Mapping of microplastics and endotoxins as a potential workplace hazard

Alessio Gomiero, Kjell Birger Øysæd, Emily Lyng, Bjørg Meling, Geir Skogerbø

Report 11-2020, NORCE Environment

Report title	Mapping of microplastics as a potential workplace hazard
Project No	297059
Institution	NORCE Environment
Client	IVAR IKS
Classification:	Open
Report No.	11-2020
ISBN	978-82-8408-117-5
No. of pages	25
Month of publ.:	10/2020
Citation	Gomiero A., K.B. Øysæd, E. Lyng, B. Meling, G. Skogerbø. "Mapping of microplastics and endotoxins as a potential workplace hazard". NORCE Environment report 11-2020.

Acronyms used

EVA	Ethylene vinyl acetate
FTIR	Fourier Transform Infrared Spectroscopy
HDPE	High density polyethylene
LDPE	Low density polyethylene
LOQ	Limit of Quantification
PA	Polyamide (nylon)
PAN	Polyacrylonitrile
PC	Polycarbonate
PE	Polyethylene
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
POM	Polyoxymethylene
PP	Polypropylene
PS	Polystyrene
PSUL	Polysulfone
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl Chloride
Pyr-GCMS	Pyrolysis gas chromatography mass spectrometry
SDS	Sodium dodecyl sulfate

Contents

Participants list

Name	Affiliation	Role
Alessio Gomiero	NORCE AS -Norwegian Research Centre	Project Leader, proposal developer analysis, data interpretation, reporting
Kjell Birger Øysæd	NORCE AS -Norwegian Research Centre	Method development, analysis, data interpretation
Emily Lyng	NORCE AS -Norwegian Research Centre	Sample preparation, reporting
Bjørg Meling	IVAR Iks	Sample collection coordination
Geir Skogerbø	IVAR Iks	Project Owner, reporting

Abstract

Humans are potentially exposed to microplastics through several exposure pathways including food, drink, and air. So far little is known about the occurrence of microplastics in indoor environments especially in connection with industrial activities dealing with the use of raw materials potentially enriched with micron sized plastics. In the present study, the indoor air of an industrial unit turning sewage sludge into fertilizer products has been characterized for microplastics content. Collected samples have been investigated by both a thermo-analytical method (pyrolysis-GCMS) and a vibrational microscopy (μ-FTIR) method. Synthetic fragments and fibers accounted, on average, for 9% of the total identified particles in the analyzed air. Polyester was the predominant synthetic polymer in all samples (31-61%), followed by polyethylene (4-58%), polypropylene (1–15%), nylon (2–34%) and acrylic paints (1–20%). Microplastics typically ranged from 10 μm (size limit of the current spectroscopic method) to 270 μm. As the identified microplastics can be inhaled, these results highlight the potential direct human exposure to microplastic contamination via indoor air in the working environments addressed in this study.

1. Introduction

The increasing use of plastic items in everyday products has resulted in the widespread presence of plastic waste in the natural environment (Horton et al. 2017). Plastics are one of the major waste articles globally and constitute 12% of solid municipal waste. The increasing presence and accumulation of plastics in the environment inevitably leads to demonstrably increased unwanted exposures and thus unknown and unforeseeable toxicological consequences. Although visually the most apparent form of plastics pollution are macroplastics, the highest health concern has been attributed to smaller fractions - microplastics (1 μm - 5 mm) and even more so, to nanoplastics (≤1 μm).

Potential health risks related to microplastics are not limited to indirect exposure via food and water, but more importantly airborne micro- and nanoplastics in both inside and outdoor environments pose a risk to humans directly through inhalation. Indeed, recent evidence indicates that the number of plastic particles inhaled is even higher than the amount ingested by a human through food or water. Although some data suggest biological adverse effects of microplastics in controlled conditions, these data originate from highly artificial model systems. Consequently, their translation to actual exposure conditions, including relevant particle types and sizes, exposure scenarios and durations, is lacking. An additional level of complexity arises from chemicals present as additives/contaminants/vibrios and pathogens in/on the plastics. Collectively, these unknowns do not allow for the full understanding and estimation of health risks caused by microplastics for humans. The scientific community considers micro- and nanoplastics exposure related health hazards likely.

Many industrial and construction sites have the potential to expose their employees to harmful dust, vapours and gases. Inhalation is usually the most significant route of entry into the body and so monitoring the air they inhale is vitally important. The utilization of

biological waste products (wastewater sludge, food waste, and spent digestate from biogas production) as a source of agricultural fertilizer and soil amendments has high priority in national and European circular economy strategies (EU, 2018). However, reutilization of biological waste materials commonly requires extensive processing, causing substantial exposure in the work environment to dust containing potential health hazards, and the sustainability of such approaches has thus been questioned (Heldal, 2015). As a responsible operator in this field, the IVAR IKS planned to carry out a pilot study of potential health hazards linked to microplastics in the work environment connected to the biological waste processing.

Thus, the aim of the present study was: a) to validate an analytical method to characterize the occurrence of airborne plastics in correlation with selected working environments where the occurrence of plastic and endotoxins is expected; b) develop a suitable procedure to integrate the sampling and the analysis of both microplastics and endotoxin content in indoor air within the same representative sample enabling expansion of knowledge regarding the occurrence and potential interaction between these two human health stressors.

2. Materials and methods

Origin of tested samples

The indoor air sampling was performed at IVAR's fertilizer production facility located in Mekjarvik, Randaberg. The production consists of several consecutive processes of fermentation, volume reduction and dehydration of the sewage sludge produced in the adjacent Nord-Jæren Sewage Treatment Plant (Figure 1). The facility has a production of approximately 5000 tons DM digestate per year.

Figure 1 – Fertilizer production facility adjacent to the Nord-Jæren sewage treatment plant (Mejarvik) owned by IVAR.

Sampling equipment and sampling strategy

The sampling devices consisted of two Casella Apex2 personal sampling pumps (Casella, UK) Figure 2). In brief, a known volume of air is drawn through a suitable sampling membrane using a sampling pump. For microplastics analysis GF/C Ø 25 mm 1,2 µm mesh fiberglass filters were selected for this study. The sampling pump is connected to an aluminum sampling head containing a suitable filter holder via a length of tubing (Figure 3).

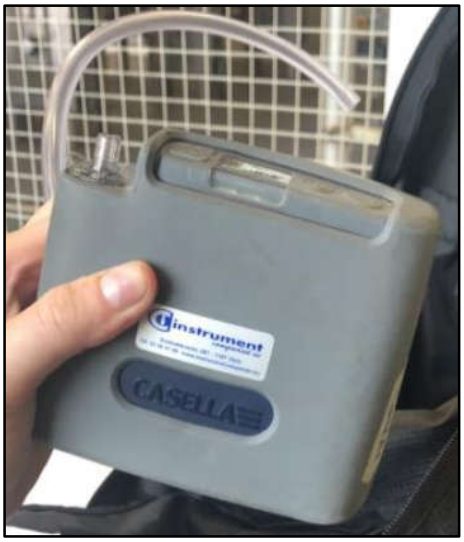

Figure 2 – Image of one of the Casella Apex2 personal sampling pumps used in the study. Credit: Geir Skoberbø, IVAR IKs

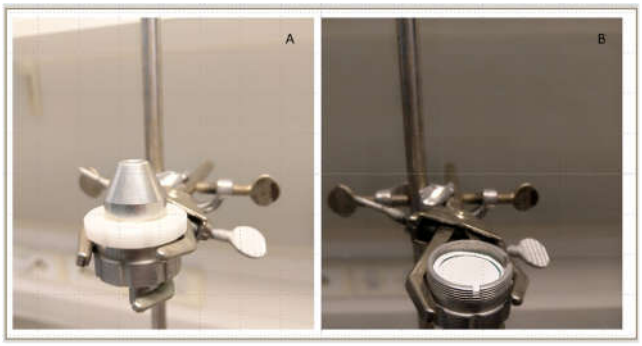

Figure 3 - Image of the aluminum sampling head (A) holding the 25 mm fiberglass filter (B).

For each of the sampling sessions one air sampling unit was worn by a volunteer operator and the second was positioned in an area of the of the facility close to the fertilized drying unit expected to be a hot spot of MPs emissions. Outdoor air samples collected 500 m away from the facility represented the control through the all the sampling sessions. The air flow through the filter was set to 2 L/min. Sampling devices were calibrated before any sampling sessions following the producer guidelines. Fiberglass filters used for air samples collection were pre-burned on muffle oven at 500 C for 3h to remove any plastics contamination in the filter surface.

Indoor air samples were collected during routine daily production between November 2019 and December 2020. A total of 17 samples were collected in the present study. Of these, 10 samples were used to test the sample preparation efficiency to remove interferents hampering the MPs characterization. The remaining 7 samples were analyzed for actual characterization of MPs in the addressed indoor environment (Table 1).

Table 1 – Description of the analyzed samples

Sampling site	Sampling date	Sampling type	Sampling time (min)	Filtered volume (m3)
E1	10.08.2020	Stationary	201	0,42
E3	19.08.2020	Stationary	361	0,72
E4	17.08.2020	Mobile (operator)	334	0,67
E5	17.08.2020	Stationary	330	0,66
E6	10.08.2020	Mobile (operator)	210	0,42
E7	19.08.2020	Mobile (operator)	331	0,66
Outdoor blank	17.08.2020	Stationary	400	0,89
Procedural Blanks	27.08.2020	Stationary		
Wet sedimentation trap	25.08.2020	Stationary	900 (two working days)	

Sample preparation for FTIR and pyr-GCMS analyses

The method developed in the present project aimed at extracting MPs from the filtered material and applying a gentle and efficient purification step before chemical identification in a way that allows for a quantitative analysis. The main interferents for a reliable quantification are the molecularly dispersed organic matter and biofilms that may aggregate the MPs and reduce the efficiency of the extraction process. Organic material may also interfere with the analysis causing both an increase in the background signal and a reduction of the signal-to-noise ratio thus hampering the characterization and quantification process. After some preliminary tests simulating different sample's incubation conditions and evaluating the resulting decrease of the background signal, a sample preparation based on a sequential on-filter incubation of 15 ml of 5% SDS, 15 ml of cellulase enzyme and 15 mL 30% H_2O_2 was established. After interferents were removed, fiberglass filters where flushed with filtered MilliQ water and sonicated on a $ZnCl_2$ (ρ= 1.85 g/cm^3) solution. The sample was transferred to pre-cleaned separator funnels and the density separation was allowed to run for 7 days. Floating particles were collected by filtration of a 1 µm mesh size stainless steel filter, washed with MilliQ water, and the filter was sonicated in an ethanol/water solution. Released particles were pre-concentrated on a RotaVapor system to a volume of 5 ml. Aliquots of 300 µl where deposited on SnSe windows for µ-FTIR scanning and the deposited material was flushed onto a fiberglass filter for the pyr-GCMS analysis afterwards. Three aliquots (replicates) were analyzed per sample.

Plastic free laboratories and contamination control

All sample preparation was performed in the NORCE Plast Lab located in Mejarvik. The dedicated area in the marine station is equipped with high efficiency low penetration HEPA filtration with an efficiency of over 99.9% for particles of 0.2-0.5 µm. Overpressure and an airlock prevent dust entry. The laboratory is entered with dedicated low-abrasion shoes and a cotton laboratory coat. Synthetic polymer fibers were avoided. Either no gloves or nitrile gloves were worn for the protection of personnel. To reduce sample contamination during sample digestion and analysis equipment in contact with the sample or solutions were made of glass or stainless steel. MilliQ water was used to prepare all solutions, and all final solutions were filtered using 0.7 µm glass fiber filters (VWR International) before use. All

tools and glass equipment were covered with aluminum foil and burned in a muffle oven at 500°C for 3 hours to remove plastic contamination and aluminum foil was used to cover processed filters during preparation and digestion when possible. An open glass jar of filtered MilliQ water was placed in the working area in the laboratory during the entire sample preparation and analyzed for MP to control for potential airborne particles. Furthermore, procedural controls consisting of blank filters processed through all steps of the sample's preparation were analyzed for plastics content. During the pyr-GCMS and µ-FTIR imaging analyses similar dust trap collectors were used to evaluate possible contamination from airborne particles. Furthermore, the steel cups used for the pyrolysis analysis were cleaned before use with a butane blow torch at 1400 °C to remove any plastic. The pyr-GCMS system was cleaned in between analyses by first running a clean empty cup containing a silylating agent to remove any polar, low-volatile contaminants from the GCMS system, followed by a second analysis performed on the same empty cup without the silylating agent to remove the excess of the derivatizing agent from the system.

Recovery tests

Recovery tests were performed by spiking fiberglass filters with approx. 100-200 particles of 2,5 µm red fluorescent beads (Cospheric, CA). The recovery rate was assessed sequentially by inverted fluorescence microscopy observations (Zeis AxioCam 100, BioNordika, Norway) and by pyr-GCMS. Overall recovery rate was 83-86%. This means that the method of extraction/purification is acceptable but not 100% and that some very fine particles likely escape extraction, which is common for all extraction procedures, including solvent extractions of chemical pollution.

Identification of MPs by vibrational spectroscopy: µFTIR

Fourier Transform Infrared spectroscopy (FTIR) is an established non-destructive technique in analytical chemistry using infrared (IR) radiation, also called vibrational spectroscopy. The FTIR instrument is equipped with a source that emits IR radiation of a spectrum of wavelengths at a known energy intensity level. The IR radiation is passed through the sample. Specific wavelengths match with atomic and molecular bond energies of a compound. The absorbed energy causes temporary charge changes within the chemical bonds and vibrating molecules. Compounds, such as natural and synthetic polymers, absorb infrared radiation at different intensities. The resulting spectrum "fingerprint" is used for molecule identification by comparison with reference databases of known compounds.

µFTIR imaging

The quantitative analysis of MP from 10-300 µm was done by µFTIR imaging (Primpke et al. 2019). With this system, both polymers and particle size distribution of an extracted sample can be determined, down to approximately 10 µm. This represents the technical limitation dictated by the analytical instrument used. Aliquots of the samples in ethanol/water solution were deposited on ZnSe windows to be imaged by the microscopic system. The windows were dried for 24 h at 40°C and placed on the IR reader plate. µFTIR imaging was

performed using a Thermo Scientific Nicolet iN10 coupled with Liquid nitrogen cooling system (Figure 4).

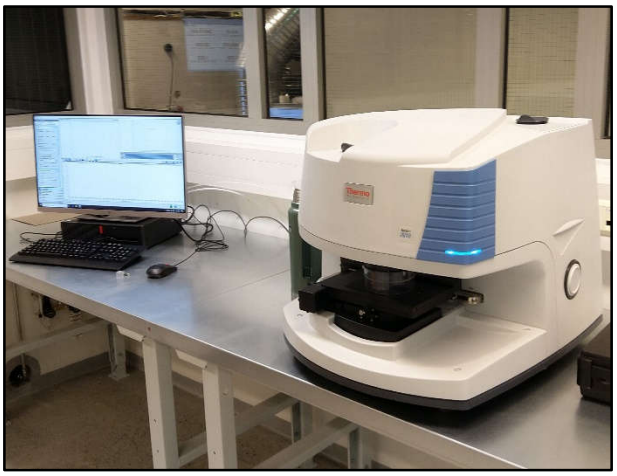

Figure 4 - µFTIR equipment at the NORCE Plast Lab (Photo: Alessio Gomiero, NORCE).

The filter was imaged in transmission mode following the procedure of (Vianello et al., 2019). During scanning, the sample was protected from contamination by a collar which is an integrated part of the equipment, and the sample kept under a constant flow of filtered air. The analysis was carried out by scanning the whole of each ZnSe window (active diameter of 10 mm, active area 78.5 mm^2). The scan time was approximately 3 hours. Automatic image processing detects the size and position of the particles, smoothest edges and assigns a polymer group to the particles. For dataset analysis, data was processed by siMPLE (v.1.0.0; simple-plastics.eu) and spectra were compared to libraries from Aalborg University and NORCE. The polymer groups included in the FTIR analysis are shown in Figure 5. The polymers that were not detected in any samples are excluded from graphs and tables. Figure 6 shows an example of the results obtained from a representative sample.

Fig. 5 - The polymer groups analyzed using the µFTIR analysis, and corresponding color codes used for the false color images.

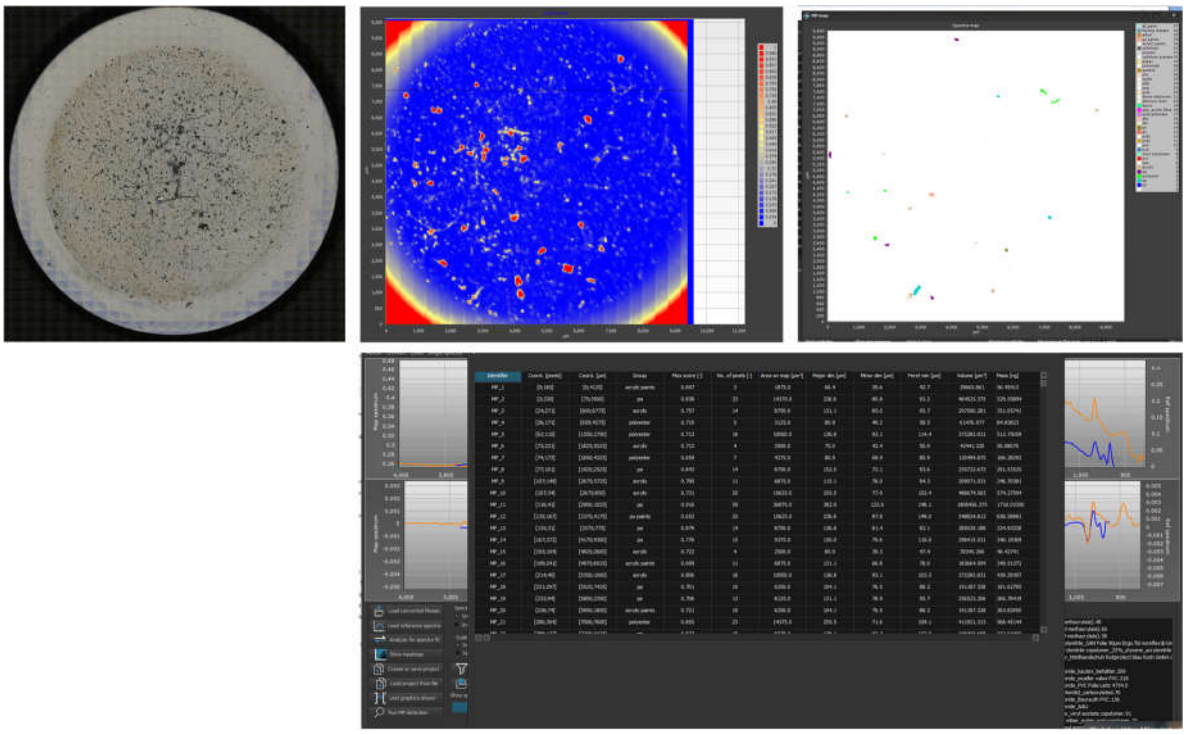

Fig. 6. Data acquisition on a representative sample investigated within the study. Visual image of the sample E3 deposited on a ZnSe window (A). Corresponding IR heatmap from blue indicating no signal to

red indicating high signal (B). Polymer type recognition and false color overlay (C), plot (MP map) of identified synthetic polymers with information on major and minor dimensions (D).

Thermal degradation analysis: Pyr-GCMS

Because the FTIR analysis method is non-destructive, the same samples have been further analyzed by pyr-GCMS after the FTIR analysis, providing direct analysis of the mass per polymer group. Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS) is a destructive method that uses thermal decomposition of materials at elevated temperatures in an inert (low-oxygen) atmosphere. Large molecules break at their weakest bonds, producing smaller, more volatile fragments. These fragments can be separated by gas chromatography and detected by a mass spectrometer. The output data can be used as a fingerprint to identify material. The obtained pyrograms, with peaks of ions appearing at different retention times, are compared with a customized database, and cross-checked with literature to identify the chemical composition of the material using recommendations and selection criteria from Gomiero et al. (2019). Standard curves with known concentrations are used to calculate the concentrations of target materials in the sample. Pyr-GCMS analyses were performed by NORCE (Stavanger) with a Shimadzu Optima 2010C GCMS controlled by GCMS solution V 4.45, equipped with a Rxi-5ms column (RESTEC, Bellefonte, PA) and coupled with Frontiers lab's Multi-Shot Pyrolizer EGA/PY-3030D with auto-shot sampler (BioNordika, Norway, Figure 7).

Figure 7 - Pyr-GCMS equipment at NORCE PlastLab (Photo: Alessio Gomiero, NORCE).

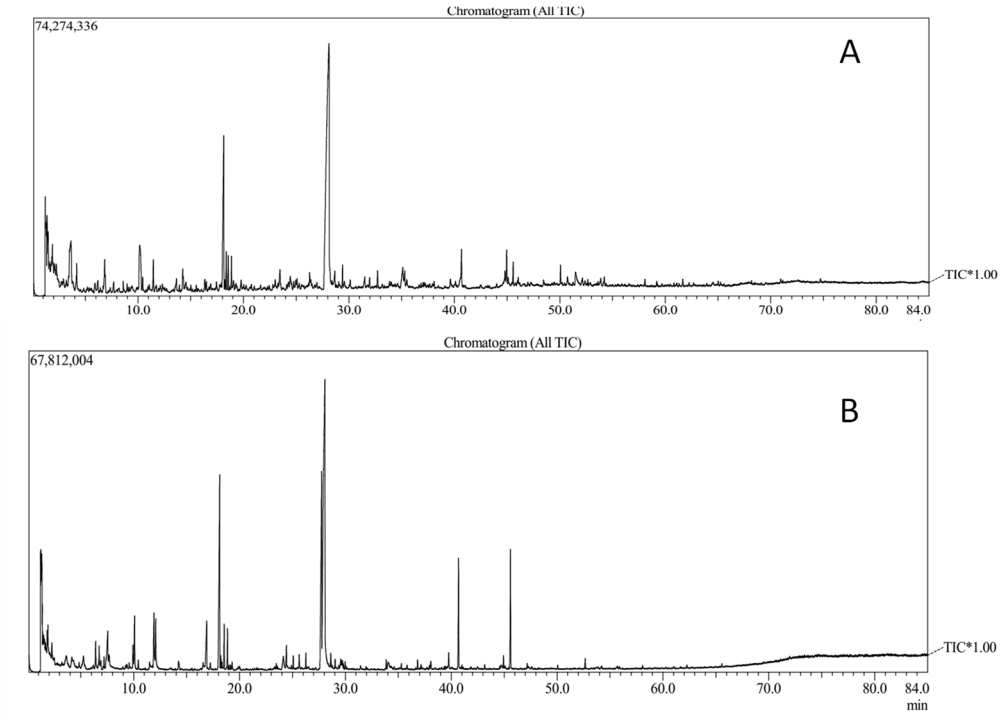

Figure 8. – Assessment interferents removal efficiency by obtained pyrograms from sequential sample preparation. Filter after SDS treatment (A); Filter after SDS and cellulase treatments (B).

Endotoxin analysis

The Endpoint Chromogenic Limulus amebocyte lysate method was used for the quantitative detection of bacterial endotoxin in the indoor air samples. After reconstructing the substrate and buffer, the samples are added. The reaction is then stopped with acetic acid. Finally, optical densities were measured. The concentrations of endotoxins obtained from the sample were converted from EU/mL to EU/m^3 using the following equation:

$$\frac{EU}{m^3} = \frac{1000\ L}{m^3}\ X\ \frac{concentration\ X\ dilution\ X\ extraction\ volume}{air\ volume\ sample}$$

Statistical analyses and graphics

Data were analyzed and graphs made using Primer 6.0, Statistica 10.0 or Excel. Analysis of variance (2-way ANOVA) was applied to test differences between sites (level of significance at $p < 0.05$). Post-hoc comparison (Newman-Keuls) was used to discriminate between means of values.

3. Results

Contamination monitoring

Three procedural blanks and six samples from the wet dust trap collectors were analyzed to monitor potential sources of contamination affecting the sample preparation and the analysis. The contamination was related to handling the sampling equipment, preparing the sample for analysis, and finally the analysis itself thus the degree of contamination was normalized against the number of blanks and not the filtered air volume.

The results showed a contamination of 2.4 ± 1.0 MPs per procedural blank sample. The polymeric composition of the contaminating MPs was 57% polystyrene, 22% polyurethane, 13% polyethylene, 4% polyester. The measured contamination of non-synthetic materials (protein-based material and cellulose) per blank dominating the particles' composition in the analyzed filters was 52 ±12 particles for protein-based material. In the meantime, the result of the wet trap collectors showed a contamination of 3.1 ± 1.5 MPs per trap located in the clean lab were the samples were processed and analyzed by µ-FTIR imaging and 4.7 ± 2.0 MPs in the room where the pyr-GCMS system is placed.

The polymeric composition of the wet trap collectors contaminating MPs were 53% polyethylene, 23% polyester, 19% nylon, 5% polyurethane. Protein-based material and cellulose were the most abundant non-synthetic materials in the analyzed filters as sample was 176 ±25 particles.

Airborne microplastic detection and quantification by FTIR and pyr-GCMS

FTIR Imaging analysis

The µFTIR-Imaging combined with automatic particle detection applying the software SimPle produced thee particle maps per sample. Seven indoor air samples, one outdoor air sample (control) and three blank type samples produced a total of thirty particle maps. The occurrence of synthetic and nonsynthetic particles was assessed by comparing the resulting IR spectra with a spectral reference database (Figure 5). All samples revealed the presence of MP as well as nonsynthetic particles, Figure 6).

The total 1823 MPs were identified among all processed indoor and outdoor (control) samples. Levels ranged from 10.4 ±1.5 MPs/m^3 in the outdoor sampling site E8 to 301.3 ±16.5 MPs/m^3 in E4 (Figure 8). On average 98.7 MPs/m^3 and 7.2 MPs/m^3 were observed in the indoor and outdoor air sampes, respectively.

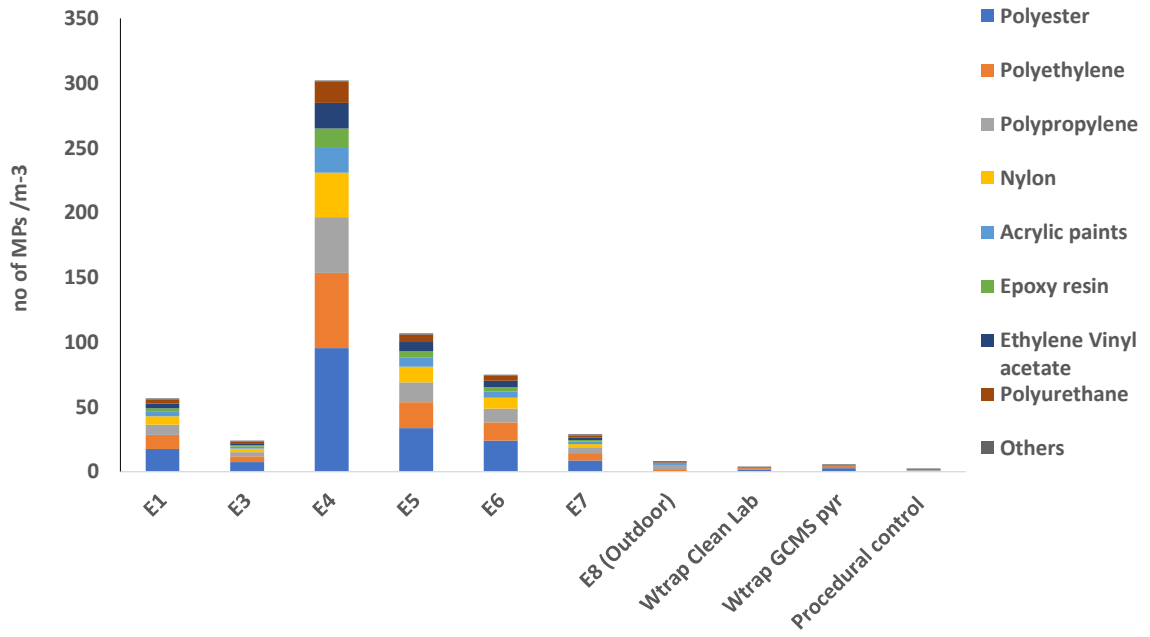

Figure 8 – Results of µ-FTIR imaging. Total and relative number of MPs in the investigated samples.

The polymer types identified in the indoor samples from both the stationary and mobile (operator) sampler were quite similar. The most abundant of the synthetic polymers was polyester (31–61%) followed by polyethylene (4–58%), polypropylene (1–15%), nylon (2–34%), acrylic paints (1–20%), epoxy resin (1–14%), ethylene vinyl acetate (1–19%) and (polyurethane (1–16%, Figure 9).

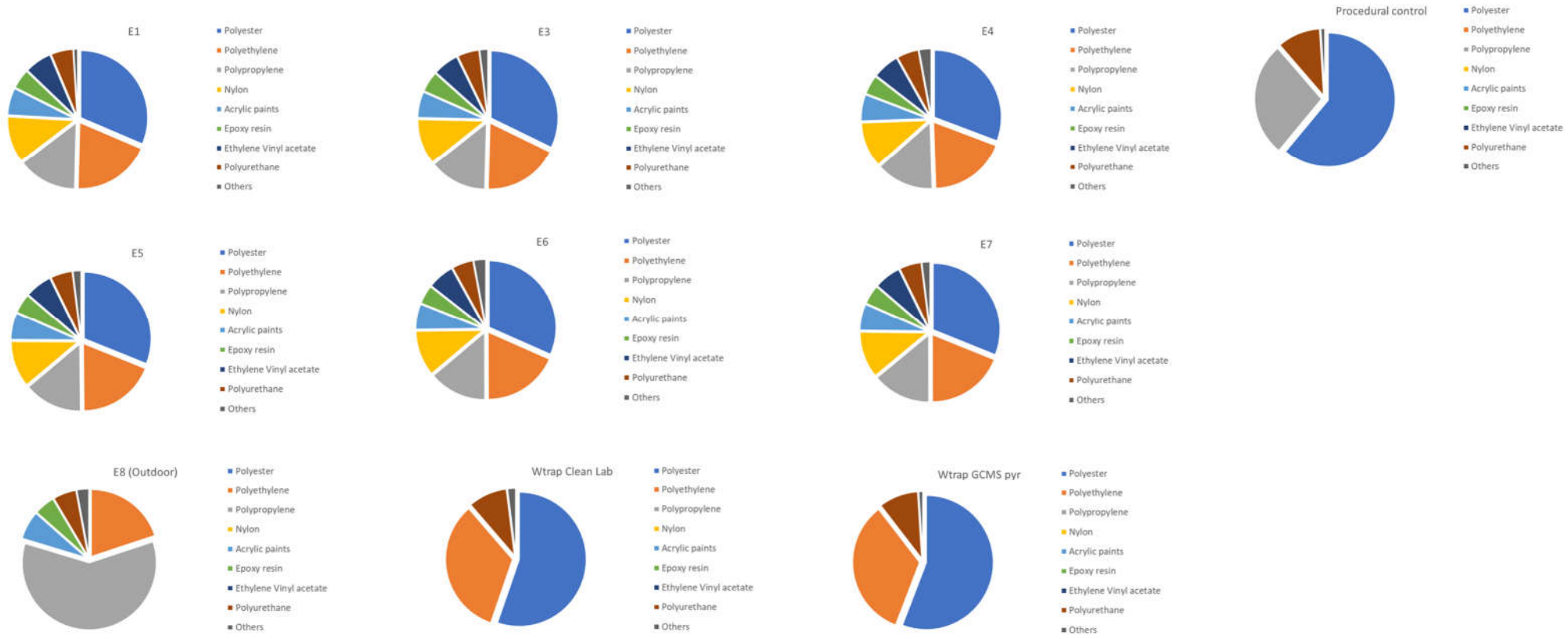

Fig. 9 – Pie charts of relative polymer distribution assessed by µ-FTIR imaging analysis. The category “Other polymers” groups the polymers present in lower percentages (polyvinyl acetate, phenoxy resin cellulose acetate and triacetate, polylactic acid, polycarbonate).

The other polymers occurred at lower percentages and were grouped as the sum of polyvinyl acetate, phenoxy resin cellulose acetate and triacetate, polylactic acid and polycarbonate. Most of the identified non-synthetic particles were cellulose-based particles (≈ 80%). The µFTIR-Imaging analysis did not allow discrimination within the non-synthetic material groups (wool or other type of protein-based material) or between cotton and other cellulose-like materials.

Particle shape

The size and shape of the particles were characterized in two dimensions. This approach was chosen as the MP were mainly irregularly shaped. Fibers were scored as having a length (M dim) to width (*m dim*) ratio >3 while fragments showed a length to width ratio ≤ 3.

The percentage of fibers ranged from 9% in E8 to 34% in E5 respectively, Overall, 26% of the identified particles were classified as fibers, while 74% were classified as fragments. To describe the overall size distribution the median values (D50) were calculated (Table 2). Overall, the size distribution of the identified MPs had D50s of 66 $\mu m_{M\ dim}$ and 27 $\mu m_{m\ dim}$ for the major and minor dimension, respectively. Significantly different D50 values were observed for the particles observed in the outdoor air (46 $\mu m_{M\ dim}$; 21 $\mu m_{m\ dim}$; $p<0,05$)

Table 2 - Calculated D10, D50, and D90 values relative to the single sample size distributions. Size distribution parameters are displayed both for the major dimension (top value at every sample/location, in bold) and minor dimension (bottom value, in italics).

Sampling site	Dimensions	MP		
		D10 (µm)	D50 (µm)	D90 (µm)
E1	**M dim**	**41**	**66**	**120**
	m dim	*22*	*30*	*42*
E3	**M dim**	**45**	**73**	**132**
	m dim	*23*	*32*	*44*
E4	**M dim**	**50**	**80**	**145**
	m dim	*27*	*36*	*51*
E5	**M dim**	**49**	**79**	**134**
	m dim	*26*	*36*	*50*
E6	**M dim**	**96**	**83**	**156**
	m dim	*28*	*38*	*53*
E7	**M dim**	**62**	**100**	**186**
	m dim	*33*	*45*	*64*
E8 (Outdoor blank)	**M dim**	**29**	**46**	**84**
	m dim	*15*	*21*	*29*
Procedural Blanks	**M dim**	**16**	**26**	**48**
	m dim	*9*	*12*	*17*
Wet sedimentation trap	**M dim**	**25**	**40**	**72**
	m dim	*4*	*6*	*8*

Pyr-GCMS analysis

The same samples were subsequently analyzed by pyr-GCMS to estimate the mass distribution of a selected group of environmentally relevant polymer types (PP, PS, PE, PVC, PET, PMMA, NY and PC). The total polymer concentrations ranged from 2.1 to 8.2 µg/m^3. Most of the investigated polymers were below the limit of quantification in most cases (LoQ < ≈1.5 µg/m^3). Based on the pyr-GCMS analysis, polyethylene and nylon were the most abundant polymer types in the analyzed air samples (Figure 10)

Overall, limited amounts of polyethylene, nylon and polypropylene were observed in few of the investigated samples. Samples such as E1, E6, E7 and E8 did not show detectable levels of the investigated eight polymer types. This may be explained by the current limitation i.e., sensitivity of the involved analytical instrument.

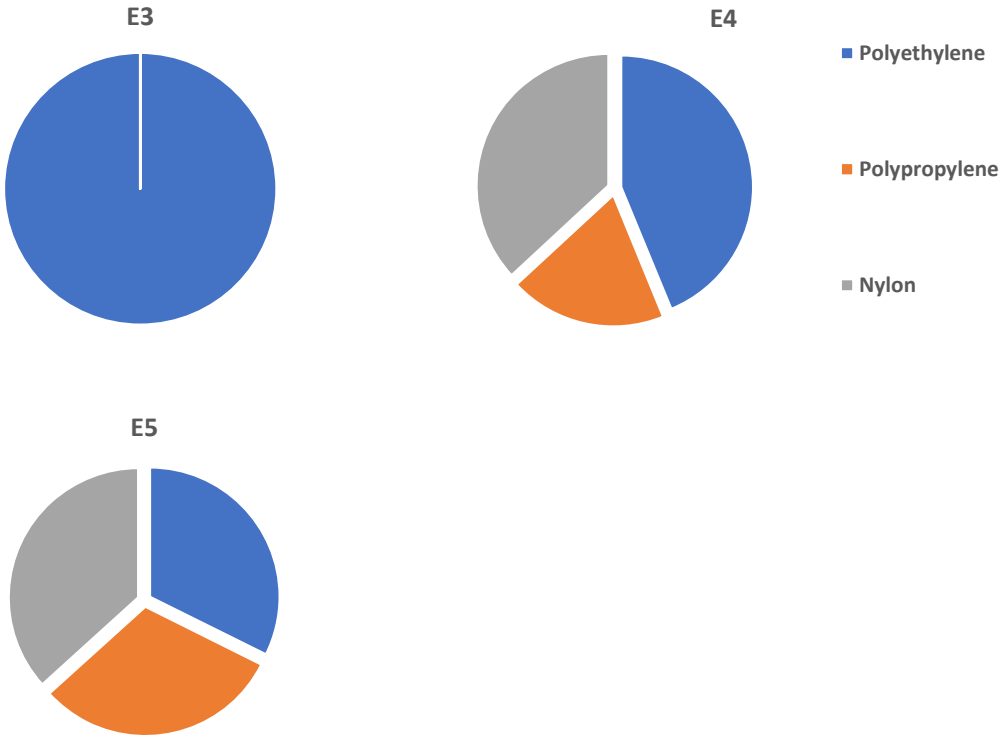

Figure 10 – Pie charts of relative polymer distribution assessed by pyr-GCMS.

Endotoxin levels

In the present study a parallel dedicated sampling device was used to investigate the occurrence of endotoxins using the same sampling strategy combining mobile (operator based) and static samplers. The results of the analysis indicated that endotoxin levels ranged from <0.7 EU/m^3 (E3) up to 45 EU/m^3 (E4). Obtained results were compared against the total content of MPs and dust. In the present study a good correlation was found

between the total amount of MPs and the endotoxins (fig. 11). In the meantime, a weaker correlation was found between MPs and dust (fig. 12) as well as between endotoxin content and dust (fig. 13).

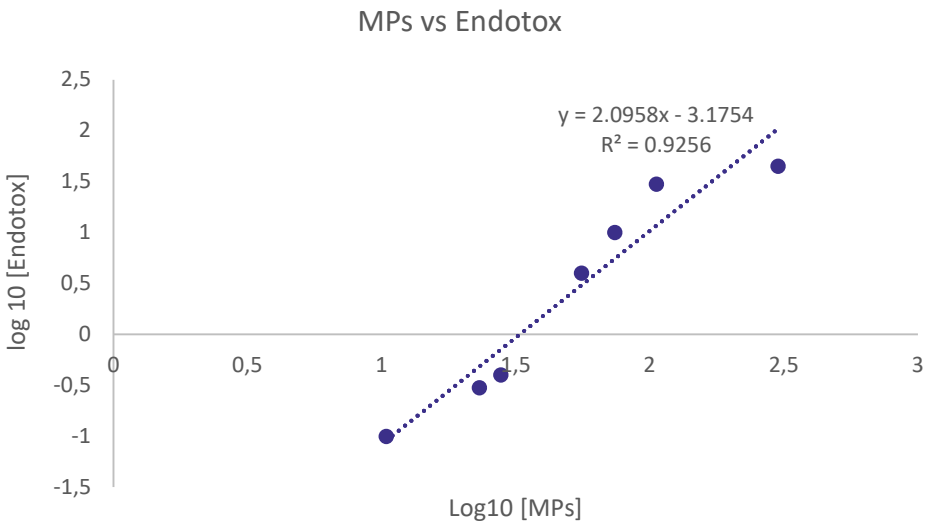

Fig. 11 - Linear correlation between the total content of MPs and endotoxins. Data are log transformed prior to analysis. The correlations were significant at $p<0.001$.

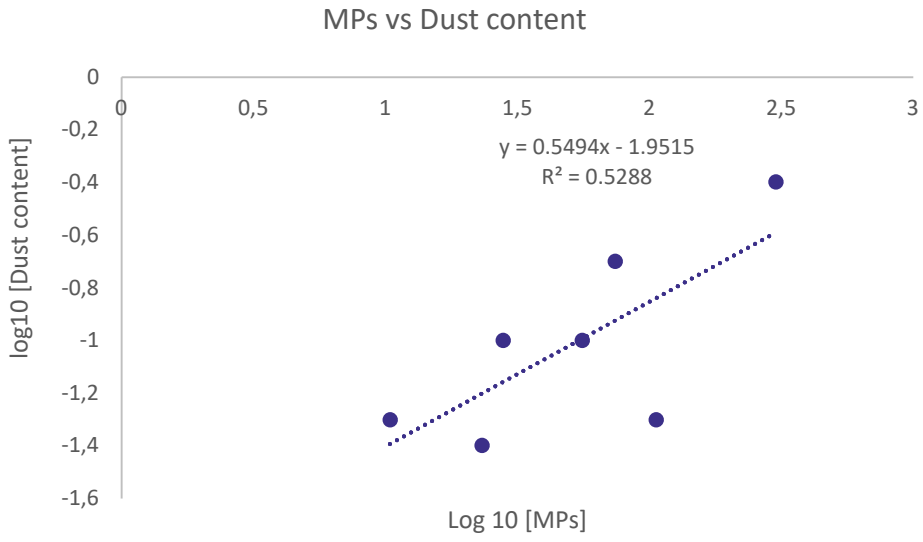

Fig. 12 - Linear correlation between the total content of MPs and dust. Data are log transformed prior to analysis. The correlations were significant at $p<0.001$.

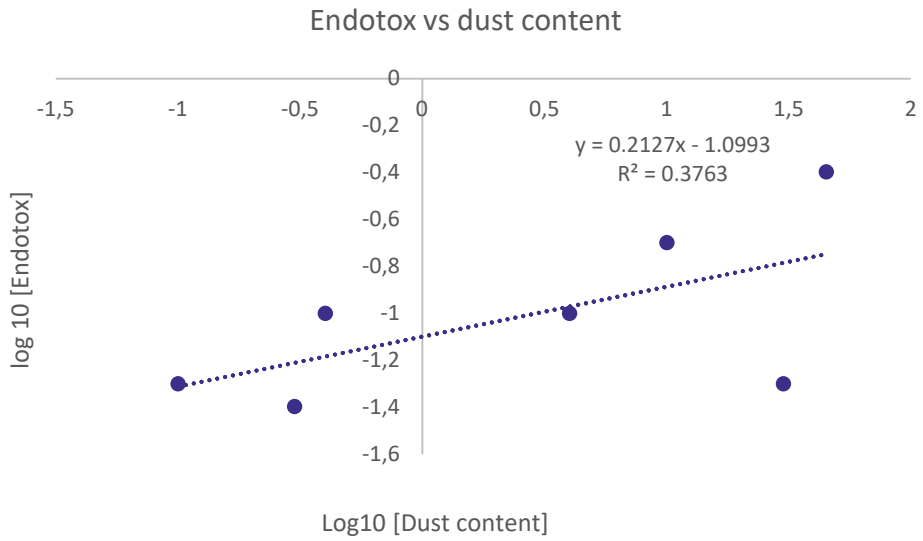

Fig. 13 - Linear correlation between the total content of endotoxins levels and total amount of dust. Data are log transformed prior to analysis. The correlations were significant at $p<0.001$.

4. Discussion

To date, the presence of small plastic particles has primarily been shown in water and sediments (Harris, 2020). However, there is increasing evidence of the occurrence of microplastics in air and soil (Vianello et al., 2019; Dris et al., 2017; Zhu et al., 2019). Nevertheless, critical information on the actual occurrence, identity, and quantity of small plastics particles in these compartments is lacking. Potential health risks related to microplastics are not limited to indirect exposure via food and water but airborne synthetic particles in both in and outdoor environments pose a risk to humans directly through inhalation. Recent evidence suggests that the number of plastics particles received by a human body via air is even higher than the amount ingested through food or water (Prata et al., 2020; Gasperi et al., 2019).

Although some data exist to provide indications of biological adverse effects of microplastics in controlled conditions, these data originate from model systems. Consequently, their translation to actual exposure conditions, including relevant particle types and sizes, exposure scenarios and durations, is complicated. An additional level of complexity arises from chemicals, pathogens and endotoxins present in/on the plastics. Collectively, these unknowns do not allow for a holistic understanding and estimation of health risks caused by microplastics for humans and other life forms. The scientific community considers micro- and nanoplastics exposure related health hazards likely. The present project aimed at preliminary assessment of the occurrence of airborne MPs in a selected case study with a potential source of MPs and endotoxins related to soil fertilizer production from sewage treatment plant sludge. It has been reported that sewage sludge concentrates large quantities of MPs from the wastewater by flocculation (Rolsky et al.,

2020). The dehydration process within the fertilizer production is suspected to facilitate the MPs aerial dispersion.

In the present study an average of 98.7 MPs/m^3 and 7.2 MPs/m^3 were observed in the indoor and outdoor air samples, respectively. The reported value in the indoor environment is one order of magnitude higher than reported in other similar studies of indoor environments which were not associated to plastics related industrial productions. Vianello et al. (2019) reported an average of 9.3 ± 5.8 MP/m^3 in three apartments located in Aarhus (Denmark) while Dris et al. (2017) reported 5.4 fibres/m^3. However, a direct comparison between these two studies is problematic, as the analytical approaches applied span from manual sorting followed by ATR-FTIR to µFTIR-Imaging as was performed in this study. The highest exposure concentration (301 MPs/m^3) was measured in sample E4, which corresponds to an inhalation rate of ≈ 200 MP per hour. At such a rate, an average male would potentially inhale up to 1600 MP during working hours. However, the overall median value of the MPs detected using this method (D50 = 66-100 µm, larger dimension) suggests that most of the particles inhaled are likely to undergo deposition by impaction, and therefore then be eliminated by the mucociliary escalator responsible for movement of mucus up and out of the respiratory tract (Houtmeyers et al., 1999). Thus, a limited number of MPs is likely to reach the deeper airways (Gasperi et al., 2018). Smaller fragments and fibres with dimension <11 µm were not detected in the present study due to the current limitations related to the filtering membranes used (GF/A; 1.2 µm mesh) as well as the analytical method. These are expected to enter the lower airways but their occurrence may also have been present in the samples indoors, however further investigation is required to test if enhanced analytical sensitivity, higher magnification capability, better resolution and particle separation may enable the characterization of smaller particles more relevant for human health.

In the present study, polyester followed by polyethylene, polypropylene and nylon were the most abundant polymer types in all the investigated samples. In contrast, polypropylene followed by polyethylene dominated the outdoor air sample (E8).

The occurrence of polyester can be linked to different sources. Among them the contribution from washing synthetic clothes to microplastic release in the sewage may have led to a significant enrichment in the sewage sludge and thus their subsequent aerial dispersion during the fertilizer production. Polyolefin fibres, such as polyethylene and polypropylene, are used for several applications in the textile industry as well. However, the reported values were probably influenced by other co-occurring sources such as fragmenting from packaging materials or other plastic items released and collected in road wastewater as well as from the wear and tear of plastic items used within the production facility such as the large bags used to collect and ship the produced fertilizer. Among the minor contributing polymers, it is worth mentioning the discontinuous presence of acrylic paint fragments. Such polymers normally contain metal based biocides, softeners, oil-based solvents and photo-stabilizers which represents a threat of the human heath *per se*.

The composition of the indoor air reported in this study is similar to what is reported by Vianello et al., 2019 but differs from the indoor MP composition reported by Dris et al., 2017, where polypropylene was the most abundant polymer, while no polyester was found.

In both cases the size distribution of the reported MPs diverges with the observations presented in this study. Overall, this highlights the influence of the characteristics of the indoor environment, the associated activities, source and inputs of plastics contamination and fragmentation patterns.

The level of MPs was compared with the occurrence of endotoxins and the amount of collected dust. Endotoxin is a component of bioaerosols that can cause symptomatic effects in exposed individuals and is associated with negative health outcomes. They are found in high concentrations in the air at sites that handle organic material such as composting facilities and wastewater operations where concentrations regularly exceeded 100 EU m^3 (Aghaei et al., 2020).

In the present study the endotoxins levels ranged from <0.7 EU/m^3 up to 45 EU/m^3. To date, there are no exposure limits for endotoxins in Norway, in the Netherlands an occupational health limit of 90 endotoxin units/m^3 has been suggested by the Dutch Heath authorities. Overall, this indicates a low level of exposure to endotoxins (lipopolysaccharides) in the investigated working environment. Interestingly, a good correlation was found between the total amount of MPs and both the endotoxins while a weaker correlation between the MPs content and the total amount of collected dust was observed. This may be partially explained by a plastic fragments/endotoxins binding phenomena already occurring during the sewage treatment processes in the aerobic reactor prior to the sewage sludge dehydration processes.

5. Conclusion

- This study has analyzed a selection of six indoor and one outdoor air samples. MPs have been found in both indoor and outdoor environments highlighting the omnipresence of plastics contamination. The results suggest that MPs are present in both indoor and outdoor environments.
- Levels of MPs in the investigated indoor air was 100 times higher than in the outdoor environment and 10 times higher than in a regular household situation
- Particles of 66-100 µm (larger dimension) were the most prevalent sizes in the analyzed indoor air samples while 21-46 µm dominated the MPs size distribution of the outdoor air.
- The overall median MP size values of the detected MPs suggests that most of the particles inhaled are likely to undergo deposition by impaction, and that the MPs will be eliminated by the mucociliary escalator responsible for movement of mucus up and out of the respiratory tract.
- The most frequently detected polymer types in the indoor air were polyester, polyethylene, polypropylene, nylon and acrylic paints. In the outdoor air sample, however, polyethylene and polypropylene dominated the MPs composition.
- The dominant polymers were not found in the control samples to the same degree as in the air samples, and for pyr-GCMS the control samples were below LOQ. The observation of MP occurrence is therefore considered reliable, yet the actual concentrations still carry a degree of uncertainty due to the limited number of

analyzed samples. The methods are more likely to underestimate than overestimate the concentrations, based on loss of material during filtration and high limits of quantification.

- The Pyr-GCMS technique has room for improvement by increasing the selection of detectable polymer types as well as the sensitivity, which is currently strongly linked to the amount of sample deposited in the filtering membrane.
- The µ-FTIR imaging technique showed good potential in providing information about the total and relative number of plastic particles as well as the size and particles. This type of data may be crucial for occupational and human health specialists to develop tailored human health risk assessment procedures.
- µFTIR-Imaging spectroscopy is a suitable technique for identifying particles potentially down to a few micrometers, and providing information about particles abundance, shape, size and polymer type composition useful to epidemiologist and HSE specialists to develop the human health risk assessment associated to MPs inhalation in indoor working environments.
- The sample preparation tailored to the µ-FTIR imaging acquisition showed the potential to perform assessment of MPSs and endotoxin levels within the same sampling membrane.
- This is the first time that the occurrence of airborne MPs has been reported from this type of indoor working environment. The correlation between the plastic content and the levels of endotoxins is also interesting, although the low number of analyzed samples does not allow conclusions to be drawn regarding human health safety. More frequent sampling and a larger sample set is recommended.

6. Acknowledgements

This study was financially supported by the Regionale forskningsfond (RFF Vestlandet, grant # 297059). Thanks to IVAR IKS for support and assistance during collection of samples at the Nord-Jæren Sewage Treatment Plant.

7. References

Aghaei, M., Yaghmaeian, K., Hassanvand, M. S., Hedayati, M. H., Yousefian, F., Janjani, H., Yunesian, M. (2020). Exposure to endotoxins and respiratory health in composting facilities. Ecotoxicology and Environmental Safety, 202, 110907.

Dris, R. et al. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. 221, 453–458, https://doi.org/10.1016/j.envpol.2016.12.013 (2017)

Gasperi, J. et al. Microplastics in air: Are we breathing it in? Curr. Opin. Environ. Sci. Heal. 1, 1–5, https://doi.org/10.1016/j. coesh.2017.10.002 (2018).

Gomiero, A., et al. First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using a thermal degradation method. Chemosphere, 227, 705-714 (2019)

Harris, P. T. (2020). The fate of microplastic in marine sedimentary environments: A review and synthesis. Marine Pollution Bulletin, 158, 111398.

Houtmeyers, E., et al. Regulation of mucociliary clearance in health and disease. European Respiratory Journal, 13(5), 1177-1188 (1999)

Prata, J. C., et a. Environmental exposure to microplastics: An overview on possible human health effects. Science of the Total Environment, 702, 134455 (2020)

Primpke, et al., Automated identification and quantification of microfibres and microplastics. Anal. Methods 11, 2138–2147 (2019).

Rolsky, C., et al. Municipal sewage sludge as a source of microplastics in the environment. Current Opinion in Environmental Science & Health, 14, 16-22 (2020)

Vianello, A., et al Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Scientific reports, 9(1), 1-11 (2019)

Zhu, F., Zhu, C., Wang, C., & Gu, C. (2019). Occurrence and ecological impacts of microplastics in soil systems: a review. Bulletin of environmental contamination and toxicology, 102(6), 741-749.