Annals of Work Exposures and Health, 2024, **68**, 58–77
https://doi.org/10.1093/annweh/wxad069
Advance access publication 23 November 2023
Original Article

A cross-sectional study on occupational exposure to microorganisms, endotoxin, hydrogen sulfide, and dust during work at drilling waste treatment plants

Hanne Line Daae[1], Pål Graff[1,*], Oda Astrid Haarr Foss[1], Victor Carp Kofoed[2], Anani Komlavi Afanou[1], Margit Wagtberg Frederiksen[2] and Anne Mette Madsen[2]

[1]National Institute of Occupational Health (STAMI), Department of Chemistry and Toxicology, Gydas vei 8, Majorstuen, 0363 Oslo, Norway
[2]National Research Centre for the Working Environment, Department of Chemistry and Microbiology, Lersø Parkallé 105, 2100 Copenhagen Ø, Denmark

[*]Corresponding author: National Institute of Occupational Health (STAMI), Gydas vei 8, Majorstuen, 0363 Oslo, Norway. Email: pal.graff@stami.no

Abstract

This cross-sectional study aims to obtain knowledge about workers' exposure to airborne dust, bacterial and fungal species, endotoxin, biofilm formation, and hydrogen sulfide (H_2S) in drilling waste treatment plants. In total, 408 full-shift personal samples, 66 work areas, 40 drilling waste, and reference (outdoor air and seawater) samples were analyzed. Some workers were exposed to high levels of endotoxin (207 EU/m^3), bacteria (3.8×10^4 colony forming units (CFU)/m^3 and 9.8×10^4 DNA copies/m^3), or fungi (1.4×10^7 CFU/m^3 and 3,600 copies/m^3). The exposure levels to endotoxin, bacteria, and peaks of H_2S were dependent on the treatment technique. All types of drilling waste contained large concentrations of bacteria compared to the seawater references. Elevated concentrations of airborne bacteria were found close to drilling waste basins. In total, 116, 146, and 112 different bacterial species were found in workers' exposure, work areas, and the drilling waste, respectively. An overlap in bacterial species found in the drilling waste and air (personal and work area) samples was found. Of the bacterial species found, 49 are classified as human pathogens such as *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella oxytoca*. In total, 44 fungal species were found in the working environment, and 6 of these are classified as human pathogens such as *Aspergillus fumigatus*. In conclusion, across the drilling waste treatment plants, human pathogens were present in the drilling waste, and workers' exposure was affected by the drilling waste treated at the plants with elevated exposure to endotoxin and bacteria. Elevated exposure was related to working as apprentices or chemical engineers, and working with cleaning, or slop water, and working in the daytime.

Key words: *Aspergillus fumigatus*; Bioaerosol; Green transition; Risk group 2 pathogens; Slop water; Wastewater.

What's Important About This Paper?

The study assessed occupational exposures during the treatment of oil drill waste. Pathogenic microoganisms were present in drilling waste, and elevated exposures to some bacteria and endotoxins were identified. Overall, exposures varied between job tasks, and also with the treatment technique. These data may help the industry in evaluating microbiological risks in the oil drill waste treatment.

Received: August 2, 2023. Accepted: October 30, 2023.

Introduction

Although attempts are made to reduce the use of oil, global oil production is rising (Statista 2023). Large amounts of different types of drilling waste and wastewater (hereafter referred to as drilling waste) are generated from the oil industry during production. Drilling waste discharge in the sea causes elevated levels of anaerobic bacteria in the seafloor with the presence of the *Clostridiales* (Nguyen et al. 2018). The drilling waste generated offshore has to be transported to the coast by companies specialized in treating it to reduce the amount of waste and to recover combustible components. Waste and water that remains stagnant may cause the growth of microorganisms (Shuster et al. 2013; Madsen et al. 2021), and this may also occur during the time between the generation and treatment of drilling waste. In line with this, our previous study of the working environment in 2 plants treating offshore drilling waste showed that human bacterial and fungal pathogens were present in the drilling waste. Among the pathogens were *Candida* species, which can cause skin infections, *Escherichia coli* and *Bacillus cereus*, which can cause gastrointestinal infections, and *Stenotrophomonas maltophilia*, which is an opportunistic pathogen (Daae et al. 2019). It is not known whether pathogens, in general, are present in drilling waste and whether the drilling waste workers are exposed to them.

Many microorganisms are able to form biofilm (Douglas 2003; Di Bonaventura et al. 2004; Beloin et al. 2008; Majed et al. 2016), which is a protective mechanism for survival under stressful conditions. In this study, we are interested in biofilm formation by microorganisms found in drilling waste workers' exposure and in the waste, because it may affect the survival of microorganisms in the waste, in aerosols generated, for example, under high-pressure cleaning of drilling waste basins, and also in the workers' airways where it may contribute to protection from the human immune system and contact with antimicrobials.

The fungal species *Aspergillus fumigatus* has been found in different working environments, and it is on the list of the World Health Organization with fungicide-resistant species defined as the critical priority group (Fisher and Denning 2023). Therefore, we include fungicide resistance of this species in the microbial risk evaluation of drilling waste workers' exposure.

In a previous study, drilling waste workers were exposed to elevated levels of endotoxin (Daae et al. 2019). Occupational exposure to endotoxin has been associated with inflammation and health symptoms of the airways in, for example, wastewater treatment plant workers (Heldal et al. 2019; Madsen et al. 2023). Therefore, it is relevant to measure whether the exposure to endotoxin is generally elevated for drilling waste workers. In contrast, the exposures to oil mist and oil vapor were below 10% of the current Norwegian occupational exposure limits (OEL) (Daae et al. 2019). Furthermore, drill floor workers' exposure to oil mist and chemical drilling mud components seem not to be associated with inflammatory reactions (Kirkhus et al. 2019).

The plants that receive offshore drilling waste use different techniques to treat the waste to evaporate the liquid from the solids and then separate the oil and water. The different techniques used at the different plants may form the basis for different working environments. Furthermore, work was also performed in the nighttime, and nighttime work can lead to worker fatigue and impair judgment, coordination, and reaction time; night shifts often have fewer workers on duty compared to daytime shifts. Therefore, it is relevant to study whether nighttime work was associated with higher exposure than daytime work. This cross-sectional study investigates workers' exposure to dust, bacterial and fungal levels and species, endotoxin, biofilm formation, and hydrogen sulfide (H_2S) at 7 drilling waste treatment plants as well as the presence of bacteria and fungi in the drilling waste. The aim of the study is to obtain knowledge about whether (i) the presence of human pathogens in waste is a general problem for drilling waste plants and waste types, and whether they become airborne; (ii) workers are exposed to pathogens and problematic levels of microorganisms, endotoxin, dust, and H_2S; (iii) these exposures are related to specific techniques of drilling waste treatment or specific job titles or tasks.

Material and methods

Plants and participants

Included in the study were 7 out of 8 different plants in Norway, working with recycling offshore drilling waste. This study was conducted during the period from October 2019 to November 2021 with samplings in October (Plant 1), November (Plant 2 and 7), March (Plant 3), and September (Plant 4 and 6). During sampling on all plants, except Plant 7, it was rainy. The average temperatures were from 1°C to 12°C with the lowest temperature in March. The eighth plant, which was not included in this study, was only partially operational during the sampling period.

Plants 1 and 7 treat the waste using a technique called Resoil, which is based on a thermal process in a heat exchanger in ovens. Plants 2, 3, and 5 use the technique Thermomechanical Cuttings Cleaner (TCC), to treat drill cuttings, and to convert kinetic energy into heat. The TCC technique is sometimes used in combination

with Water Oil Solid Separation (WOSS) technology to separate mud slop into water, oil, and solids. Plant 4 treats the waste with Fluidized Bed Combustion (FBC) technique, which is a complete combustion of drill cuttings in a reactor.

The plants differed in size and had different variations of shift arrangements. All employees working in the production at the time of measurements were offered to take part in the study. This resulted in a total of 53 workers, corresponding to 75–100% of those working in the productions at the time of measurements, participating in full-shift measurements (40 persons in 2 days, 8 persons in 1 day, and 5 persons in 3 consecutive days). In total, 408 full-shift personal samples were collected. The participants were asked to take note of the different work tasks which were performed. The measurements were conducted on employees with 6 different job titles: 1 process operator; 2 apprentices; 3 mechanics/electricians; 4 chemical engineers; 5 tank operators; 6 managers. For job descriptions see Table S1 and Fig. S1. Night shifts had fewer workers on duty compared to daytime shifts, and we measured exposure on 14 nighttime workers.

Personal exposure and references

In general, each worker was equipped with 4 air sampling cassettes, placed near the breathing zone. In addition, 3–13 of the workers at each plant were equipped with a direct reading, H_2S logger. The personal air samples were collected during the entire work shift, which varied between 8 and 12 h; however, the sampling was stopped if some had to leave the facility (e.g. off-site meetings, etc.). The sampling time for reference samples was 7–12 h. During the air sampling, the authors responsible for the data collection were at the sampling sites throughout the sampling period to follow the work and track the measuring equipment.

Endotoxin and airborne dust

In total, 96 personal samples were taken for endotoxin analysis (average sampling time, 463 min) using 25 mm glass fiber filters with pore size 1.6 µm (GF/A, Whatman, Maidstone, UK) mounted in PAS-6 aerosol samplers (University of Wageningen, The Netherlands). Airborne dust was collected using a 25 mm antistatic polypropylene air monitoring cassette (Pall Laboratories, Port Washington, NY, USA) equipped with 0.8 µm hydrophilic polycarbonate membrane filter (Merck KgaA, Darmstadt, Germany). A total of 110 personal full-shift samples were collected, of these, 68 samples were parallels (average sampling time, 455 min). Both the 25 mm antistatic polypropylene air monitoring cassettes and PAS-6 aerosol samplers were attached to sampling pumps (SG5200 GSA, Messgerätebau GmbH, Ratingen, Germany, TUFF4 Casella, Bedford, UK) operated at a flow rate of 2 L/min. Sampling flow rates were measured before and after sampling, using a flowmeter (Bios Defender 510, Bios Int. Corp., NJ, USA).

Hydrogen sulfide (H_2S)

H_2S was measured with portable personal direct-reading logging instruments: Dräger PAC 6500 and Dräger PAC 7000 (Drägerwerk AG & Co. KGaA, Lübeck, Germany) (0.1–200 ppm; resolution 0.1 ppm) on 58 workers. The instruments recorded 10 s concentration average, in a continuous log. The number of peaks and the highest concentration level were used to evaluate the H_2S exposure.

Microorganisms

Airborne inhalable microorganisms were sampled at the 7 plants. A total of 144 personal and 11 outdoor references, full-shift measurements were conducted (average sampling time 478 and 424 minutes respectively), using Conical Inhalable Samplers (CIS) (JS Holdings, Hertfordshire, UK) and Casella Apex2 sampling pumps (Casella UK, Wolseley Rd, Kempston, Bedford, UK), airflow rate of 3.5 L/min. All samplers were mounted with polycarbonate filters (pore size 1 µm, Frisenette (DK),-GVS Filter Technology, ME 04073-USA). After sampling, 57 polycarbonate filters (including 6 outdoor reference filters from 6 plants) were transported to the laboratory at NRCWE in Denmark, where the dust on the filters was immediately extracted, while 98 filter samples (including 5 outdoor reference filters from 4 plants) were transported to the laboratory at NIOH in Norway for DNA extraction and analysis.

Environmental sampling

MAS100 air samples of microorganisms.

A MAS100 NT (Merck, Germany) with a flow rate of 100 L/min was used to sample in working areas; the samples were taken in areas where workers usually had work tasks such as close to basins, hoppers, and on footbridges. In total 123 samples were taken in working areas and 39 outdoors. The sampler was mounted with Nutrient agar (NA; Thermo Fisher Scientific Oxoid) for culturing bacteria or with Dichloran Glycerol agar (DG-18 agar; Thermo Fisher Scientific Oxoid) for fungi. At each sampling location repeated samplings were done using different sampling times (from 2 to 8 min), to ensure an optimal sampling time, to avoid overloading the agar plates with microorganisms. Post-sampling, the agar plates were transported to NRCWE in Denmark. At arrival, they were incubated at 25°C. Plates that were not overgrown and had a sufficient amount of colonies were used for counting and identification. The data are presented

as time-weighted average (TWA) exposures in colony forming units (CFU)/m^3 air.

Waste material for microorganisms

At each plant, waste materials were sampled. Approximately 20 mL were taken from the mud waste, slop waste, and drill cuttings in duplicates, and a reference sample was taken from the sea. The samples were taken in the middle of the workday and kept at +4°C until arriving at NRCWE in Denmark the next day.

Analysis

Gravimetry.

Determination of the mass of the collected airborne dust was performed by weighing the filters before and after exposure using a microbalance (Sartorius AG, MC210, Göttingen, Germany) in a temperature- and humidity-controlled room (20 ± 1°C, 40 ± 2% relative humidity). The LOD was 0.01–0.02 mg/filter, estimated as three times the standard deviation of blank filters. For further details, see supplementary file (Methods section).

Endotoxin

The filters were extracted using 5 mL pyrogen-free water with 0.05% Tween-20 solution by orbital shaking (500 rpm for 1 h), followed by centrifugation (1000 G/rcf for 15 min). Supernatants were stored at −20°C until analysis. The extracts were diluted 20 times and analyzed in duplicates (with and without 50 EU/mL spike-in control) using the kinetic Limulus amebocyte lysate (LAL) assay according to the manufacturer's description (Lonza Ltd., Basel Switzerland). Endotoxin concentrations were determined by comparison with a five-point standard curve (0.005–50 EU/mL) obtained from *E. coli* O55:B5. The limit of detection (LOD) was 0.25–0.5 EU/filter across the analytical series.

Microorganisms—cultivation

The bacteria and fungi collected on polycarbonate filters were extracted in 5.5 mL sterile solution (0.05% Tween 80 and 0.85% NaCl) by orbital shaking (500 rpm) for 15 min at room temperature. The dust suspensions were plated in different dilutions on three types of agar media: NA for the quantification and identification of bacteria; DG18 agar and Sabouraud agar medium (SA, Oxoid, Basingstoke, UK) for quantification of fungi. The plates were incubated at different temperatures with or without oxygen.

The drilling waste samples were after arrival at the laboratory shaken (500 rpm) for 15 min at room temperature. The samples were plated and incubated as described in the supplementary file (Methods section). Part of each suspension was stored with 33% glycerol at −80°C for re-plating and biofilm assay.

Bacteria able to grow aerobically at 25°C are called bacteria, and bacteria grown anaerobically are called anaerobic bacteria. The number of fungi on DG18 and SA did not differ significantly, and counting numbers are presented for DG18 data. The data on airborne microorganisms are presented as TWA (CFU)/m^3 air, and microorganisms in the drilling waste samples as CFU/mL.

MALDI–TOF MS for species identification

When the bacteria and fungi appeared on the agar plates they were identified using the MALDI–TOF MS Biotyper System (Bruker Daltonics, Bremen, Germany) with a bacterial and fungal library as described previously (Madsen et al. 2023). However, the sampling occurred over 2 years, and the library was updated during that period; therefore, we had to reanalyze old spectra for unidentified isolates against the updated libraries (Bruker BDAL v.9 library for bacteria and Filamentous library 4.0 for fungi). A bacterial test standard (Bruker Daltonics) was used to calibrate the instrument. Agar plates of each sample and each growth condition with 20–45 bacterial colonies were selected for identification. All fungal isolates from each sample and growth condition were selected for identification.

Biofilm

Dust suspensions from the 51 personal CIS samples, the 6 outdoor reference CIS samples, the 40 drilling waste samples, and 14 reference (seawater) samples with the glycerol were added to tubes with Tryptic soy broth (Oxoid, United Kingdom) in the ratio of 1:10. After vortexing, aliquots from each suspension were dispensed into 8 wells in a flat-bottom, clear microtiter plate (Corning, USA) and incubated at 37°C for 24 h as described previously (O'Toole 2011; Madsen et al. 2023). For further details see supplementary file (Methods section).

Antifungal resistance testing

Susceptibility testing was carried out using broth microdilution according to guidelines from EUCAST (Guinea et al. 2022). Fungal isolates were tested against the polyene Amphotericin B, and the triazoles: Itraconazole, Voriconazole, and Posaconazole, with concentration ranges of 0.0016–8 mg/L for Amphotericin B, Itraconazole and Voriconazole and 0.0008–4 mg/L for Posaconazole. For Quality Control purposes strains *A. flavus* CNM-CM 1813 and *A. fumigatus* ATCC 204305 were used. Prior to susceptibility testing, isolates of *A. fumigatus* were screened for triazole resistance using screening agar plates (VIPcheck™, MediaProducts, Groningen, Netherlands).

Bacterial and fungal DNA

Exposed polycarbonate filters were transferred to sterile 15 mL tubes and stored at 4°C until DNA extraction and analysis. DNA was extracted by cell lysis and spin-column separation using a DNeasy plant kit (Qiagen GmbH, Hilden, Germany) (Straumfors et al. 2019). Quality scores of PCR products were evaluated with a Bio-Rad QX200 droplet reader (Bio-Rad Laboratories Inc., CA, USA). For further details see supplementary file (Methods section).

Treatment of data

All exposure data were log transformed. Data were analyzed in one model (general linear models, GLM) with the three factors: Plant, job title, and night versus day. Pearson correlation between exposures was analyzed. Concentrations of airborne bacteria close to versus not close to basins were also analyzed as GLM.

The study was approved by the Regional committees for medical and healthcare research ethics (Ref: 2019/853/REK nord). Prior to participating in the study, each participant signed an informed consent.

Results

Personal exposure—plants and job titles

Airborne dust.

The mean (AM) exposure to airborne dust was 0.30 mg/m^3 (GM; 0.19 and Min–Max = 0.01–1.84, Table 1). Dust exposure was associated with plant ($P < 0.0001$) and day versus night ($P = 0.023$) with the highest exposure in the day and for Plant 1. Dust exposure was not associated with job title ($P = 0.31$), but the comparison of the individual tasks showed that employees with job title 3 (mechanic/electrician) were exposed to more dust ($P < 0.001$) than employees with other job titles. The highest single exposures were measured in connection with maintenance work performed by an apprentice and a mechanic.

Hydrogen sulphide. A total of 58 personal measurements were carried out during 1–4 shifts at each plant. None of the measurements was above the Norwegian OEL value of 5 ppm, measured over a working day. Short-term peaks of 7.2–7.7 ppm were recorded in connection with the slop water cleaning process in Plants 1 and 7. H_2S is a volatile gas that occurs and disappears quickly. Short-term peaks can be difficult to capture, and concentration can vary widely within the same industry and work operations. At Plant 7, 9 out of 13 recorded measurements had H_2S peaks above the detection limit (0.1 ppm) and the total number of H_2S peaks varied from 7 up to 218. This plant was only partially operational at the time and resumed production after a 3-week shutdown. The exposure to H_2S was above the odor threshold (0.001–0.13 ppm) at Plants 1, 2, 4, and 7.

Endotoxin

A total of 96 personal measurements of airborne endotoxin were performed with concentrations below the detection limit in 26% of the collected samples. The mean exposure to endotoxin was 8.9 EU/m^3 (GM; 2.1 EU/m^3, Table 1). The personal exposure was associated significantly with the plant ($P < 0.0001$), day versus night ($P = 0.0012$), and job title ($P = 0.0041$), with high exposure for Plants 1, 2, and 7, daytime, and job titles 2, 3, and 4. Two workers were exposed to high concentrations of endotoxin (101 and 207 EU/m^3), and they worked with slop water cleaning as apprentices. Tasks such as flushing and maintenance also caused higher exposure to endotoxin (22–51 EU/m^3, $n = 12$, different job titles). At the plants using the Resoil technique, endotoxin exposure was significantly higher than those using TCC/WOSS ($P < 0.001$) and FBC ($P = 0.001$).

Endotoxin exposure correlated significantly with dust ($r = 0.41$, $P = 0.0002$ $n = 96$), bacterial (CFU: $r = 0.36$, $P = 0.012$, $n = 48$; DNA copies: $r = 0.56$, $P < 0.0001$, $n = 85$), and fungal DNA copies ($r = 0.32$, $P = 0.0027$, $n = 85$) exposure.

CFU of bacteria

In total 116 different bacterial species were found in workers' exposure (Table S2). The exposure to bacteria was between 24 and 3.8×10^4 CFU/m^3 with a GM of 626 CFU/m^3 and lower exposure to anaerobic bacteria (Table 1). Personal exposures were higher than outdoor concentrations of both bacteria and anaerobic bacteria ($P < 0.05$). The exposure differed between plants ($P = 0.011$), job titles ($P = 0.025$), and day vs night ($P = 0.031$). It was highest for workers at Plants 1 and 7 using Resoil technique (Table 1), and job title apprentice was associated with higher exposure than the titles process operators, chemical engineers, and tank operators. The worker with the highest exposure worked as an apprentice in Plant 1, and, in general, cleaning and flushing were associated with the highest exposures. Exposure was highest in the daytime compared to nighttime. For anaerobic bacteria, it also differed between plants ($P = 0.0001$) with the highest exposure in Plant 1. Personal exposure to bacteria correlated significantly with anaerobic bacteria ($r = 0.43$, $P = 0.0018$, $n = 52$) and endotoxin, and not with other exposures.

CFU of fungi

In total 29 different fungal species were found in workers' exposure (Table S3). The exposure was between 4 and 1.4×10^7 CFU/m^3 with a GM of 78 CFU/m^3 (Table 1), and it was not significantly different from

Table 1. Personal exposure (averages, in brackets geometric mean) to dust, endotoxin, bacteria and fungi and stationary measures of outdoor concentrations and concentrations of bacteria and fungi in work areas (MAS100 samples).

Plant	1	2	3	4	5	6	7	Across plants
Process type	Resoil	TCC/WOSS	TCC/WOSS	FBC	TCC/WOSS	TCC/WOSS	Resoil	
Dust mg/m³	*n* = 16	*n* = 14/2	*n* = 17	*n* = 9	*n* = 32	*n* = 3	*n* = 19	*n* = 110/2
Persons	0.79^{a} (0.66)	0.15^{bc} (0.09)	0.10^{c} (0.08)	0.19^{b} (0.16)	0.25^{b} (0.19)	0.22^{b} (0.21)	0.30^{b} (0.20)	0.30 (0.19)
Outdoors	nm	<0.01	nm	nm	nm	nm	nm	-
Endotoxin EU/m³	*n* = 18	*n* = 11	*n* = 12	*n* = 18	*n* = 21	*n* = 3	*n* = 13	*n* = 96
Persons	17.6^{a} (11.0)	9.8^{a} (5.7)	1.1^{b} (1.1)	2.3^{b} (1.1)	0.75^{b} (0.67)	1.6^{b} (0.88)	27.4^{a} (2.7)	8.9 (2.1)
Bacteria CFU/m³	*n* = 10/0	*n* = 7/1	*n* = 6/1	*n* = 9/1	*n* = 11/1	*n* = 3/1	*n* = 5/1	*n* = 51/6
Persons	5873^{a} (1765)	486^{bc} (292)	2456^{abc} (795)	520^{c} (268)	1017^{ab} (782)	1079^{abc} (576)	3873^{ab} (1444)	2255 (626)
Outdoors	nm	bd	314	bd	6.9	197	805	265 (67)
Anaerobic bacteria CFU/m³	*n* = 10/0	*n* = 7/1	*n* = 6/1	*n* = 9/1	*n* = 11/1	*n* = 3/1	*n* = 5/1	*n* = 51/6
Persons	450^{a} (243)	185^{b} (52)	108^{b} (55)	40^{c} (15)	159^{a} (90)	128^{a} (94)	128^{b} (57)	183 (55)
Outdoors	nm	7	88	bd	bd	13	bd	19 (6.3)
Fungi CFU/m³	*n* = 10/0	*n* = 7/1	*n* = 6/1	*n* = 9/1	*n* = 11/1	*n* = 3/1	*n* = 5/1	*n* = 51/6
Persons	342^{a} (90)	347^{a} (43)	198^{a} (30)	208^{a} (45)	1.3x10$^{6\ a}$ (227)	188^{a} (151)	88^{a} (71)	2.7x10^{5} (78)
Outdoors	nm	529	127	12	14	33	64	153 (72)
Biofilm OD/m³	*n* = 10/0	*n* = 7/1	*n* = 6/1	*n* = 9/1	*n* = 11/1	*n* = 3/1	*n* = 5/1	*n* = 51/6
Persons	2.07^{c} (1.62)	4.37^{b} (4.02)	21.5^{a} (17.1)	1.36^{c} (1.32)	1.67^{c} (1.50)	1.51^{c} (1.47)	2.88^{bc} (1.97)	4.50 (2.33)
Outdoors	nm	3.93	2.80	1.29	bd	1.33	bd	1.76 (1.38)
Bacteria copies/m³	*n* = 13/0	*n* = 12/1	*n* = 12/0	*n* = 18/0	*n* = 22/1	*n* = 3/1	*n* = 13/2	*n* = 93/5
Persons	2978^{bc} (1238)	2.1 × 10$^{4\ a}$ (5821)	89^{d} (80)	5116^{b} (1391)	2308^{b} (358)	114^{cd} (110)	1.4 × 10$^{5\ ab}$ (1119)	2.5 × 10^{4} (738)
Outdoors	nm	27	nm	Nm	175	32	114	92 (72)
Fungi copies/m³	*n* = 13/0	*n* = 12/1	*n* = 12/0	*n* = 18/0	*n* = 22/1	*n* = 3/1	*n* = 13/2	*n* = 93/5
Persons	189^{b} (78)	1543^{a} (647)	8^{c} (4)	720^{a} (279)	40^{c} (18)	4^{c} (4)	821^{b} (70)	490 (57)
Outdoors	nm	7	nm	nm	313	139	23	101 (40)
Work areas and outdoors MAS100								
Bacteria CFU/m³	*n* = 8/2	*n* = 9/3	*n* = 9/3	*n* = 9/3	*n* = 8/2	*n* = 9/3	*n* = 9/3	*n* = 61/19
Work areas	729^{a} (724)	173^{c} (158)	136^{bc} (133)	490^{b} (255)	75^{d} (67)	156^{c} (144)	161^{c} (149)	241(149)
Outdoors	83 (51)	50 (20)	147 (135)	487 (480)	24 (19)	28 (23)	60 (54)	141 (77)
Fungi CFU/m³	*n* = 8/2	*n* = 9/3	*n* = 9/3	*n* = 9/3	*n* = 9/3	*n* = 9/3	*n* = 9/3	*n* = 62/20
Work areas	26^{b} (25)	31^{b} (14)	17^{b} (16)	80^{a} (78)	28^{b} (27)	64^{a} (62)	39^{b} (35)	43 (33)
Outdoors	75 (73)	50 (20)	28 (25)	35 (35)	33 (33)	60(54)	28 (23)	48 (38)

n = number of samples taken using personal sampler/outdoor samplers. Bacteria and fungi measured as CFU (colony forming units), DNA copies and dust for biofilm were sampled using CIS samplers; dust was sampled using antistatic polypropylene air monitoring cassettes and endotoxin was sampled using PAS-6 aerosol samplers, bd = below detection level, nm=not measured. Numbers in the same row followed by the same letter(s) (a, b, and/or c) are not significantly different ($P > 0.05$).

the outdoor concentrations ($P = 0.64$) and did not correlate with other exposures. The exposure did not differ between plants ($P = 0.40$), job titles ($P = 0.38$), and day versus night ($P = 0.52$). For Plant 5, an apprentice and a tank operator were exposed to very high concentrations of fungi (3.2×10^5 and 1.4×10^7 CFU/m^3).

Biofilm formation

The biofilm formation tended to be higher for the workers' exposure than for the outdoor measurements ($P = 0.078$), (Table 1). The biofilm formation differed between plants ($P < 0.0001$), not between job titles ($P = 0.58$), but between day versus night ($P = 0.0084$). The highest biofilm formation was found for samples from workers at Plants 2 and 3 and for day samples. The biofilm formation did not correlate with any single exposure.

Bacterial DNA

The exposure to bacterial DNA copies was between 51 and 9.8×10^4 copies/m^3 (GM = 738 copies/m^3; Table 1) and was lower than the outdoor references ($P = 0.037$). The exposure differed between plants ($P < 0.0001$), job titles ($P = 0.0051$), and day versus night work ($P < 0.0001$) with high exposures for Plants 2, 4, and 7 (Table 1), job title 2, 4, and 5, and the daytime. The exposure to DNA copies of bacteria correlated significantly with DNA copies of fungi ($r = 0.78$, $P < 0.0001$, $n = 91$), and as mentioned with endotoxin exposure.

Fungal DNA

The exposure to fungal DNA copies was between bd and 3,600 copies/m^3 (GM = 57 copies/m^3; Table 1), and it was not significantly higher than outdoor references ($P = 0.43$). The exposure differed significantly between plants ($P < 0.0001$) and job titles ($P = 0.023$) with high exposure at Plants 2 and 4 (Table 1) and for job title 4 (higher than titles 1, 2, 5 and 6, and 5 higher than 1).

Bacteria and fungi in work areas

Bacteria

In work areas (MAS100 sampler), a total of 146 bacterial species were found while 51 species were found in outdoor samples (Table S2). The concentrations of bacteria were between 20 and 1,400 CFU/m^3. Different concentrations were found between plants ($P < 0.0001$) with Plant 1 having the highest concentrations (Table 1). If the samples were divided into close to basins versus not close to basins versus outdoor, the highest concentrations were found close to basins ($P < 0.0001$) while there was no difference between outdoors and the areas not close to basins (examples in **Fig. 1**).

Fungi

In work areas (MAS100 sampler), a total of 31 fungal species were found while 20 species were found in outdoor samples (Table S3). The concentrations of fungi were between 4 and 160 CFU/m^3, and different concentrations were found between plants ($P < 0.0001$) with the highest concentrations in Plant 4 (Table 1). If the samples were divided into close to basins versus not close to basins versus outdoor no significant differences were found ($P = 0.67$). Fungal and bacterial concentrations measured in the same areas did not correlate significantly ($r = -0.10$, $P = 0.38$).

Drilling waste—microorganisms and biofilm formation

Different types of drilling waste and seawater were analyzed for microorganisms (Tables 2 and 3 and Table S4) and biofilm formation (Fig. S2). In total, 112 different bacterial and 20 fungal species were found in the drilling waste, and 113 bacterial and 7 fungal species in the seawater (Tables S2 and S3). The waste is categorized into drilling mud, slop water, and the water fraction from the drill cuttings (WDC).

Drilling mud

The drilling mud contained 10^3–10^{10} CFU of bacteria/mL and 10^2–10^6 CFU of anaerobic bacteria/mL. Most of the bacteria were *Aerococcus viridans* (Table S4). The bacteria were able to form biofilm, and the largest amounts were found for Plant 1. The mud from Plant 1 had been in the basin for a long time. The studied mud samples contained low concentrations of fungi (<300 CFU/mL), and *Candida boidinii*, *Penicillium chrysogenum*, and *P. commune* were found repeatedly. The pathogen *A. fumigatus* was also found.

Slop water

The slop water contained 10^2–10^6 CFU of bacteria/mL and 10^2–10^6 CFU of anaerobic bacteria/mL, including the species: *A. viridans, Citrobacter* species, *Halomonas aquamarina, Klebsiella oxytoca, Lelliottia amnigena, Raoultella ornithinolytica*, and *Shewanella baltica*—and in Plant 7 also *E. coli* (Table S4).

The slop water contained significantly fewer bacteria, had a lower species richness, and formed less biofilm after cleaning (*P*s < 0.0001) with 40 times less bacteria and 3 times less biofilm after chemical treatment in Plant 2 (Table 2). In Plant 4, it contained 3 times less bacteria and formed 8 times less biofilm after cleaning (Fig. S2). The slop water contained a few *Fusarium solani* and *A. fumigatus* isolates.

Waste drill cuttings (WDC)

The water fraction from the WDC contained 10^2 to 10^6 CFU of bacteria/mL and 10^2 to 10^4 CFU of

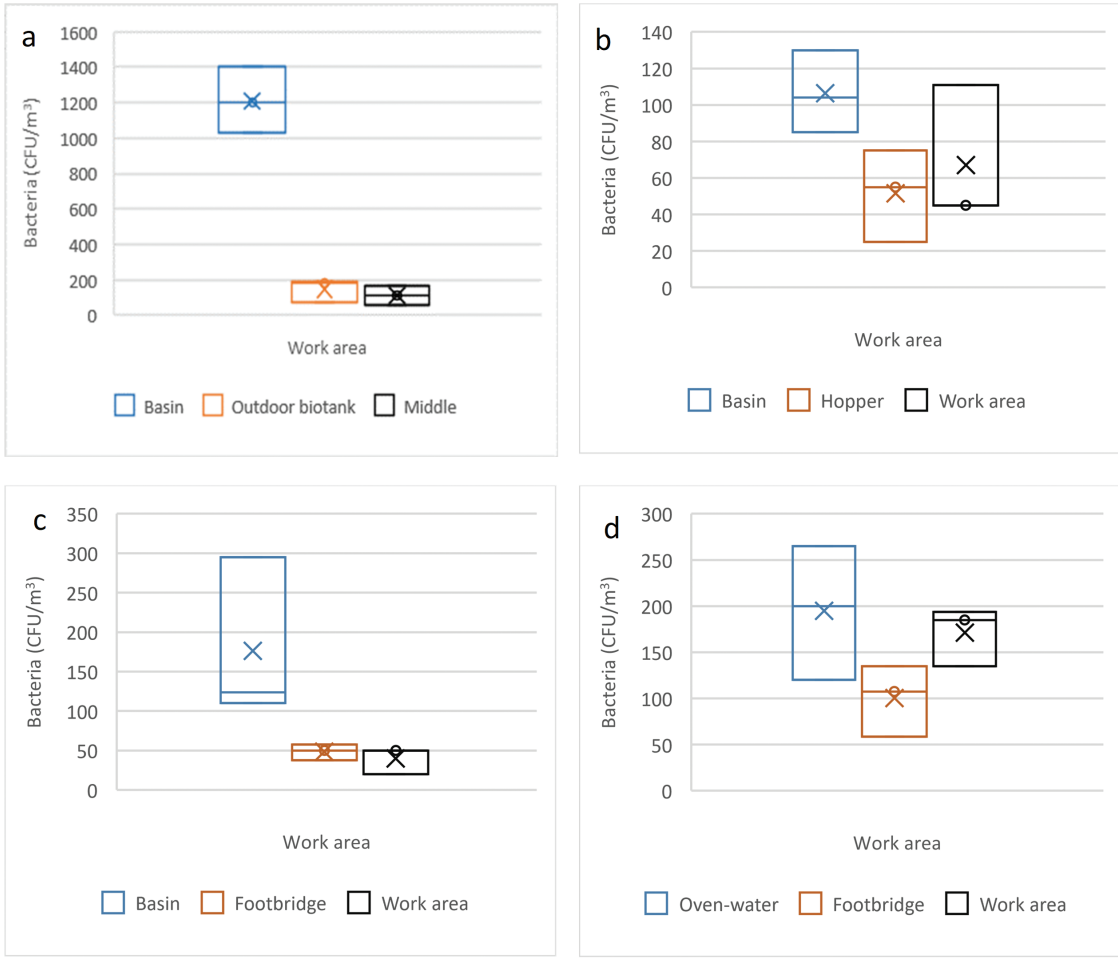

Fig. 1. Concentrations of airborne bacteria in: Plant 4 (FBC) close to a skimming basin, close to an outdoor Biotank, and in the middle of the plant (a); Plant 5 (TCC/WOSS) close to a TTC basin, close to the hopper (mix drilling fluid and additives), and a WOSS outside a control room (b); Plant 6 (TCC/WOSS) close to a basin, on a footbridge between a process engineering room and the basin, and in the middle of the plant behind the process engineering room (c); Plant 7 (Resoil) between an oven and water cleaning in the middle of the plant, a footbridge in the water cleaning area, and close to a basin; all gates were open during sampling (d). The *x*'s are the averages, the horizontal lines the medians, and the top and bottom lines the maximum and the minimum measured concentrations (n = 3).

anaerobic bacteria/mL and the bacteria formed biofilm (Fig. S2). The following species were found repeatedly: *A. viridans*, *Arthrobacter* species, *Citrobacter braakii*, *C. freundii*, *Citricoccus nitrophenolicus*, *Dietzia natronolimnaea*, *Pseudomonas veronii*, *P. xanthomarina*, and *Sphingomonas faeni*. The drill cuttings contained a few *Penicillium* isolates.

Seawater

The seawater contained from bd to 10^3 CFU bacteria/mL; it contained several *Pseudomonas* species (Table 3), and no or very little biofilm was formed by the bacteria in the seawater (Fig. S2). The seawater contained a few *Penicillium* isolates (Table 4).

Microbial species across sample types

In total 320 different bacterial and 44 fungal species were found in the working environment and reference samples (Tables S2 and S3), and an overlap in species between sample types is found (Fig. 2). Across the seven plants, 71% of the tested microorganisms were identified to species level.

Bacteria

The bacterial species found most frequently are presented as a frequency table in a heatmap separated into personal exposure, drilling waste samples, stationary MAS100 samples in work areas and outdoors, and seawater samples (Table 3). Some species were dominating

Table 2. Bacterial and fungal species in slop water before and after cleaning and in a seawater reference (n = 2, average concentrations, CFU/mL). Samples from Plant 2.

Bacteria	Slop	Slop-clean	Seawater
Aerococcus viridans	45 000	183	2
Citrobacter amalonaticus	4,500		
Citrobacter braakii	550		
Citrobacter farmeri	600		
Citrobacter freundii	200		
Citrobacter gillenii	500		
Citrobacter koseri	550		
Corynebacterium glutamicum	33 400	2,267	
Enterobacter cloacae	550		
Hafnia alvei	10 000	100	
Klebsiella oxytoca	5,050	400	
Kluyvera ascorbata	1,100		
Kluyvera intermedia	11 620	100	700
Lactobacillus coryniformis	200	250	
Leclercia adecarboxylata	3,000		
Lelliottia amnigena	5,300		1,100
Propionibacterium acnes	2,100		
Pseudomonas anguilliseptica			2
Pseudomonas koreensis			2
Pseudomonas fluorescens			2
Psychrobacter sp			
Raoultella ornithinolytica	7,167		
Raoultella planticola	5,000		
Vibrio rumoiensis	100		5
Total bacteria	**13 6487**	**3,300**	**1,813**
Fungi			
Candida boidinii	10	Bd	Bd
Fusarium solani	2	Bd	bd

Bd = below detection level.

in workers' exposure as well as in the drilling waste: *A. viridans*, *B. cereus*, *B. pumilus*, *Brevibacterium linens*, *Dietzia maris*, *Micrococcus luteus*, and *S. faeni*, and some species were found both in drilling waste and in the air in work areas. Many workers were exposed to *D. maris* (bd to 316 CFU/m^3) or *D. natronolimnaea* (bd to 198 CFU/m^3), and these species were also found in the drilling waste and the air around the biotanks. Most workers were exposed to *Rhodococcus fascians* (bd to 735 CFU/m^3), and this species was found in some working areas, for example, next to the biotank, next to a tank where organic material was getting removed, and close to a centrifuge.

Some bacteria found in workers' exposure are human skin-related species such as *M. luteus*, *Staphylococcus epidermidis*, and *Cutibacterium acnes*. Examples of bacterial species present in the exposure of colleagues in the same plant are given in Figs. S3–S5.

Forty-nine bacterial species found in personal, work area, drilling waste, seawater, or outdoor samples are classified in Risk group 2 (Gestis). In addition, several species also known to cause health problems were found repeatedly. Most different Risk group 2 species were found in waste from Plants 1 and 7 followed by Plant 2 (Table 5), and the highest exposures were also found in Plants 1 and 7.

Fungi

Some species were found in all sample types (Table 4). Six different fungal species classified in Risk group 2

Table 3. Number of plants (maximum 7) in which the most frequently found bacterial species were present.

Frequently found bacterial species	Personal air	Drilling waste	MAS100 work	MAS100 outdoor	Seawater
Aerococcus viridans	2	7	1	1	
Arthrobacter flavus	5	1	4	1	2
Bacillus cereus	5	3	1		
Bacillus pumilus	2	3	2	1	1
Brevundimonas intermedia	1	1	3		1
Cutibacterium acnes	4				
Dermacoccus nishinomiyaensis	5		2		
Dietzia maris	3	3	3		
Dietzia natronolimnaea	4	1	2	1	
Exiguobacterium aurantiacum	2	1	3	1	2
Janthinobacterium lividum			2		2
Kocuria palustris	3		1	1	
Microbacterium maritypicum		2	1		1
Microbacterium phyllosphaerae	2	1	3		
Micrococcus flavus	5		3	1	
Micrococcus luteus	7	3	6		
Micrococcus terreus	5	1	1		
Paracoccus yeei	3		2		
Planomicrobium okeanokoites	1		4	2	
Pseudarthrobacter polychromogenes	2		4		1
Pseudomonas anguilliseptica		3	1	1	4
Pseudomonas antarctica			2		1
Pseudomonas fragi		1	2		3
Pseudomonas fluorescens		1	2	2	3
Pseudomonas koreensis			2	1	3
Pseudomonas stutzeri		4	2		
Pseudomonas xanthomarina	1	2	3		
Psychrobacter sp	2	2	2	1	1
Rhodococcus fascians	6	3	6	2	2
Shewanella baltica		3	1		2
Sphingomonas aerolata	5	2	3	2	1
Sphingomonas faeni	5	1	2	1	1
Staphylococcus capitis	3		3		
Staphylococcus epidermidis	5		3		
Staphylococcus hominis	6		3	2	
Tsukamurella paurometabola	2	2	3	1	1

Drilling waste includes drilling mud, slop water, and drill cuttings. For all species see Table S2.

were found in the personal exposures and work areas (*Aspergillus* species), mud (mainly *Fusarium* species), and mud and slop water (*A. fumigatus*); the Risk group 2 species were found in low concentrations (Table 6).

The four *A. fumigatus* isolates and the *Lichtheimia corymbifera* isolate were not resistant to Amphotericin-B, Itraconazole, and Posaconazole. Furthermore, *A. fumigatus* was not resistant to Voriconazole while *L. corymbifera* was resistant (MIC value above 8 mg/L). However, *L. corymbifera* is intrinsically resistant to Voriconazole.

Discussion

In this study, exposure was measured by obtaining 408 full-shift personal samples collected for workers in 7

Table 4. Personal exposure to fungi (CFU/m³) in work areas and outdoor references as measured using MAS100 (CFU/m³), and in drilling waste and seawater (CFU/mL).

Fungal species	Personal air	Drilling waste[a]	MAS100, work	MAS100, outdoor	Seawater
Alternaria alternata	0.25	0.80	0.83	0.25	
Aspergillus candidus		6.0			
Aspergillus fumigatus	3	1.7			
Aspergillus niger	29		1.0		
Aspergillus versicolor	33		2.2	1.7	
Aspergillus candidus		11			
Aspergillus glaucus	145		5.0	3.8	
Aspergillus nidulans	1809		3.9	1.3	
Aureobasidium pullulans			3.5		
Botrytis cinerea	33		3.6		0.67
Candida boidinii[b]		62			
Cladosporium spp.	17		47	5.0	0.40
Cladosporium herbarum	6		1.9	0.25	
Cryptococcus magnus[b]	62				
Epicoccum nigrum	0.39		0.6		
Fusarium incarnatum			2.5		
Fusarium oxysporum		10			
Fusarium solani		50			
Fusarium dimerum		10			
Fusarium tabacinum		10			
Lichtheimia corymbifera		6.0			
Paecilomyces farinosus			2.5	0.25	
Penicillium brevicompactum	278	0.40	3.1	4.4	0.40
Penicillium camemberti	268		0.9	5.8	
Penicillium chrysogenum	356	50	1.5	0.25	
Penicillium citrinum			1.3		3.0
Penicillium commune	70	9.0	1.3	1.3	
Penicillium corylophilum	20		0.25	1.9	
Penicillium dierckxii	0.88	0.40			
Penicillium digitatum	1.1	0.80	0.6	2.3	0.40
Penicillium expansum	33		5.0		
Penicillium italicum		10	0.83	0.63	
Penicillium glabrum	8.8	3.3	6.2	2.0	0.50
Penicillium olsonii	114	2.5	0.25	3.3	
Penicillium roqueforti	0.33	3.0			
Penicillium rugulosum	35		3.3	2.5	
Phoma herbarum	4.0		0.25	0.25	
Phoma glomerata	28			0.50	
Phoma sorghina			2.5		
Scopulariopsis brevicaulis	5.5				
Scopulariopsis brumptii			5.0		
Thanatephorus cucumeris	35				
Wallemia sp.	105	0.40	1.2	1.6	
Yarrowia lipolytica[b]		3.3			

[a]Drilling waste includes the drilling mud, slop water, and drill cuttings.
[b]Yeast.

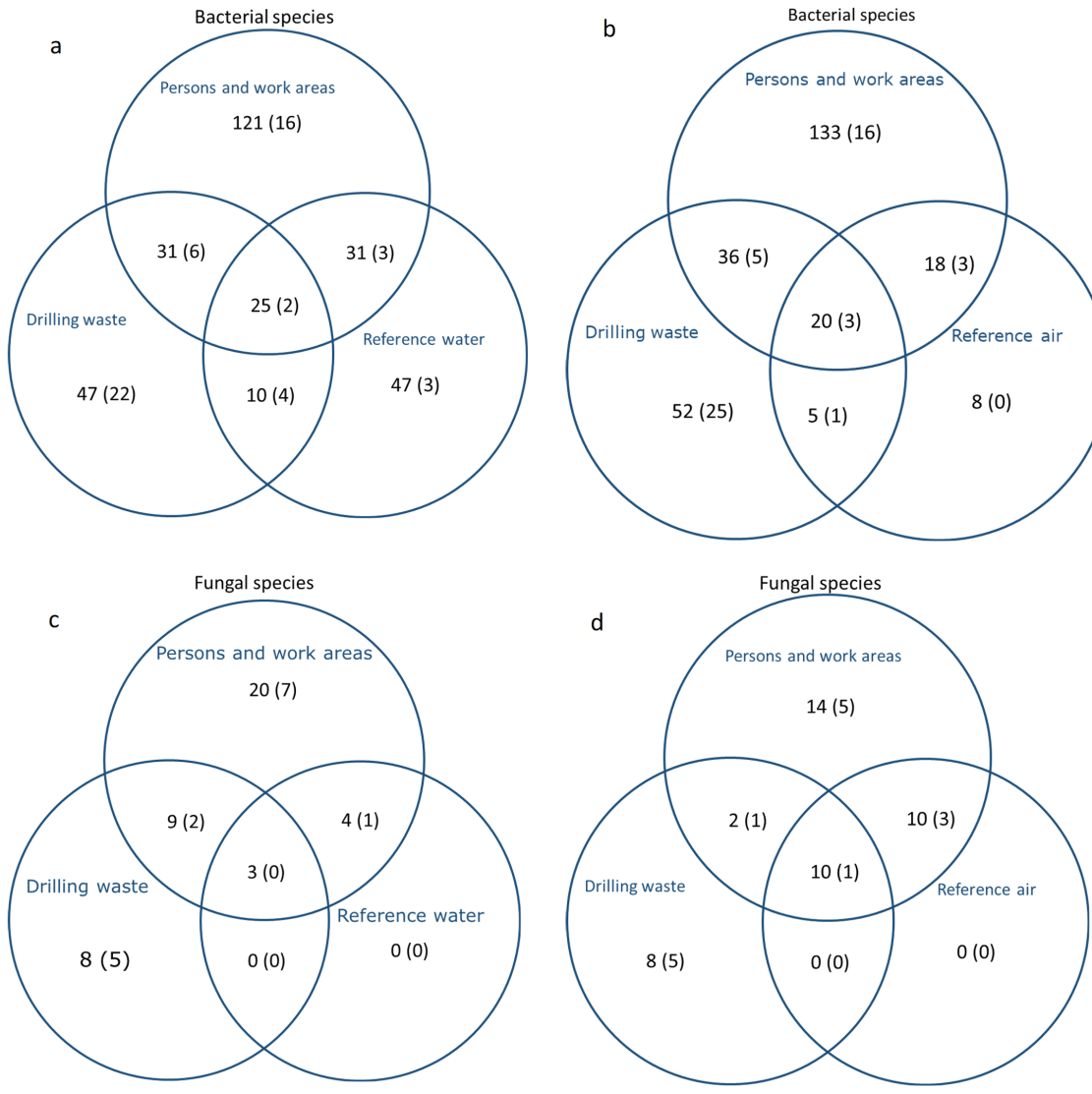

Fig. 2. Venn diagram on numbers of bacterial (a and b) and fungal (c and d) species present in the personal and work areas samples, in the drilling waste samples, reference water (seawater), and/or reference air. In brackets are the numbers of pathogen species defined in Tables 5 and 6.

drilling waste plants. In addition, stationary air samples were collected in work areas and outdoors, and drilling waste and seawater samples were collected and characterized. Based on these measurements, we will in the following discuss the results to interpret the findings concerning the microbiological working environment of drilling waste plants.

Drilling waste as a source of exposure

Risk group 2 fungal pathogens were found in drilling waste in 6 of the 7 plants, with *A. fumigatus* and *A. niger* also detected in one worker's exposure. Other *Aspergillus* species were only found in the drilling waste or the air, while some *Penicillium* species were found in both the drilling waste samples and workers' exposure. *P. chrysogenum* and *A. fumigatus* have been described as oil-degrading fungi (Ahmad and Ganjo, 2020) and were found in both workers' exposure and waste, but only in low concentrations. *Aspergillus nidulans* was found in very high concentrations in some workers' exposure. This species has been studied for its potential use in bioremediation of crude oil spills in soil (Nrior and Mene 2017), but it is unclear from this study whether it grew in the drilling waste.

Table 5. Bacterial species potentially causing health problems, maximum concentrations measured, and positive plants.

Problematic bacteria	Person CFU/m^3	Work area CFU/m^3	Outdoors CFU/m^3	Waste CFU/mL	Sea CFU/mL	Positive plants[a]	Risk group[b]	Reference[c]
Acinetobacter johnsonii			20	560	4	3, 4, 7	2	
Acinetobacter lwoffii	330	254	3			1, 4, 5, 7	2	
Acinetobacter junii		8				1	2	
Actinomyces neuii	29					7	2	
Aerococcus viridans	330	22	3	5×10^6		1, 2, 3, 4, 5, 7	2	
Alcaligenes faecalis					40	6	2	
Arthrobacter gandavensis		29				4, 6, 7	2	
Bacillus cereus	133	13	bd	4,500		1, 2, 4, 5, 7	2	
Bacillus mycoides	10		bd	200		1, 2, 3	2	
Brevundimonas vesicularis	14					7	2	
Chryseobacterium indologenes		3				5	2	
Citrobacter amalonaticus				4,500		1, 2, 7	2	
Citrobacter braakii				1×10^6		1, 2, 6, 7	2	
Citrobacter farmeri				1,000		1, 2, 7	2	
Citrobacter freundii				2×10^6		2, 6	2	
Citrobacter gillenii				1×10^4		1, 2, 6, 7	2	
Citrobacter koseri				1,000		2, 7	2	
Clostridium bifermentans				15		1	2	
Clostridium intestinale				20		4	2	
Corynebacterium tuberculostearicum	15					7	2	
Cutibacterium acnes	116					1,3, 4, 5, 6, 7	2	
Delftia acidovorans		1			40	1, 6	1	Bilgin et al. (2015)
Dietzia maris	316	20		1,000	4	1, 2, 3,4, 5, 6, 7	1	Koerner et al. (2009)
Dietzia natronolimnaea	197	15	1	6		1, 3, 4, 5, 7	1	Koerner et al. (2009)
Enterobacter asburiae				1,000		1	2	
Enterobacter aerogenes				100		2	2	
Enterobacter bugandensis				100	2	7	2	
Enterobacter cloacae				10 000	2	1, 2, 3, 7	2	
Enterobacter kobei				2,000		7	2	
Enterococcus casseliflavus		5		450		1, 3, 6	2	
Enterococcus faecium				150		1, 4	2	
Escherichia coli				2,000		7	2	
Gordonia otitidis	7	3	7		4	3, 4, 5	2	
Klebsiella oxytoca				80 000		1, 2, 6	2	

Table 5. Continued

Problematic bacteria	Person CFU/m³	Work area CFU/m³	Outdoors CFU/m³	Waste CFU/mL	Sea CFU/mL	Positive plants[a]	Risk group[b]	Reference[c]
Kluyvera cryocrescens				100		7	2	
Kluyvera intermedia				40 000		1, 2, 7	2	
Leclercia adecarboxylata				3,000		2	2	
Lelliottia amnigena				80 000	40	6, 7	2	
Massilia timonae	110	6				1, 7	1	Lindquist et al. (2003)
Moraxella osloensis	24	1				1, 2, 5	2	
Oerskovia turbata		1				3	1	Thomas et al. (2007)
Pantoea agglomerans					200	6	2	
Pantoea septica	48	5				1	2	
Paracoccus yeei	99	1				3, 7	2	
Propionibacterium avidum	19			200		1, 2	2	
Propionibacterium granulosum	11					1	2	
Pseudomonas stutzeri		5		8×10^5		1, 3, 4, 5, 6, 7	1	Foote et al. (2017); Canada GO (2018)
Psychrobacter pulmonis				3×10^4		2	2	
Raoultella ornithinolytica				2×10^5		1, 2, 6, 7	2	
Raoultella planticola				100		2	1	Ershadi et al. (2014); Hajjar et al. (2018)
Rhodococcus erythropolis	72	1	5			1, 2, 3, 5, 6	1	Roy et al. (2009)
Roseomonas mucosa	24					2, 3, 5	2	
Serratia fonticola		3	3		40	5, 6	2	
Serratia liquefaciens					2	7	2	
Serratia proteamaculans				24 000		6, 7	2	
Wautersiella falsenii					10	5	2	
Yersinia intermedia				1×10^6		6	2	

[a]Plants where the bacteria has been found in a personal (CIS), work area (MAS100, stationary), or an outdoor (MAS100, stationary) air sample, or in drilling waste or a seawater reference.
[b]Risk classification according to Gestis.
[c]Reference to a paper describing the species as a pathogen if it is classified in Risk group 1. Several *Staphylococcus* species belonging to Risk group 2 were found in workers' exposure, but they are not included in this table as we consider them as human-related bacteria.

Another oil-degrading fungus, *Yarrowia lipolytica* (Beopoulos et al. 2009), was found in the drilling waste in low concentrations, but not in the air. *Fusarium* species were also found in the drilling waste, and previous research has found *Fusarium* in metalworking fluid (Dahlman-Höglund et al. 2022), but whether it grew in the drilling waste is not known. *Fusarium* species were only rarely detected in the air.

The exposure to CFU of fungi was not related to job title or working area, and exposure to CFU of fungi and fungal DNA was not higher than outdoor references. Except for *A. nidulans* no single species was found in high concentrations in the drilling waste or the air. Based on these findings, we conclude that there was no clear evidence of the airborne fungi originating from the drilling waste.

Table 6. Fungal species potentially causing health problems, maximum concentrations measured, and positive plants.

Problematic fungi	Person CFU/m³	Work area CFU/m³	Outdoors CFU/m³	Waste CFU/mL	Sea CFU/mL	Positive plants[a]	Risk group[b]	Occupational problems[c]
Alternaria alternata	5					7	1	Yes (Baur et al. 1992)
Aspergillus candidus				11		1	1	Yes (Krysińska-Traczyk and Dutkiewicz 2000)
Aspergillus fumigatus	5			10		1, 5, 7	2	Yes (Poole and Wong 2013)
Aspergillus glaucus	281	5	4			2,3	1	Yes (Yoshida et al. 1990)
Aspergillu nidulans	8×10^5	15	3			2, 3, 5, 6, 7	1	No (Henriet et al. 2012)
Aspergillus niger	44	1				2,3,6	2	Yes Pinckard et al. 2003)
Aspergillus versicolor	75	5	5			1, 3,6, 7	1	Yes (Barnes et al. 2018)
Botrytis cinerea	63	5			2	1, 6, 7	1	Yes (Jürgensen and Madsen 2009)
Epicoccum nigrum		7				7	1	Yes (Hogan et al. 1996)
Fusarium dimerum				10		3	1	Yes (Uemura et al. 2022)
Fusarium incarnatum		3				3	2	No (Gupta 2017)
Fusarium oxysporum				10		3	2	Yes (Chi et al. 2005)
Fusarium solani				20		2, 7	2	Yes (Dalphin and Gondouin 2015)
Lichtheimia corymbifera				4		1	2	Yes (Bellanger et al. 2010)
Scopulariopsis brevicaulis	5	5				6	1	Yes (Lander et al. 1988; Cuenca-Estrella et al. 2003)

[a]Plants where the fungus has been found in a personal (CIS), work area (MAS100, stationary) or an outdoor (MAS100, stationary) air sample, or in drilling waste or a seawater reference.
[b]Risk classification according to Gestis.
[c]References to papers describing cases where the fungus has caused occupational health problems; no means that it has caused health problems, but not related to occupation.

Bacteria classified as Risk group 2 pathogens were found in the drilling waste in all plants. The Risk group 2 pathogens, *A. viridans* and *B. cereus*, were found frequently in the drilling waste and workers' exposure. Of these, *A. viridans* was found in both drilling mud, slop water, and the water fraction from drill cuttings, and it was the species found in the highest concentrations. The species *A. viridans* and *B. cereus* have previously been found as airborne bacteria in other occupational settings, but due to the high concentration in the drilling waste and their ability to degrade oil (Boboye et al. 2010), as well as their absence in seawater, it is likely that the drilling waste is the source of exposure.

The slop water in Plant 7 contained several gram-negative bacteria including *E. coli* and five *Citrobacter* species, *Enterobacter kobei*, and *L. amnigena*. Of these, the *Citrobacter* species and *L. amnigena* were also found in the drilling waste in other plants. These species were not found in the air, and this may be because they were not aerosolized, or they might have lost their cultivability in the air or during sampling. Other Gram-negative species such as *H. aquamarina* and *H. elongata* and some *Pseudomonas* species were found as cultivable bacteria in both drilling waste and the air. We expect that bacteria aerosolized as biofilm are better protected and therefore resist aerosolisation and sampling better than planktonic bacteria.

Dietzia maris and *D. natronolimnaea*, found in the drilling waste, were also found in the air around

biotanks, in other work areas, and workers' exposure. These species can also degrade oil (Gharibzahedi et al. 2014). Even though concentrations of bacteria were in general low, higher concentrations were found close to drilling waste basins. Based on this and the overlap in species between drilling waste and the air we conclude that bacteria from the drilling waste were aerosolized and contributed to the workers' exposure.

Plant, job, waste, and day versus night—workers' exposure

Even though the variation in concentrations of bacteria, endotoxin, and fungi between plants and within plants was small, the personal exposures varied significantly between plants using different process types. The bacterial (CFU) and endotoxin exposure were highest at plants using Resoil, and bacterial DNA was high in Plant 7 using Resoil. The drilling waste in these plants also contained most different bacteria classified as pathogens. As an example, the pathogen, *E. coli*, was only found in a plant using Resoil. Interestingly, this species was found in the slop water in the same plant 6 years ago (Daae et al. 2019). Bacterial exposure and the number of species classified as pathogens, and the amount of biofilm formed from workers' exposure were in general low at the plant using the FBC technique. However stationary measurements close to a basin in the plant using the FBC technique showed high concentrations of airborne bacteria. The exposure to H_2S was above the odor threshold (0.001–0.13 ppm) at the two plants using Resoil (Plants 1 and 7), Plant 2 (TCC/WOSS), and Plant 4 (FBC).

The dust exposure was in general low, but the highest amounts of airborne dust were measured in connection with maintenance work and work as a mechanic/electrician. Personal exposure to CFU bacteria was affected significantly by job title, and working as an apprentice was associated with higher exposure than working as a process operator, chemical engineer, and tank operator. Two workers were exposed to high concentrations of endotoxin, and they worked with slop water cleaning as apprentices. Work as an apprentice has in other environments been associated with high risk of accidents (Grytnes et al. 2021), and attempts to reduce exposure could focus on apprentices. For bacterial DNA and endotoxin, the highest exposures were found for chemical engineers. Peaks of H_2S were associated with the slop water cleaning process, several short-time peaks were measured.

Bacteria seem to be aerosolized from the drilling waste; therefore, it is relevant to compare the different types of drilling waste as potential sources of exposure. The drilling waste was divided into three types, drilling mud, slop water, and the water fraction from drill cuttings with seawater as reference. All waste types contained high concentrations of bacteria including many different gram-negative species. The species *A. viridans* was found in the highest concentrations and in all waste fractions. The cleaned slop water contained fewer bacteria and formed less biofilm unaffected by whether it was treated at a TCC/WOSS plant or a plant using the FBC technique. This shows that work with untreated slop water, in general, poses a higher risk of bacterial exposure than treated slop water.

The exposure to dust, bacteria, and endotoxin was highest in the daytime and hence night work seems not to be more problematic in terms of microbial exposure level. More people work during the daytime and more exposure assessments were done during the daytime, thus covering the possibility of larger variations in exposure. It is worth highlighting that workers' lung function seems to be more negatively affected by endotoxin during nighttime compared to daytime exposure (Zock et al. 1999).

Risk evaluation of workers' exposure

Airborne dust exposure was higher than an outdoor reference but was in general low and below the Norwegian OEL of 5 mg/m^3. The dust exposure was in the lower range of what has been measured for inhalable dust for sewage treatment plant workers in the Netherlands (AM = 0.4 mg/m^3) but higher than for the fraction of sewage treatment plant workers working in offices (AM = 0.1 mg/m^3) (Spaan et al. 2008).

The exposure to endotoxin was higher than what has been measured in outdoor air (Madsen 2006). The average endotoxin exposure was low as compared to wastewater treatment plant workers (Spaan et al. 2008; Madsen et al. 2023), although for two workers the recommended 90 EU/m^3 (Nordic Expert Group, 2011) was exceeded. Handling of drilling fluids has been associated with pulmonary symptoms (IPIECA 2009), but whether endotoxin exposure contributes to the symptoms was not studied. Even though the average exposure to endotoxin was low, experimental studies indicate that repeated exposure to low but elevated concentrations of endotoxin has a negative impact on the airways (Lai et al. 2012; Janssen et al. 2013).

The workers were exposed to CFU of aerobic and anaerobic bacteria, and bacterial DNA levels were higher than those found outdoors. While there is no OEL for bacteria, the relevance of setting one can be questioned due to the bacterial diversity. However, we can compare the exposure levels and the species to previous findings. The GM exposure to bacteria was low when compared to work with waste collection (Madsen et al. 2020), but similar to work at a wastewater treatment plant (Madsen et al. 2023), and at the higher end of levels found in normal indoor air in homes (Frankel et

al. 2012). The workers appear to be exposed to bacteria from the drilling waste, but based on the species composition also to species previously found in indoor air in homes such as *M. luteus* and *Staphylococcus hominis* (Madsen et al. 2018). This is not surprising as the workers spend a large part of their workday in control rooms. It is not known if exposure to these species, which workers inhale daily during work in drilling waste plants as well as in normal homes, has a negative effect on the airways.

The workers were exposed to several Risk group 2 pathogens via air, and they may also be exposed to Risk group 2 pathogens on the skin from, for example, splashes from the drilling wastewater. The bacterial species *E. coli* and *B. cereus* can also belong to Risk group 3, depending on other characteristics of the isolates, which were not studied in this paper. Of the Risk group 2 pathogens, *A. viridans* was frequently found in workers' exposure and drilling waste. It has previously been found in high concentrations in turkey (Fallschissel et al. 2010) and pig farms (White et al. 2019). *Bacillus cereus* was also frequently found in workers' exposure and drilling waste, and if swallowed, it may cause gastrointestinal problems. The several different *Enterobacter* species found in the drilling waste; *Enterobacter* species have previously been found in the working environment in wastewater treatment plants (Lu et al. 2020) and have caused soft tissue infection in relation to wastewater exposure (Baker and Gardner 2021).

An apprentice and a tank operator were exposed to very high concentrations of fungi (10^5 and 10^7 CFU/m^3). The exposure was dominated by *A. nidulans*, *P. brevicompactum*, and *P. olsonii*. Some of their colleagues were also exposed to these fungi but in lower concentrations. The exposure of 10^7 CFU/m^3 is considered as high, and health effects such as cough can be expected (Eduard 2009). *Aspergillus nidulans* belongs to Risk group 1 (Unfallversicherung 2017) but can grow at 37°C, and cases of infections have been reported (Henriet et al. 2012).

Other workers were exposed to only low concentrations of fungi, and except at Plant 2, outdoor concentrations were also low. Thus, the drilling waste workers were exposed to lower concentrations of fungi than workers in waste plants (Rasmussen et al. 2021; Eriksen et al. 2023), and in the lower end of wastewater treatment plant workers (Madsen et al. 2023), but at the level found in outdoor air in Finland (Kiviranta et al. 1999). Besides *A. nidulans*, fungal species which have previously caused occupational health problems including allergic reactions or are classified in Risk group 2 were found only in low concentrations in the exposures, and they were not fungicide resistant, and hence are not expected to cause health problems in healthy workers.

Microorganisms in workers' exposure and the drilling waste were able to form biofilm. Splashes of water with, for example, *Fusarium* (Nucci and Anaissie 2002) and several of the found bacterial species should be taken seriously, as they may cause skin infections, particularly in workers with existing skin problems. Dermatitis has been reported after repeated exposure to drilling fluids (IPIECA 2009). In wounds, biofilm formation may inhibit the effectiveness of antibiotics and physical presence may obstruct wound healing (Vestby et al. 2020). The capacity of the microorganisms to form biofilm was studied in an *in vitro* model, which has some limitations. However, for some bacterial species, *in vitro* biofilm-forming capacity has been shown to correlate well with measurements in patients (Bendouah et al. 2006).

The time-weighted average exposure to H_2S was <0.1 ppm, but the exposure was above the odor threshold (0.001–0.13 ppm) at four of the seven plants, which can lead to uncomfortable working conditions. Higher H_2S concentrations (7.2–7.7 ppm) were also recorded, and exposure to several short-time peaks of H_2S may lead to adverse health effects even though the average exposure was low (Austigard et al. 2018). In the previous study, H_2S was measured above the Norwegian ceiling value of 10 ppm (Daae et al. 2019).

Conclusions

This study shows that human pathogens were present in the drilling waste and work in the drilling waste treatment industry is associated with exposure via the air and potential exposure via splashes to bacterial pathogens, which was related to job title, techniques used at the plants, and area at the plants. Certain tasks were also associated with elevated levels of endotoxin and H_2S. Therefore, efforts should be made to reduce exposure associated with:

- Working as apprentices. It is crucial to determine whether the higher exposure of apprentices is due to the tasks they perform or how they perform them.
- Plants that use the Resoil technique, as these plants were associated with the highest exposures and most pathogens.
- Working close to basins because bacterial pathogens were found in the drilling waste and the air around the basins.
- Working with uncleaned slop water rather than cleaned slop.
- Cleaning processes such as flushing and high-pressure cleaning.

Strategies to reduce exposure could include the training of apprentices in occupational hygiene, marking

of zones in the plants where one can stay for an extended period, and use of masks during cleaning processes.

Fungi in the working environment seem not to derive from the drilling waste, and most workers were exposed to levels similar to those found in outdoor air.

Acknowledgements

The workers in the plants are highly acknowledged for their participation. Emma Enshelm Tranchant at NRCWE-dk is highly acknowledged for highly skilled laboratory work with the MALDI-TOF MS identifications. Thilde Sofie Nygård, formerly at STAMI, is acknowledged for invaluable help during the field work and with endotoxin analysis.

Author contributions

All the authors listed have read and approved the manuscript and agreed to authorship and submission of the manuscript for peer review.

Funding

The main funding of this project was The Confederation of Norwegian Enterprises grant number S-3104, S-3105 and S-3108. MST-DK grant 2020-6748 funded the fungicide resistance testing. The funding sources had no involvement in study design; collection, analysis and interpretation of data or in the writing of the article.

Conflict of interest

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

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *Annals of Work Exposures and Health* online.

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