EC, 1986). The EU has also published the Fertilising Products Regulation ((EU) 2019/1009) in 2019, but microplastics are not taken into account in this legislation either (EU, 2019).

One of the main reasons for the lagging regulation can be assumed to be the lack of standardized methods to quantify the concentration of microplastics in organic-rich matrices. The need for the unification of microplastics analysis has frequently been identified in recent studies (Hurley et al., 2018; Li et al., 2019; Petersen and Hubbart, 2020; Sun et al., 2019). The objectives for such analysis methods include accuracy, reliability, ease of use and minimised duration (He et al., 2020; Prata et al., 2019a). When considering sample matrices of high organic matter content, such as sludge, fulfilling these requirements becomes highly challenging. The extraction of microplastics from the organic matter increases the complexity of the process, adding to the duration and labour-intensity of the analysis. Moreover, due to the large volume of the organic matrix, the ability to analyse representative sample sizes may be compromised, resulting in impaired reliability of the results (Prata et al., 2019a; Raju et al., 2020). Depending on the used method to extract microplastics from the organic-rich matrix, the properties of microplastics may also be affected (Lessa Belone et al., 2021).

A common procedure for the extraction of microplastics from sludge includes either a separation of microplastics solely by density (Corradini et al., 2019; Mahon et al., 2017), or the combination of an oxidative digestion of organic matter and density separation (Edo et al., 2020; Hurley et al., 2018; Li et al., 2019; Xu et al., 2020; Zhang et al., 2020). These methods, however, involve certain limitations. The reliability of separating microplastics by density has been criticised because microplastics exist in a range of densities depending on the polymer (Lares et al., 2019). In addition, the actual density of plastics depends on the used additives (Turner and Filella, 2020), further widening the density range of microplastics. Another disadvantage encountered in the density-based separation is the lower recovery rate of fibrous microplastics compared to that of microplastics with more spherical form (Hurley et al., 2018; Li et al., 2020; Xu et al., 2020). In organic-rich matrices, microplastics are also susceptible to biofouling that may change their intrinsic density or affect their floating velocity (Ballent et al., 2016; Li et al., 2020; Munno et al., 2018). Indeed, Li et al. (2020) discovered that the extraction efficiency was improved when an oxidative digestion was conducted before the density separation. Therefore, it can be assumed that the extraction of microplastics is more reliable when an oxidative or other type of chemical digestion is included, and the digestion should be conducted before a density-based separation step to decrease the effects of biofouling on the extraction efficiency.

A major drawback of the chemical digestion, on the other hand, is the potential of affecting the microplastics or even destroying them (Lessa Belone et al., 2021; Munno et al., 2018). An optimised digestion procedure requires a careful choice of the digestion agent and the digestion temperature. Hydrogen peroxide (H_2O_2), either used on its own or combined with an iron catalyst (Fenton's reagent), has often been considered as the most optimal digestion agent when comparing its efficiency in removing organic matter to its potential effects on microplastics (Hurley et al., 2018; Pfeiffer and Fischer, 2020). When considering the digestion temperature, Munno et al. (2018) recommend maintaining the temperature below 60 °C to minimise the potential losses of microplastics. However, Lessa Belone et al. (2021) noticed that several commodity plastics were affected in a digestion at incubation temperatures of 40–50 °C. Hurley et al. (2018) also suggest maintaining the digestion temperature below 40 °C to optimise the extraction process.

Despite being commonly used, there is limited knowledge on the actual efficiency of H_2O_2 digestion in removing organic matter in the context of microplastic analysis from sludge matrix (Hatinoğlu and Sanin, 2021). In fact, the oxidative digestion has quite often been optimised in terms of minimum effect on microplastics (Li et al., 2020; Sujathan et al., 2017; Tagg et al., 2017), whereas the degree of oxidation of organic matrix has been more seldomly studied (Hurley et al., 2018; Lavoy and Crossman, 2021). However, an optimisation of the organic matter reduction can be assumed to increase the accuracy of the microplastic analysis from organic-rich matrices by increasing the liberation of microplastics from organic aggregates (Li et al., 2019). Therefore, in this paper, we optimised the reduction of sewage sludge organic matter by varying some of the digestion process conditions, while aiming for an increased loss of total solids (TS) and carbon content of the sludge matrix. The varied process conditions were the use of sodium dodecyl sulphate (SDS) as a pretreatment, and the duration of the first oxidation step conducted with H_2O_2. According to our knowledge, an analysis of carbon content has not been used before to optimise the digestion of sludge for microplastic analysis. After the optimisation phase, the possible effects of the digestion on microplastics were examined and finally the extraction efficiency was estimated by using fluorescent reference microplastics.

2. Materials and methods

2.1. Wastewater treatment plant and sludge collection

The sewage sludge was collected from Viinikanlahti WWTP in Tampere, Finland. The plant operates with the unit processes of screening, grit removal, primary sedimentation, activated sludge process and secondary sedimentation. The average flow rate to the WWTP is approximately 70,000 m^3/d. The sewage sludge originating from primary and secondary sedimentation was collected after thickening in a steel canister from a tap and transported to the laboratory where the sludge batch was thoroughly mixed and samples of approximately 20 mL were taken into glass bottles. In addition, separate samples of each sludge batch were taken for the determination of TS and volatile solids (VS), as well as for the determination of carbon content. The bottled sludge samples were stored at 4 °C for maximum three weeks. Altogether five sludge batches were collected during the period from March to August 2021. The sludge batches were always collected in midweek and approximately at the same time of the day to minimise the day-of-week/time-of-day variation in the consistency of sludge. The average TS and VS (± standard deviation, S.D.) of the collected sludge batches were 4.80 ± 0.50% and 3.17 ± 0.34%, respectively.

2.2. Extraction of microplastics from the sludge

2.2.1. Prefiltration

The extraction process began with a prefiltration of sludge in a filtering system based on the filter device designed by Talvitie et al. (2015), where three filters connected by tubes were stacked on top of each other. In this study, the diameter of the tubes was 102 mm and a vacuum gas pump (VWR, United States) was applied to accelerate the filtration. The nominal mesh sizes of the stainless steel filters (The Mesh Company, United Kingdom) were 360 μm, 123 μm and 26 μm from top to bottom. The two uppermost filters were used to reduce clogging of the lowest filter, the mesh size of which determined the approximate minimum size for the captured particles. Hence, no size fractioning was performed.

Prior to the prefiltration, 20 mL of sludge (dry weight of approximately 1 g) was diluted to 200 mL with ultrapure water. The diluted sludge was

then filtered, after which the filters with retained solids were removed one by one from the stacked filter system. Upon removing a filter and the tube section above it, the tube walls below the removed filter were rinsed with ultrapure water to the next filter to minimise any sample losses. The removed filters were placed on top of each other in a beaker, where the first step of the digestion was conducted.

2.2.2. Digestion

The digestion procedure principally followed the method developed by Lessa Belone et al. (2021). As a pretreatment for the oxidative digestion, the filters with retained sludge were immersed in SDS (Acros Organics, United States) in the beaker for an average (± S.D.) of 24 ± 1.3 h at 40 °C. SDS concentrations of 1% and 1.5% were used as variables.

Next, the contents of the beaker, along with the filters from prefiltration, were diligently rinsed with ultrapure water onto a 47 mm glass vacuum filtration unit (Merck Millipore, Germany) employing a new stainless steel filter of 26 μm mesh size. The filter was replaced upon clogging and each used filter was placed in a beaker. As a first digestion step, 40 mL of 30% H_2O_2 (Merck, Germany) was added, and the digestion was let to proceed for an average of 24 ± 0.8 h, 47 ± 1.8 h, or 72 ± 1.3 h at 40 °C in the beaker covered with perforated aluminium foil. After the first digestion step, the vacuum filtering procedure was repeated and used filters were again placed in a beaker, to which 30 mL of 30% H_2O_2 was added. The duration of the final digestion step was 24 ± 0.6 h. Lastly, the filters used in the final digestion were rinsed to new filter(s), which were dried at 105 °C when used for TS and organic content analysis, and at 40 °C when employed in imaging. The dry weight of each filter was determined before use and after rinsing to determine the TS losses on filters. Fig. 1 illustrates the whole extraction process along with the sampling stages.

Cotton lab coats and glass or metal equipment were used throughout the experiments whenever possible, but no other special precautions to avoid microplastic contamination were taken because the effects of such contamination on the results were considered negligible, and analysis of indigenous microplastics was not the objective of this study.

For the examination of TS and carbon content reduction along the treatment process, samples were collected at several stages of the treatment procedure. The first sample was taken after the primary filtration (stage 1), the second after the SDS treatment (stage 2), the third after the first H_2O_2 digestion (stage 3) and the fourth after the second H_2O_2 digestion (stage 4), i.e., at the end of the experiment (Fig. 1). Each experiment, done in duplicate or triplicate, was terminated at the point of sample acquisition to enable the determination of the complete TS of the remaining sample mass. It was assumed that drying the sample at 105 °C for TS analysis would change the susceptibility of organic matter for degradation in later treatment stages (Al-Azzawi et al., 2020), and therefore continuing the treatment after drying the sample was not considered as an option.

The remaining TS at each stage was compared to the calculated original TS of the untreated sludge. The remaining TS included both the retained material on the filter at a certain stage (e.g., on stage 3) as well as the residues remaining on the rinsed filters from the previous stage (e.g. stage 2). This way the TS reduction caused only by the digestion and filtration were considered, neglecting the TS reduction caused by mass losses due to material remaining on filters after rinsing. However, the mass losses on filters were found to be minor, contributing to maximum of 3.5% of the remaining TS per stage.

The relation between the remaining TS and the original TS is denoted as remaining TS (%). Similarly, for the assessment of carbon content reduction, the carbon content per TS after each treatment stage was compared to the initial carbon content per TS of the untreated sludge and referred to as remaining carbon (%).

Used variables in the optimisation of the TS and carbon content reduction were 1) concentration of the SDS solution (1% or 1.5%) or exclusion of the SDS treatment, and 2) duration of the first digestion step (1, 2 or 3 days).

Table 1 summarises the process steps and used parameters.

2.3. Reference microplastics

Reference microplastics were employed to demonstrate the efficiency of the extraction process and the potential effects of the oxidative digestion on the integrity of microplastics. Fluorescent polyethylene (PE) microspheres in nominal size range of 125–150 μm (Cospheric, CA, USA) were selected of the available grades based on their density (0.995 g/cm^3) that was closest to the density of typical unfilled PEs, i.e. less than 1 g/cm^3 (Malpass, 2010). The size was selected in accordance with a typical size range of non-fibrous microplastics detected in sewage sludge in past studies. For

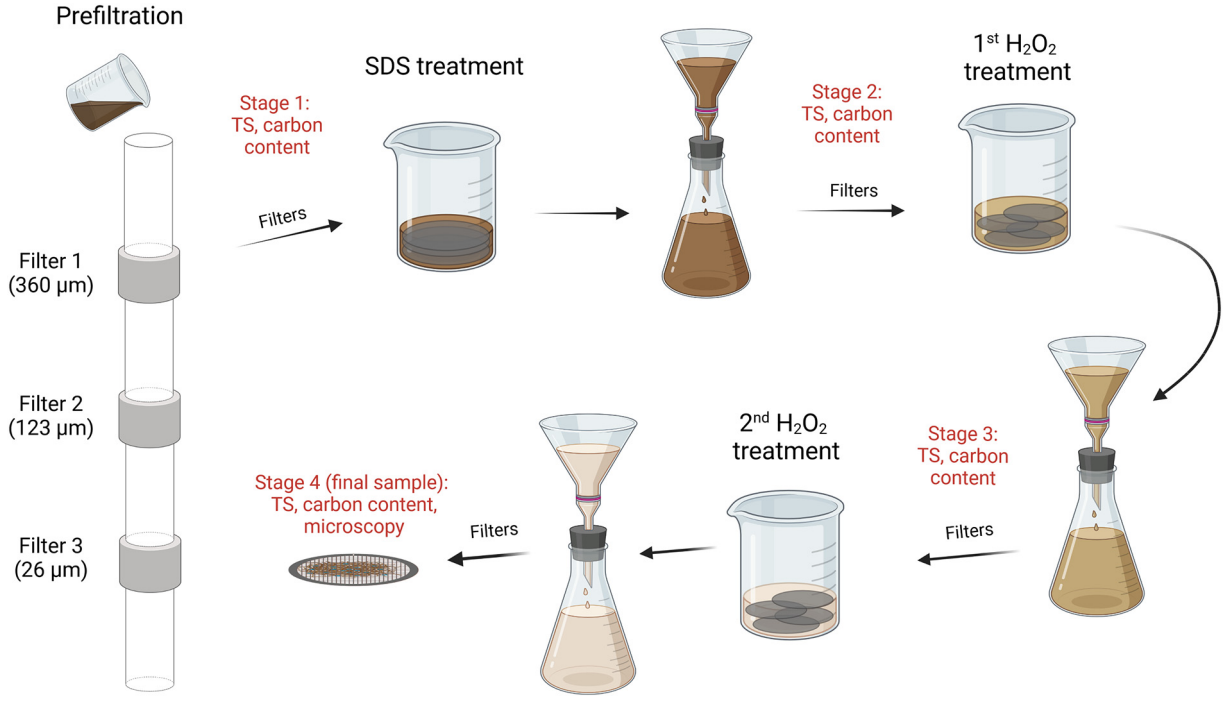

Fig. 1. Steps of the digestion process and stages where analyses were performed. (Created with BioRender.com).

Table 1
Description and parameters of each process step. Studied variables are presented in bold.

Process step	Description	Concentration of the chemical	Duration	Incubation temperature
Prefiltration	Filtration with three stacked filters (360 μm, 123 μm and 26 μm)	N.A.	N.A.	N.A.
SDS treatment	Prefilters and retained solids immersed in SDS solution	**None**[a]**/1%/1.5%**	24 h	40 °C
First digestion	26-μm filters and retained solids immersed in H_2O_2	30%	**1 d/2 d/3 d**	40 °C
Second digestion	26-μm filters and retained solids immersed in H_2O_2	30%	24 h	40 °C

N.A. = not applicable.
[a] Step excluded.

instance, Magni et al. (2019) and Raju et al. (2020) observed that a majority of the microplastics detected in return activated sludge belonged to the size group of 100 μm to 500 μm and 125 μm to 250 μm, respectively

Fibrous reference particles made of polyamide 6.6 (PA66) were purchased as 0.7 mm long flock fibres in fluorescent pink colour (Campbell Coutts Ltd., Hampshire, UK). The nominal diameter of the fibres was 1.5 decitex, i.e., approximately 13 μm for PA66. The diameter of the fibres was chosen in accordance with the average diameter of fibres detached from textiles upon washing, which has been observed to range from 12 to 18 μm (De Falco et al., 2019; Napper and Thompson, 2016). The variation in the diameter has been discovered to be somewhat larger, from 5 μm up to 34 μm, in fibres detected in sludge (Edo et al., 2020). According to Corradini et al. (2019), however, fibres belonging to the size group 10–20 μm in diameter were detected most frequently in sludge and soil samples.

For the extraction efficiency tests, a known number of both microspheres (approximately 45 pcs) and fibres (approximately 30 pcs) were introduced to the sludge samples after their dilution. The sludge samples were then treated by the optimised extraction procedure and the extraction efficiency was determined in duplicate based on the number of reference particles detected in the final digested samples.

2.4. Analysis methods

TS and VS content of the untreated sludge were determined in triplicates following the standard SFS 3008. In short, the procedure involved drying the samples at 105 °C for 24 h (TS), followed by heating in a muffle furnace at 550 °C for 2 h (VS). TS was also determined in the samples collected at different stages of the treatment process, and after the TS determination, carbon content was analysed from these samples by an organic elemental analyzer (OEA, Thermo Scientific FlashSmart Elemental Analyzer (CHNS/O)). Due to the high fibre content of the samples in the early stages of the extraction process, a pair of forceps was used to rip the dried samples into small pieces for the OEA. Samples of 2–3 mg were weighed with a microgram balance (Mettler Toledo WXTS Microbalance) in tin cups. Calibration was performed by applying 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl)thiophene (BBOT) as a standard. Helium was used as a carrier gas and oxygen was used for sample oxidation. The analysis was performed with 3–5 parallel samples.

For the determination of the extraction efficiency, a fluorescence microscope (Zeiss Axio Observer Z1, filter set 00) was employed to detect the fluorescent reference microplastics after the digestion process. In addition, reference microplastics were imaged before and after the digestion using a scanning electron microscope (SEM, JSM-IT500, JEOL) to detect possible effects on the particle size and surface topography induced by the digestion. The observation of the effects of the digestion on microplastics was restricted to visual examination only, because in our concurrent study, Lessa Belone et al. (2021), a wide range of analysis methods was employed to examine the effects of a similar digestion process on different polymers. To determine whether there were statistically significant differences between the measured particle sizes before and after the digestion process, Welch's *t*-tests were conducted in Analysis ToolPak of Microsoft Excel (*t*-Test: Two-Sample Assuming Unequal Variances, $\alpha = 0.05$). Prior to SEM imaging, samples were coated with a thin metallic coating to increase their conductivity. Imaging was conducted in a low-vacuum mode of 15 Pa pressure using an acceleration voltage of 10 kV.

3. Results and discussion

3.1. Optimisation of the reduction of TS and organic content

3.1.1. Prefiltration and SDS treatment

Approximately 25% of the original TS and carbon were removed already in the prefiltration step (Fig. 2), which indicates that approximately one fourth of the TS in the untreated sludge constitutes of particles smaller than 26 μm (the mesh size of the bottom filter). When considering the optimal SDS concentration, 1% SDS resulted in higher loss of TS and carbon

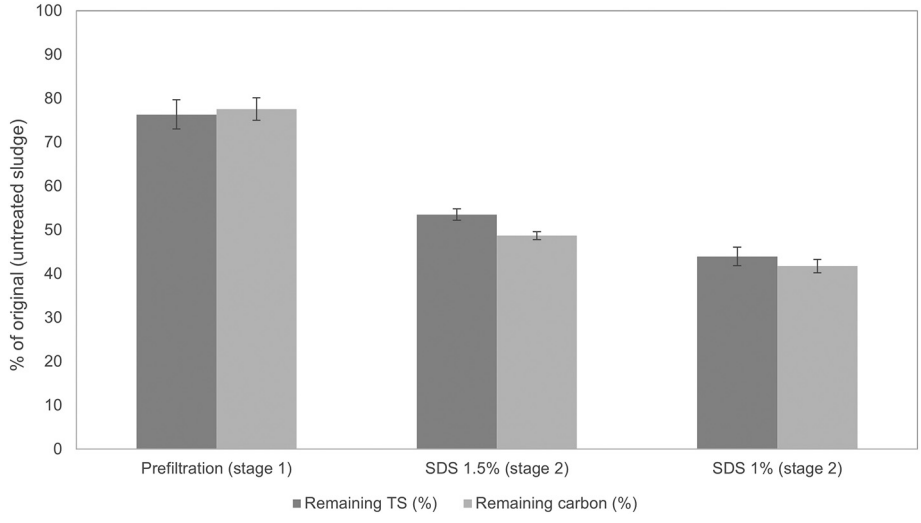

Fig. 2. Average remaining TS (%) and carbon (%) (± S.D., $n = 2$ or 3) in relation to the original untreated sludge (100%) determined after the prefiltration (stage 1) and after the SDS treatment (stage 2) with SDS concentrations of 1% and 1.5%.

content than 1.5% SDS (stage 2). This was assumed to be due to the increased foaming during the filtration following the SDS treatment when using the higher SDS concentration, which made the filtration less efficient and therefore caused more sample mass to be retained on the filters.

The effect of the exclusion of SDS treatment was examined by comparing the remaining TS (%) and carbon (%) in the final stage of the extraction process. The exclusion of the SDS treatment clearly increased the average remaining TS and carbon content (14.1 ± 3.2% and 8.5 ± 4.0%, respectively) compared to the use of 1% SDS (4.5 ± 1.3% and 1.9 ± 0.3%, respectively) when other process parameters were kept the same (Fig. S1 in supplementary information). The benefit of the SDS treatment seen on stage 4 may partly be explained by an increase in the effectiveness of digestion: when the SDS treatment was included, the first H_2O_2 addition resulted in foaming, which was not observed when the SDS step preceded the H_2O_2 addition (Fig. S2). The foaming may have led to a decreased contact between the filters and H_2O_2, resulting in reduced digestion of organic matter.

After the SDS treatment, it was first assumed that all mass loss was due to the filtration because no oxidative digestion had yet been applied. The assumption was supported by the observations of Prata et al. (2019b), according to which the digestion efficiency of 10% SDS solution on various natural organic matter samples was similar or lower to water in varying temperatures and treatment times. The benefit of SDS was expected to rise from the fact that as a surfactant, SDS would increase the surface area of the organic matter in sludge, thus making it more susceptible to digestion by H_2O_2 in later stages (Lessa Belone et al., 2021; Prata et al., 2019b). However, in the current study, SDS resulted in reduced TS and carbon content already before the oxidative digestion. The reduction was associated with a decrease in the particle size of the sludge matrix which led to the elimination of the smallest particles during the filtration following the SDS treatment (Fig. 2). On the other hand, Löder et al. (2017) also observed a significant loss in the dry weight of plankton samples upon SDS treatment, attributing the weight loss to the degradation of proteins. This attribution can be assumed legitimate as SDS is commonly employed in protein biochemistry studies to solubilise cell membrane proteins (Seddon et al., 2004). As proteins form one of the main components of the organic matter in sewage sludge, especially in the sludge from secondary settling (Jimenez et al., 2013), it can be assumed that the advantage of the SDS treatment in the context of sewage sludge would also partly derive from the solubilisation of proteins.

Based on the results, the SDS step was considered advantageous and was thus included in the further experiments. The SDS concentration of 1% was chosen based on the increased loss of TS and carbon and because the diminished foaming improved the feasibility of the method by reducing the duration and laboriousness of the following filtration step. Decreased chemical consumption was also considered as a benefit of the smaller SDS concentration. Before the current study, SDS has rarely been used as a pretreatment for a chemical digestion of sewage sludge, except for Lessa Belone et al. (2021). However, Xu et al. (2020) have used SDS to promote the separation of microplastics in flotation, whereas in other studies an SDS treatment has been applied prior to enzymatic digestion of sludge (Chand et al., 2021; Rasmussen et al., 2021; Sakali et al., 2021).

3.1.2. Duration of the first digestion step

The effect of the duration of the first digestion step was examined by investigating the TS and carbon content reduction after stages 3 and 4. After the first digestion, the lowest average remaining TS (%) was achieved with the shortest duration (Fig. 3). On the other hand, the remaining carbon (%) was rather similar (4.7% ± 0.2%) irrespective of the first digestion time. While it was hypothesised that the longer treatment time would result in smaller remaining TS (%) and carbon (%), it was observed that the three-day digestion caused the liquid to be completely evaporated, which questions the comparability of the obtained result. However, after the second digestion step, clearly less TS and carbon remained when a two-day treatment time had been used in the first digestion step. Because no variables were introduced in the second digestion, it can be assumed that these changes were induced by the variation in the treatment time of the first digestion step. This implies that, when optimising the digestion process, it is important to conduct all steps to be able to conclude which change of process parameter is ultimately advantageous.

Based on the results (Figs. 2 and 3), incorporating an SDS treatment step with a concentration of 1% into the digestion process and selecting a duration of two days for the first digestion step were considered optimal for the reduction of TS and carbon content in the sludge matrix. Fig. 4 presents the evolution of the remaining TS and carbon in grams along the consecutive

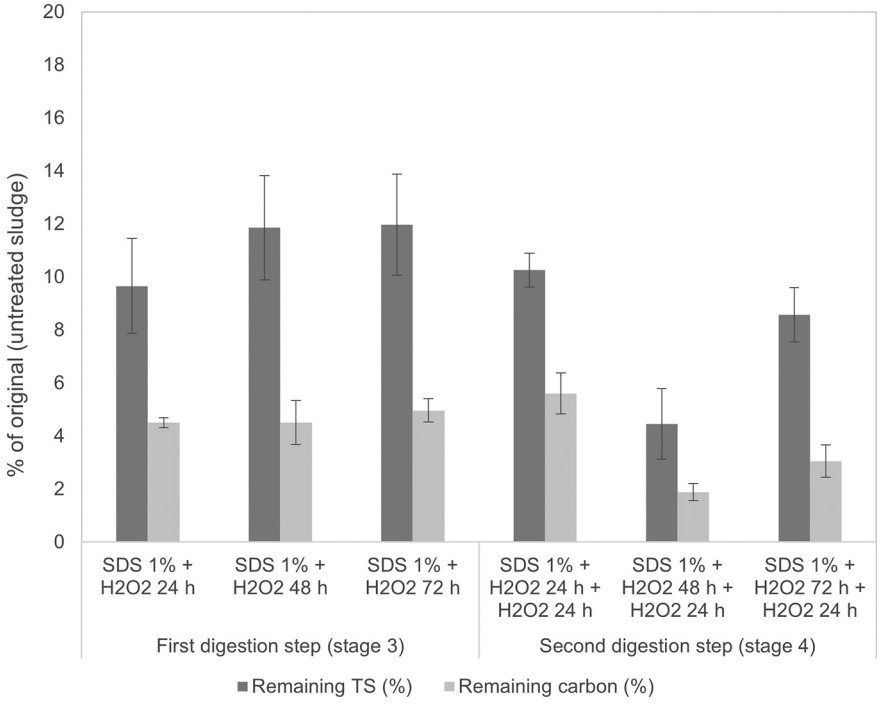

Fig. 3. Average remaining TS (%) and carbon (%) (± S.D., n = 2) in relation to the original untreated sludge (100%) determined after the first (stage 3) and the second digestion step (stage 4).

stages of the optimised digestion process. Until stage 3, both TS and carbon content decreased steeply, by 89.1% and 95.7%, respectively, compared to those of untreated sludge. The carbon content reduction can be compared to the mass loss and organic content removal of 41% and 80%, respectively, determined by Hurley et al. (2018) after a digestion employing 30% H_2O_2 at 60 °C. If assumed that the oxidative digestion decreases carbon content and organic matter in the same proportion, the higher removal rate obtained in the current study, especially in the case of TS reduction, can be accounted for the repeated filtration steps that removed some portion of particles smaller than 26 μm irrespective of their nature. Lavoy and Crossman (2021) combined a catalysed H_2O_2 oxidation with the use of a commercial enzymatic septic tank cleaner and achieved reductions in sludge TS by 93% at 55 °C and 89% at 40 °C. They also reported that the proportion of organic matter remaining after the digestion was 3.9% at 55 °C and 5.6% at 40 °C. However, the mass of remaining organic matter was not compared against the original sample organic matter, but to the original sample TS, and thus these values are not directly comparable to the results of the current study. Nevertheless, the results of Lavoy and Crossman (2021) offer an interesting comparison point to this study, because similar original sample TS (approximately 1 g), mesh size (approximately 20 μm) and temperatures were employed in both studies. The results of these studies support the idea that a step-wise oxidation, whether solely chemical or chemical-enzymatic, offers an approach to maximise the TS and organic content reduction in a relatively short time.

Bretas Alvim et al. (2020) achieved a reduction of 92% in total suspended solids of activated sludge during an oxidative treatment involving a digestion by 30% H_2O_2 at 60 °C for four hours. However, even though the volume ratio of H_2O_2 to sludge was the same as in the current study (2:1, when stage 4 is excluded), the VS content in the sludge treated by Bretas Alvim et al. (2020) was significantly smaller than in the thickened sludge used in the current study, approximately 2.1 vs. 30 g/L, respectively. Thus, the H_2O_2 dosage in relation to the sample VS applied by Bretas Alvim et al. (2020) was about 14-fold compared to the dosage used in the current study, when stage 4 is excluded. In another study, the total dosage of 200 mL of 30% H_2O_2 with iron catalyst was added in two steps for 10 g (wet weight) sewage sludge, allowing a two-day digestion (Cunsolo et al., 2021). However, no details of the sludge TS or VS were provided. In the current study, altogether 70 mL of 30% H_2O_2 was used for 20 g (wet weight) sewage sludge. These comparisons suggest that by greatly increasing the H_2O_2 dosage in relation to the sample VS, the duration of the digestion can be significantly reduced. Nevertheless, the trade-offs with sample representativeness, laboratory safety and expenses need to be carefully evaluated.

When stage 4 is included, the reduction was 95.6% in TS and 98.1% in carbon content (Fig. 4). The result suggests that a possible third digestion step would only nominally reduce the remaining carbon content while adding to the laboriousness of the extraction procedure. Therefore, incorporating a third digestion step is not recommended based on the present results. On the contrary, the remaining TS and carbon after stage 3 are already lower than, or comparable to, the results reported by Hurley et al. (2018) and Lavoy and Crossman (2021), and therefore, depending on the further extraction and analysis methods, it may be sufficient to terminate the digestion process already after stage 3.

3.2. Effects on microplastics

The digestion process did not have a notable effect on the size or surface topography of the reference microspheres (Fig. 5). The average diameter (± S.D.) of pristine microspheres was 149.1 ± 6.6 μm (n = 10), whereas after the digestion process the diameter was 151.7 ± 3.6 μm (n = 11). There was no statistically significant difference between the results and thus it was concluded that the digestion process did not induce a loss of material in the PE reference spheres. After the digestion process, some residual organic and inorganic matter could be observed on the surface of the spheres. The surface topography seemed slightly rougher in pristine spheres compared to spheres undergone the extraction process. This observation is in line with the findings of Lessa Belone et al. (2021) who noticed that the surface topography of PE samples became smoother after a digestion process where similar treatment stages and parameters were used.

The average length and width of pristine PA66 microfibres were 650.1 ± 27.8 μm (n = 23) and 13.9 ± 0.7 μm (n = 11), respectively, whereas after the digestion process the length was 619.2 ± 30.1 (n = 30) μm and width 13.8 ± 0.9 μm (n = 15). The width of reference fibres therefore remained unchanged (no significant difference), but the reduction in the length was statistically significant (p = 0.0004) and could be attributed to the digestion process. PA66 is known to be prone to thermo-

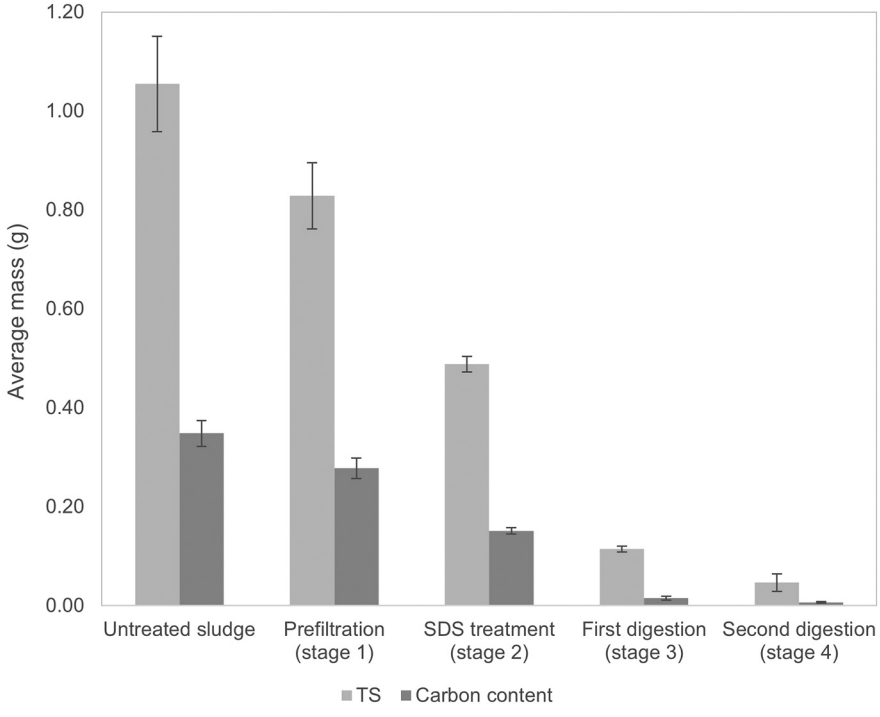

Fig. 4. Evolution of TS and carbon content (± S.D.) along the stages of the optimised digestion process.

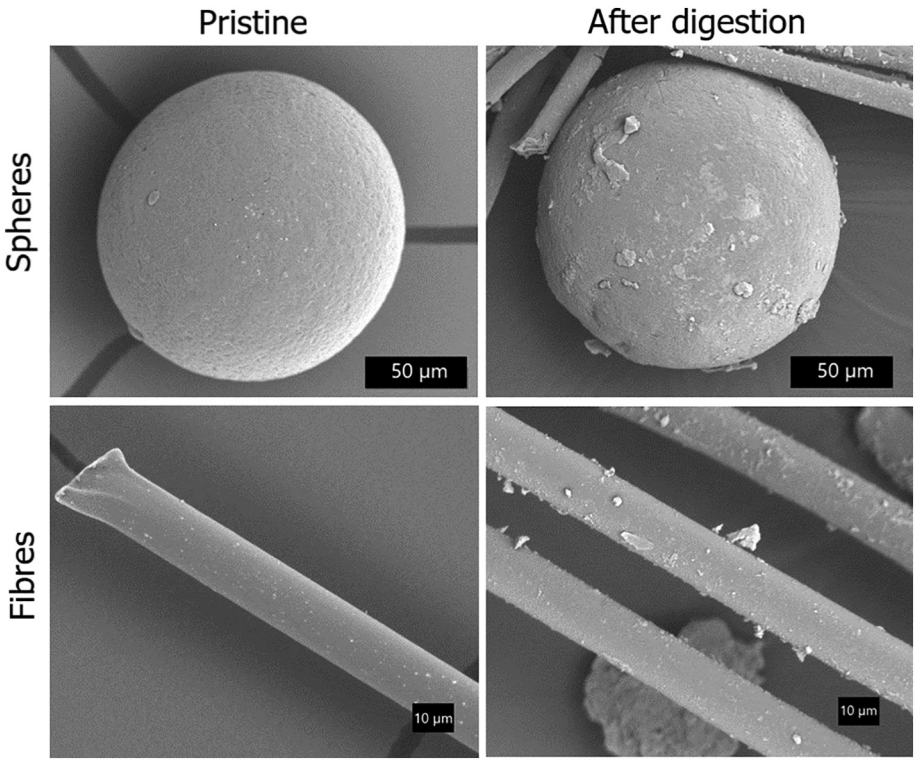

Fig. 5. SEM images of microspheres and -fibres used as reference microplastics. Left column: pristine reference microplastics. Right column: reference microplastics after the optimised digestion process.

oxidation in the presence of water and elevated temperatures (Lessa Belone et al., 2021), which could explain the reduction in length. On the other hand, the identification of reference microfibres in SEM in this study was only based on their distinctive profile (rather straight shape and known width), and due to the abundancy of indigenous microfibres in the processed sewage sludge samples, it is possible that some of the measurements were performed on other than reference microfibres.

The surface topography of reference PA66 fibres seemed unaffected by the digestion process: fibres exhibited a smooth surface before and after the process (Fig. 5), excluding particles of residual material from sludge attached to the surface after the digestion. The possible oxidation of PA66 reference fibres was therefore not confirmed by the examination of surface topography in this study, whereas Lessa Belone et al. (2021) discovered severe surface cracking in PA66 after a digestion process that involved maximum incubation temperatures of 50 °C. In this study, the incubation temperature was 40 °C, but as the temperature of the digestion was not controlled, it may have reached higher values (Munno et al., 2018). For comparison, Li et al. (2019) reported no effect on polyamide in H_2O_2 digestion at 70 °C, whereas Hurley et al. (2018) discovered that H_2O_2 digestion at an incubation temperature of 60 °C did not cause notable effects on studied microplastics, including PA66, but at an incubation temperature of 70 °C the effects on PA66 varied from complete fragmentation to no effects at all. In light of these results, the incubation temperature of 40 °C can be considered relatively safe for PA66, although controlled or recorded digestion temperatures would allow more precise conclusions.

3.3. Extraction efficiency

The extraction efficiency (± S.D., $n = 2$) was 84.0% ± 6.9% for reference spheres and 72.2% ± 6.6% for fibres. The nominal diameter of reference fibres (13 μm) was deliberately chosen smaller than the smallest mesh size (26 μm) of filters to examine the probability of fibres to pass through the filters vertically. When comparing the extraction efficiency of fibres to that of spheres (average diameter 149 μm) it may be assumed that some portion of reference fibres escaped through the filters. The escaping of fibres through the filters is more likely in later stages of the extraction process, when the sample volume and thus the accumulation of the organics on the filter has decreased. Despite thorough rinsing, the losses of both reference particle types can also be accounted for particles sticking to the walls of the used laboratory equipment (Möller et al., 2020). In addition, a possible factor decreasing the extraction efficiency can be an unsuccessful identification of reference particles by fluorescent microscopy because reference microplastics may have been covered by residual material or actual microplastics of larger size. Encountered examples of these kind of obstructing materials in the samples in the current study were pieces of films and nets of fibres. Furthermore, some uncertainty was related to the identification of visible fluorescent reference microplastics because, besides the reference particles, there were also other fluorescent particles in the treated samples originating from sludge.

The extraction efficiency obtained in this study is not directly comparable to many other studies because only digestion was considered here, and it is seldomly used as the only extraction method for sludge samples. However, Lares et al. (2019) compared different extraction methods and a wet H_2O_2 oxidation was one of the examined methods. The extraction efficiencies obtained in their study were over 99% for non-fibrous particles and 91% for fibres in the case of oxidative digestion. In studies involving both an oxidative digestion of sludge and a density-based separation of microplastics, extraction efficiencies of approximately 70–85% for fibres and over 95% for spheres and granules have been achieved (Hurley et al., 2018; Li et al., 2020). However, the comparison of the extraction efficiencies between studies is further complicated by factors such as sizes and shapes of reference microplastics. For instance, Lares et al. (2019) employed several shapes of non-fibrous particles, the longest dimension of which varied from 300 μm to 3400 μm, and fibrous particles with length of 600–6700 μm, whereas Hurley et al. (2018) used microspheres in two size classes, 425–500 μm and 850–1000 μm, and fibres ranging from 322 to 395 μm in length. Apart from the length of fibres employed by Hurley et al. (2018), the dimensions of reference microplastics used in these two studies are larger than those used in the current study. Handling larger reference particles is less burdensome, and their identification and counting

more reliable, but they can be assumed to facilitate attaining extraction efficiencies which are not necessarily in line with the extraction efficiencies obtained with actual microplastics. An important aspect in the extraction method optimisation is ensuring an appropriate representativity of the determined extraction efficiencies in relation to extracting actual microplastics. This is best achieved by selecting reference microplastics that reflect those found in the respective environments.

3.4. Implications for the oxidative digestion of sludge and the extraction of microplastics

This study demonstrated the analysis of carbon content by OEA as a method to monitor the digestion efficiency. The analysis of carbon content by OEA offers a quick and easy alternative to quantify the removal of organic content compared to other methods, such as the measurement of chemical oxygen demand (COD) suggested by Nguyen et al. (2019). Even though the carbon content is not a direct measure of the total amount of organics in the sample, we believe that the measured decrease in carbon content depicts the decrease in organic content more accurately than associating the total mass loss after digestion with the loss of organic content (Hurley et al., 2018). The problem in the latter method is, according to Al-Azzawi et al. (2020), that some part of the inorganic content may also dissolve during the digestion, resulting in additional weight loss that would be incorrectly attributed to the removal of organic content.

To enable the application of digestion procedures to different (sludge) samples, the volumes and types of digestion agents should be set in proportion to certain quantitative parameters describing the organic content of the sample (Nguyen et al., 2019). In the digestion process optimised in this study, the average chemical consumption per gram of original sample VS (±S.D.) was 2.93 ± 0.27 g of SDS, 59.7 ± 6.0 mL of 30% H_2O_2 in the first digestion step, and 44.1 ± 3.8 mL of 30% H_2O_2 in the second step. While the use of SDS clearly enhanced the TS and carbon removal from sewage sludge, thus contributing to the aim of the present study, in other studies the foaming effect of SDS has been considered as a barrier for its use (Salmi et al., 2021). On the other hand, the concentration of the SDS solution largely affects the foaming effect, as discussed in Section 3.1.1. To further improve the feasibility of the optimised digestion method, decreasing the SDS concentration even further could be examined. For example, the SDS concentration could be set in proportion to the amount of proteins in the sludge. It has been shown in biochemistry research that 1.4 g of SDS is needed to bind 1 g of most proteins (Otzen, 2011; Reynolds and Tanford, 1970). This information could be used in optimising the SDS concentration, while also considering the complexity of components in sludge and other treatment conditions that may interfere with the theoretical binding ratio. In addition, while SDS is known to be highly effective in solubilising proteins (Seddon et al., 2004), other surfactants than SDS could be explored in order to identify a possible alternative with a lower foaming tendency.

In the described digestion process, the minimum dimension of extractable microplastics is principally defined by the mesh size of the filter, 26 μm. The mesh size was chosen based on notions that 20 μm is approximately the limit for microplastics identification by infrared spectroscopy, which is commonly used in microplastics analysis (Zarfl, 2019). The high organic content of sludge restricted from selecting smaller mesh sizes for this study. However, as was seen in the case of reference fibres, majority of them (72%) were retained despite having a diameter smaller than the mesh size of the filter, possibly because their length was significantly larger than the mesh size. On the other hand, microplastics with all dimensions smaller than the mesh size may be retained on filters, if attached to other, larger particles (Lares et al., 2018). The mesh size of filters should therefore not be considered as a strict boundary for the size of extractable microplastics. This also implies that a size distribution of analysed microplastics should rather be reported based on actual measurements, as conducted for example by Bretas Alvim et al. (2020), than on sequential filtering only.

The sample size in this study was 20 g of wet sludge, indicating a sample TS of approximately 1 g. Dry weights of sludge samples reaching 90 g have been reported (Wei et al., 2022), but more commonly sample dry weights of maximum 10 g have been employed (Edo et al., 2020; Hurley et al., 2018; Lares et al., 2018; Lavoy and Crossman, 2021; Xu et al., 2020; Zubris and Richards, 2005). The drying of sludge appears to be one method to increase the sample size (Li et al., 2019; Wei et al., 2022), but it may lead to a formation of hard sludge cakes of decreased digestibility (Lavoy and Crossman, 2021). Moreover, if the samples are ground after drying, the microplastics in them may disintegrate, leading to a false increase in the number of microplastics in results. In the current study, the sample size was determined by the capacity of prefiltration, the purpose of which was to remove the size fraction that was out of interest as discussed above. The elimination of the smallest size fraction by filtration in the beginning of the digestion procedure, corresponding to 23% of the original carbon content, also meant that SDS was only consumed by the organic size fraction included in the next treatment stage.

A comparison of both the extraction efficiencies and the effects of digestion on microplastics between different studies is hindered by the variety of used reference microplastics, which decelerates the development of microplastic extraction processes. To alleviate the problem, a set of uniform reference microplastics made of defined polymers and additives in defined shapes and sizes should be agreed on in the scientific community. This need has frequently been recognised in the field of microplastics toxicology (Hong et al., 2017; Shim and Thomposon, 2015; Siri et al., 2021). It is important to ensure that such a kit of reference microplastics would, at least to some extent, represent the characteristic distribution of actual microplastics encountered in different environmental matrices. For instance, including fibres in reference materials for sewage sludge is important because fibres are the most abundant shape of microplastics detected in sludge (Li et al., 2019; Raju et al., 2020; Ziajahromi et al., 2021). Moreover, a wide range of dimensions of reference particles, as used for example by Lares et al. (2019), would assumably serve the environmental representativity better than distinct size classes, especially if the size distribution is known.

4. Conclusions

The accuracy of a microplastic analysis from matrices of high organic content, such as sewage sludge, benefits from an optimised digestion of the organic matter. The objectives for such a digestion process include maximised organic matter reduction, conservation of microplastics and a high extraction efficiency. In this study, a reduction of TS by 95.6% and carbon content by 98.1% was achieved by applying 1% SDS solution as a pretreatment, followed by two-step digestion with 30% H_2O_2 at 40 °C. The optimised digestion procedure provided an extraction efficiency of 84% for 150 μm reference spheres and 72% for 650 μm long fibres and maintained the integrity of the tested reference microplastics. This procedure can therefore be used as a microplastics-conserving but effective means to reduce organic matter prior to other separation methods, such as density-based separation, allowing to considerably diminish the disturbing effect of the organic matrix on the separation efficiency of microplastics. The digestion procedure is also potentially applicable to other types of sludges of known organic content.

CRediT authorship contribution statement

Elina Yli-Rantala: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing – review & editing.

Maria Clara Lessa Belone: Conceptualization, Investigation, Methodology, Validation, Writing – review & editing.

Essi Sarlin: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Marika Kokko: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft, Writing – review & editing.

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.

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Appendix A. Supplementary data

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