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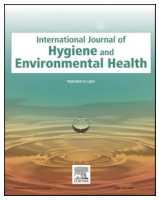

Occupational exposure to inhalable pathogenic microorganisms in waste sorting

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ABSTRACT

This study assessed microorganisms in personal inhalable work air samples aiming to identify potential human pathogens, and correlate exposure to adverse health outcomes in waste workers. Full-shift personal exposure was measured in six different waste sorting plants. Microbial concentrations in inhalable air samples were analysed using MALDI-TOF MS for cultivable, and next generation sequencing (NGS) for non-cultivable microorganisms. Concentrations of bacterial and fungal CFUs varied substantially within and between waste sorting plants, ranging from no identifiable organisms to a maximum concentration in the order of 10^5 CFU/m^3. *Bacillus* and *Staphylococcus* were among the most abundant bacterial genera, whilst fungal genera were dominated by *Aspergillus* and *Penicillium*. Approximately 15% of all identified species were human pathogens classified in risk group 2, whereas 7% belonged to risk group 1. Furthermore, significant correlations between concentrations of fungi in risk group 1 and self-reported adverse symptoms, such as wheezing were identified in exposed workers. The combination of culture-based methods and NGS facilitated the investigation of infectious microbial species with potential pathophysiological properties as well as non-infectious biological agents in inhalable work air samples and thereby contributed to the risk assessment of occupational exposure in waste sorting.

1. Introduction

Global sustainability goals promote technological progresses in the waste management sector in various way, such as the introduction of new work operations and the implementation of fully automated waste sorting machines that increases the overall waste treatment capacity and reduces the need for manual labour and thus potential exposure moments. However, exposure during cleaning and maintenance of waste sorting machines is an underestimated health challenge. Previous studies have reported differences in exposure levels at automated waste sorting plants and identified cleaning with compressed air as potential high exposure moment (Eriksen et al., 2022, 2023a).

Changes in sorting processes and the introduction of new waste fractions affect pre-sorting routines and waste collection intervals of domestic waste with a general decrease in collection frequencies. Despite dedicated sorting strategies for various waste fractions, about 50% of all food waste is discarded as residual waste (Haslegaard, 2019; ROAF, 2023). Organic material that contaminates residual waste not only reduces the recyclability but also poses potential exposure risk for workers, as prolonged waste collection intervals may promote microbial growth under beneficial conditions (Madsen et al., 2019). Furthermore, effects of climate change need to be considered, as increased temperature and humidity are beneficial for microbial growth in waste bins (Gladding and Gwyther, 2017).

During the handling and sorting of residual waste biological agents, such as microorganisms are aerosolised and dispersed as bioaerosols. Exposure to bacteria and fungi as well as associated toxins such as endotoxins and mycotoxins have repeatedly been linked to occupational diseases of the respiratory tract leading to decline in lung function, the gastrointestinal tract, eyes and skin (Bolund et al., 2017; Hambach et al., 2012; Heldal et al., 2001; Poulsen et al., 1995). As inhalation often is considered the main exposure route in an occupational setting, it is of interest to investigate the biodiversity of inhalable fungi and bacteria to identify potential human pathogens. However, so far there is limited knowledge on the composition of microbial communities in bioaerosols emitted in waste sorting plants.

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Traditionally, workplace assessments investigate the exposure to microorganisms by using culture-based methods, which capture cultivable fungi and bacteria only and potentially underestimate the exposure to non-infectious biological agents with pathophysiological properties. Molecular-biological approaches, such as next generation sequencing (NGS), on the other hand, provide tools for a holistic assessment of microbial work air contaminants (Duquenne, 2018). Degois et al. (2017) conducted a metagenomics analysis in a waste sorting plant and identified *Enterobacteriaceae*, *Staphylococcus*, and *Acinetobacter* to be among the most prevalent bacterial genera, whereas fungal genera were dominated by *Penicillium, Aspergillus* and *Rhizopus*. However, the authors reported their findings on the genus level, and it remains unclear if the samples contained potentially pathogenic species. The present study attempted to combine NGS and culture-based methods to identify non-cultivable potential pathogens on the species level as pathogenicity within a genus can vary substantially. Species in the genus *Aspergillus*, such as *A.fumigatus* have the potential to colonise the airways and cause pulmonary infections. Furthermore, *Aspergillus* spp. have been reported to be problematic in a clinical setting as they develop azole resistance (Chowdhary et al., 2013; Prigitano et al., 2019; Vermeulen et al., 2013). Due to its relevance as human pathogen, *Aspergillus* has been proposed as potential bioindicator for health risks assessment in waste management (Viegas et al., 2015a).

The present study aimed to 1) identify airborne inhalable bacteria and fungi at species level, 2) to identify variability in microbial communities that can be attributed to seasonal variation and different waste sorting plants, 3) to identify human pathogens and potentially harmful non-pathogenic microorganisms in the waste sorting work environment and 4) to evaluate the relationship between concentrations of biosafety level classified microorganisms and the symptoms reported by workers.

2. Material and methods

2.1. Study design

Six Norwegian waste sorting plants that treat residual waste from private homes and small businesses were visited between June 2020 and November 2021 for working environment air sampling. Two waste sorting facilities were in the western part of Norway, and 4 facilities were located in the greater Oslo area. Three of the facilities used fully automated waste sorting lines that sort automatically waste products from private homes, whereas the remaining three facilities use manual labour and excavators for sorting waste from industry and gastronomy (Eriksen et al., 2023a). A total of 59 workers (58 males, 1 female) participated voluntarily and helped recover 114 and 47 samples for NGS and MALDI-TOF MS, respectively (Table 1). Sampling was conducted on two consecutive days (NGS).

Table 1
Overview: Number of participants and collected samples b waste sorting plant.

WSP	season	participants n	NGS n (personal + stationary)		MALDI n (personal + stationary)
			Monday	Tuesday	Tuesday
A	summer	8	8 + 2	8	9[a] + 1
A	autumn	8	8 + 2	8	8 + 1
A	summer	9	9 + 2	9	8 + 1
B	autumn	8	8 + 2	8	5 + 1
B	summer	6	5 + 2	6	2 + 1
C	autumn	5	5 + 2	5	5 + 1
D	autumn	6	6 + 2	6	6 + 1
E	autumn	3	3 + 1	0	0
F	autumn	6	6 + 2	6	4 + 1
Total		**59**	**58 + 17**	**56**	**47 + 8**

[a] One participant was equipped with two samplers that were carried as parallels.

2.2. Sampling methods

Airborne microorganisms were sampled on 37 mm PC membrane filters with a pore size of 1 μm (Frisenette, DK) using personal and stationary conical inhalable air samples (CIS, JS Holdings, Hertfordshire, UK) that were operated at an average airflow of 3.5 L/min. Stationary samples were placed in previously selected spots in the respective waste sorting plant (WSP). Outdoor references were sampled at all sampling sites (approximately 50–100 m from main buildings). For personal samples, sampling devices were placed in the workers' breathing zone and carried for a full shift. Mean sampling time for personal samples was 7.1 h (min: 5.1, max: 8.7). Each participant was equipped with parallel air sampling sets, one dedicated to CFU count and MALDI-TOF MS analyses, the other for DNA extraction and sequencing. At plant E no samples for MALDI-TOF MS analysis were collected. Stationary samples were collected at selected sites of each respective WSP for an average of 27.8 h (min: 6.67, max: 35.3). Filter cassettes were exchanged after approximately 10 h of operation and DNA from filters collected at the same site were pooled during the DNA extraction step. Filter elution and DNA extraction were performed as previously described by Straumfors et al. (2019).

The DNA yield was measured spectrophotometrically on a Qbit 4 Fluorometer (Thermo Scientific, DE, USA) and varied between 0.021 and 15 ng/μL.

2.3. DNA amplification, sequencing, and data analysis

DNA samples were pre-amplified on an Eppendorf Mastercycler X50s (Eppendorf SE, Hamburg, Germany) under the following conditions: Initial denaturation at 90 °C for 30 s, followed by 30 cycles of amplification (98 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s). A final elongation step at 72 °C for 5 min was added. Each reaction contained 5 μL 5x HF PCR buffer, 0.5 μL dNTPs (40 mM), 5 μL of each the forward and reverse primer, 0.25 μL Phusion polymerase (2U/μL), 7.25 μL PCR-grade water and 2 μL template DNA. For bacterial amplification the primer set 515FB/926R that targets the 16S rRNA V4–V5 region was used (Parada et al., 2016; Walters et al., 2016) (Table S1). Fungal amplification was achieved using the fungal specific primer set ITS86(F)/ITS4(R) that targets the ITS2 region as recommended by Op De Beeck et al. (2014). PCR reactions were carried out in duplicates (25 μL each) and pooled after amplification. Subsequently PCR products were purified using a PCR Purification kit (Norgen Biotek Corp., Thorold, Canada) following the manufacturer's recommendations.

Sequencing was performed by the IMR sequencing service (Halifax, Canada) using the same primer sets as had been used in the pre-amplification step. Raw amplicon sequences (fastq) were processed using the DADA2 pipeline (Callahan et al., 2016). Primers were removed prior to downstream analyses using the package's remove primer function allowing for 0 unidentified (N) bases. Further filtering steps were applied allowing a maximum expected error rate of 2 in both forward and reverse bacterial sequences and 2 and 5 errors in fungal sequences, respectively. The minimum sequence truncation length was defined based on the consent quality scores for bacterial amplicons at 230 bases for forward and 180 bases for reverse sequences, respectively. For fungal sequences a minimum sequence length of 50 bases was used due to large variation in intra-specific ITS sequences. Sequences with a quality (phred) score above 30 were included in further analysis. Forward and reverse reads were matched allowing 0 mismatches between sequences and enforcing a 12bp minimum overlap for bacterial sequences and 5bp overlap for fungal sequences. Chimeric sequences were removed based on consensus sequences for each sequence variant. Taxonomy was assigned using the SILVA138.1 database for bacteria (Quast et al., 2012; Yilmaz et al., 2013) and the UNITE database for fungi (Kõljalg et al., 2020), respectively. A mock community with known community composition was included in the analysis (from filter extraction to sequencing) to evaluate the accuracy of the sequences. A

bootstrap cut off at 80 on the species level was used to remove low support amplicon sequence variants (ASV). ASV analysis was conducted using the phyloseq package (McMurdie and Holmes, 2013), and the vegan package (Oksanen et al., 2022). A Permutational multivariate analysis of variance (PERMANOVA) was applied to investigate the differences in clustering of microbial communities between WSP during summer and autumn. The PERMANOVA was based on ordination using principal coordinate analysis (PCoA) and a bray distance matrix.

2.4. Analyses of bacteria and fungi using MALDI-TOF MS

2.4.1. Filter extraction

Filters dedicated to CFU count and MALDI-TOF MS analyses were transferred to sterile tubes and extracted in 5 mL sterile MilliQ +0.85% NaCl and 0.05% Tween 80 by orbital shaking at 500 rpm for 15 min at room temperature. Filter eluates were aliquoted, and glycerol was added. Samples were kept at −80 °C until analyses.

2.4.2. Cultivation

Cultivable bacteria and fungi were grown on nutrient agar (NA; Thermo Fisher Scientific Oxoid, Basingstoke, UK) and Dichloran Glycerol agar (DG18; Dichloran-Glycerol Agar Base; Thermo Fisher Scientific Oxoid, Basingstoke, UK) medium, respectively in a serial dilution as previously described in Madsen et al. (2016). NA plates were incubated at 25 °C ($bacteria_{25}$) and DG18 plates were incubated at 25 °C ($fungi_{25}$) and 37 °C ($fungi_{37}$) for four to seven days, respectively. Colony forming units were counted on day four and seven. 50% of autumn samples collected at WSP A did not contain any microorganisms. Re-plating of the samples at higher dilution produced identical results. It is unsure whether this is due to biases in sampling, handling, or extracting the filters. Samples containing zero CFUs were not included in the summary statistics.

2.4.3. MALDI-TOF MS

DG18 and NA plates with optimal coverage and separation of colony forming units were chosen for analyses. Bacterial colonies were directly transferred to MALDI target plates and analysed on the MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) as previously described by Madsen et al. (2016). Fungal colonies were identified based on the ethanol extraction protocol as described by (Madsen et al., 2015). In short, fungal material was transferred to sterile Eppendorf tubes containing Sabouraud agar (Sabouraud Dextrose Liquid Medium, Oxoid, Basingstoke, United Kingdom, 2% agar) under sterile conditions and cultivated overnight. Subsequently, isolates were extracted in alcohol, formic acid, and acetonitrile before they were transferred to the MALDI target plate.

MALDI-TOF MS analyses were performed on a Microflex LT mass spectrometer (Bruker Daltonics, place). All isolates were analysed in duplicates. Bacterial and fungal identification was based on the BDAL standard library and filamentous library 1.0. included in the Bruker Biotyper 3.1 software. A cut off of 1.70 was used for positive identification of isolates (Stein et al., 2018). Isolates with a score between 1.70 and 1.79 were identified on the genus level, whereas identification on the species level was assigned at a score above 1.80. The GESTIS database (IFA, 2023) was used as reference tool to identify human pathogens in work air samples.

2.5. Questionnaire

Self-reported health data were collected using an adapted version of the questionnaire published by Susitaival and colleagues (Eriksen et al., 2023a; Susitaival et al., 2003) and health outcomes in exposed workers were described in Eriksen et al. (2023b). The present study used the data to investigate the correlation between the prevalence of health symptoms and exposure to viable microorganisms.

2.6. Data analysis

All data analyses were executed in R (version 4.2.2) and RStudio (version 2022.07.2) using the following packages: statix (Kassambara, 2022) for statistical analysis, the ggplot2 for graphics (Wickham, 2016), phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2022) for community analyses and lme4 (Bates et al., 2015) for model fitting. Log transformed exposure levels were reported as time weighted average (TWA) for bacterial and fungal CFU/m^3, respectively. The levels were calculated based on average air follow, sampling time and dilution (filter and inoculation). Results were considered as statistically significant at a p value below 0.05 and borderline significant at a p value between 0.05 and 0.1.

2.6.1. Modelling

A linear mixed effect model stratified by growth medium/temperature was applied to investigate the effect of season (categorical: summer vs autumn) and exposure time (categorical: over vs under 360 min in sorting hall) on personal exposure levels. The model allowed for random intercepts between waste sorting plants,- $\beta_{1\ and}\ \beta_2$ the respective slopes, and Ɛ the residual error.

$$\log(\mathrm{CFU/m^3}) = \beta_1\ \mathrm{season} + \beta_2\ \mathrm{exposure\ time} + (1|\mathrm{WSP}) + Ɛ \quad [1]$$

Furthermore, a logistic regression model was used to investigate the effect of microbial concentrations in risk group 1, 2, and “no risk group”, respectively on the occurrence of health symptoms in exposed workers. The probability of having symptoms is linked to a linear predictor by means of a logistic link function:

$$\mathrm{logit}(\mathrm{Prob}(\mathrm{symptom} = 1)) = \beta\ \mathrm{pathogen\ frequency} \quad [2]$$

where symptom = the symptom prevalence in exposed waste workers (categorical, 0 = no symptoms, 1 = symptoms), β = slope, and pathogen frequency = the concentration of human pathogens in personal air samples (continuous, measured as concentration of pathogens in risk group 1, 2 and “no risk group”).

3. Results

3.1. Exposure to bacteria and fungi

The time-weighted geometric mean (GM) CFU/m^3 levels in personal samples across all samples were 8.1×10^3 CFU/m^3 (range: 1.5×10^1–8.5×10^5) for $fungi_{25}$, 1.8×10^3 CFU/m^3 (range: no CFU – 1.0×10^5) for $fungi_{37}$, and 2.7×10^3 CFU/m^3 (range: no CFU – 3.9×10^5) for $bacteria_{25}$, respectively. Average CFU/m^3 levels in outdoor references were 6.0×10^2 CFU/m^3 (range: 1.8×10^2–6.3×10^3), 4.5×10^1 CFU/m^3, and 7.5×10^1 CFU/m^3 (range: no CFU – 1.3×10^3), for $fungi_{25}$, $fungi_{37}$, and $bacteria_{25}$, respectively (Table S2). Four of the 8 samples collected at WSP A during autumn months did not contain viable microorganisms, these samples were excluded from further analyses.

Significant differences in microbial concentrations were identified between seasons and WSP (Fig. 1, Fig. S1, Table S4). At plant A, levels of $fungi_{25}$ differed significantly between seasons with elevated levels during summer months. In plant B, autumn levels tended to be higher than summer levels. Autumn $fungi_{25}$ CFU/m^3 concentrations were significantly elevated at WSP B (p-value <0.001), and WSP C (p-value = 0.023) compared to plant A. Bacterial CFU levels were significantly different between plant A and B during autumn months with highest levels at the latter. At WSP A average CFU/m^3 concentration were generally lover compared to summer concentrations, whereas autumn concentration in WSP B were on average higher compared to summer CFU/m^3 levels.

The exposure to microorganisms in personal samples was higher than the outdoor background samples during both seasons at each plant, respectively, however only in a few cases the difference was statistically

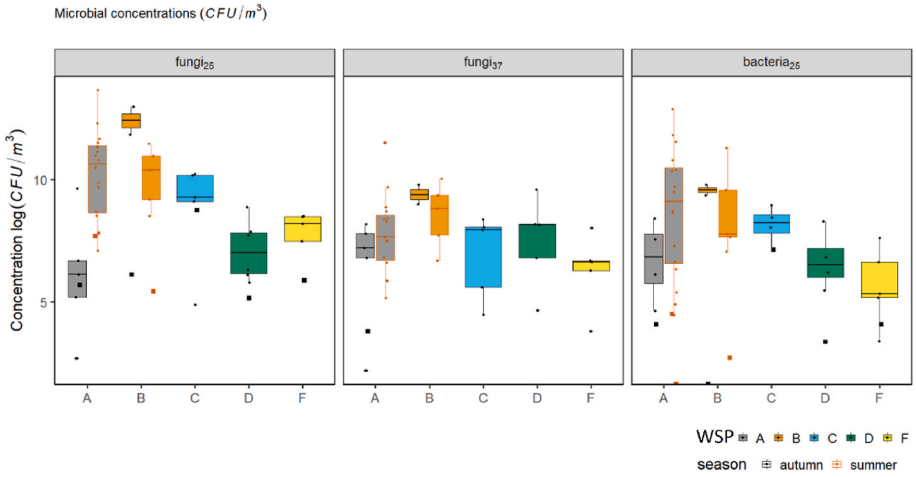

Fig. 1. Microbial concentrations in personal samples (boxplot and dots) and background samples (squares) by WSP (A–F) and season (black: autumn, orange: summer). No samples were collected at plant E. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

significant (WSP A: bacteria$_{25}$ summer, WSP B: fungi$_{25}$ and bacteria$_{25}$, bacteria$_{25}$ autumn, WSP F: fungi$_{25}$). Average autumn exposure levels of fungi$_{25}$ were 5x higher (p-value <0.001) in personal samples compared to outdoor references, whereas fungi$_{37}$ levels were 25x higher (p-value <0.001) and bacteria$_{25}$ levels were 11x higher (p-value <0.001) compared to outdoor references. Summer exposure levels in personal samples were, however, 38x higher for fungi$_{25}$ (p-value <0.001) and as much as 1600x higher (p-value <0.001) for bacteria$_{25}$ (Table S3).

3.2. Modelling determinants of CFU concentrations in personal samples

Average summer CFU levels were statistically higher compared to autumn levels for fungi$_{25}$ as well as bacteria$_{25}$ (Equation (1), Table 2). Time spent in waste sorting did not affect any of the assessed exposure levels. The proportion of the within-WSP variance reported as inter class coefficient (ICC) was relatively low in all cases. Variance within-WSP accounted for 9% for fungi$_{25}$, 4% for fungi$_{37}$ and 1% for bacteria$_{25}$ of the total variance observed in the data set. Thus, the variance in the exposure levels could largely be attributed to different WSP.

3.3. Microbial species in personal air samples identified with MALDI-TOF MS

A total of 42% of bacterial and 78% of fungal CFUs were identified on the species level (MALDI score ≥ 1.8), and 47% of bacterial and 86% of fungal CFUs were identified on the Genus level (MALDI score ≥ 1.7), respectively (Table S5). Thirty three percent of the analysed bacterial CFUs had specific spectra, were however not found in the reference library, whereas 8% of all fungal sequences were unidentifiable. Personal exposure was on average 2.7 × 10^3 CFUs/sample (GSD: 9.4) in bacterial samples, 8.0 × 10^3 (GSD: 10.2) CFUs/sample in fungi$_{25}$ and 1.8 × 10^3 (GSD: 6.0) CFUs/sample for fungi$_{37}$. The average number of identified species in outdoor references was 6.0 × 10^2 (GSD: 3.8) CFUs/sample for fungi$_{25}$ and 7.5 × 10^1 (GSD: 4.6) CFUs/sample for bacteria$_{25}$.

3.3.1. Bacterial diversity

A total of 82 bacterial species were identified in personal air samples belonging to the most abundant genera *Bacillus* (29%), *Staphylococcus* (24%), *Streptomyces* (13%) and *Enterobacter* (8%). Among bacteria, *Bacillus pumilus* (11%), *Streptomyces albidoflavus* (9%), *Staphylococcus equorum* (9%), *Bacillus subtilis* (8%), *Staphylococcus saprophyticus* (7%) and *Enterobacter cloacae* (6%) were among the most prevalent species (Table S6). Bacterial richness was generally higher at automated WSP (A:C) compared to manual WSP (D,F) (Fig. 2). Species in the genus *Bacillus* and *Staphylococcus* were dominant in personal samples collected at automated WSP, whereas *S.albidoflavus*, *Bacillus* spp. and *A.johnsonii* were identified in manual WSP. *E.cloacae* was identified in WSP C only, where it accounted for 32% of the bacteria. The composition of bacterial CFUs in personal samples varied somewhat between seasons with *B. pumilus*, *S.saprophyticus*, *S.albidoflavus* and *S.equorum* dominating

Table 2
Model output of linear mixed effect model accounting for season (autumn as reference) and time spent in waste sorting plant (exposure time <360 min per workday as reference) on fungal and bacterial CFU/m^3 concentrations. The model allows for variation in baseline exposure between plants (WSP included as random effect).

	fungi$_{25}$		fungi$_{37}$		bacteria$_{25}$	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Intercept	7.5	**<0.001**	7.2	**<0.001**	6.8	**<0.001**
season (ref 'autumn')	2.5	**<0.001**	0.96	0.12	1.7	**0.02**
exposure time (ref '<360min')	0.51	0.69	−0.15	0.89	0.22	0.87
Random Effects						
σ^2	4.0		3.0		4.5	
τ_{00}	0.38 $_{WSP}$		0.11 $_{WSP}$		0.03 $_{WSP}$	
ICC	0.09		0.04		0.01	
N	5 $_{WSP}$		5 $_{WSP}$		5 $_{WSP}$	
Observations	48		48		48	
Marginal R^2/Conditional R^2[a]	0.26/0.32		0.073/0.11		0.14/0.14	

N = number of grouping variables in random intercept model.
τ_{00} variance of random intercepts.
[a] Marginal R^2 accounts for variance in fixed effects. Conditional R^2 accounts for variance of fixed and random effects.

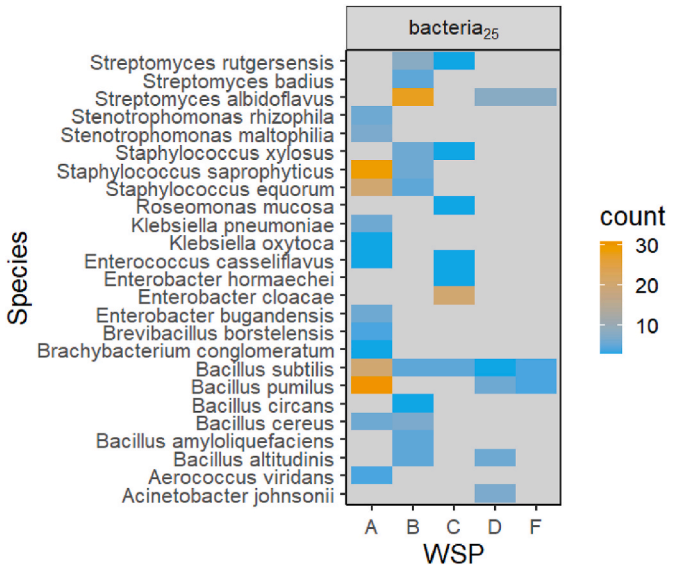

Fig. 2. Most abundant bacterial species in personal air samples stratified by WSP (A:F). Species with abundance greater than 2 CFU/sample are included in the graph.

summer samples (41% of all CFUs in summer samples), whereas *E. cloacae*, *S.xylosus*, *S.equorum* and *S.albidoflavus* dominated autumn samples and accounted for 38% of all CFUs identified in autumn samples (Table S7). Bacterial species profiles were unique for each WSP with highest number of unique species at WSP D (16 species) followed by WSP C (8 species), F (6 species) and B (4 species), whereas only one unique species was identified at WSP A. No bacterial species were common in all WSP (Fig. S4 A). For $fungi_{37}$ and $fungi_{25}$ the species profiles were less distinct and many common species were identified between WSP (Fig. S4 B&C).

3.3.2. Fungal diversity

In total, 36 different fungal species were identified in personal air samples. 60% of the identified fungal species belonged to the genus *Aspergillus*, followed by species in the Genus *Penicillium* that accounted for 33% of the fungal biodiversity. The most abundant species were *A. niger* (26%), *A.fumigatus* (15%), *A.flavus* (8%) and *P.commune* (6%) (Table S7). The species *A.niger* and *P.commune* dominated $fungi_{25}$ in personal samples, accounting for 21% and 12% of all identified fungal CFUs, respectively, were however absent in samples collected at WSP F (Fig. 3). The dominating $fungi_{37}$ in personal samples were *A.niger,* and *A. fumigatus*, and *A.flavus*, accounting for 32%, 27% and 16% of all fungal CFUs, respectively (Table S7).

3.3.3. Seasonal variation in plants A and B

Bacterial diversity in identified isolates was significantly higher during summer months in both WSP with 90% and 74%, respectively (Fig. S2). Only 6% and 13% of all isolates were present during both seasons at plant A and B (Fig. S2). Fungal species richness was generally higher during summer months ranging from 14% to 43% (Fig. S3). Approximately 50% of the fungal species were present during both seasons (Fig. S3).

3.4. Human pathogens in personal and background samples

A total of 14 bacterial species in risk group 2, and 5 species in risk group 1 that have been reported as potential human pathogens in association with occupational exposure were identified in personal air samples (Table 3, Table S6). *B.cereus*, *E.casseliflavus*, and *S.maltophilia* and *A.viridans* were among the most abundant pathogens and were present in between 13% and 23% of personal air samples. Furthermore, 4 fungal species assigned to risk group 2, as well as 3 fungal species assigned to risk group 1 were identified (Table 3, Table S7). Among these, *A.niger* and *A.fumigatus* were among the most prevalent species and were found in 66 and 51% of all personal samples. *A.terreus* and *R. oryzae* were found in a few samples only. Pathogenic species were substantially less present in background samples.

The proportion of fungal species that belong to risk group 2 varied substantially between WSP and seasons, with higher pathogen prevalence at WSP A (72%), B (54%) and C (34%) compared to WSP D (10%) and F (0%), as well as increased levels of risk group 2 species during autumn months (Fig. 4, Fig. S5). The proportion of pathogenic bacterial species in risk group2 in personal air samples was, however, rather similar at all five WSP during both seasons (min: 6%, max:18%).

3.5. Amplicon variants

A total of 1110 bacterial and 1049 fungal ASVs were identified. Principal coordinate analysis (PCoA) of the distance matrix of personal air samples identified clusters of WSP-specific bacterial taxa in autumn samples (Fig. S6). Fungal taxa, however, were largely common between WSP. No distinct clustering was visible for summer samples (Fig. S7, Fig. S8). However, the PERMANOVA analysis indicated that about 40% (p-value <0.001) of the clustering of both fungal and bacterial communities during autumn months was due to differences in WSP.

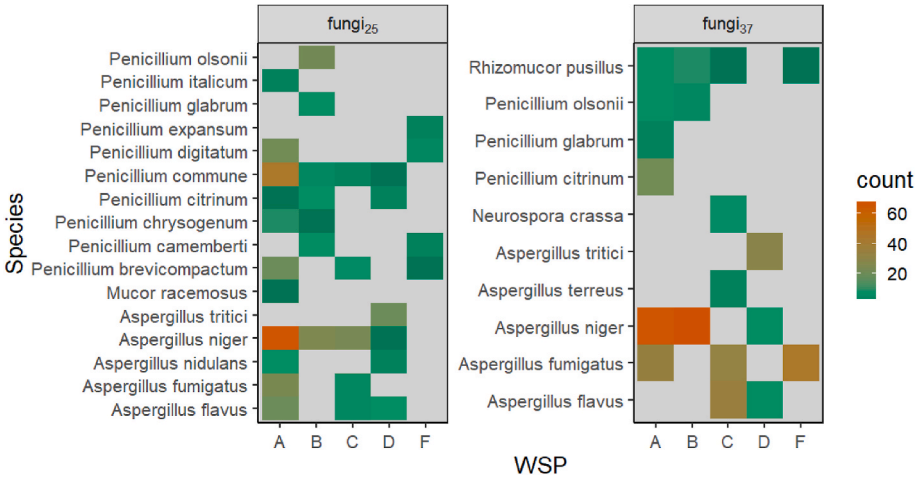

Fig. 3. Most abundant fungal species in personal air samples stratified by WSP (A:F). (Left panel: $fungi_{25}$, right panel: $fungi_{37}$). Species with abundance greater than 2 CFU/sample are included in the graph.

Table 3
Human pathogens identified in personal and background samples. Risk group classification in reference to GESTIS.

	Species	risk group	identified in # personal samples	identified in # stationary samples	Ref
Bacteria	*Bacillus cereus*	2	11	1	
	Enterococcus casseliflavus	2	7	0	
	Aerococcus viridans	2, ht	6	0	
	Staphylococcus saprophyticus	2, ht	6	0	
	Stenotrophomonas maltophilia	2	6	1	
	Enterobacter cloacae	2	3	0	
	Acinetobacter lwoffii	2	2	0	
	Enterobacter bugandensis	2	2	0	
	Enterococcus gallinarum	2, ht	2	0	
	Klebsiella pneumoniae	2	2	0	
	Acinetobacter johnsonii	2	1	0	
	Enterobacter hormaechei	2	1	0	
	Enterobacter ludwigii	2	1	0	
	Enterococcus faecalis	2	1	0	
	Enterococcus faecium	2	1	0	
	Klebsiella oxytoca	2, ht	1	0	
	Kosakonia cowanii	2	1	0	
	Leclercia adecarboxylata	2	1	0	
	Lelliottia amnigena	2	1	0	
	Pantoea eucrina	2	1	0	
	Pantoea septica	2	1	0	
	Pseudomonas putida	2, ht	1	0	
	Roseomonas mucosa	2	1	0	
	Bacillus licheniformis	1	3	0	Haydushka et al., 2012
	Lysinibacillus fusiformis	1	2	0	Wenzler et al., 2015
	Rhodococcus fascians	1	1	0	Austin et al., 2016
	Acinetobacter baylyi	1	1	0	Chen et al., 2008
	Acinetobacter radioresistens	1	1	0	Wang et al., 2019
Fungi	*Aspergillus niger*	2	31	3	
	Aspergillus fumigatus	2	24	0	
	Aspergillus flavus	2	23	1	
	Aspergillus terreus	2	2	0	
	Paecilomyces variotii	2	1	0	
	Rhizopus oryzae	2	1	0	
	Penicillium commune	1	24	4	
	Penicillium brevicompactum	1	22	4	
	Rhizomucor pusillus	1	20	0	St-Germain et al., 1993
	Penicillium digitatum	1	19	5	Oshikata et al., 2013
	Penicillium camemberti	1	17	2	
	Penicillium olsonii	1	17	5	
	Penicillium chrysogenum	1	15	4	
	Penicillium citrinum	1	14	1	Beena et al., 2021
	Penicillium glabrum	1	10	2	
	Neurospora crassa	1	9	0	
	Aspergillus tritici	1	7	0	

ht = Pathogenic for humans and vertebrates, but normally no transmission between the host groups.
Ref = reference articles that have identified the organism as potential human pathogen.
[a] Risk group classification in accordance with GESTIS database (IFA, 2023).

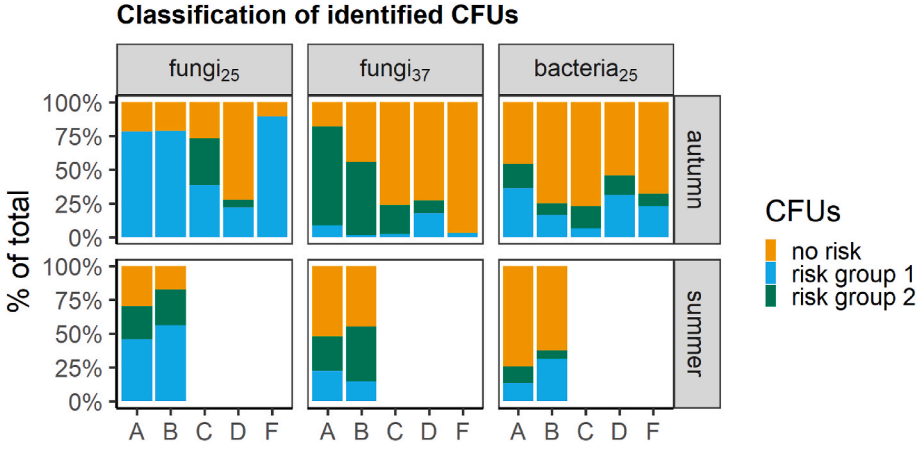

Fig. 4. Classification of identified microbial CFUs in personal air samples by WSP (A:F) and season. No risk group assigned (orange), CFUs in risk group 1 including species that are suspected of being pathogenic for humans and vertebrates in individual cases (blue), CFUs in risk group 2 (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Furthermore, differences in WSP accounted for 12% (p-value <0.001) and 22% (p-value <0.001) of the clustering of bacterial and fungal communities during summer, respectively.

3.5.1. Pathogenic species in NGS samples

A total of 838 taxa (bootstrap of 80% on species level) were identified in NGS samples. Of these, 78 were fungal taxa (13 in risk group 2) and 760 bacterial taxa (136 in risk group 2) (Table S8, Fig. S9). There was substantial variation in the composition of risk assessed microorganisms in personal filter samples between WSP and individuals (Fig. S9). The abundance of risk group 2 classified species was generally low in individual samples. However, the number of bacterial pathogens was somewhat higher at AWSP compared to MWSP, whereas fungal pathogens appeared at similar frequency.

3.5.2. Species present in MALDI and NGS

22 species (10 fungi, 12 bacteria) were identified in both MALDI and NGS samples on the species level. Seven bacterial taxa belonged to risk group 1, and five (*Acinetobacter johnsonii, Acinetobacter lwoffii, Klebsiella pneumoniae, Kosakonia cowanii, and Pantoea septica*) were classified in risk group 2. Seven of the identified fungi were in risk group 1, and three (*Aspergillus flavus, Aspergillus fumigatus,* and *Paecilomyces variotii*) risk group 2.

3.6. Microbial exposure in correlation to symptom frequency

The odds ratio (OR) for experiencing wheezing was positively and significantly correlated to increased levels of risk group 1 fungi, whereas the OR for doctor diagnosed asthma was positively and significantly associated with increased risk group 2 pathogen concentrations in personal samples (Equation (2), Table 4). The OR for exposure to risk assessed bacterial pathogens was borderline significant for coughing (risk group 2, p-value = 0.066) and nausea (risk group 1, p-value = 0.074). No significant impact of bacteria and fungi without risk classification was identified on the symptom prevalence in waste workers.

4. Discussion

This study assessed the microbial burden in personal air samples to identify potentially pathogenic species that may promote adverse health outcomes in susceptible individuals. The results show large seasonal variation in bacterial and fungal CFU concentrations within and between waste sorting plants as well as substantial variation in plant specific microbial species composition (Fig. 1, Tables S2–S4). *Aspergillus* was the most prevalent fungal genus accounting for 58% of all identified fungal CFUs, whereas dominating bacterial species belonged to the genus *Bacillus* and *Staphylococcus* accounting for 29% and 26% of all identified bacterial CFUs, respectively. Furthermore, significant correlation between self-reported symptoms as well as doctor diagnosed asthma and concentrations of risk assessed pathogens was observed in waste workers (Table 4).

4.1. Microbial concentrations in work air

The global shift towards greener societies and the sustainable use of natural resources has caused changes in national waste management regimes. In Norway, this is expressed in the segregation of household waste in different fractions, such as paper and cardboard, glass and metal, organic food waste, plastic and residual waste that are collected with different frequencies with varying time intervals. Hence, residual waste may remain in waste collection containers for up to 4 weeks before it is collected and processed. This prolonged storage contributes, especially during summer months, to accelerated microbial growth and thus to increased exposure levels during collecting and handling when microorganisms are aerosolised (Madsen et al., 2021; Viegas et al., 2016). However, concentrations of air-borne microorganisms have been reported to vary substantially regardless of environmental factors. This may be due to differences in exposure intensities during various non-comparable work tasks but also due to non-standardised sampling and evaluation protocols (Reinthaler et al., 1999).

Fungal and bacterial CFU levels presented in the current study were on average a 10-fold higher (Tables S2–S3, Fig. 1) compared to microbial concentrations reported in a Danish study that investigated occupational exposure to microorganisms in cardboard waste sorting (Madsen et al., 2019), and a Spanish exposure study in household waste sorting conducted by Solans et al. (2007). Results in the present study were comparable to levels reported for Polish waste soring plants (Szulc et al., 2022), whereas Kontro et al. (2022) reported air-borne concentrations of *Aspergillus fumigatus* and *Streptomyces* spp. measured in biowaste processing plants in Finland with concentrations as high as $10^5/m^3$ and $10^6/m^3$, respectively. However, these studies were conducted in manual WSP, comparable to plant D & F assessed in the present study. Microbial concentrations were generally higher in automated WSP (WSP A, B & C) compared to manual WSP (WSP D & F), with significantly higher bacterial CFU levels during autumn (Fig. S1). This supports results presented in a previous study that investigated exposure levels of dust, endotoxins and microbial DNA in the same population (Eriksen et al., 2023a). Seasonal variation in CFU concentrations was observed with contradicting trends at WSP A& B (Fig. 1). Summer levels were generally higher at WSP A, whereas autumn levels were higher at WSP B (Figs. S2 and S4). However, the number of autumn samples was lower in both cases. Furthermore, results from WSP A included two independent samplings during summer months, as well as samples collected during autumn. 50% of the autumn samples did not contain any viable cultivable microorganisms. This is most likely due to biases introduced during the handling of exposed filters, such as filter elution. Variance in CFU levels was generally higher between WSP than within WSP, which can be explained by differences in plant type (automated WSP versus manual WSP) (Fig. S1), observed work operations, as well as geographic location. The large variance in CFUs at plant A and B can largely be explained by the investigated work tasks. Samples with highest concentrations were collected on workers who used compressed air during cleaning operation, which has previously been reported as high exposure moment for various agents (Eriksen et al.,

Table 4
Model output generalised linear model investigating the effect of risk group levels (1, 2, no risk) on odds ratio (OR) of symptom occurrence in exposed waste workers. Only symptoms with significant (<0.05) or borderline significant (0.05–0.1) p values are shown.

	doctor diagnosed asthma		sore eyes		wheezing		coughing		nausea	
Predictor	*OR*	*p*	*OR*	*p*	*OR*	*p*	*OR*	*p*	*OR*	*p*
(Intercept)	0.008	0.002	0.081	0.001	0.022	0.001	0.032	0.001	0.042	<0.001
fungi risk group 1			1.09	0.052	1.1	**0.035**				
fungi risk group 2	1.1	**0.043**								
bacteria risk group 1									1.1	0.074
bacteria risk group 2							1.1	0.066		
Observations	40		41		41		41		41	
R^2	0.19		0.098		0.15		0.099		0.12	

2023a).

Microbial concentrations in personal air samples tended to be higher compared to outdoor samples, however, the differences were only in a few cases statistically significant (Fig. 1). This may be because the background samples were collected too close to the respective WSP in a distance of approximately 50–100 m. However, Cyprowski et al. (2021) reported significant differences in microbial concentrations between work-environmental and background samples that were collected approximately 50 m from the respective waste sorting plants, suggesting that 50 m should be enough distance between the main source of exposure in order to avoid contamination of reference samples. Furthermore, concentrations of airborne microorganisms generally increase in areas where waste with potentially high microbial content is handled (Tables S2–S4). The presence of pathogens, such as *Aspergillus* in background samples may result from transfer from the waste sorting plants to the surrounding environment and indicates that microbial contamination can be of concern for workers and residents in neighbouring areas (Schlosser et al., 2016).

4.2. Community composition and pathogenic potential of microorganisms

The assessment of the microbial community on the species level is crucial to adequately risk assess occupational exposure and potential exposure related health risks, as not all species within a genus may be pathogenic to humans (Duquenne, 2018). A total of 28 cultivable bacterial and 17 cultured fungal species with potentially pathophysiological properties were identified in personal air samples using culture-based methods in combination with MALDI-TOF MS (Table 3), whereas analysis of NGS data identified 136 biosafety level 2 pathogenic bacterial species and 13 fungal species with potentially pathogenic effects (Table S8). However, abundance of risk group 2 classified pathogens varied substantially between WSP and seasons with generally higher pathogen burden at automated WSP (A, B & C) compared to manual WSP (D & F) indicating that each WSP had characteristic microbiomes (Fig. S2:S5). These results were supported by WSP-specific clusters of bacterial communities (Figs. S6–A). Fungal taxa however, appeared to be largely shared between WSP (Figs. S6–B). Species in the genus *Aspergillus* were among the prevalent risk classified fungal pathogens observed in the present study and accounted for approximately 50% of the fungal exposure. Occupational exposure to various species in the genus *Aspergillus* has been associated with adverse health outcomes in susceptible individuals (Bafadhel et al., 2014; Bush et al., 2006; Greenberger, 2002). The presence of *Aspergillus* in the work environment has been proposed as bioindicator for pathogenic fungal exposure with toxicological potential (Sabino et al., 2019). *A.niger, A.fumigatus* and *A. flavus* were among the most abundant *Aspergillus* species, each of which is classified as risk group 2 pathogen that are associated with human respiratory disease. *A.flavus* is one of the main aflatoxin-producing fungi with carcinogenic properties (IARC, 2012) whose prevalence is suspectedly affected by climate change related increase in humidity and temperature (AssunJo et al., 2018; Viegas et al., 2015b). In addition to *Aspergillus*, a large number of species belonging to the genus *Penicillium* and *Rhizomucor* that are classified in risk group 1 was identified. Although classified in risk group 1, such microorganisms may affect the respiratory system and reduce pulmonary function in susceptible individuals (Beena et al., 2021; Oshikata et al., 2013) as well as contribute to antibiotic resistance by transferring antibiotic resistant genes to pathogenic species, as has been reported for bacteria (Jiang et al., 2017).

The most abundant cultured bacterial species belonged to the genus *Bacillus*, *Enterococcus*, *Staphylococcus* and *Streptomyces*, most of which are commonly found in soil, plant material and the natural skin-biota. Some of these were classified in risk group 2 or have been assessed as potential causative agents for human disease or opportunistic pathogens that can elicit an immune response in immunocompromised individuals (Table 3, Table S8). *Bacillus cereus* was the most abundant cultured bacterial species in personal air samples, and appears to be a common contaminant in waste as it was also reported as most prevalent species in cardboard waste sorting in a Danish study (Madsen et al., 2019). *B.cereus* has been discussed as opportunistic human pathogen and causative agent for gastrointestinal symptoms (Messelhäußer and Ehling-Schulz, 2018). The presence of *B.cereus* in combination with other human pathogens in the inhalable fraction indicates that occupational exposure during waste handling and sorting may elicit an immune response in exposed workers with compromised immune systems.

4.3. Fast emerging pathogens

The present study revealed the presence of three fungi with high pathophysiological potential in inhalable work-air samples (Table 3, Table S8). *A.fumigatus* was among the most prevalent fungal species identified in personal air samples. This mould is ubiquitous in the environment and has been reported as causative agent of pulmonary disease such as aspergillosis (Latgé and Chamilos, 2019). Due to its invasiveness and anti-fungal resistance *A. fumigatus* is classified as critical fast emerging pathogen and included the WHO's fungal priority pathogen list (WHO, 2022). Furthermore, we identified eight different species in the genus *Fusarium* that were highly prevalent in personal air samples (Table S8). Some of these filamentous fungi are predominantly found in decomposed organic material are classified as high emerging human pathogens due to their ability to cause serious infections of the respiratory tract especially in immune-compromised individuals (Nucci et al., 2021). Other members of WHO priority list, such as *Candida albicans* and *C. tropicalis* were identified at rather high frequencies in the present study. The opportunistic pathogenic yeast *Pichia kudriavzeveii* was identified in one personal air sample (Table S8). Albeit common in the human microbiome, infections of the skin or mucosa may cause serious health outcomes, such as invasive or oropharyngeal candidiasis (Coronado-Castellote et al., 2013; Pappas et al., 2018). The presence of relatively high levels of fast emerging pathogens in the work air samples implies that the waste sorting industry potentially provides the perfect breeding ground for proliferating microorganisms.

4.4. Exposure-related health outcomes

A great variety of symptoms that are related to exposure to bioaerosols have been reported in waste workers by (Schlosser, 2019; Wikuats et al., 2022). An increased prevalence of respiratory and gastrointestinal symptoms was identified among exposed waste workers in previous studies (Eriksen et al., 2023a, 2023b). The present study revealed correlations between concentrations of cultivable human pathogens in the inhalable work air and the frequency of self-reported health outcomes (Table 4). Significant correlation between symptoms of the respiratory tract, such as wheezing can be explained by high concentrations of infectious biological agents such as species in the genus *Aspergillus* and *Stenotrophomonas* (Chawla et al., 2014; Walsh et al., 2008). Symptoms such as diarrhoea and nausea have been reported in association with exposure to *Bacillus cereus*, a pathogen commonly causing gastrointestinal symptoms (Griffiths and Schraft, 2017). However, despite relatively high concentrations of *B.cereus* measured in the present study, correlations to health effects were non-significant. The prevalence of nausea was, however, borderline significantly correlated to exposure level of bacteria in risk group 1. Even though the frequency of health effects coincided with levels of infectious biological agents in the present study, the effects of non-infectious biological agents with pro-inflammatory and allergenic potential, such as endotoxins and β-glucans, remain unclear (Heldal et al., 2003; Straumfors et al., 2016). The importance of these in regards to occupational exposure in waste sorting cannot be disregarded.

4.5. Limitations of the study

Microorganisms were cultivated on a limited number of culture

media, selected temperature, and oxygen levels. Thus, only fungi and bacteria capable of growing on the provided substrate under the constraining conditions could be cultivated. The use of different culture media and growth conditions may have promoted the growth of other species, such as anaerobic bacteria. Furthermore, only a subfraction of the filter extracts was used for cultivation. Thus, it remains uncertain to what extent rare species were identified.

Identification of viable cultivable fungi at species level using MALDI-TOF MS technology was successful in 78% of all analysed CFUs, however only 42% of the analysed bacterial colonies could be identified on the species level using the reference database provided by the manufacturer. It can be assumed that the unidentified CFUs contained actinobacteria that are difficult to identify. Furthermore, the large differences in the number of unidentified CFUs in MALDI-TOF MS may be due to limitations of the reference database that predominantly included clinically relevant pathogens and may thus have underestimated the presence of potentially harmful species in the analysed work air samples.

In order to risk assess microorganisms that are contained in work air, different tools need to be considered as national and international directives for exposure to microbial agents vary considerably. This study used the GESTIS database (IFA, 2023) for classification of potential human pathogens, which is more detailed than the reference database used in Norway (Arbeidstilsynet, 2020). However, the use of other reference databases might have produced different results. This shows that there is a need for harmonised classification and legislation on microbiological exposure.

5. Conclusions

The present study contributed to increasing the knowledge on occupation exposure to microorganisms and the prevalence of potential exposure related health effects in contemporary waste sorting plants. Microbial biodiversity in personal air samples identified with NGS was higher compared to culture-based methods, indicating that the work environment contained substantial concentrations of not only infectious but also non-infections biological agents. The predominant cultured bacterial species belonged to the genus *Bacillus* and *Staphylococcus*, whereas fungal species were dominated by species in the genus *Aspergillus* and *Penicillium*. However, microbial CFU levels varied substantially between seasons and waste sorting plants. Twenty-three percent of bacterial, as well as 19% of fungal species were classified as human pathogens or biological agents that are suspected of being pathogenic to humans (Arbeidstilsynet, 2020; IFA, 2023). Large variation in the microbial community was identified between WSP as well as between seasons. Furthermore, fast emerging pathogens, such as *A.fumigatus*, were relatively abundant in personal samples. Due to its prevalence and significance as human pulmonary irritant, allergen, and toxin, *Aspergillus* has been proposed as sentinel species for occupational exposure to fungi and *Aspergillus* surveillance in the work air may provide means for occupational risk assessment and health promotion. The presence of risk group 1 assessed fungi was positively and significantly associated to the occurrence of wheezing in exposed workers. This implies that waste workers were potentially exposed to high levels of microbial organisms and metabolites thereof, with pathophysiological potential. However, the discordance between reference databases concerning pathogen classification and national legislations provide challenges in terms of risk assessment of the microbial exposure in waste sorting plants. Nonetheless, the results presented in this study indicate the need for measures to reduce exposure, such as personal protective equipment, to promote workers' health and prevent occupational disease.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

Elke Eriksen: Methodology, Investigation, Data curation, Data curation, Visualisation, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114240.

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