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Occupational Exposure to Microorganisms When Using Biological Degreasing Stations

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IRSST – Communications and Knowledge Transfer Division
505 De Maisonneuve Blvd. West
Montréal, Québec
H3A 3C2
Phone: 514 288-1551
publications@irsst.qc.ca
www.irsst.qc.ca

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Carol-Anne Villeneuve[1], Geneviève Marchand[2],
Marie Gardette[1], Jacques Lavoie[2],
Denis Bégin[1],
Maximilien Debia[1]

[1] Département de santé environnementale et santé au travail, École de santé publique, Université de Montréal

[2] IRSST

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[1] For the role of the follow-up committee consult http://www.irsst.qc.ca/en/ohs-research/knowledge-translation

ABSTRACT

Biological degreasing stations, also called bioremediating parts-washing systems, biological parts washers or simply biowashers, use a degreasing agent containing bacteria that break down fats, oils and greases (FOG) by mineralization. The manufacturers of these agents claim the microorganisms used are harmless, since they are classified as Risk Group 1 according to the four-group infection risk ranking system. Some researchers, however, identified a number of Risk Group 2 bacteria (moderate individual risk, low community risk), such as *Pseudomonas aeruginosa,* in biological degreasing station solutions, but no metrological data were available for assessing the occupational risk of exposure through inhalation. The purpose of this study was to provide such data.

Five biological degreasing stations were monitored for a year. Bioaerosols were sampled every two months using an Andersen single-stage impactor and three-piece polystyrene filter cassettes. Sterile tubes were used to collect 50-mL samples of degreasing fluid from the biological degreasing stations. In addition, for each biological degreasing station, a 50-mL sample of unused degreaser was collected straight from its container at the first visit. The samples were used to count and identify culturable bacteria, either directly by incubation of the agar medium from the Andersen impactors or using 200-µL smears of extracts from the polycarbonate filter or the liquid samples. Several methods were used to identify the bacteria: Gram staining, catalase test, oxidase test, MicroScan plate reading, fatty acid profile analysis and mass spectrometry analysis.

The year-long monitoring of liquids from the five biological degreasing stations demonstrated that culturable microorganism concentrations ranged from 3.6×10^4 to 2.6×10^7 CFU/mL. Sixty species of bacteria classified as Risk Group 1 or Risk Group 2 were identified, including Gram-positive as well as Gram-negative bacteria. Several bacteria genera were found, including *Bacillus, Pseudomonas, Citrobacter, Burkholderia, Staphylococcus* and *Stenotrophomonas*, though only the species *Bacillus subtilis* was found in the unused solutions for all five biological degreasing stations. In other words, the biological degreasing stations were rapidly colonized by exogenous microorganisms such as *Pseudomonas aeruginosa*. The main risk with skin contact is wound infection or accidental ingestion—should the mouth come in contact with the hand or with a contaminated object, for example. Strict personal hygiene measures, including wearing gloves and hand-washing before and after using the biological degreasing station, are therefore necessary.

This study shows that workers using a biological degreasing station have very low exposure to bioaerosols. While recommended intervention levels for occupational exposure to bioaerosols are around 10^4 CFU/m³, the average ambient concentrations measured during this study were all below 480 CFU/m³. Moreover, use of an air blower to dry parts degreased in the biological degreasing stations did not significantly increase worker exposure to culturable microorganisms. No respiratory protection is therefore recommended during biological degreasing station use.

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LIST OF ACRONYMS AND ABBREVIATIONS

$\bar{x}$:	sample mean
σ:	standard deviation
#:	number of particles
DNA:	deoxyribonucleic acid
rRNA:	ribosomal ribonucleic acid
SDS:	safety data sheet
log:	decimal logarithm
MAC:	MacConkey agar
p:	significance level
pH:	potential of hydrogen
sp:	species
TSA:	trypticase soy agar
CFU:	colony-forming unit
UV-APS:	ultraviolet aerodynamic particle sizer

1. INTRODUCTION

1.1 Background

The toxicity of many organic solvents is well established (Bruckner, Anand and Warren, 2013). In addition, their flammability, the explosiveness of their vapours and their use in confined spaces can lead to fatal accidents (Beaudette and Marquis, 2014-05-08; Chester and Rosenman, 2012). More than 300,000 Québec workers are frequently or continuously exposed to organic solvent vapours on the job (Vézina et al., 2011). There is good reason, as a result, for companies to replace them with less hazardous products or processes.

The mechanical maintenance industry is a major user of solvents, for degreasing metal parts (Guillemin and Lupin, 2008). Water-based cleaners are also used to degrease metal parts, and have been for a long time (Spring, 1974). At first, these water-based products were all highly alkaline and thus corrosive. Thanks to innovations in surfactant chemistry, however aqueous degreasers that are only mildly alkaline but perform just as well have been available for a number of years. Water-based degreasers containing bacteria that break down fats, oils and greases (FOG) by mineralization have also been on the market since the 1990s. These biotechnological water-based degreasers, used in biological degreasing stations (also called biowashers) are now common in the mechanical maintenance industry (Bégin, Gérin and Lavoie, 2014a).

1.2 Microorganisms used for bioremediation

The manufacturers of bioremediating degreasers for parts washers claim that the microorganisms used in their products are harmless since they are classified as Risk Group 1 according to the four-group infection risk ranking system used in most countries around the world. According to the Public Health Agency of Canada, Risk Group 1 (RG1) microorganisms pose a low risk to the health of individuals and to public health (PHAC, 2015).

However, under certain conditions, a number of RG1 microorganisms can pose occupational health risks other than infection to those exposed to them. Endotoxins are associated with airway disease in workers exposed to them (Rylander, 2006), and the proliferation of Gram-negative bacteria increases the likelihood of finding endotoxins in ambient air in the workplace (Marchand, 1996a). Though the etiological agents have not been identified, exposure to high levels of microorganisms is also associated with non-allergic respiratory disorders, such as organic dust toxic syndrome (ODTS) and hypersensitivity pneumonitis (Douwes, Thorne, Pearce and Heederik, 2003).

1.3 State of the art

David et al. studied the bacterial flora in the degreasing solutions used in seven biological degreasing stations in France (David, Boucher, Duquenne and Brugnot, 2009). In addition to RG1 bacteria, these researchers identified several Risk Group 2 (RG2) bacteria, including *Pseudomonas aeruginosa* (PHAC, 2012) and *Klebsiella pneumoniae* (PHAC, 2011). These RG2 microorganisms pose a moderate infection risk to individuals and a low risk to public health (PHAC, 2015). The biological degreasers may have been contaminated by the environment or users (David et al., 2009). Furthermore, David et al. (2009) and Boucher et al. (2011) noted major temporal variations in bacterial composition in the French biological degreasing stations from one brand to the next, from one biological degreasing station to the next and even with the same biological degreasing station. The French researchers report an average culturable bacteria concentration of 3.4×10^5 CFU/mL in the biological degreasers (David et al., 2009). They suggest that workers who use biological degreasing stations may be exposed to aerosols generated by the brush used to apply the product to the part to be degreased. In addition, they noted that many workers did not wear protective gloves (Boucher et al., 2011). The French study, however, includes no bioaerosol measurements.

For an environmental engineering design project at the École polytechnique fédérale de Lausanne, Bodin and Larivé (2013) visited five Swiss companies that use biological degreasing stations. They report presence of culturable bioaerosols from the biological degreasers at four of the five companies. These bioaerosols contained the original microorganisms as well as others that had contaminated the biological degreasing stations.

Bégin et al. (2014a) visited four Québec companies where they saw a blower being used to dry parts that had been degreased in biological degreasing stations. They suggest the possibility that this could lead to presence of bioaerosols in the workers' breathing zone.

1.4 Bacteria identified in European studies

Bacteria of the genus *Bacillus* colonize diverse habitats because of their ability to adapt to a variety of temperatures, pH levels and salinity levels (Holt, Krieg, Sneath, Staley and Williams, 1994). Some species are pathogenic and can produce extracellular toxins, but most are harmless (Holt et al., 1994).

Bacteria of the genus *Pseudomonas* are also found in a variety of habitats and some species are pathogenic for humans (Holt et al., 1994). For example, prolonged exposure to water contaminated by *Pseudomonas aeruginosa* ($> 10^6$ CFU/mL) can cause skin infections such as folliculitis, dermatitis and otitis externa (Pitt and Simpson, 2006). *Pseudomonas aeruginosa* is an opportunistic bacterium that can infect intact skin (Agger and Mardan, 1995).

Stenotrophomonas maltophilia, which belongs to the family *Pseudomonadaceae*, is widespread and can cause a variety of diseases (Bartelt, 2000). Most infections, however, are nosocomial and in immunodepressed patients (Bartelt, 2000).

Though *Citrobacter* is part of the normal intestinal flora of humans, some species can contaminate soil, water or food (Holt et al., 1994). Some species are opportunistic pathogens.

For example, *Citrobacter amalonaticus* can potentially cause gastroenteritis (Lipsky, Hook, Smith and Plorde, 1980).

Most bacteria of the genus *Staphylococcus* are part of the natural flora of the skin and some species can be found in food, dust and water (Holt et al., 1994). A few species are opportunistic pathogens that can cause infections in predisposed individuals—those with burns or wounds, for example (Bartelt, 2000).

Bacteria of the genus *Klebsiella* are found in human excrement, soil, water, grains, fruit and vegetables (Holt et al., 1994). Some species, such as *Klebsiella pneumoniae* and *Klebsiella oxytoca*, are opportunistic pathogens that can cause nosocomial infections (Holt et al., 1994).

Infections associated with bacteria of the genus *Providencia* are uncommon. However, some species can cause nosocomial infections (Ovchinnikova, Rozalski, Liu and Knirel, 2013).

Bacteria of the genus *Enterococcus* are found in a wide variety of environments, generally in the excrement of vertebrates (Holt et al., 1994). In fact, *Enterococcus faecalis* and *Enterococcus faecium* are commensal species commonly found in the human gut (Gilmore, Coburn, Nallapareddy and Murray, 2002).

2. RESEARCH OBJECTIVES

The purpose of this research is to fill the gap in measurement data concerning occupational inhalation exposure to bioaerosols generated by degreasers when using biological degreasing stations. The research objectives are as follows:

1- Identify culturable bacteria in virgin biological degreasers and in these same degreasers during use

2- Measure bioaerosol concentrations and identify airborne culturable bacteria near biological degreasing stations in use

3- Characterize in real time and determine the particle-size distribution of biological particles using an ultraviolet aerodynamic particle sizer (UV-APS)

4- Issue recommendations on using biological degreasing stations

3. METHODOLOGY

3.1 Study environments

Participating companies were selected in cooperation with the follow-up committee (see Acknowledgements) and through contacts established during a previous research project (Bégin et al., 2014a). Four companies agreed to take part in this research project: an aircraft manufacturer (B1), a telecommunications company (B2), a public transport company (B3 and B4) and a manufacturer of recreational vehicles (B5). The alphanumeric codes in parenthesis identify the biological degreasing station studied.

3.2 Collecting degreaser samples (virgin and used) and inoculation filters

At each visit, a 50-mL sample of degreasing fluid was collected from the biological degreasing station at the end of a washing period using a sterile tube. Immediately after collecting this fluid, its temperature was taken with an infrared thermometer (Fluke, Everett, WA) and its pH was measured by dipping Whatman indicator strips directly in the fluid (Fisher Scientific, Waltham, MA). In addition, a 50-mL sample of virgin fluid was collected at the first visit directly from the container of the degreaser used for each biological degreasing station, to obtain the bacterial load of the virgin product.

For company B2, which uses Brand B technology (a filter transmits bacteria to the degreasing fluid), a single sample of a new filter was obtained and analyzed.

Figure 1 shows the two biological degreasing station models (Brand A and Brand B; see Table 2 in Section 4) and the measuring instruments (Andersen impactors on the left and UV-APS on the right) used in each of the sampling sessions. For a description of the biological degreasing stations and more information about how they work, consult the paper by Bégin et al. (2014a).

Figure 1. Biological degreasing stations and measuring instruments.

3.3 Bioaerosol sampling

Two sampling technologies were used to collect bioaerosol samples:

- Andersen N6 single-stage impactors (Thermo Fisher Scientific, Franklin, MA). Two different collection media were used simultaneously: trypticase soy agar (TSA) (Oxoid,

Ottawa), a general culture medium for culturable heterotrophic bacteria; and MacConkey agar No. 3 containing a triphenylmethane dye (MAC) (Oxoid, Ottawa), a selective growth medium that makes it possible to collect culturable Gram-negative bacteria. The impactors work with Gast 1531 pumps (Gast, Benton Harbor, MI) calibrated to 28 L/min as per the manufacturer's recommendations using a TSI model 4043 mass flowmeter (Shoreview, MN). Analytical performances are reported in the IRSST MA-264 standard method (Marchand, 2009).

- Three-piece polystyrene cassettes (SKC, Eighty Four, PA) with polycarbonate filter 37 mm in diameter and 0.8-µm porosity in accordance with IRSST method MA-368. The cassettes were connected to GilAir 5 pumps (Sensidyne, St. Petersburg, FL) calibrated to 2 L/min using a volumetric Defender 510 flow meter (Mesa Labs, Butler, NJ). Analytical performances were reported in IRSST research report R-125 and the IRSST MA-368 standard method (Marchand, 1996b, 2011).

Table 1 shows detection limits of each method and operating flow rates, sampling times and number of samples collected at each visit.

The sampling period was one year, with samples collected every two months at each of the five biological degreasing stations, for a total of 30 visits. This strategy is similar to that described by David et al. (2011) and Boucher et al. (2009).

Table 1. Sampling conditions for different devices used

Device	Flow (L/min)	Detection limit (CFU/m^3 of air)	Sampling time (minutes)	Number of samples
Andersen impactor (TSA + MAC)	28	7	5	5 + 5
Cassette	2	420	30	2

During the sampling days, workers washed different soiled parts in the biological degreasing station. At each visit, samples were collected over a period of about 30 minutes, including disassembly, cleaning and drying of parts.

The workers wore two sampling cassettes in their breathing zone. The impactors were installed at a fixed station on a cart near the biological degreasing stations.

At each visit, the following information was obtained from the workers or supervisors: the biological degreasing station model used and its maintenance, any particular problems since the last visit, the nature of the soiling and the parts washed and use of individual protective equipment.

To establish the background, samples were collected with the Andersen impactors and the UV-APS at each visit before using the biological degreasing stations.

3.4 Identification of microorganisms

The samples collected were used to count and identify culturable bacteria, either directly by incubation of the agar medium from the Andersen impactors or using 200-µL smears of extract from the polycarbonate filters or the degreaser samples (Marchand, 2011). The culture media were incubated for 24 hours at 37°C.

The following methods were used to identify the bacteria: wet mounting, differential Gram staining, catalase test, oxidase test, MicroScan identification panels and fatty acid profile analysis of all strains isolated in pure culture (Marchand, 2009). For certain strains which proved hard to identify, a MALDi-TOF[2] (Levesque 2015) mass spectrometer (VITEK® MS, bioMérieux, Marcy-l'Étoile, France) and/or 16S sequencing was used. Sequencing of the 16S fragment was performed by McGill University's Genome Québec Innovation Centre using primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). Consensus sequences obtained were compared with the Ribosomal Database Project (RDP) to identify problematic strains (PHAC, 2011).

The German regulation on bacteria (Ausschuss für Biologische Arbeitsstoffe, 2015), which includes a larger number of species than its Canadian equivalent (Government of Canada, 2013), was used for infection risk group classification of the bacteria identified.

3.5 Concentrations and particle-size distributions of fluorescent and non-fluorescent particles

Concentrations and aerodynamic diameters of aerosols emitted during biological degreasing station use were measured with stationary sampling in real time using an ultraviolet aerodynamic particle sizer (UV-APS) spectrometer Model 3314 (TSI, Shoreview, MN). This spectrometer operates at an excitation wavelength of 355 nm and measures ultraviolet fluorescence between 420 and 575 nm (TSI, 2010). This wavelength is considered appropriate for microorganisms. The UV-APS allows the user to differentiate the biological fraction associated with fluorescence from the non-biological fraction. The device establishes the particle size of an aerosol in real time for particles whose aerodynamic diameter is between 0.5 and 15 µm. This wide range of diameters corresponds to the diameters of particles likely to reach the different parts of the lungs (Lavoie et al., 2015). With the help of the UV-APS, the number of particles per cubic centimetres of air (#/cm^3) over one minute was determined. At each sampling session, measuring duration was 40 to 70 minutes.

3.6 Data analysis

Variance analyses were performed comparing log-transformed concentration values for airborne culturable bacteria and yeasts around the different biological degreasing stations while in use as well as before and during their use.

Morisita-Horn similarity indices were calculated to estimate the similarity between the bacterial flora of the samples. Indices were calculated for the culturable bacterial flora species isolated in

[2] Matrix-assisted laser desorption/ionization – time of flight mass spectrometer.

the fluid and air samples. Morisita-Horn index values range from 0 to 1, with 1 signifying great similarity between populations compared and zero signifying none: in other words, the closer the index is to zero, the greater the difference in biodiversity between the populations compared (Horn, 1966; Morisita, 1962).

EstimateS (Cowell, 2013) and NCSS (Hintze, 2013) software were used for the calculations.

4. RESULTS

4.1 Conditions of biological degreasing station use

Table 2 shows the conditions of use of the five biological degreasing stations studied and the sampling dates. The companies had to wash metal and plastic parts (for example, nuts and bolts, tools and bearings) soiled mainly by lubricating oils and greases. Frequency of biological degreasing station use ranged from several times a month to several times a day. Degreasing solution temperatures recorded ranged from 30.6 to 40.8°C, and pH was between 7.3 and 8.5. The workers wore gloves and goggles.

Table 2. Conditions of use of biological degreasing stations sampled

Biological degreasing station (degreasing system)	Use frequency	Soil	Type of material cleaned	Use of blower	Average pH	Average temperature (˚C) (min-max)	Sampling date
B1 (Brand A)	Several times a day	Hydraulic oil, light mineral oil, cutting fluid, extreme pressure grease, bearing grease, molybdenum disulfide (MoS_2) lubricant	Mild steel, aluminium	Yes, occasionally	7.5	36.4 (34.6 - 37.4)	2015: May, July, September, November 2016: January, March
B2 (Brand B)	A few times a /week	Engine oil, transmission oil, bearing grease	Hardened steel, plastic	No	8.0	35.5 (30.6 - 37.4)	2015: June, July, August, November 2016: January, March
B3 (Brand A)	Several times a day	Extreme pressure grease, bearing grease, engine oil, hydraulic oil, cutting fluid, synthetic oil, gunk	Mild steel, stainless steel, aluminium, copper, brass	No	7.6	37.6 (35.6 - 38.8)	2015: July, September, November 2016: January, March, April
B4 (Brand A)	A few times a month	Diesel, motor oil, transmission oil, synthetic oil, extreme pressure grease, MoS_2 lubricant, bearing grease, gunk	Stainless steel, copper, mild steel, brass	No	7.5	37.3 (35.4 - 40.8)	2015: July, November,, December 2016: February, March, May
B5 (Brand A)	Several times a day	Bearing grease, extreme pressure grease, light mineral oil, pneumatic tool oil	Aluminium, mild steel, brass, plastic	Yes	7.3	38.1 (37.0 - 39.2)	2015: July, September, November 2016: January, March, May

4.2 Culturable bacterial flora in biological degreasing station fluids

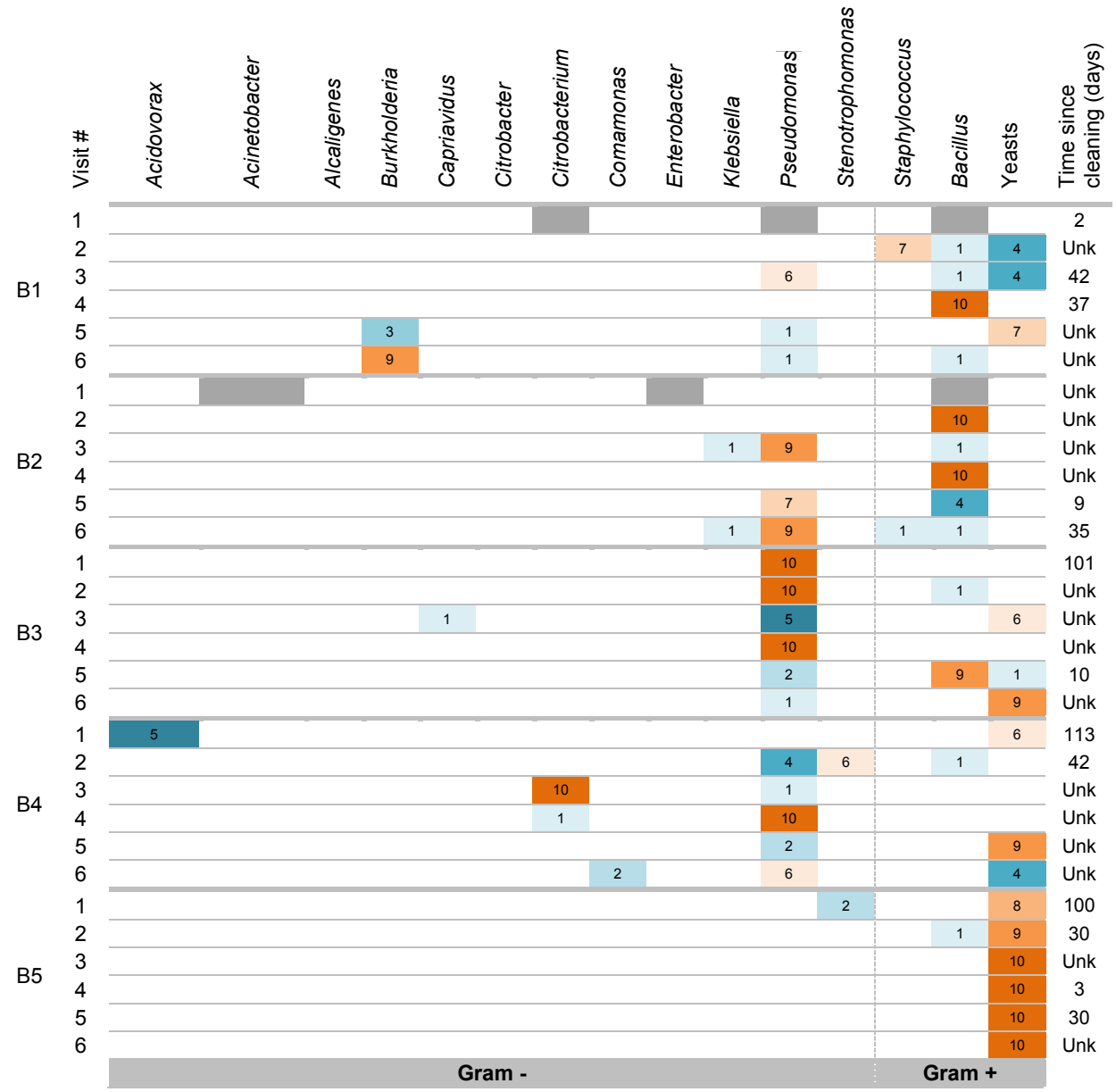
Table 3 shows average concentrations of bacteria and yeasts in biological degreasing station fluid samples at the end of the sampling period and in the virgin fluid. Average microorganism concentrations ranged from 2.7×10^5 to 5.3×10^6 CFU/mL. In the virgin degreasers, concentrations ranged from 1.4×10^5 to 2×10^7 CFU/mL. Average microorganism concentrations were higher when the biological degreasing stations were in use than those measured in the virgin fluid, except in the case of biological degreasing station B2.

Table 3. Average culturable bacteria and yeast[1] concentrations (CFU/mL) in biological degreasing station fluid and virgin fluid

Biological degreasing station	Average (CFU/mL) (n=6) (min; max)	Virgin fluid (CFU/mL) (n=1)
B1	1.2×10^6 (1.2×10^5; 3.3×10^6)	5.7×10^5
B2	5.3×10^6 (2.7×10^5; 2.6×10^7)	2.0×10^7
B3	2.7×10^5 (3.6×10^4; 8.3×10^5)	1.4×10^5
B4	2.9×10^6 (4.7×10^4; 1.5×10^7)	1.4×10^5
B5	8.2×10^5 (4.4×10^4; 2.1×10^6)	8.1×10^5

[1]Culturable yeast concentrations reported are for information purposes only, since TSA is not the standard medium for satisfactory quantitative yeast analysis by cultivation.

Figure 2 gives a percentage breakdown of the culturable microorganisms detected in the fluids at each visit. Bacterial diversity in the fluids varied from one biological degreasing station to the next and even in the same biological degreasing station from one visit to the next. Though *Bacillus* was found systematically in the virgin fluid it was only found in 15 of the 30 samples from the biological degreasing stations in use. Bacteria of the genus *Pseudomonas* were found in 18 of the 30 fluids sampled. In addition, the number of days between visits and the number of days since last cleaning of the different biological degreasing stations are shown on the right in Figure 2.



The method of analysis used for visit 1 to B1 and B2 did not allow quantification.
Unk: unknown

Figure 2. Percentage breakdown of culturable microorganisms found in fluid samples at each of 30 visits.

Table 4 shows the bacterial species identified in the biological degreasing station fluids after purification on TSA agar. Sixty distinct strains of bacterial species or genera were identified, 37 Gram-positive and 23 Gram-negative, including 23 bacterial species belonging to infection Risk Group 2.

Table 4. Infection risk groups* of 60 culturable bacteria species found in fluid from five biological degreasing stations

Species	Group	Gram	Species	Group	Gram
Acidovorax delafieldii	1	-	*Bacillus pumilus*	1	+
Pseudomonas balearica	1	-	*Bacillus silvestris*	1	+
Pseudomonas boreopolis	1	-	*Bacillus simplex*	1	+
Pseudomonas oleovorans	1	-	*Bacillus subtilis*	1	+
Pseudomonas pseudoalcaligenes	1	-	*Bacillus thuringiensis*	1	+
Pseudomonas stutzeri	1	-	*Corynebacterium lubricantis*	1	+
Acinetobacter baumannii	2	-	*Dermabacter hominis*	1	+
Acinetobacter johnsonoli	2	-	*Desemzia incerta*	1	+
Acinetobacter lwoffii	2	-	*Exiguobacterium aurantiacum*	1	+
Alcaligenes faecalis	2	-	*Exiguobacterium sibiricum*	1	+
Alcaligenes xylosoxidans	2	-	*Luteococcus japonicus*	1	+
Burkholdea cepacia	2	-	*Lysinibacillus massiliensis*	1	+
Burkholderia ambifaria	2	-	*Microbacterium aurum*	1	+
Burkholderia multivorans	2	-	*Microbacterium hydrocarbonoxydans*	1	+
Citrobacter amalonaticus	2	-	*Micrococcus luteus*	1	+
Comamonas terrigena	2	-	*Micrococcus lylae*	1	+
Cupriavidus pauculus	2	-	*Paenibacillus lautus*	1	+
Enterobacter cloacae	2	-	*Staphylococcus auricularis*	1	+
Klebsiella oxytoca	2	-	*Staphylococcus capitis*	1	+
Pantoea agglomerans	2	-	*Staphylococcus cohinii*	1	+
Pseudomonas aeruginosa	2	-	*Staphylococcus sciuri*	1	+
Pseudomonas oryzihabitans	2	-	*Staphylococcus warneri*	1	+
Stenotrophomonas maltophilia	2	-	*Staphylococcus xylosus*	1	+
Arthrobacter nicotianae	1	+	*Bacillus cereus*	2	+
Bacillus circulans	1	+	*Corynebacterium amycolatum*	2	+
Bacillus firmus	1	+	*Staphylococcus aureus*	2	+
Bacillus humi	1	+	*Staphylococcus epidermidis*	2	+
Bacillus licheniformis	1	+	*Staphylococcus hominis*	2	+
Bacillus megaterium	1	+	*Staphylococcus pasteuri*	2	+
Bacillus niacini	1	+	*Bacillus longisporus*	NC	+

** According to the German classification regulation (Ausschuss für Biologische Arbeitsstoffe, 2015). NC: Not classified*

Table 5 shows calculated Morisita-Horn similarity indices for the culturable bacterial flora species isolated in the biological degreasing station fluids at each visit. Only 16 of 65 indices exceeded 0.75. The low indices confirm the variation in the bacterial populations present in the biological degreasing station fluids. Biological degreasing stations B2 and B5 seem to show less variation over time, with average indices of 0.62 and 0.89 respectively. Note that low indices were calculated for biological degreasing station B3 despite substantial presence of the genus *Pseudomonas* (Figure 2). These low indices are explained by the presence of many different species of *Pseudomonas* during the different visits.

Table 5. Morisita-Horn indices calculated for culturable bacterial flora in biological degreasing station fluids

Biological degreasing station	Visit	2	3	4	5	6	Average
B1*	2	-	0.24	0.11	0.08	0.00	
	3	-	-	0.03	0.27	0.00	0.08
	4	-	-	-	0.00	0.03	
	5	-	-	-	-	0.00	
B2*	2	-	0.57	1.00	0.64	0.08	
	3	-	-	0.58	1.00	0.82	0.62
	4	-	-	-	0.65	0.08	
	5	-	-	-	-	0.77	
B3	1	0.01	0.64	0.93	0.43	0.09	
	2	-	0.02	0.00	0.03	0.00	
	3	-	-	0.63	0.10	0.05	0.21
	4	-	-	-	0.14	0.08	
	5	-	-	-	-	0.07	
B4	1	0.02	0	0	0.74	0.37	
	2	-	0.02	0.66	0.20	0.58	
	3	-	-	0.15	0.02	0.05	0.30
	4	-	-	-	0.25	0.75	
	5	-	-	-	-	0.66	
B5	1	0.67	0.67	0.67	0.67	0.67	
	2	-	1.00	1.00	1.00	1.00	0.89
	3	-	-	1.00	1.00	1.00	
	4	-	-	-	1.00	1.00	
	5	-	-	-		1.00	
Index	> 0.75						

* The method of analysis used to produce the results for visit 1 to biological degreasing stations B1 and B2 did not allow for quantitative analysis.

4.3 Culturable bacterial flora in the ambient air

Table 6 shows airborne concentrations of bacteria and yeasts during the 30 sampling sessions. Average concentrations ranging from less than 7 to 160 CFU/m³ and from less than 420 to 480 CFU/m³ were measured with the Andersen impactor and the cassettes respectively. Concentrations measured at biological degreasing station B5 are significantly different from those measured at the other biological degreasing stations with the Andersen impactors before and after use. There were no significant differences between concentrations measured with the impactors before (background) and during biological degreasing station use, including at biological degreasing station B5. Note that most of the cassette sampling yielded non-detected values. Use of a blower to dry the parts was noted only on one occasion at biological degreasing station B1 but at every visit at biological degreasing station B5.

Table 6. Average airborne bacteria and yeast concentrations (CFU/m^3) with Andersen impactor (n = 5/visit)[1] and polycarbonate filter cassettes (n = 2/visit)

Biological degreasing station	Visit	Background	During use	
		Impactor	Impactor	Cassette
B1	Visit 1*	30	110	< 420
	Visit 2	60	70	< 420
	Visit 3	10	30	< 420
	Visit 4	< 7	30	< 420
	Visit 5	10	20	< 420
	Visit 6	< 7	10	< 420
B2	Visit 1	10	20	< 420
	Visit 2	10	< 7	< 420
	Visit 3	< 7	20	< 420
	Visit 4	30	20	480
	Visit 5	50	20	< 420
	Visit 6	20	20	< 420
B3	Visit 1	40	20	< 420
	Visit 2	30	60	< 420
	Visit 3	250	120	< 420
	Visit 4	60	30	< 420
	Visit 5	10	20	< 420
	Visit 6	100	70	< 420
B4	Visit 1	10	60	< 420
	Visit 2	60	80	< 420
	Visit 3	10	10	< 420
	Visit 4	10	< 7	< 420
	Visit 5	40	20	< 420
	Visit 6	40	100	< 420
B5	Visit 1*	160	160	< 420
	Visit 2*	100	120	< 420
	Visit 3*	160	70	< 420
	Visit 4*	40	60	< 420
	Visit 5*	60	30	< 420
	Visit 6*	170	120	< 420

* Blower used.

[1] No culture was obtained from the MacConkey agars after 24 hours of incubation, hence n = 5 in this table instead of n = 5 + 5 as in Table 1.

As in the fluid samples, the bacterial flora obtained with the Andersen impactor shows great variability in airborne microorganisms over time. As Figure 3 shows, for example, the dominant microorganisms were different at each visit at biological degreasing station B5: *Staphylococcus hominis, Bacillus* sp*, Staphylococcus pasteuri, Staphylococcus* sp*, Bacillus subtilis* and *Staphylococcus epidermidis*. Appendix A includes similar figures for the four other biological degreasing stations.

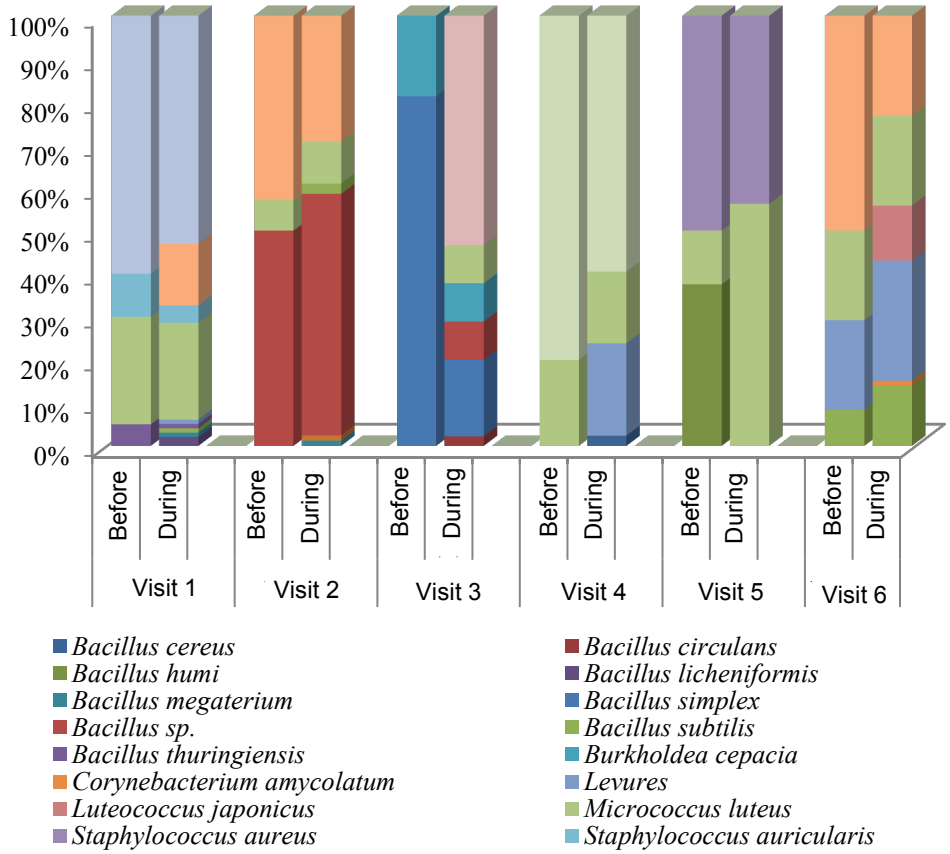


Figure 3. Percentage breakdown of airborne culturable microorganisms collected with an Andersen impactor before and during use of biological degreasing station B5.

Despite the differences observed over time, in some visits there was great similarity between the flora present before use of the biological degreasing station (background) and during its use (Figure 3 and Table 7). During three of the 30 visits, no bacteria were detected in the nutrient media; it was thus not possible to check for similarity. Lastly, at 63% of the visits, the main microorganism found in the background was also the main microorganism found when the biological degreasing station was in use. To quantify these observations, Morisita-Horn similarity indices were calculated for the bacterial flora present before and during biological degreasing station use (Table 7), and for close to half of the visits the indices calculated were greater than 0.75—confirming there is little difference between the bacterial flora present before and during biological degreasing station use.

Table 7. Morisita-Horn indices for culturable bacterial flora present before and during biological degreasing station use

Biological degreasing station	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
B1	NC	0.78	0.71	NA	0.76	NA
B2	NC	0.86	NA	0.04	0.74	0.90
B3	0.91	0.49	0.74	0.00	0.97	0.25
B4	0.76	0.00	0.18	1.00	0.30	0.31
B5	0.98	0.99	0.31	0.91	0.63	0.83

Index > 0.75
NC: Not calculated. The method of analysis used to obtain the results for visit 1 to biological degreasing stations B1 and B2 did not allow quantitative analysis.
NA: Not analyzable. There was only one species in the sample.

4.4 Comparison of culturable bacterial flora in ambient air and in biological degreasing station fluids

Table 8 compares bacterial species and yeasts accounting for more than 2% of colonies identified in the air (background and during biological degreasing station use) and in fluids (virgin and used fluids) during the 30 visits. The airborne bacterial species are more diversified than the species found in the biological degreasing station fluids. For example, *Bacillus cereus* (Gram-positive, Risk Group 2) and *Staphylococcus hominis* (Gram-positive, Risk Group 2) were detected only in the air, whereas in the virgin fluid, only *Bacillus subtilis* was identified. This bacteria was also detected in a new filter for biological degreasing station B2 (part of the technology of this biological degreasing station). In the biological degreasing station fluids, 16% of the bacteria were *Pseudomonas aeruginosa* (Gram-negative, Risk Group 2), 20% were *Bacillus subtilis* (Gram-positive, Risk Group 1) and 29% were yeasts. The two groups most commonly found in fluids, *Bacillus subtilis* and yeasts, are also the groups most frequently found airborne. However, this phenomenon of aerosolization was not noted in the case of the bacterium *Pseudomonas aeruginosa* which, though it accounted for 16% of the colonies in the fluids, was never detected in the ambient air. This lack of aerosolization was also observed in a study conducted in composting centres (Marchand et al., 2017) and merits study in greater depth. Yeasts were found in large quantities in the degreasers in the biological degreasing stations in use, though these microscopic mycetes were not specifically characterized because the methods used, which are appropriate for bacterial flora, are not suitable for a rigorous analysis of yeasts. In some cases, only identification of the genus of the bacteria was possible, despite the numerous methods of identification used in the analytical process.

Table 8. Comparison of bacteria and yeasts constituting over 2% of colonies identified in the air before and during biological degreasing station use and in virgin and used fluids

Bacterial species (Risk Group)	Percentage of isolated airborne colonies (%)		Percentage of isolated colonies in fluids (%)	
	Before degreasing	During degreasing	From virgin fluids	From biological degreasing stations
Bacillus cereus (2)	4.0	0.6	-	-
Bacillus licheniformis (1)	1.3	3.6	-	2.2
Bacillus megaterium (1)	2.5	5.3	-	-
Bacillus simplex (1)	4.4	0.8	-	-
Bacillus sp	3.3	3.1	-	0.3
Bacillus subtilis (1)	5.1	10	100	20
Burkholderia multivorans (2)	-	-	-	3.0
Citrobacter amalonaticus (2)	-	1.7	-	3.4
Corynebacterium lubricantis (2)	2.6	1.4	-	-
Yeasts	6.3	9.1	-	29
Micrococcus luteus (1)	12	21	-	-
Micrococcus sp	4.6	7.3	-	-
Pseudomonas aeruginosa (2)	0.7	-	-	16
Pseudomonas oleovorans (1)	2.6	-	-	2.8
Pseudomonas oryzihabitans (2)	-	0.5	-	3.1
Pseudomonas sp	1.3	-	-	4.3
Staphylococcus epidermidis (2)	5.7	4.9	-	2.2
Staphylococcus hominis (2)	12	6.8	-	-
Staphylococcus sp	11	3.7	-	0.1
Stenotrophomonas maltophilia (2)	-	0.5	-	2.2

To determine if the flora in the biological degreasing station fluids during biological degreasing station use influence those the airborne flora, Morisita-Horn indices comparing the two were calculated. Table 9 shows the values obtained for each visit. These results demonstrate that there is little similarity between the flora in the two environments, confirming that the fluid flora had little impact on airborne flora during 25 of the 28 visits for which more than one type of bacteria were identified.

Table 9. Morisita-Horn indices calculated for culturable bacterial flora present in biological degreasing station fluids and airborne during biological degreasing station use

Biological degreasing station	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
B1	NC	0.84	0.05	0.00	0.16	0.00
B2	NC	0.00	0.25	0.95	0.00	0.17
B3	0.02	0.30	0.49	0.53	0.18	0.01
B4	0.00	0.00	0.42	0.00	0.84	0.25
B5	0.01	0.00	0.00	0.29	0.43	0.57

Index > 0.75
NC: Not calculated. The method of analysis used to obtain the results at visit 1 to biological degreasing stations B1 and B2 did not allow for quantitative analysis.
NA: Not analyzable. There was only one species in the sample,

4.5 Fluorescent and non-fluorescent aerosols measured by UV-APS

For all visits taken together, the concentrations measured using a UV-APS ranged from 1.0×10^4 #/m^3 (0.001 #/cm^3) to 9.3×10^6 #/m^3 (9.3 #/cm^3) for the fluorescent fraction and from 4.4×10^5 #/m^3 (0.44 #/cm^3) to 1.9×10^8 #/m^3 (190 #/cm^3) for the non-fluorescent fraction. Table 10 summarizes the UV-APS results (averages per biological degreasing station). Appendix B gives detailed results and Appendix C concentration profiles for each visit. Figure 4 shows four examples, with the background during the first few minutes of sampling graphically represented. Notes taken during the sampling make it possible to explain the concentration profiles measured with the UV-APS. Figure 4 B-C thus shows UV-APS concentrations when splashing was noted during the parts washing. Increases in concentrations were noted for the fluorescent as well as the non-fluorescent fraction. In the case of the fluorescent particle peaks in Figure 4B (splashing), concentrations were ten times the background concentration. The same phenomenon was noted when the blower was used (Figure 4 A-D). However, the impact on concentrations was greater during drying with the blower—as much as 100 times background concentrations for both fluorescent and non-fluorescent fractions (Appendix D, B5-1 to B5-6).

Table 10 and Appendix B show ratios (quotients) of averages obtained before biological degreasing station use (background) and those measured during biological degreasing station use. These ratios help in understanding the increase in concentration attributable to the washing activity. Of the non-fluorescent fraction, seven ratios exceeded 2, and some were as high as 12, all from the seven occasions on which the blower was used. For the fluorescent fraction, the ratios were higher, as much as 59 for biological degreasing station B5 (average ratio = 21, Table 7). There again, the ratios were highest when the blower was used, though ratios as high as 6 were calculated in workplaces where a blower is not used.

Table 10. Summary of particle concentrations measured by UV-APS

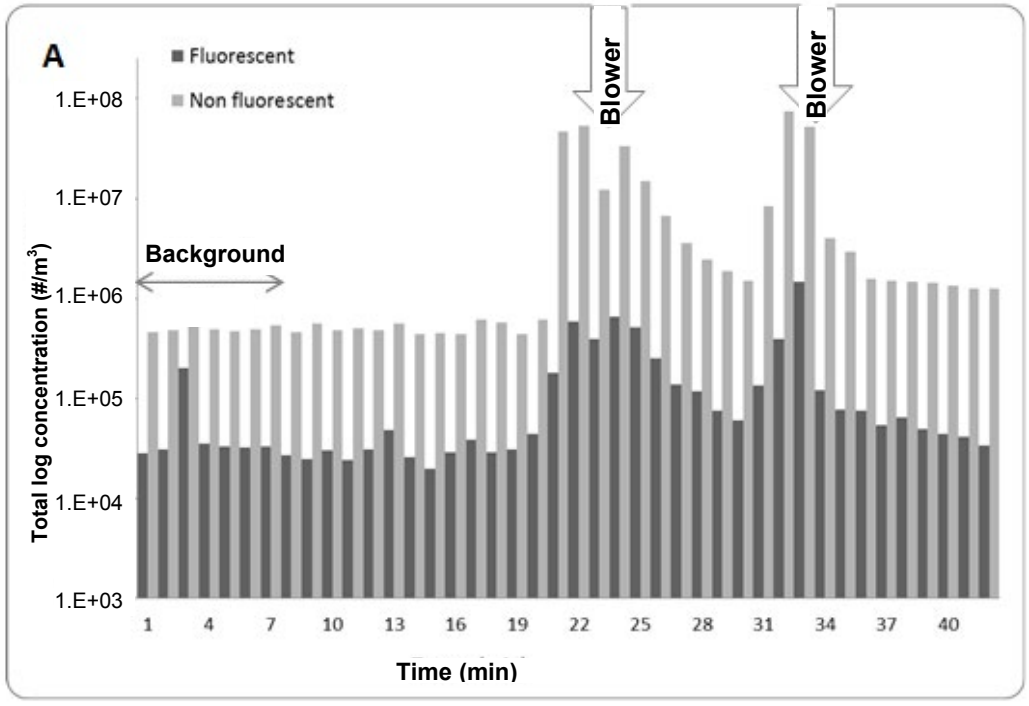
Fluorescent Particles

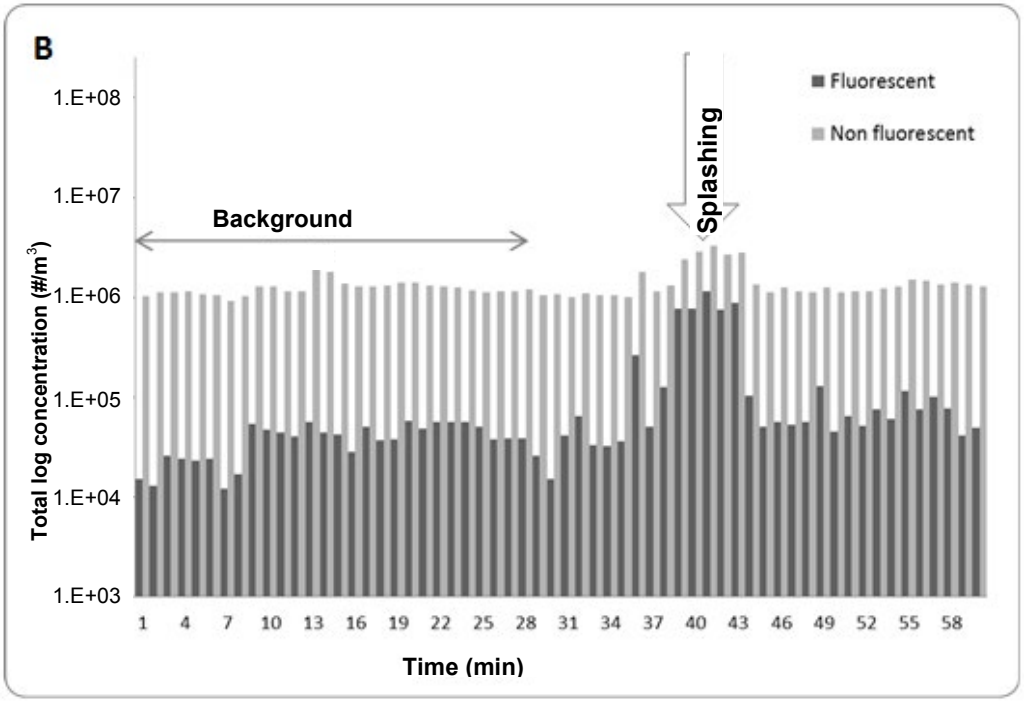
	During biological degreasing station use				Background				
Biological degreasing station	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Ratio*
B1	0.08	0.13	0.01	1.46	0.06	0.03	0.00	0.20	1.33
B2	0.15	0.16	0.01	1.14	0.06	0.03	0.01	0.22	2.50
B3	0.10	0.06	0.04	0.36	0.12	0.15	0.00	0.86	0.83
B4	0.13	0.10	0.01	0.55	0.09	0.11	0.00	0.66	1.44
B5	2.10	1.97	0.02	9.34	0.10	0.11	0.00	0.57	21.0

Non-Fluorescent Particles

	During biological degreasing station use				Background				
Biological degreasing station	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Ratio*
B1	5.23	8.35	0.44	75.4	4.20	2.25	0.46	12.7	1.25
B2	4.08	2.18	1.01	13.1	3.52	2.15	0.91	15.1	1.16
B3	4.74	1.34	1.98	8.05	5.32	1.51	1.51	7.86	0.89
B4	7.41	4.50	3.07	26.5	6.60	3.57	2.67	17.3	1.12
B5	58.0	39.4	4.46	195	12.7	6.30	4.33	40.8	4.57

* Quotient of average concentration during use and average background concentration.


A
Fluorescent
Non fluorescent
Blower
Blower
Background
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
Total log concentration (#/m3)
1 4 7 10 13 16 19 22 25 28 31 34 37 40
Time (min)


B
Fluorescent
Non fluorescent
Splashing
Background
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
Total log concentration (#/m3)
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Time (min)

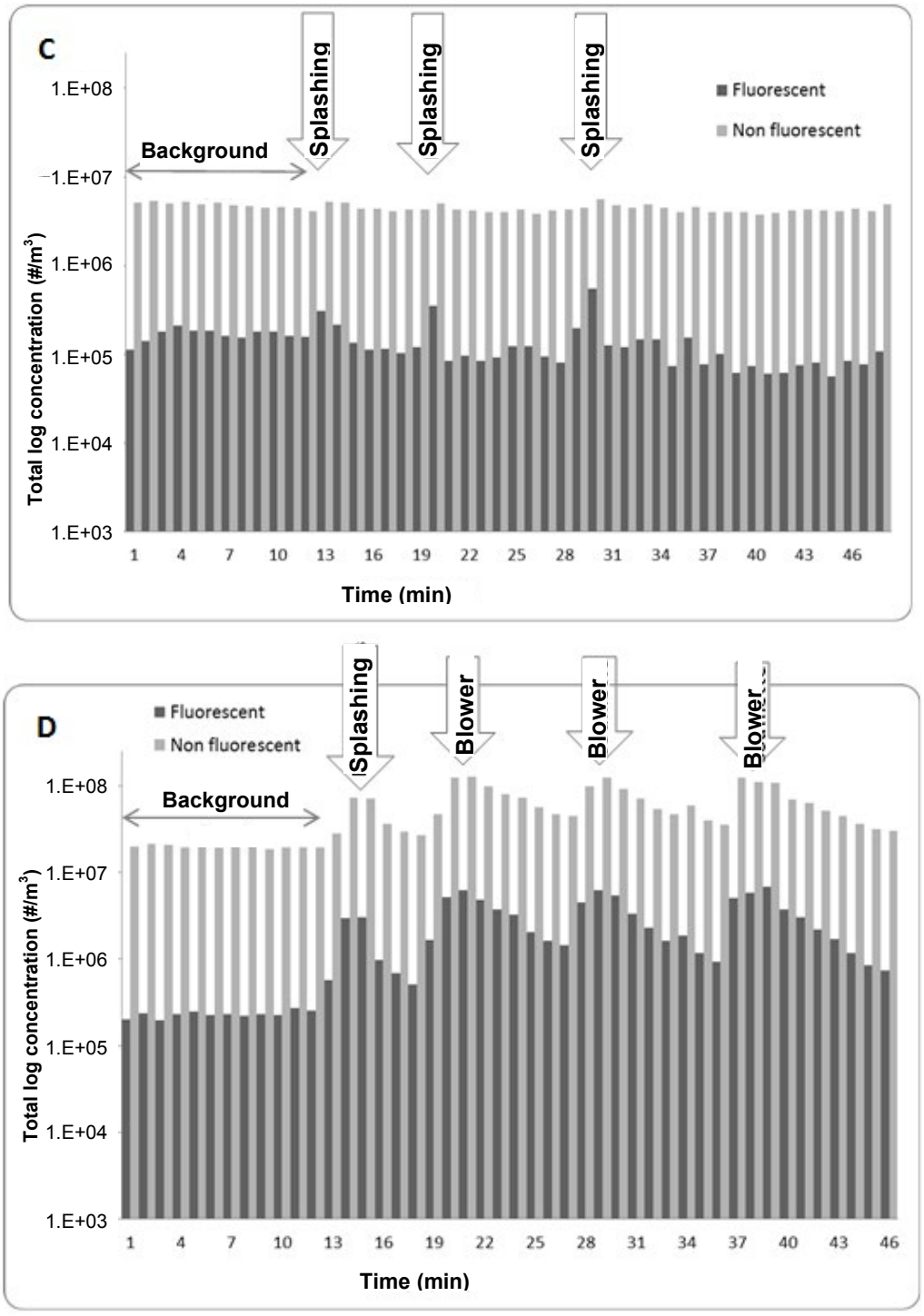
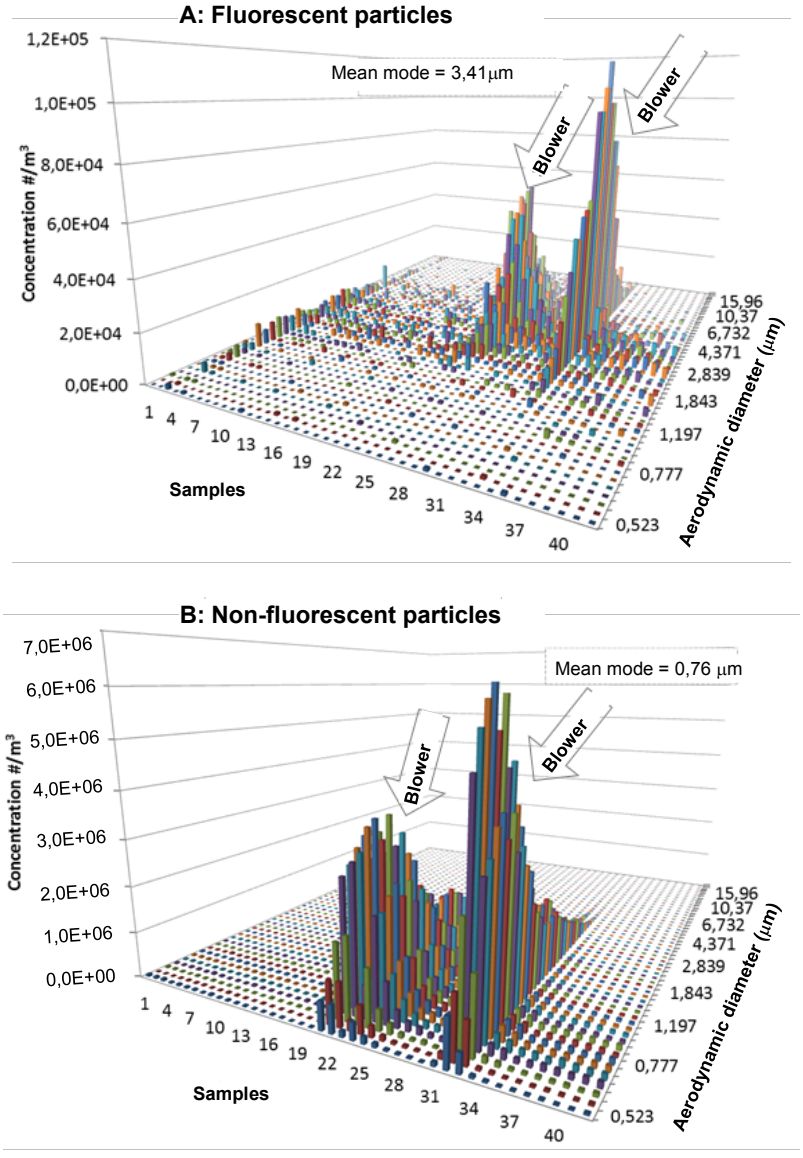


Figure 4. Concentration peaks in fluorescent and non-fluorescent particles measured at the first visit during use of biological degreasing stations B1(A), B2 (B), B4 (C) and B5 (D).

Figure 5 is a 3D representation of a breakdown of concentrations measured with the UV-APS by particle aerodynamic diameter. Different diameters were noted for the fluorescent as well as the non-fluorescent fractions. For all biological degreasing stations taken together, average aerodynamic diameter in the fluorescent fraction was 2.96 µm, compared to 0.76 µm in the non-fluorescent fraction. Concentrations peaked during activities that generate bioaerosols (blower use), in the case of the fluorescent as well as the non-fluorescent particles. These peaks were present at every visit during which a blower was used. Noteworthy is the repeatability of the particle-size distribution in successive uses of the blower.



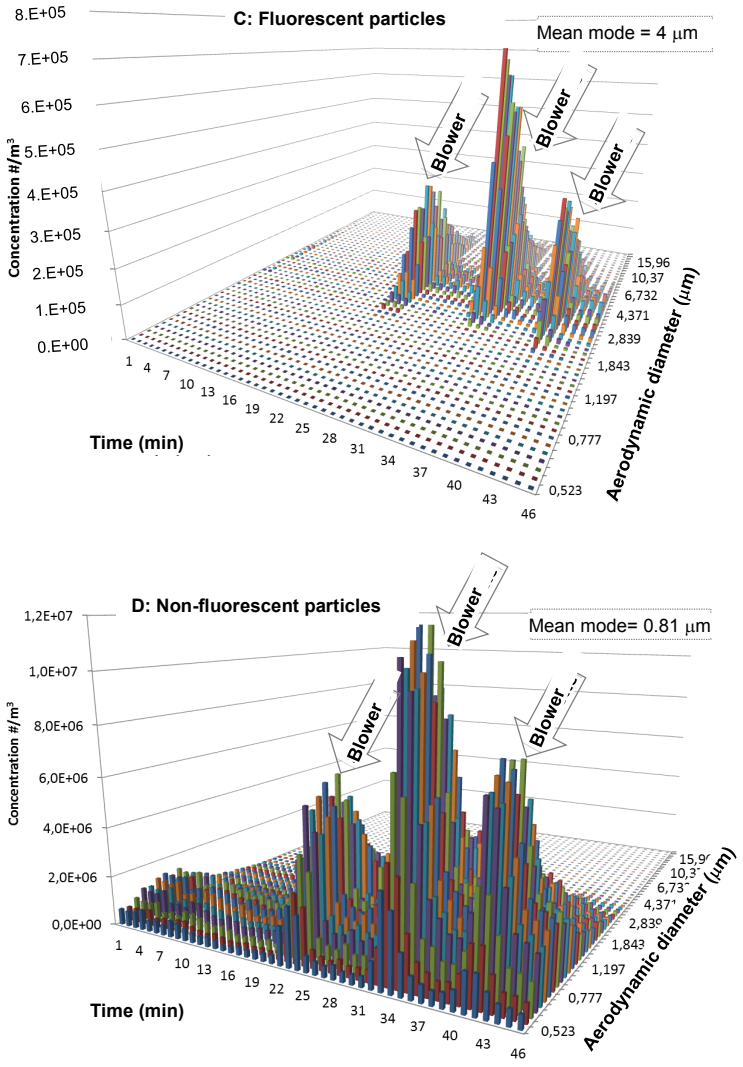


Figure 5. Particle-size distribution of fluorescent and non-fluorescent particle concentrations measured in the visit 1 to biological degreasing station B1 (A-B) and the visit 5 to biological degreasing station B5 (C-D).

5. DISCUSSION

5.1 Culturable microorganism composition of fluids

The measurements taken during this research project indicate that numerous bacterial species and yeasts can be found in biological degreasing station fluids during biological degreasing station use. These microorganisms are different from those found in virgin fluid, where only the species *Bacillus subtilis* was detected. During one visit, which took place only three days after cleaning of the biological degreasing station and replacement of its fluid, *Bacillus subtilis* was not detected in the biological degreasing station fluid (Figure 2). Rapid colonization by other microorganisms is clearly possible. Conditions favorable for growth of *Bacillus* sp seem also to promote growth of many other microbial species. Nelson et al. maintain that the environment in a biological degreasing station may be able to support proliferation of exogenous microorganisms (Nelson, Tkachenko, Delawder and Marsch, 2004), a hypothesis confirmed by the research of David et al. (2009) and Boucher et al. (2011).

For four of the five biological degreasing stations, average microorganism concentrations measured in the used fluids were higher than those in the virgin fluid. Average microorganism concentrations in the biological degreasing station fluids varied by one order of magnitude from one biological degreasing station to the next, whereas in time they varied (measurements taken every two months over one year) by two orders of magnitude in four of the five biological degreasing stations. Nelson et al. studied seven biological degreasing stations used in five vehicle maintenance shops in the United States over eight weeks (Nelson et al., 2004), measuring microorganism concentrations in the fluids weekly using Oxoid™ dip slides (Nelson and Tkachenko, 2001). They report concentrations (CFU/mL) that varied by one order of magnitude in five of the biological degreasing stations, by two orders of magnitude in the sixth biological degreasing station and by three orders of magnitude in the seventh. Average microorganism concentrations measured in the present study in the company using the same technology are of the same order of magnitude as those measured in most of the biological degreasing stations studied by Nelson et al. (2001).

In the present study, 100% of the bacteria identified in the virgin fluids were *Bacillus subtilis,* while in the fluids collected during operation of the biological degreasing stations, 59.5% of the bacteria found belonged to the genera *Bacillus, Pseudomonas, Citrobacter, Burkholderia, Staphylococcus* and *Stenotrophomonas,* and 29% of the microorganisms detected were yeasts. The microorganisms found in the fluids included a number of infection Risk Group 2 bacteria, such as *Pseudomonas aeruginosa, Pseudomonas oryzihabitans, Stenotrophomonas maltophilia* and *Staphylococcus epidermidis*, suggesting a possible health risk.

David et al. report that bacterial populations fluctuate tremendously with the smallest change to the environment and that it is impossible to determine bacterial composition in biological degreasing stations over time (David et al., 2009). The low similarity indices calculated in our study for the different visits to the same biological degreasing station confirm what David et al. report. However, the variation was not as marked at biological degreasing stations B2 and B5, and a number of indices above 0.75 were calculated (Table 5). The marked recurrence of *Bacillus subtilis* in biological degreasing station B2 accounts for these high similarity indices, whereas at biological degreasing station B5 the very strong presence of yeasts during visits 2, 3, 4 and 5 account for the high similarity indices (Figure 2). Although the recurrent presence of yeasts in biological degreasing station B5 cannot be easily explained, one hypothesis explaining

the recurrence of *Bacillus subtilis* in biological degreasing station B2 is the continuous inoculation of bacteria by the filter in the biological degreasing station fluid.

5.2 Airborne culturable microorganisms found close to the biological degreasing stations and in workers' breathing zones

The air samples taken during use of the biological degreasing stations made it possible to characterize a number of bacterial genera and species and several yeasts. The four main microorganisms found after culture on agar and accounting for 76% of the microorganism identified are, in decreasing order of frequency, *Micrococcus, Bacillus, Staphylococcus* and yeasts. In the background measurements taken before use of the biological degreasing stations, accounting for 77% of the microorganisms, listed in decreasing order of frequency, were *Staphylococcus, Bacillus*, yeasts, *Micrococcus*, *Corynebacterium* and *Pseudomonas*. At two-thirds of the visits (63%), the main bacterial species found in the background were the same as those detected during use of the biological degreasing station. Calculation of Morisita-Horn indices confirmed this strong similarity between airborne flora before and during biological degreasing station use. In addition, no significant difference in airborne microorganism concentrations (CFU/m³) was noted in air samples taken before (background) and during biological degreasing station use. These results suggest that the bioremediating degreasing activity had little impact on the concentration or composition of culturable airborne microbial flora.

Use of a blower during seven visits, however, demonstrated that blower use generates aerosols (figures 4 and 5 and Appendix B). Fluorescent fraction averages measured by UV-APS during biological degreasing station use were as much as 59 times background averages. Aerodynamic diameter modes of the fluorescent particles generated during blower use were ≤ 4 µm, that is, within the inhalable fraction (< 100 µm). However, the use of a blower to dry the degreased parts in the biological degreasing stations did not appreciably increase worker exposure to culturable microorganisms. In fact, for biological degreasing station B5, the only biological degreasing station where a blower was systematically used at each visit, there was no significant difference between calculated airborne concentrations before and during biological degreasing station use. In addition, all average ambient concentrations measured during this study, even when a blower was used, were below 480 CFU/m³, that is, well below those reported in many workplaces and even in fresh air. Goyer et al. (2001), for example, report concentrations of 10^2 CFU/m³ outside; 10^4 CFU/m³ in wastewater treatment facilities, household waste collection and sorting plants, and sawmills; 10^5 CFU/m³ in composting centres; 10^6 CFU/m³ in paper mills and hog houses; and 10^7 to 10^9 CFU/m³ in agriculture. Recommended activity levels for occupational exposure to bioaerosols are about 10^4 CFU/m³ (Goyer et al., 2001). The concentrations reported in our study are thus quite low compared to these levels.

5.3 How the biological degreasing stations work

According to the user manuals, the degreasing solution for the Brand B biological degreasing stations must be maintained at a temperature of 40 ± 3°C, while the solutions for the Brand A biological degreasing stations must be maintained at a temperature of 41 ± 1°C. Our results indicate that these conditions were not respected, that on average the temperatures were too low (see Table 2). This means that the degreasing conditions were probably not optimal for

maintaining the growth of the seeded bacteria (*Bacillus subtilis*). However, we noted that it was more difficult to obtain information concerning use of the biological degreasing stations (for example, when they were emptied, when the degreaser was added or replaced) when numerous workers made sporadic use of the same biological degreasing station. Among other things, the vast majority of the workers had never received any training when the biological degreasing stations were introduced at their shops, and the user manuals were not available in the workplace.

Several months after our study, the aircraft manufacturer studied (B1) decided to stop using the biological degreasing stations, because the technology did not meet its cleaning effectiveness criteria. In addition, the degreaser often emitted a fetid smell. Incorrect use of the biological degreasing stations in this company may have been the reason for the unpleasant odour: noncompliance with optimal degreaser temperature, for example, and washing of parts soiled by contaminants incompatible with the bacterial flora of the degreaser. Bégin et al. (2014a), for example, report that the bacterial culture of the degreaser in a Brand A biological degreasing station used in a machine shop was destroyed by a hydraulic fluid. In fact, as shown in Table 2, hydraulic oil was among the soils on the parts to be degreased.

Brand A is certified under UL standard 2792 (UL, 04/05/2016), issued under the ECOLOGO Certification Program managed by the US company Underwriters Laboratories (UL), an independent certification company. Theoretically, this third-party certification program ensures the user that a commercial preparation meets minimum requirements for health and environmental sustainability. UL 2792, for example, requires absence of pathogenic microorganisms and certain toxic substances, such as 2-butoxyethanol and aromatic solvents (UL, 04/05/2016). The results of our study show that virgin samples of Brand A collected in the workplaces studied did not comply with the minimum value of 10^7 CFU/mL required under UL 2792 (see Table 3). One possible explanation is failure to comply with required storage temperatures for the original containers (≥ 5 °C, ≤ 25 °C) or that the storage period (one year) was exceeded (storage requirements taken from the fact sheet for the Brand A degreaser). A below-standard bacterial concentration could lead to less efficient bioremediation of soils.

The technology of Brand B differs from that of Brand A. The manufacturer confirmed that it is the filter through which the degreaser passes in the biological degreasing station that releases the bacteria. *Bacillus subtilis* was in fact detected in the filter, but it was also found in the virgin degreasing solution, and at a higher concentration than in any of the unused Brand A fluids analyzed. This finding is difficult to reconcile with the manufacturer's technical literature, which does not mention presence of bacteria in the virgin degreasing solution.

5.4 Prevention

Given the numerous exogenous bacteria and yeasts identified in the biological degreasing station fluids, the presence of Risk Group 2 bacterial species, Gram-negative bacteria and a large number of yeasts, the risk of occupational exposure through skin contact must be considered. The risk with skin contact is mainly related to wound infection or ingestion by cross contamination. Kohli (2013) claims that individual protective equipment is not needed when using a biological degreasing station. We, other the other hand, like Bégin et al., recommend protecting skin and eyes by wearing a faceshield if splashing is a possibility and preventing contact with wounds (Bégin, Gérin and Lavoie, 2014b). David et al. also insist on the need for strict personal hygiene and wearing of gloves. These researchers recommend hand washing

after removing gloves and before bringing the hands to the mouth or handling objects brought to the mouth (David, 2005; David et al., 2009).

This metrological study established that there is little inhalation exposure to bioaerosols by workers using a biological degreasing station to degrease soiled parts in mechanical maintenance shops. Maximum level measured on a worker during a degreasing operation was 480 CFU/m^3. In addition, use of a blower to dry the degreased parts in the biological degreasing station did not appreciably increase worker exposure to microorganisms. Levels measured in the situations studied were very far below the action threshold of 10^4 CFU/m^3 suggested by Goyer et al. (2001) for total culturable bacteria. As a result, no respiratory protection is recommended when using a biological degreasing station.

5.5 Limitations of this study

The analytical methods based on cultivation on a nutrient medium used in this study are limited because only culturable microorganisms likely to develop in the applied cultivation conditions are identified (Eduard, Heederik, Duchaine and Green, 2012). The TSA agars used in this study to cultivate bacteria can also be used to grow certain yeasts, which is why we found yeasts. Nonetheless, the yeast results of this study must be considered with caution, because they underestimate yeast presence both quantitatively and qualitatively.

No measurements of background concentrations were taken after biological degreasing station use or in other locations in the companies studied. Backgrounds were characterized before biological degreasing station use to assess the effect of their use.

A UV-APS measures everything of biological origin, dead or alive, in real time, but it does not distinguish between microorganisms and certain other biological content. In addition, this study did not research contamination of surfaces around the biological degreasing stations, which are potential sources of skin contact.

The recommendations in this report are based solely on observations and measurements made when monitoring the five biological degreasing stations. The measurements were taken while the biological degreasing stations were in use for 30 consecutive minutes. Note that users reported in some cases that the biological degreasing stations were used very infrequently (several times a month). Other particular situations should be examined in depth by a professional.

Lastly, possible of exposure of workers to toxins produced by the microorganisms under certain conditions were not examined. The proliferation of Gram-negative bacteria increases the possibility of detecting airborne endotoxins (Marchand, 1996a), which can cause respiratory problems in workers exposed to them (Rylander, 2006). *Bacillus subtilis* can produce certain enzymes—such as subtilisin, a protease in aerosol form that triggered allergic reactions in workers in plants manufacturing laundry detergent powders made with crystalline subtilisin (AMFEP, 2007). Subtilisin also caused respiratory problems in workers at hospitals where surgical and diagnostic instruments are washed with a subtilisin-based cleanser (Lemière, Cartier, Dolovich and Malo, 1996).

6. CONCLUSION

Monitoring of fluids in five biological degreasing stations over one year showed microorganism concentrations in these fluids ranging from 3.6×10^4 to 2.6×10^7 CFU/mL. Sixty bacterial species were identified in the fluids. These species are Risk Group 1 and Risk Group 2 species and include Gram-positive as well as Gram-negative bacteria. Several bacteria genera were identified, including *Bacillus, Pseudomonas, Citrobacter, Burkholderia, Staphylococcus* and *Stenotrophomonas*. On the other hand, only the species *Bacillus subtilis* was identified in virgin fluids for the five biological degreasing stations. In other words, the biological degreasing stations were rapidly colonized by different exogenous microorganisms such as *Pseudomonas aeruginosa*. The main hazard of skin contact is the possibility of wound infection or ingestion from hand-to-mouth events or handling items put in the mouth. Strict individual hygiene measures, wearing of gloves and handwashing before and after biological degreasing station use is thus required.

This study established that there is very little inhalation exposure to bioaerosols when workers use a biological degreasing station. Use of a biological degreasing station does not seem to affect ambient culturable bacterial flora. In addition, though airborne microorganism concentrations increase when a blower is used to dry degreased parts in a biological degreasing station or when splashing occurs, this does not seem to appreciably affect worker exposure to culturable microorganisms. As a result, no respiratory protection is recommended when using a biological degreasing station.

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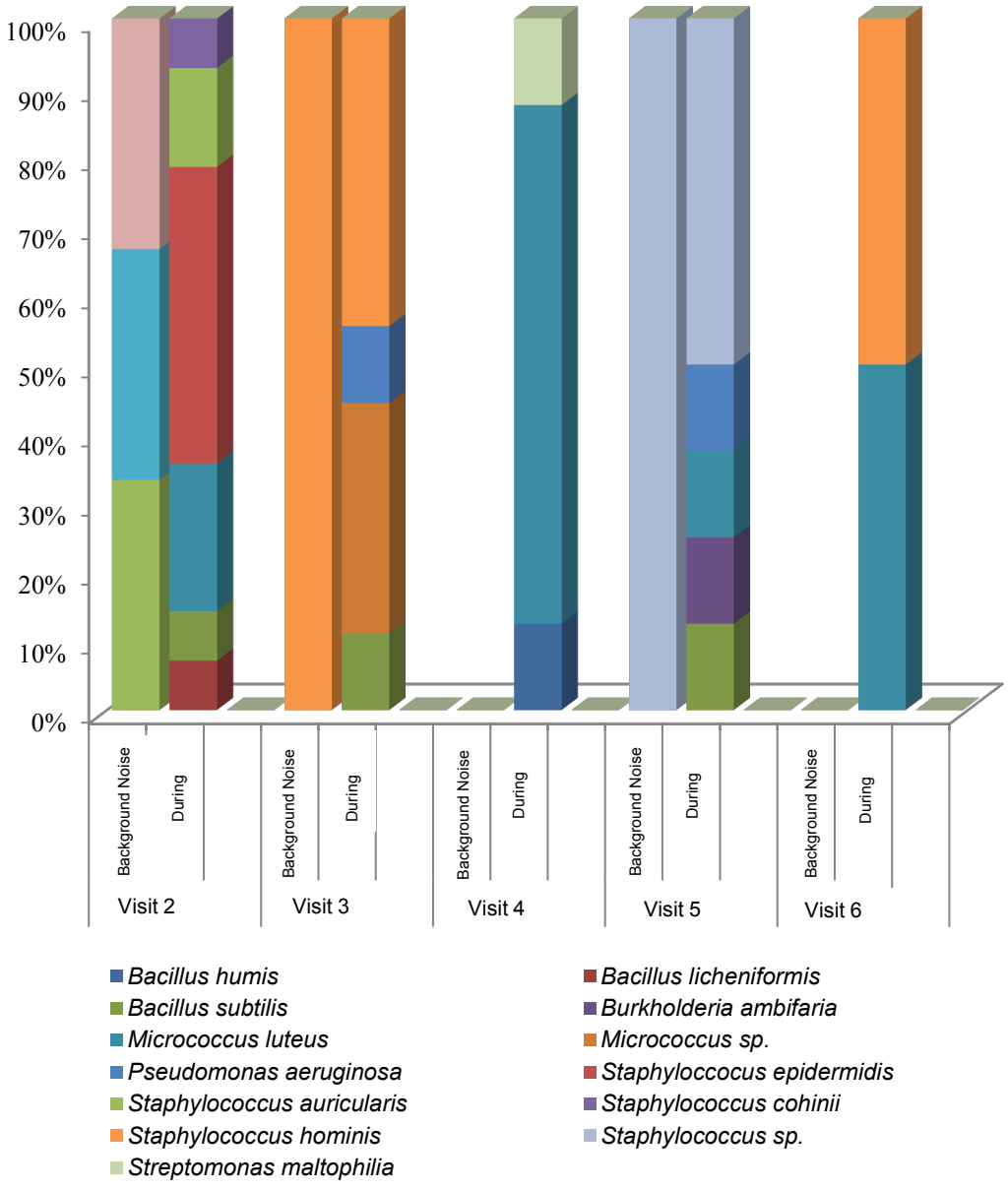
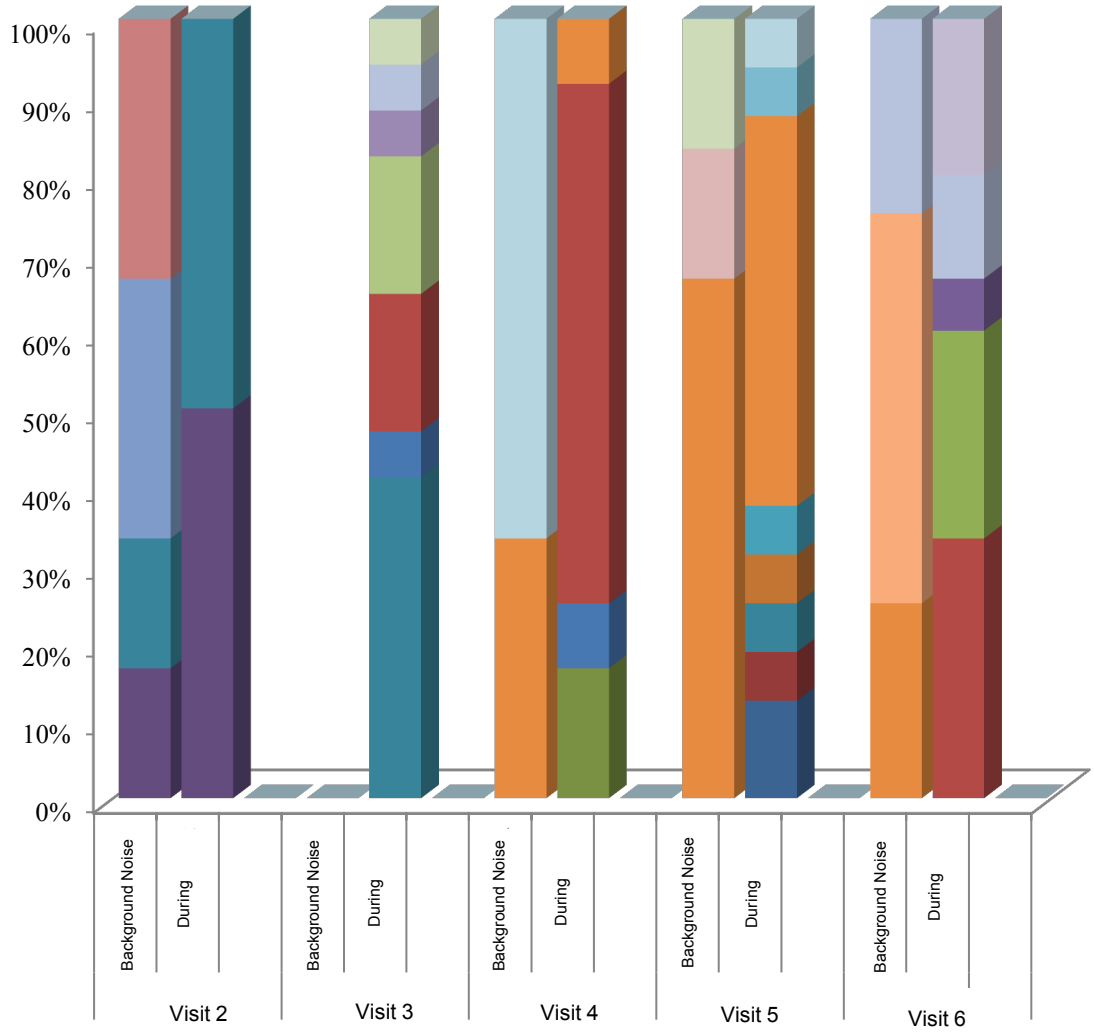
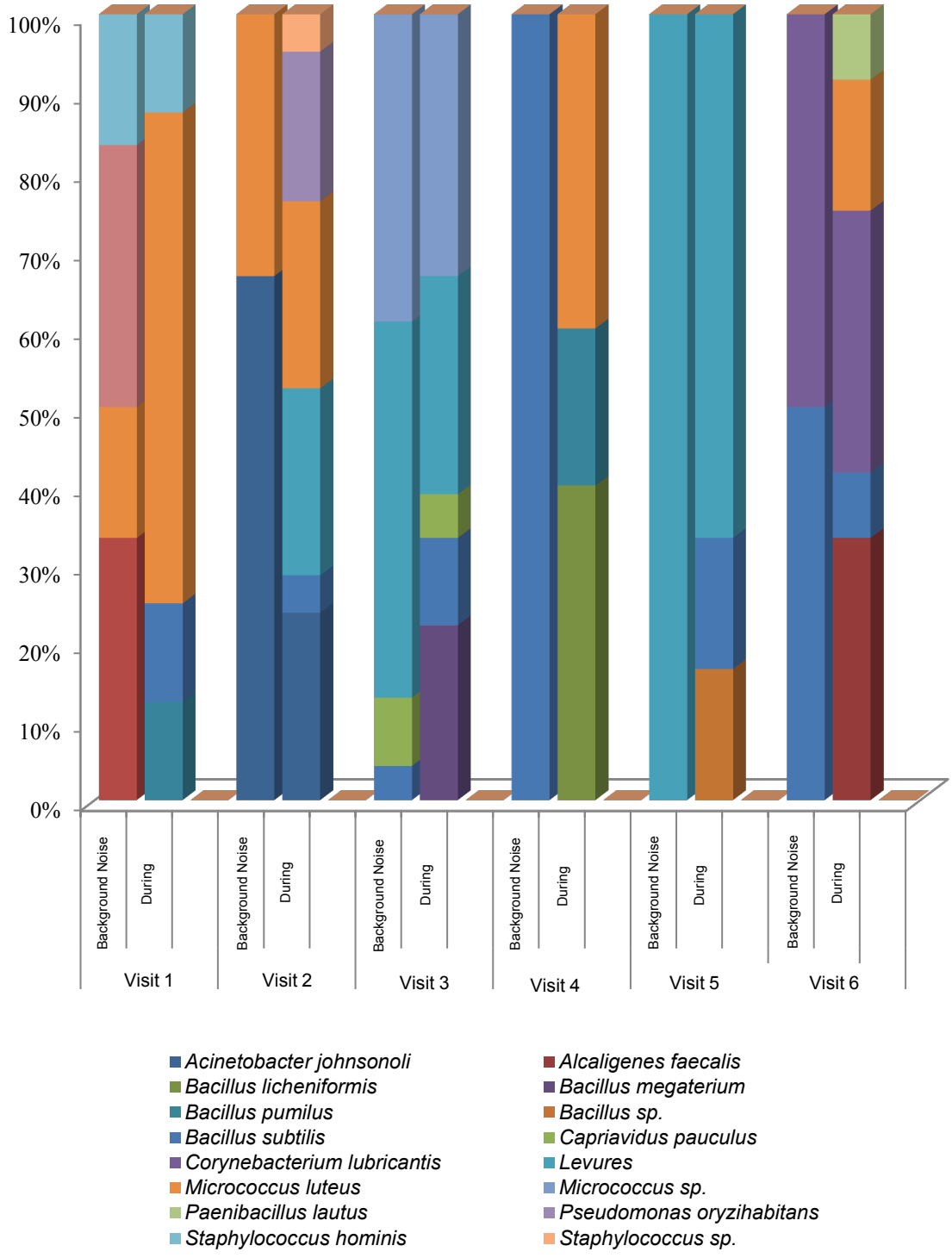
APPENDIX A – PERCENTAGE BREAKDOWN OF AIRBORNE MICROORGANISMS IN SAMPLES COLLECTED WITH AN ANDERSEN IMPACTOR BEFORE AND AFTER USE OF BIOLOGICAL DEGREASING STATIONS 1, 2, 3 AND 4



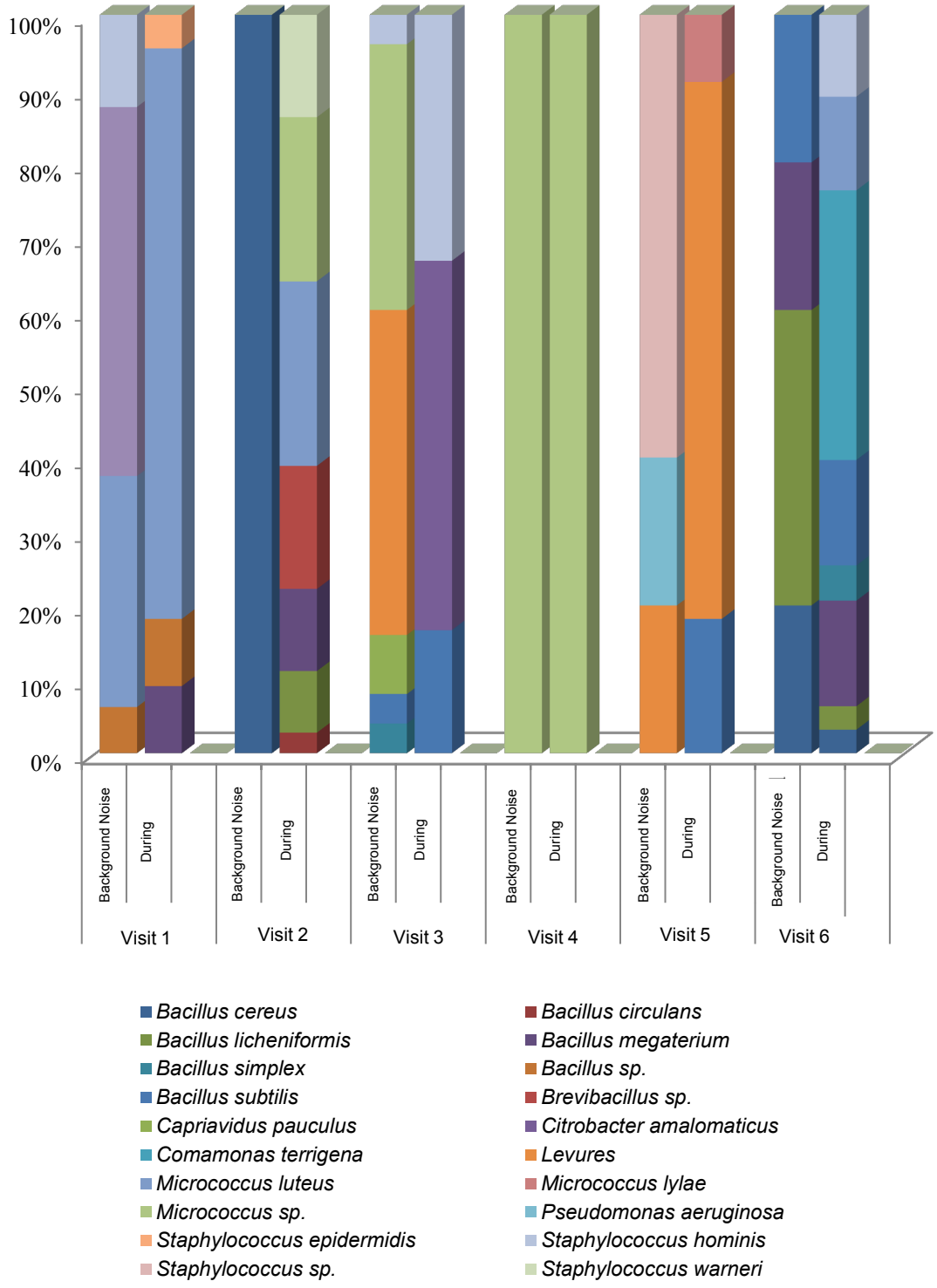
Biological degreasing station 1



Biological degreasing station 2



Biological degreasing station 3



Biological degreasing station 4

APPENDIX B – PARTICLE CONCENTRATIONS MEASURED WITH A UV-APS

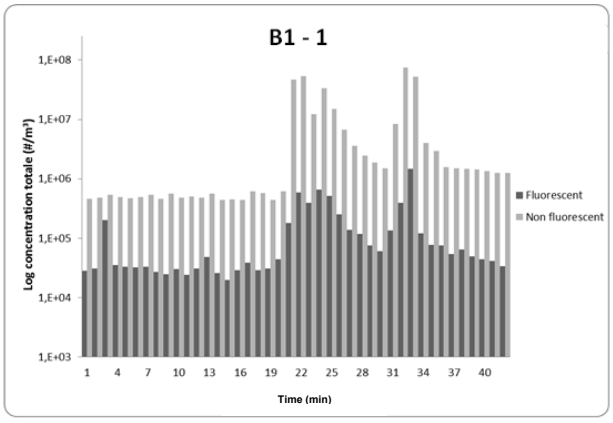
Fluorescent Particles									
	During biological degreasing station use				Background				
Visit	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Ratio*
B1 - 1	0.17	0.29	0.02	1.46	0.06	0.06	0.03	0.20	2.83
B1 - 2	0.05	0.02	0.02	0.09	0.05	0.02	0.03	0.11	1.00
B1 - 3	0.06	0.02	0.03	0.11	0.05	0.01	0.03	0.07	1.20
B1 - 4	0.10	0.01	0.08	0.16	0.10	0.02	0.07	0.17	1.00
B1 - 5	0.05	0.01	0.04	0.08	0.05	0.01	0.03	0.06	1.00
B1 - 6	0.02	0.01	0.01	0.06	0.01	0.00	0.00	0.02	2.00
B2 - 1	0.18	0.29	0.01	1.14	0.04	0.02	0.01	0.06	4.50
B2 - 2	0.16	0.11	0.06	0.68	0.08	0.04	0.05	0.22	2.00
B2 - 3	0.14	0.07	0.08	0.34	0.10	0.01	0.08	0.12	1.40
B2 - 4	0.11	0.04	0.06	0.21	0.05	0.02	0.03	0.10	2.20
B2 - 5	0.19	0.15	0.05	0.77	0.03	0.02	0.01	0.08	6.33
B2 - 6	0.06	0.03	0.03	0.14	0.05	0.01	0.03	0.07	1.20
B3 - 1	0.06	0.02	0.04	0.12	0.05	0.01	0.03	0.08	1.20
B3 - 2	0.19	0.06	0.10	0.36	0.17	0.07	0.03	0.28	1.12
B3 - 3	0.08	0.02	0.05	0.14	0.06	0.02	0.00	0.08	1.33
B3 - 4	0.09	0.04	0.04	0.19	0.06	0.02	0.03	0.11	1.50
B3 - 5	0.13	0.05	0.04	0.21	0.39	0.26	0.06	0.86	0.33
B3 - 6	0.07	0.02	0.05	0.12	0.07	0.02	0.00	0.10	1.00
B4 - 1	0.14	0.10	0.06	0.55	0.14	0.05	0.06	0.21	1.00
B4 - 2	0.12	0.02	0.09	0.18	0.24	0.20	0.00	0.66	0.50
B4 - 3	0.13	0.05	0.06	0.22	0.07	0.01	0.05	0.11	1.86
B4 - 4	0.27	0.09	0.12	0.54	0.06	0.01	0.04	0.07	4.50
B4 - 5	0.05	0.03	0.01	0.13	0.01	0.01	0.00	0.05	5.00
B4 - 6	0.06	0.07	0.02	0.46	0.04	0.02	0.02	0.07	1.55
B5 - 1	2.93	1.90	0.51	6.86	0.26	0.10	0.20	0.57	11.27
B5 - 2	2.93	1.75	0.15	6.42	0.13	0.05	0.00	0.19	22.54
B5 - 3	2.52	1.03	0.09	4.29	0.10	0.04	0.00	0.16	25.20
B5 - 4	1.77	2.11	0.05	9.34	0.03	0.01	0.00	0.06	59.00
B5 - 5	1.23	1.87	0.02	6.81	0.13	0.21	0.01	0.56	9.46
B5 - 6	1.09	2.12	0.02	7.82	0.03	0.01	0.01	0.06	36.33

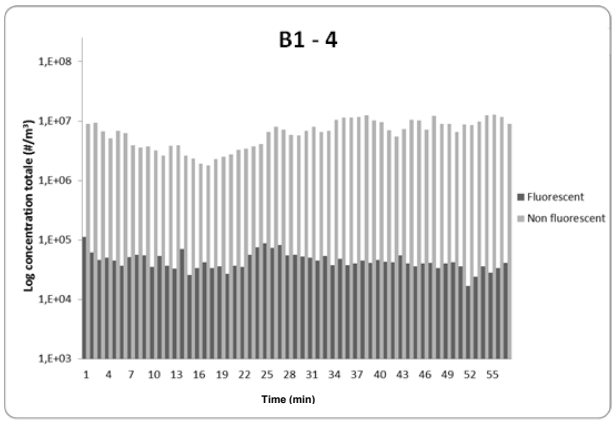
* Quotient: Average during biological degreasing station use divided by average background.

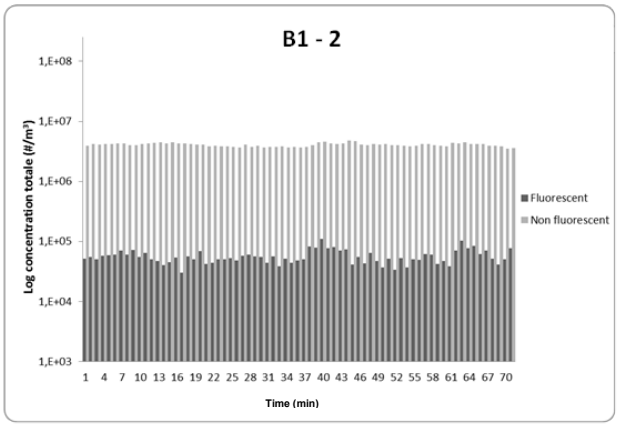
Non-Fluorescent Particles									
	During biological degreasing station use				Background				
Visit	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Average (#/cm^3)	Standard deviation (#/cm^3)	Min (#/cm^3)	Max (#/cm^3)	Ratio*
B1 - 1	10.02	19.25	0.44	75.39	0.97	0.45	0.46	1.45	10.33
B1 - 2	7.73	2.79	2.50	12.51	5.68	3.55	1.81	12.70	1.36
B1 - 3	4.12	0.27	3.53	4.75	4.02	0.25	3.63	4.52	1.02
B1 - 4	4.63	0.12	4.39	4.94	5.94	1.68	4.75	11.18	0.78
B1 - 5	2.45	0.09	2.29	2.60	2.60	0.15	2.25	2.80	0.94
B1 - 6	2.03	0.16	1.81	2.41	2.38	0.44	1.74	3.07	0.85
B2 - 1	1.45	0.61	1.01	3.31	1.26	0.22	0.91	1.95	1.15
B2 - 2	7.10	2.00	5.37	13.10	7.92	3.12	5.71	15.09	0.90
B2 - 3	5.20	1.82	3.49	10.21	4.17	0.84	3.39	6.96	1.25
B2 - 4	4.11	0.26	3.73	4.69	3.28	0.47	2.49	4.12	1.25
B2 - 5	3.20	0.56	2.61	5.66	3.00	0.19	2.61	3.41	1.07
B2 - 6	3.33	0.39	2.95	4.58	3.30	0.19	2.98	3.65	1.01
B3 - 1	2.63	0.36	1.98	3.11	1.80	0.18	1.51	2.07	1.46
B3 - 2	4.87	0.64	3.90	6.66	5.54	0.54	5.03	7.16	0.88
B3 - 3	6.13	0.79	5.07	8.05	6.86	0.66	5.80	7.86	0.89
B3 - 4	5.38	0.73	4.36	6.91	4.90	0.40	4.06	5.96	1.10
B3 - 5	4.23	0.75	3.23	5.63	4.70	0.60	3.75	5.97	0.90
B3 - 6	5.36	0.55	4.45	6.27	6.25	0.63	4.59	7.24	0.86
B4 - 1	4.37	0.41	3.79	5.57	4.73	0.42	4.09	5.34	0.92
B4 - 2	3.63	0.41	3.07	4.61	7.61	4.32	3.18	16.64	0.48
B4 - 3	6.32	1.48	4.03	8.84	4.70	1.25	3.82	6.98	1.34
B4 - 4	4.52	0.59	3.72	6.07	3.96	0.99	2.67	5.80	1.14
B4 - 5	8.79	1.56	6.42	12.96	7.95	1.42	5.92	10.53	1.11
B4 - 6	15.60	2.22	13.01	26.48	14.90	1.91	12.09	17.26	1.05
B5 - 1	67.25	31.39	26.72	126.5	20.5	2.5	18.6	28.3	3.28
B5 - 2	79.19	37.37	17.04	147.7	14.5	1.2	12.0	16.3	5.48
B5 - 3	67.65	23.65	4.46	107.2	5.7	0.7	4.3	6.6	11.87
B5 - 4	44.59	24.75	15.55	127.6	12.9	1.8	9.3	15.6	3.46
B5 - 5	42.63	40.09	8.68	156.9	18.6	10.4	10.8	40.8	2.29
B5 - 6	45.35	56.50	6.38	195.2	9.9	2.3	6.3	13.2	4.56

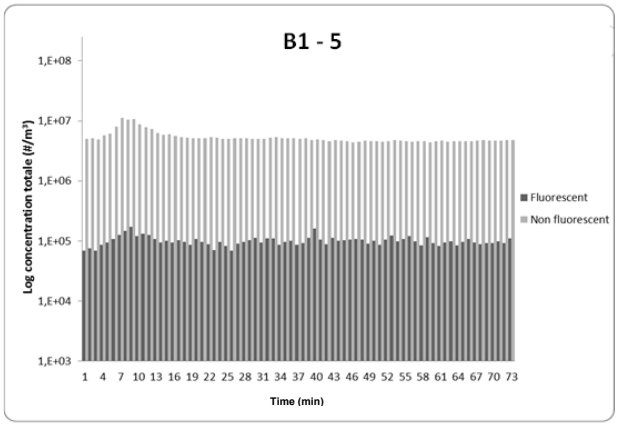
* Quotient: Average during biological degreasing station use divided by average background.

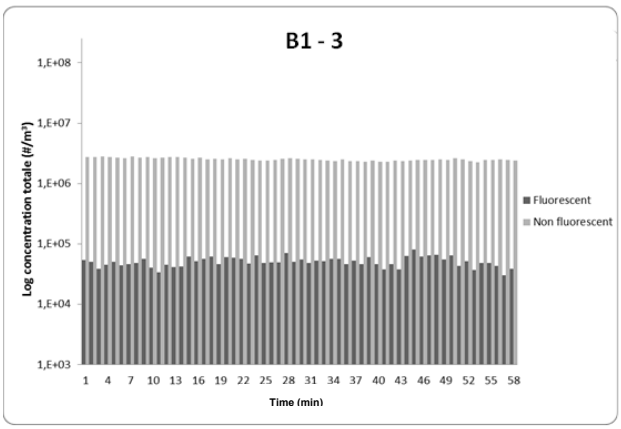
APPENDIX C – FLUORESCENT AND NON-FLUORESCENT PARTICLES MEASURED DURING BIOLOGICAL DEGREASING STATION USE

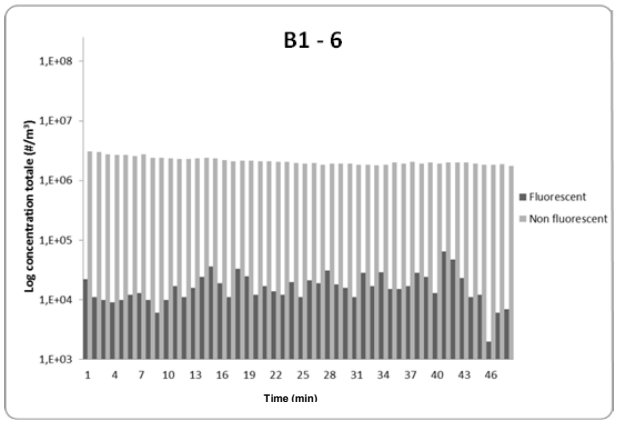


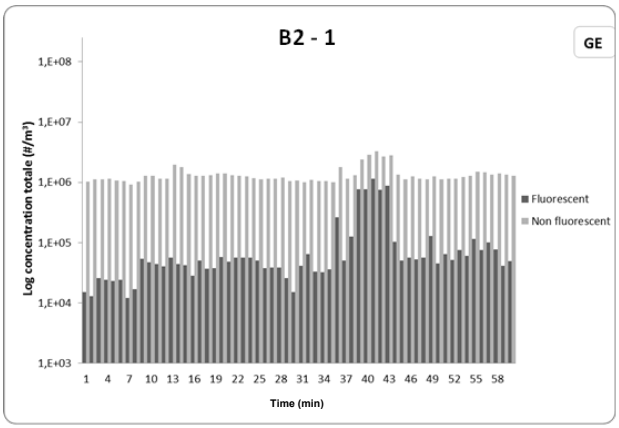


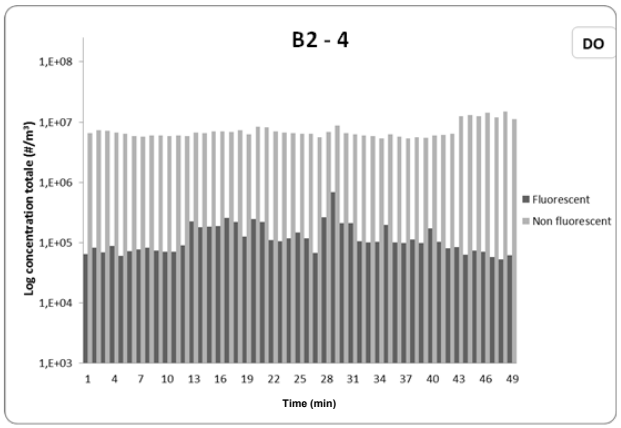


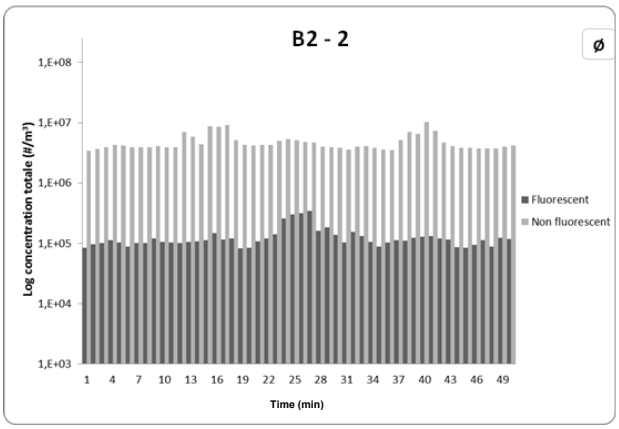


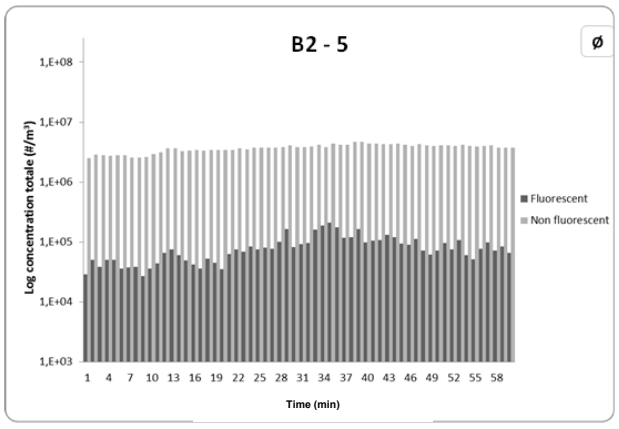


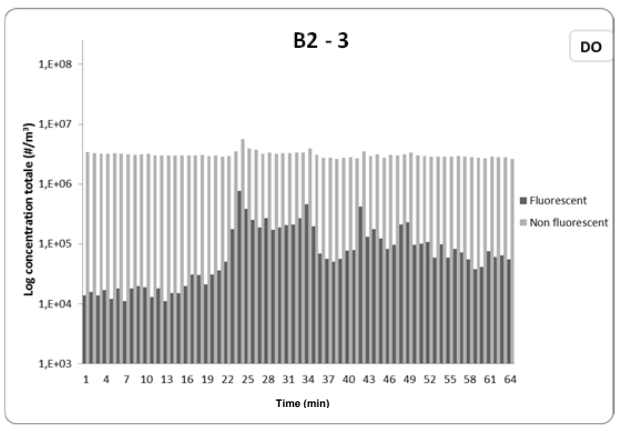


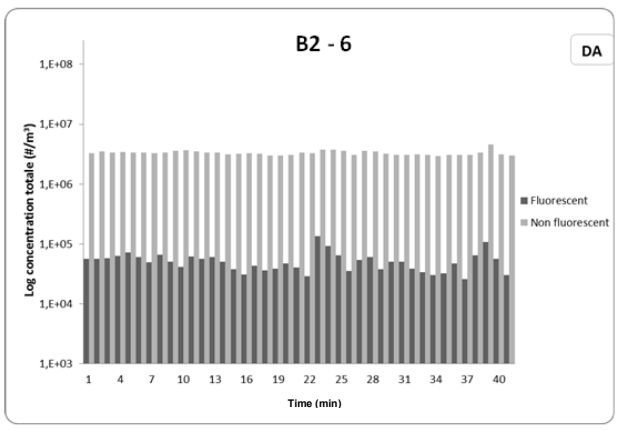

B2 - 1
GE
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Time (min)
Fluorescent
Non fluorescent

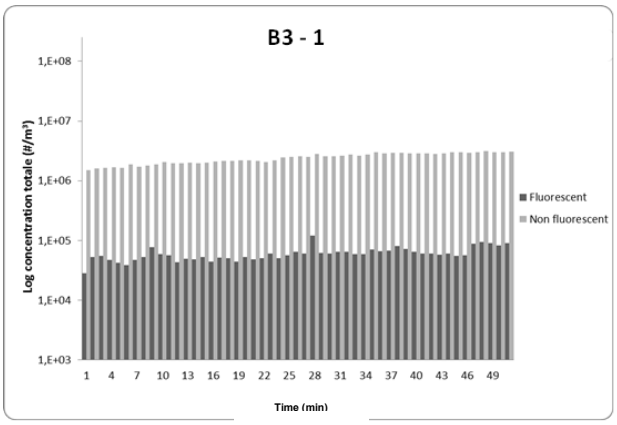

B2 - 4
DO
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

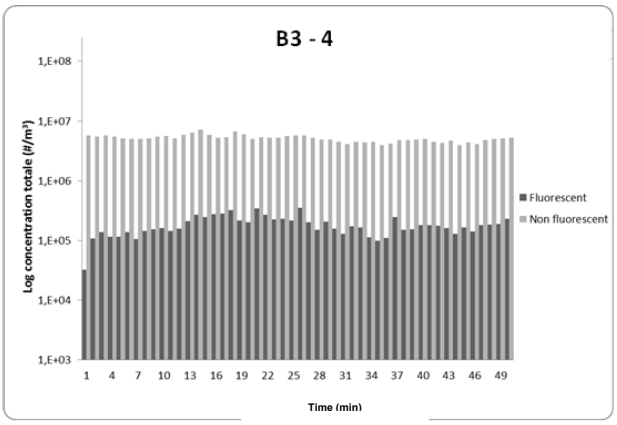

B2 - 2
Ø
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

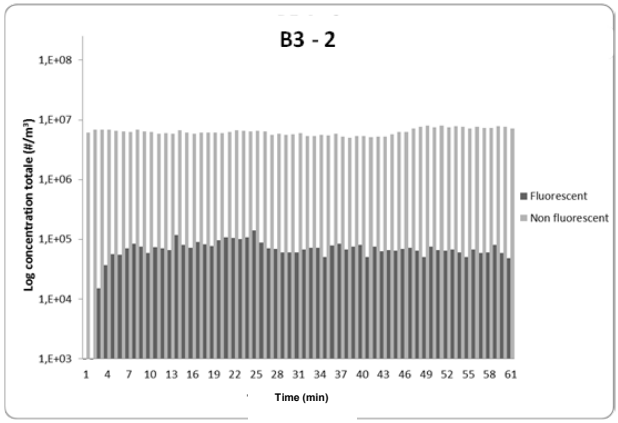

B2 - 5
Ø
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Time (min)
Fluorescent
Non fluorescent

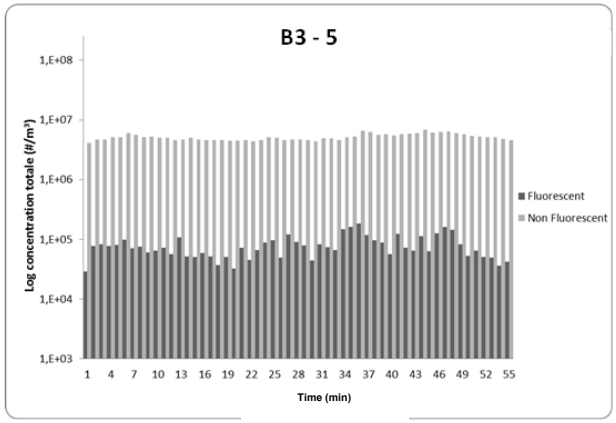

B2 - 3
DO
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64
Time (min)
Fluorescent
Non fluorescent

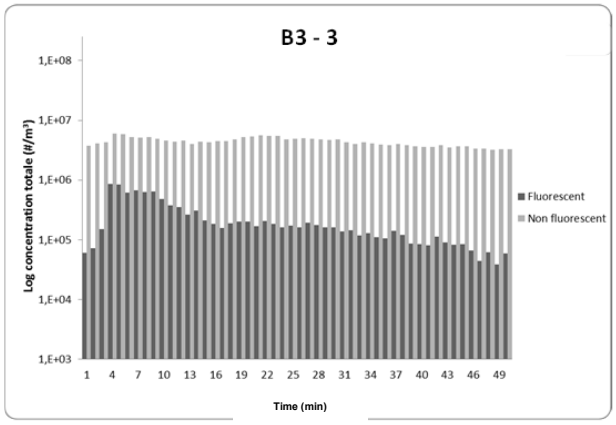

B2 - 6
DA
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40
Time (min)
Fluorescent
Non fluorescent

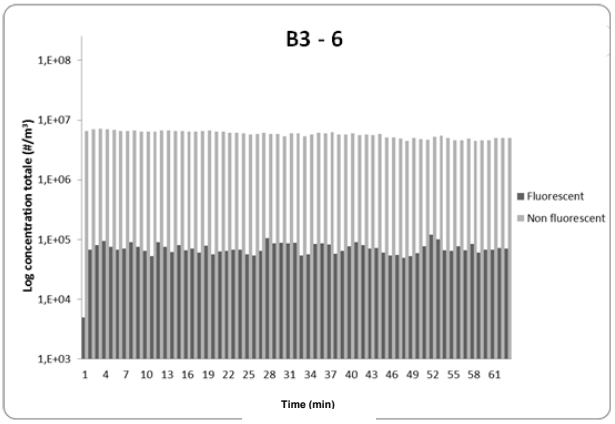

B3 - 1
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

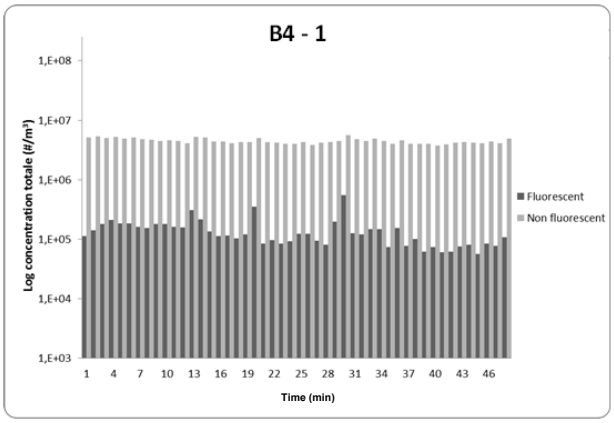

B3 - 4
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

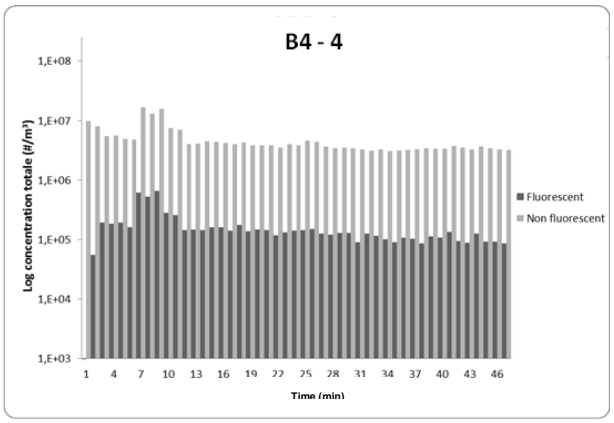

B3 - 2
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61
Time (min)
Fluorescent
Non fluorescent

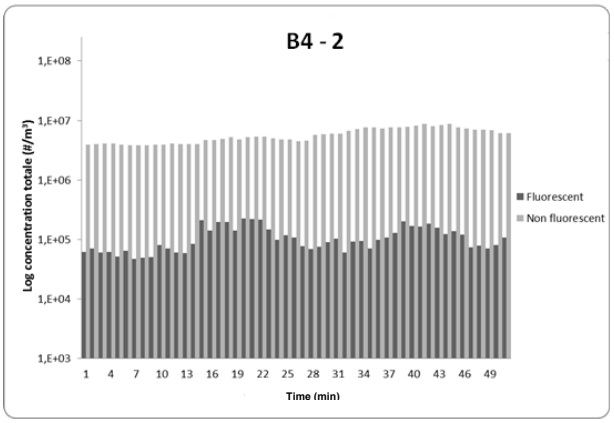

B3 - 5
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55
Time (min)
Fluorescent
Non Fluorescent

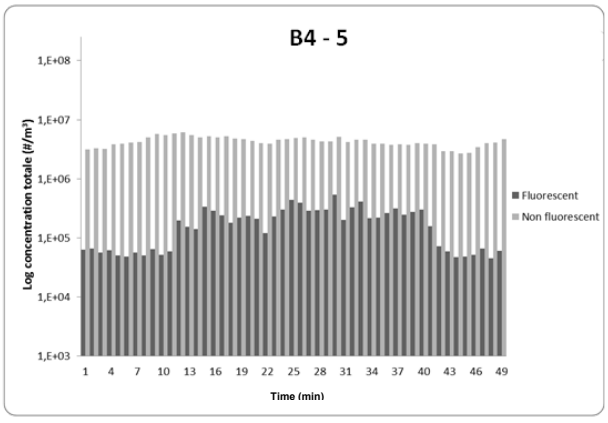

B3 - 3
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

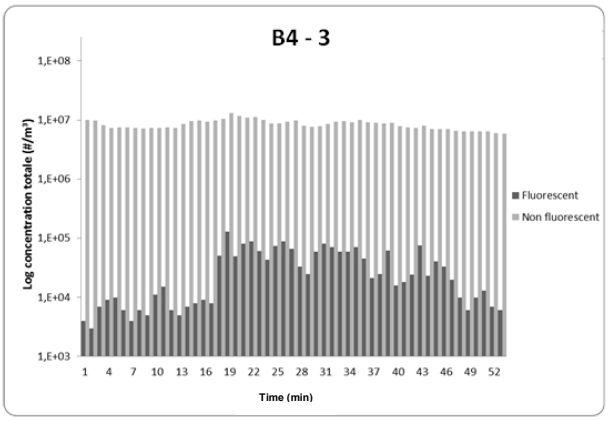

B3 - 6
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61
Time (min)
Fluorescent
Non fluorescent

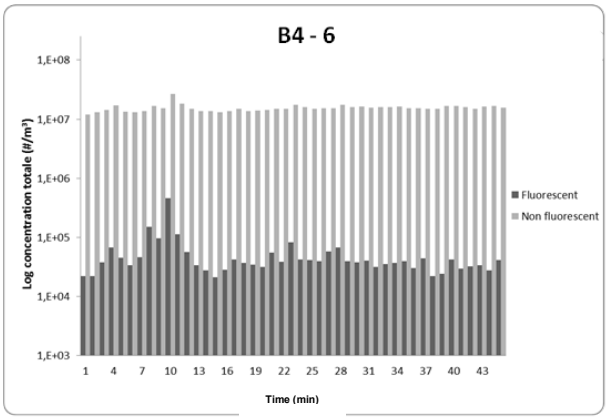

B4 - 1
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
Time (min)
Fluorescent
Non fluorescent

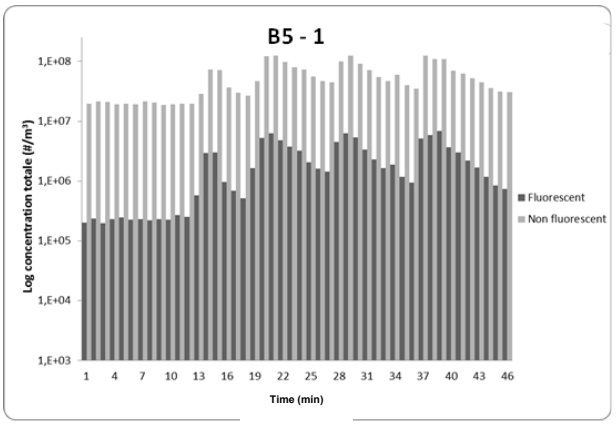

B4 - 4
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
Time (min)
Fluorescent
Non fluorescent

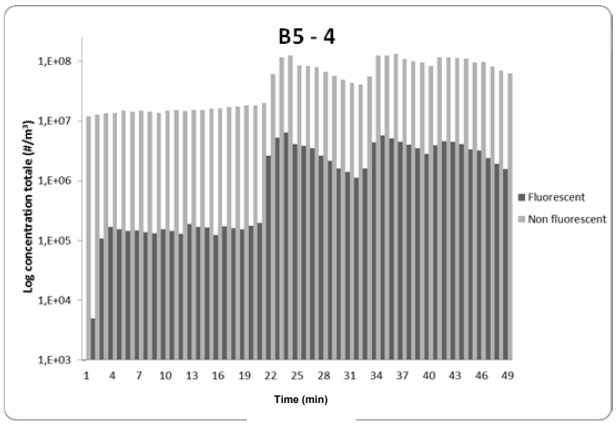

B4 - 2
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

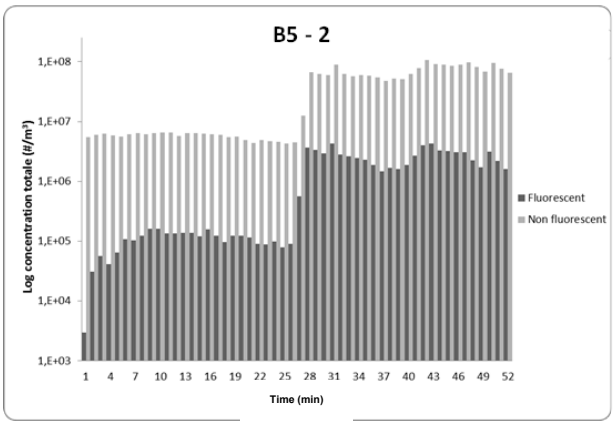

B4 - 5
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent

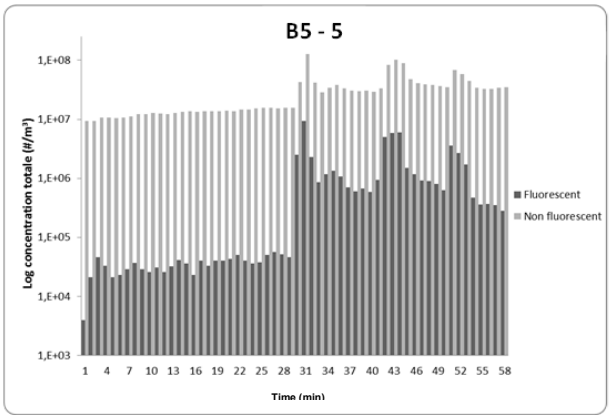

B4 - 3
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52
Time (min)
Fluorescent
Non fluorescent

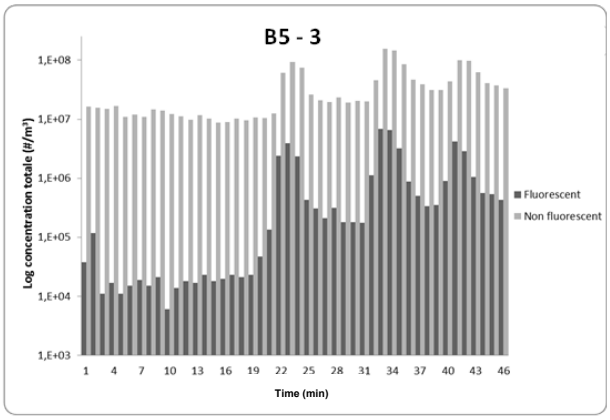

B4 - 6
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
Time (min)
Fluorescent
Non fluorescent

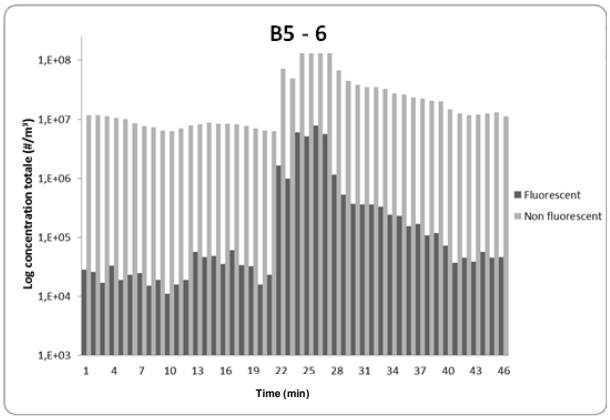

B5 - 1
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
Time (min)
Fluorescent
Non fluorescent


B5 - 4
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Time (min)
Fluorescent
Non fluorescent


B5 - 2
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52
Time (min)
Fluorescent
Non fluorescent


B5 - 5
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Time (min)
Fluorescent
Non fluorescent


B5 - 3
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
Time (min)
Fluorescent
Non fluorescent


B5 - 6
Log concentration totale (#/m³)
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
Time (min)
Fluorescent
Non fluorescent