A study of the potential release of bioaerosols from containers as a result of reduced frequency residual waste collections

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ABSTRACT

Microorganisms have the potential to grow within waste containers if waste is stored for longer periods as a result of an extended residual waste collection cycle. Release of microorganisms as bioaerosols during waste collection and processing may be an occupational risk to workers within the industry. There may be many constituents of the bioaerosol that may be of concern, however, there are currently only workplace exposure limits proposed for endotoxin (90 EU m^{-3}). A field-scale trial was established to determine the concentration of mesophilic bacteria, Gram-negative bacteria, *Listeria monocytogenes*, thermotolerant fungi, *Aspergillus fumigatus*, endotoxin and (1→3)-β-D-glucan in air within bins containing either bagged or loose residual waste, in warm (23 °C) or cold (7 °C) conditions, to simulate an extended collection cycle. Fresh waste was added during the first four weeks, with an additional 'missed collection' phase of a further four weeks where no more waste was added. A second trial examined the microbiological components of bioaerosols associated with 'tipping' the bins, simulating the moment when bins are emptied into waste collection vehicles. The majority of

mesophilic bacteria, fungi and *A. fumigatus* concentrations were recorded when fresh material was added to the bins, with only mesophilic bacteria recorded up to week 6 during the 'missed collection' phase. (1→3)-β-D-glucan concentrations were variable throughout the first trial, (geometric mean range 0.4-13.8 ng m^{-3}). Perhaps the bioaerosol component of most interest was endotoxin (geometric mean range 0.52-1288 EU m^{-3}). Elevated endotoxin concentrations were recorded during the 'missed collection' phase of the extended collection cycle and during 'tipping'. This data demonstrates significant concentrations of bioaerosols and particularly endotoxin can be generated during prolonged residual waste storage and collection. As endotoxin is a bioaerosol component of concern it can be concluded there is the potential for workplace exposure hence identifying key areas for risk assessment.

Keywords: (1→3)-β-D-glucan, *Aspergillus fumigatus*, endotoxin, waste, occupational risk.

1. Introduction

Residual waste is all the material that is left after the recyclables have been removed, and may consist of organics (including kitchen waste), plastics, cans, glass and various other recyclable and non-recyclable components. Householders may deposit this material either loose or bagged, and the size of residual waste bins are often restricted to 120 litres to encourage materials to be deposited in recycling containers. In a bid to bring the UK's household recycling rates in line with the European Union's target of 50% of household waste recycled by 2020 (EC, 2008) many UK Councils have extended residual waste collection cycles to fortnightly or three-weekly (Gladding, 2009). Indeed, this practice is not uncommon within Europe, particularly in Germany. Within the UK devolved regions, more stringent local government targets of 70% by 2025 have also been introduced (Scottish Government, 2010, Welsh Assembly Government, 2010). Monthly bin collections of residual

waste have also been suggested, although public opinion has hampered progress in this area (Yates, 2016). One reason for this lack of support is often due to the perception that bins would become a nuisance via odours but also because of public health fears regarding vermin. However, there is also concern they could also provide a breeding ground for microorganisms, particularly in bins where nappies and organic wastes are deposited.

The health effects of bioaerosols are of interest as they have the potential to be released whenever the bin is opened, such as when waste is added and when a bin is emptied. Bioaerosols potentially contain aerosolised biological material such as bacteria, fungi, viruses, endotoxins and (1→3)-β-D-glucan which may all have health impacts ranging from upper airways irritation, nausea, and fever to potential lung inflammation and respiratory illness (Gutarowska et al., 2015, Swan et al., 2003, Searl, 2008). However, only endotoxin, found in the cell walls of gram-negative bacteria, has a demonstrable increased risk of symptoms alongside increased exposure (Searl, 2008). As a result, currently, there are no agreed upon workplace exposure limits for any bioaerosol components (Walser et al., 2015). In a recent comprehensive review no suitable exposure-response relationships could be found between the microbial component of bioaerosols and human health due to insufficient and comparable data in the literature, the range of health effects, and insufficient exposure assessment (Walser et al., 2015). It has also been noted that variability in response due to individual risk factors is also a barrier to the setup of reliable exposure-response relationships for inhaled biological agents (Searl, 2008). Various authors have reported exposure thresholds in the literature ranging from 10^3 cfu m^{-3} and 10^5 cfu m^{-3} for both general bacteria and total fungi (Eduard et al., 2012; Kuijer et al., 2010; Searl and Crawford, 2012). The Health council of the Netherlands has imposed a 30 EU m^{-3} limit on endotoxin released from livestock farms for the protection of the public and an occupational limit of 90 EU m^{-3} (Health Council of the Netherlands, 2010, Health Council of the Netherlands, 2012), whilst

Rylander (1997) has suggested a 10 ng m^{-3} guideline value for exposure to (1→3)-β-D-glucan.

Although not occupational, the Environment Agency in the UK has applied precautionary environmental limits to bioaerosols that are emitted downwind of waste compost sites in order to protect the public (1000 cfu m^{-3} for bacteria, 500 cfu m^{-3} for *Aspergillus fumigatus* and 300 cfu m^{-3} for Gram-negative bacteria) (EA, 2009). In the absence of validated occupational limits, data from this study was also assessed against these more stringent reference guidelines.

Whilst some work has been undertaken to assess the health risks associated with bioaerosols emitted from general waste collection (Neumann et al., 2005, Roodbari et al., 2013, Kuijer et al., 2010, Neumann et al., 2014, Neumann et al., 2015, Schantora et al., 2015), source separated recyclable collection (Heldal et al., 1997) and indoor storage of organic waste (Wouters et al., 2000) no studies to date have detailed the risks associated with storage and collection of containers (bins) containing only the residual fraction after recycling. Therefore, the aims of this study were three-fold:

1) To explore the potential for bioaerosol emissions arising from the extended storage of residual waste material,
2) To explore the link between these emissions and potential health impacts on householders and waste collectors, and,
3) To provide further evidence to support the development of guidance to local authorities that may be considering extended waste collections in the future.

2. Material and methods

2.1. Experimental setup

Household-sized wheeled bins (240 litres) were stored under 'simulated waste disposal' and 'tipping' conditions at a dedicated indoor field site facility at the Open University (52°1'27"N, 0°42'20"W). The facility space was in two parts, and was approximately 5 m x 5 m at 23 °C in one part, with a separated refrigerated container measuring 5 m x 10 m at 7 °C in the other. Data were collected between July and September, 2013. 'Simulated waste disposal' bins were divided by waste addition method, e.g. bagged (black plastic refuse sacks tied at the top) and loose (waste material emptied straight into the bin). Each waste treatment was further subdivided into summer (warm) and winter (cold) groupings in the separated field site facility areas (23 °C and 7 °C respectively). Temperatures were checked in the airspace of the bins and in the atmosphere of these areas daily utilising calibrated digital thermometers. This culminated in four subgroups (n = 6 in each) namely, bagged, warm (BW); loose, warm (LW); bagged, cold (BC), and loose, cold (LC). Mixed residual waste (4.5 kg; see section 2.2, Table 1) was added to each bin once a week for four weeks and subsequently left for a further four weeks to simulate householder deposition and subsequent missed collection on an extended frequency. Therefore, results from week 2 could be used to assess the risk to householders and collectors after a fortnightly waste collection, week 4 could be used to assess the risk to householders and collectors after an extended four-weekly collection, and results at week 8 could be used to assess the risk to householders and collectors after a missed collection of an extended four-weekly collection cycle.

To further simulate the risks posed to waste operators involved in bin emptying, a 'tipping' scenario was established. 'Tipping' was standardised by laying the bins on their side and agitating them for 10 seconds to replicate the disturbance caused when wheeled bins are emptied or moved. Bins (n = 6) containing loose, warm waste (23 °C) (expected to be the 'worst case scenario') were left undisturbed for four weeks (LW4) and then agitated as described to mimic the activity of tipping bins into a waste collection vehicle. Subsequently,

125 the bins used in the eight-week ‘simulated waste disposal’ trial were left for a further week
126 before tipping and agitating (week 9; LW9, BW9, LC9 & BC9). In this instance, waste was
127 added at time 0 and 1 day prior to tipping for LW4. The last addition of waste to the other
128 treatments was as for the ‘simulated waste disposal’ scenario, i.e. in week 4.

129 Table 1: The composition of waste added to the bins during weeks 1 to 4.

Waste category	Source	Specific waste[1]	Week 1 (kg)	Week 2 (kg)	Week 3 (kg)	Week 4 (kg)
Food waste	Local markets, food deposited on plastics, out of date material, university food waste	Vegetables: Potatoes, onions, courgettes, carrots (uncooked), some apples Week 2 onwards	0.9	0.9	0.9	0.9
		Meat: Beef mince, chicken legs (uncooked); Week 4 cooked ham	0.1	0.1	0.1	0.1
		Fish: Fish fingers (uncooked)	0.1	0.1	0.1	0.1
		Bread: Pitta breads	0.2	0.2	0.2	0.2
Garden waste	University premises garden waste	Hay, grass	0.3	0.3	0.3	0.3
		Woodchips	0.3	0.3	0.3	0.3
		Stones: Gravel	0.56	0.56	0.56	0.56
Paper	University waste paper	Newspaper	0.3	0.3	0.3	-
Card	Primarily as packaging for end of life material and from university recycling	Cardboard cut to size	0.4	0.4	0.4	0.4
Textiles	Bought commercially	Cotton rags, Week 4 cotton t-shirts	0.2	0.2	0.2	0.2
Sanitary materials	Commercial nursery	Nappies	0.1	0.1	0.1	0.1
Plastics and plastic film	Primarily as packaging for end of life material and from University recycling	2D: Heavy duty plastic bags	0.45	0.45	0.45	0.45
		3D: Plastic bottles	0.45	0.45	0.45	0.45
Metals	University recycling	Soiled drinks cans	0.1	0.1	0.1	0.1
Total			4.46	4.46	4.46	4.16

130 [1]Vegetable category accurate to within 0.05kg, all other categories accurate to within 0.02 kg

131 Table 2: A summary of the sampling strategy

Week	Monday	Tuesday	Wednesday	Thursday	Friday
1	BW & LW	BC & LC		Waste deposited in BW & LW	Waste deposited in BC & LC
2	BW & LW	BC & LC		Waste deposited in BW & LW	Waste deposited in BC & LC, PCR
3	BW & LW	BC & LC		Waste deposited in BW & LW	Waste deposited in BC & LC
4	BW & LW	BC & LC	*LW4*	Waste deposited in BW & LW	Waste deposited in BC & LC, PCR
5	BW & LW	BC & LC			
6		BW & LW	BC & LC		
7	BW & LW	BC & LC			
8				BW & LW	BC & LC
9	*BW9, LW9*	*BC9, LC9*			

132 Trial 1, simulated extended waste collection: BW= bagged waste at 23°C, LW = loose waste at 23°C, BC = bagged waste at 7 °C & LC = loose
133 waste at 7 °C.

134 Trial 2, tipping (*in italics*): LW4 = loose waste at 23 °C, week 4; LW9 = loose waste at 23 °C, week 9; BW9 = bagged waste at 23 °C, week 9;
135 LC9 = loose waste at 7 °C, week 9; BC9 = bagged waste at 7 °C, week 9.

136 *2.2. Waste properties*

137 Waste was chosen to reflect typical waste compositions (Table 1) and were taken from Parfitt
138 and Bridgewater (2010) with slight modifications to account for 'fines' and 'other organics'
139 which were not defined, and to remove glass and replace it with other packaging in the
140 interests of safe handling. The following assumptions were made to determine the quantity of
141 material to be added to the bins per week: On average, one person generates 428 kg of waste
142 a year which, with a 43 % recycling rate (DEFRA, 2013) results in approximately 4.7 kg of
143 residual waste per person a week. Previous scoping studies from Zero Waste Scotland (ZWS)
144 (Gladding, *unpublished*) on an extended waste collection scheme in Germany showed that, on
145 average, 37 % of all waste material was deposited in the residual bin. Based on the waste
146 generation figure above, this equates to 3 kg of residual waste per person a week. Waste
147 generation is also falling by around 2 % a quarter (DEFRA, 2013). Therefore, taking all of
148 these figures into account, and assuming a two-person household, a target of 4.5 kg of
149 residual waste per bin per week was considered acceptable.

150

151 *2.3. Bioaerosol sampling*

152 A trial of 12 bins (6 using a clear plastic bag and 6 a chamber) was undertaken to identify the
153 best method of sampling the bioaerosols from the bins. All microbiological analysis in the
154 trial was as for the main study. Based upon these results it was decided that a clear plastic bag
155 (240 l) would be taped to the opening of the bin immediately after waste depositing and left
156 for approximately 4 days before sampling to catch all emissions after deposition to model a
157 'worst case scenario'. Prior to sampling air (200 l) was pumped into the bag through a
158 sealable porthole at the top of the bin. Bioaerosols were captured using liquid impingers
159 (SKC BioSamplers and Biolite air sampling pumps, SKC Ltd. Blandford Forum, UK)
160 containing 20 ml of sterile non-pyrogenic water (Associates of Cape Cod Intl. Inc.,

Liverpool, UK) at a rate of 12.5 l min^{-1} for 10 minutes through a sealable porthole in the sampling bag. Bioaerosol samples, bin mass and headspace measurements were taken on a weekly basis for the duration of the trial. The sampling strategy can be found in Table 2. Samples were transported from the field site to the laboratory and microbiological determinants were processed immediately. Aliquots (1 ml) were stored at -20 °C for subsequent endotoxin and (1→3)-β-D-glucan analysis. The remaining liquid was stored at -20 °C for molecular analysis.

2.4. Microbiological analysis

2.4.1. Enumeration

Aliquots of the impinger liquid (100 µl) were spread-plated in triplicate onto ½-strength nutrient agar containing 100 mg l^{-1} cycloheximide for total mesophilic bacterial counts, MacConkey agar No. 3 containing 200 mg l^{-1} cycloheximide for Gram-negative bacteria, *Listeria* selective media (Oxford formulation) containing *Listeria* selective supplement for *Listeria monocytogenes* and malt extract agar containing 20,000 U l^{-1} penicillin and 40,000 U l^{-1} streptomycin for total fungi favouring *A. fumigatus*. Plates were incubated for 48 hours (counted again after 7 days for *L. monocytogenes*) at 37 °C for bacteria and at 40 °C for fungi. All media were obtained from Oxoid Ltd. (Basingstoke, UK) and supplements from Fisher Scientific (Loughborough, UK) and Sigma Aldrich (Dorset, UK). Hence all bioaerosols collected were culturable, that is they were able to grow on agar. This method, including media and incubation temperatures, followed the Environment Agency (England and Wales) recommended 'AfOR Protocol' (2009). This protocol is in common use throughout the UK to assess bioaerosols from open windrow composting sites and other organic waste treatment facilities, as a result the study is not designed to identify all

mesophilic bacteria and fungi that might grow on waste but to align with the standards by which emissions are measured from UK facilities.

2.4.2. Confirmation

Colonies chosen for molecular confirmation were collected from counted plates in weeks 2, 4 and 8. Presumptive Gram-negative and *L. monocytogenes* colonies were suspended in sterile phosphate-buffered saline (PBS) and pelleted. DNA was extracted using the PureLink® Genomic DNA kit (Life Technologies, Paisley, UK) according to the manufacturer's instructions and sent to GATC Biotech AG (Köln, Germany) for PCR and sequencing using universal bacterial primers COM1 (5'-CAGCAGCCGCGGTAATAC-3') and COM2 (5'-CGTCAATTCCTTTGAGTTT-3') (Schwieger and Tebbe, 1998). Returned sequences were identified using the BLASTn tool available at http://decipher.cee.wisc.edu/.

2.5. Endotoxin and (1→3)-β-D-glucan analysis

Endotoxin and (1→3)-β-D-glucan were analysed using the kinetic Pyrochrome® and Glucatell® kits respectively (Associates of Cape Cod Inc., Liverpool, UK) following the manufacturer's instructions and analysed by a BioTek ELx808 microplate reader (BioTek Instruments Inc., Swindon, UK) according to the British Standard BS EN 14031:2003 (British Standards Institution, 2003).

2.6. Data analysis

Detection limits were 533 cfu m^{-3} for all microorganisms, 0.8 EU m^{-3} for endotoxin and 0.5 ng m^{-3} for (1→3)-β-D-glucan. Concentrations below the limit of detection were given an arbitrary value of half the detection limit. All statistical analysis was carried out in the open-source package 'R'. Normality was assessed using the Shapiro-Wilk test. Data remained

positively-skewed despite attempts to transform the data; therefore, all statistics were carried out on the original figures. Means were summarised using the geometric mean and a 95% bias-corrected accelerated bootstrapped confidence interval. The non-parametric tests Kruskal-Wallis, followed by the post-hoc Nemenyi test with Chi-squared approximation for independent samples, and Mann-Whitney were used to determine if there were differences in bioaerosol concentrations between treatments. Differences between normal collections (week 2) and missed collections (week 4) were determined using the Wilcoxon sign rank test. Correlations between determinants were determined using the Spearman Rank test.

3. Results

3.1. Trial Management

Temperature measurements taken in the airspace across a selection of bins confirmed that bins stored in the summer and winter groupings had an average temperature of 23 °C (min = 21.7 °C, max = 25.0 °C) and 7 °C (min = 6 °C, max = 8 °C) respectively. Approximately 4.5 to 5.0 kg of waste was added to each bin, once a week for four weeks. At four weeks bins were almost at capacity as can be seen from the head-space data (Figure 1A), particularly for bagged material (BW & BC). No further waste additions were made to simulate the scenario of a missed extended four-weekly collection. On average, approximately 4.0 kg was lost from each bin in the first four weeks after which time, mass remained constant (Figure 1B); this indicates that the majority of organic waste decomposition occurred within the first four weeks.

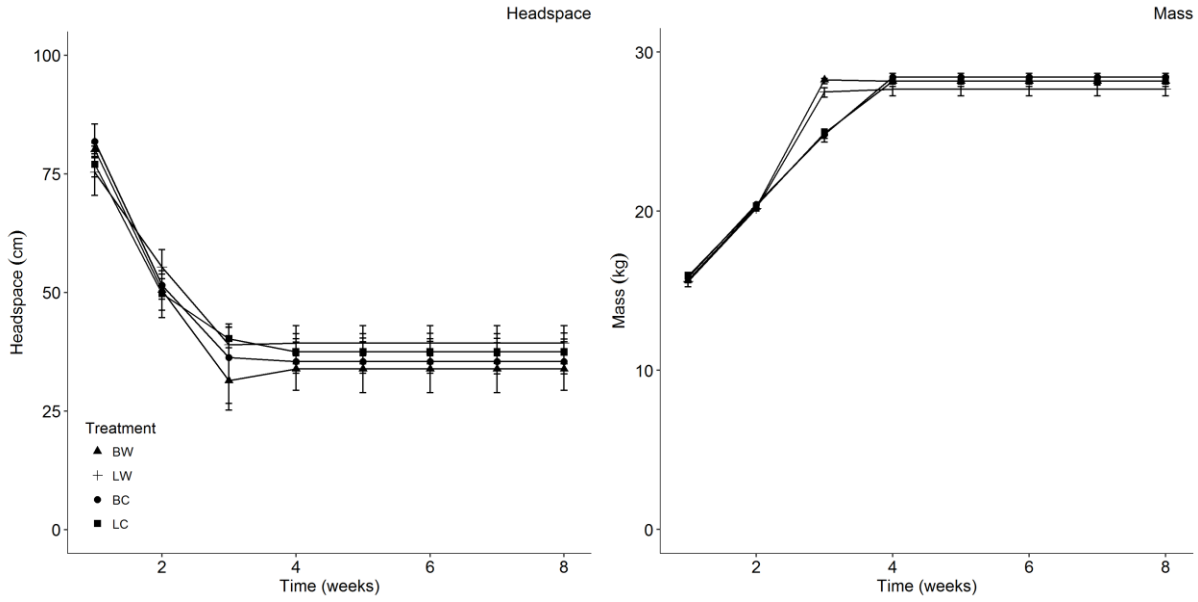


Figure 1: Weekly headspace and bin mass measurements over the 8-week 'simulated waste disposal' trial. BW= bagged waste at 23°C, LW = loose waste at 23°C, BC = bagged waste at 7 °C & LC = loose waste at 7 °C. Values represent the geometric mean of six replicates 95% confidence interval estimated from 5000 bootstrap samples.

3.2. Bioaerosol analysis

Although selective media were used, 16S rRNA sequencing identified presumptive Gram-negative colonies as being either *Streptomyces* spp., or *Staphylococcus* spp., neither of which are Gram-negative. Similarly, no *L. monocytogenes* were positively confirmed. Indeed, colonies grown on *Listeria* selective media were shown to be *Streptomyces* spp. and *Bacillus* spp. Therefore, the data relating to these culturable microorganisms were deemed unreliable and removed from further analysis.

3.2.1. Simulated waste disposal

Culturable microbial counts were restricted to the first six weeks of the trial. After this time, no bacteria or fungi were isolated from bioaerosols (Figure 2). Mesophilic bacteria concentrations differed significantly between waste treatments ($X^2 = 12.9$, $P < 0.01$), and

showed two peaks in concentration in BW bins, with the first peak at week 4, followed by a further peak at week 6. LW showed a higher concentration at week 4 ($P > 0.05$) but numbers were reduced to below detection limits by week 5. BC mesophilic bacterial concentrations were significantly lower than those of LW ($P < 0.01$) and were only recordable at concentrations higher than the detection limit in weeks 3 and 4. Similarly to BC, mesophilic bacteria in LC were only recordable in weeks 2-4. However, concentrations reached the highest recorded for all treatments in week 3 (3.47×10^4 cfu m^{-3}) and the second highest recorded in week 4 (1.47×10^4 cfu m^{-3}). *A. fumigatus* had a similar pattern of activity to mesophilic bacteria with the exception that there was no second peak in the BW treatment and concentrations fell to below detection limits at a slightly faster rate in all treatments (Figure 2). As for mesophilic bacteria, the highest *A. fumigatus* count was recorded in LC at week 3 (2.79×10^4 cfu m^{-3}). Fungi other than *A. fumigatus* growing at the 40°C incubator temperature were only recorded in one incidence (BW, week 3, 4.14×10^2 cfu m^{-3}; data not shown).

Figure 3 shows the concentration of endotoxins released in the bioaerosols from the different waste storage treatments. Endotoxin concentration showed a similar pattern in all treatments with a slight peak at week 1 and a much higher peak in week 7 (Figure 3). Overall endotoxin concentrations were not significantly different between treatments ($X^2 = 3.962$, $P > 0.05$). However, during week 7, BC was significantly lower than both LC & LW ($P < 0.05$ for both; Figure 3). It is notable that endotoxin concentrations were detected consistently above the 30 EU m^{-3} exposure guideline (Health Council of the Netherlands, 2012) at week 1 and from week 5 until the end of the trial, with the highest endotoxin concentration recorded in LW in week 7 (1,313 EU m^{-3}). The mean concentration of (1→3)-β-D-glucan showed more variability throughout the trial (data not shown) with the highest concentration recorded in LC, in week 5 (14 ng m^{-3}). In fact, the data is almost cyclical. However, whilst the

Kruskal-Wallis tests indicated a significant difference between treatments (X^2 = 9.048, P < 0.05), the post-hoc Nemenyi with chi squared distribution test failed to distinguish where the differences lay. Nevertheless, geometric mean (1→3)-β-D-glucan concentrations only exceeded the proposed 10 ng m^{-3} exposure limit (Rylander, 1997) at two time points; LW, BC & LC in week 5 (11, 12 & 14 ng m^{-3} respectively) and LW (10 ng m^{-3}) in week 8.

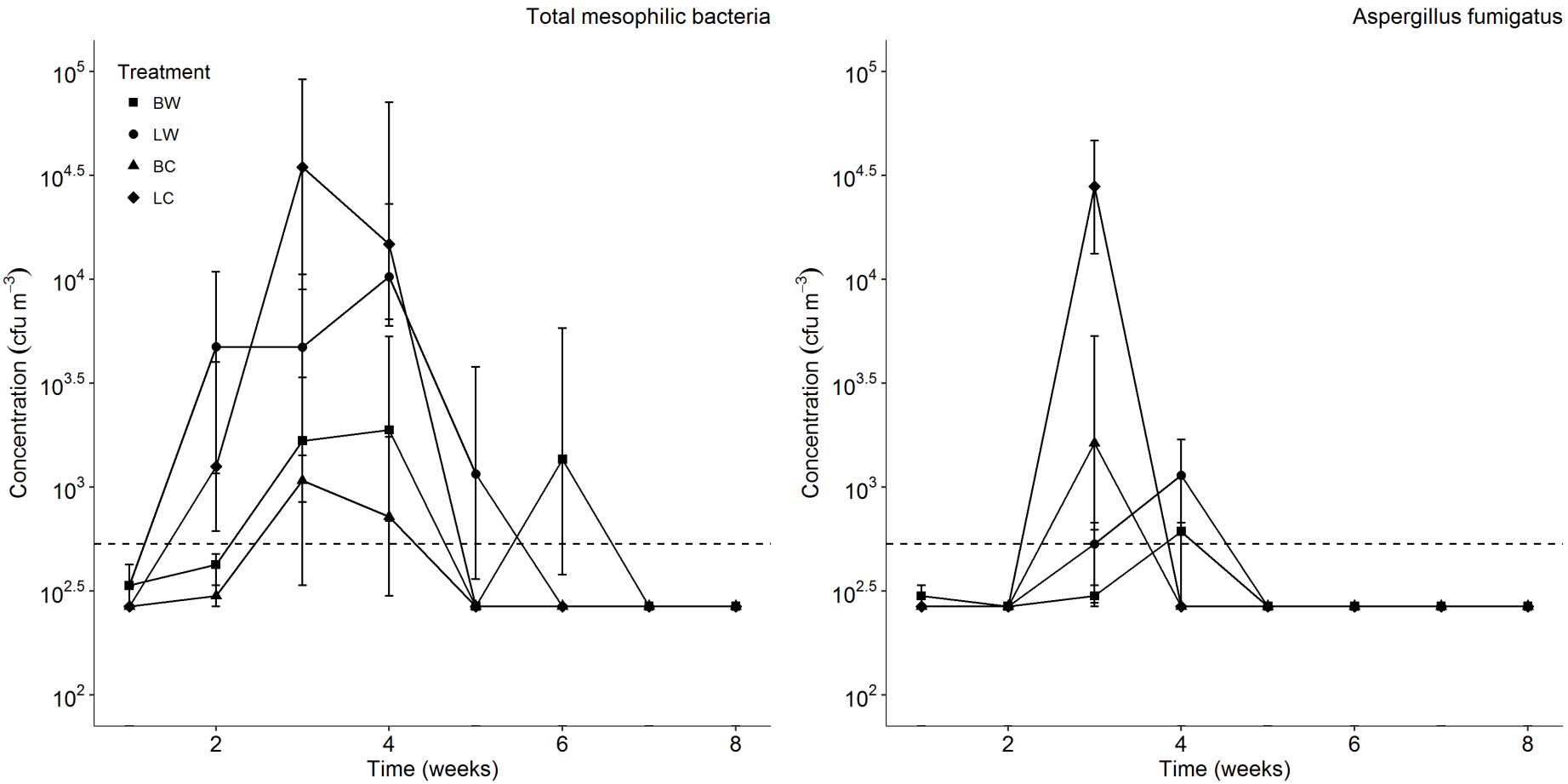


Figure 2: The concentrations of microorganisms emitted as bioaerosols during the 'simulated waste disposal' scenario. BW= bagged waste at 23°C, LW = loose waste at 23°C, BC = bagged waste at 7 °C & LC = loose waste at 7 °C. Values represent the geometric mean of six replicates 95% confidence interval estimated from 5000 bootstrap samples. The dashed line represents the detection limit.

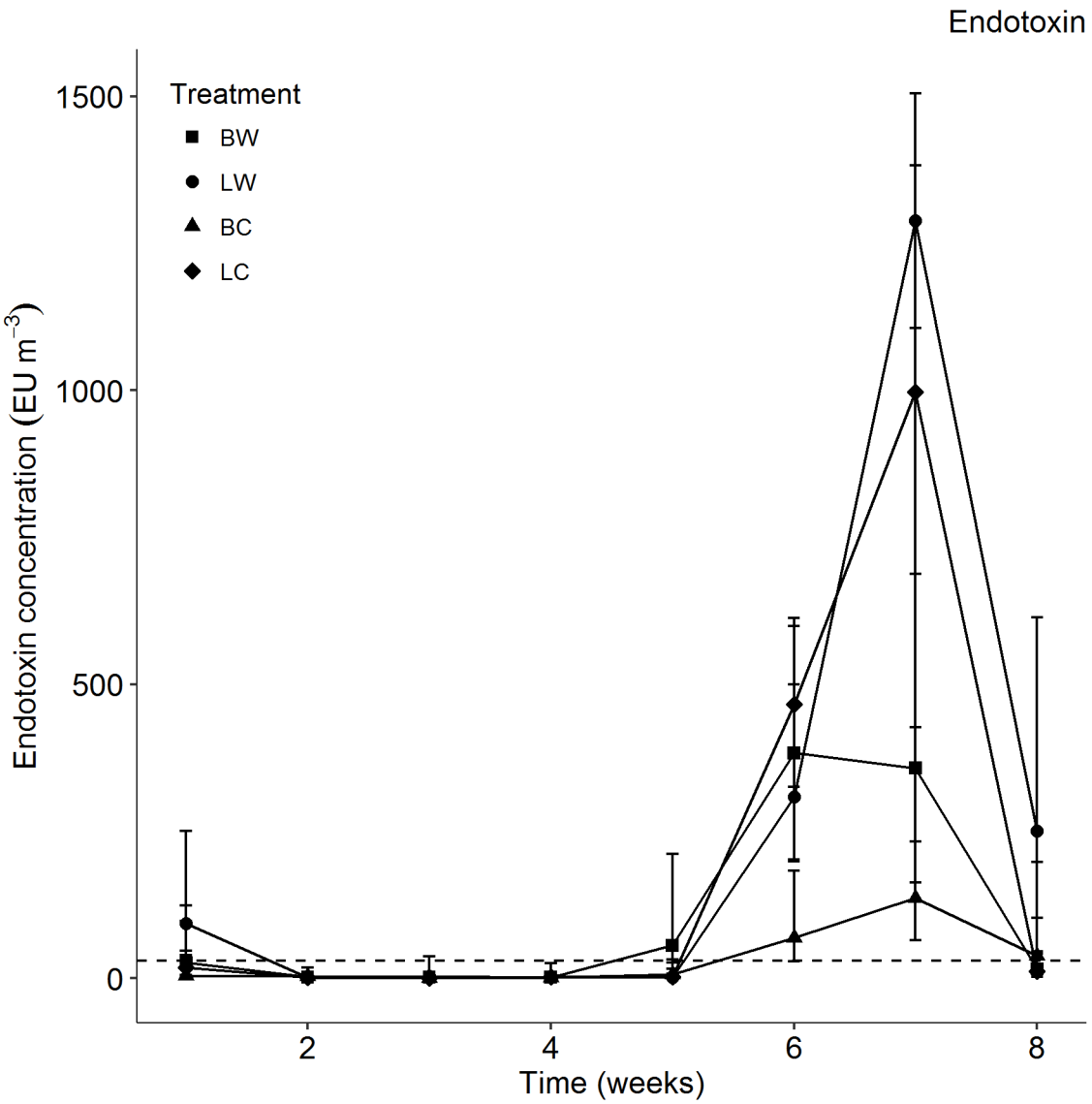
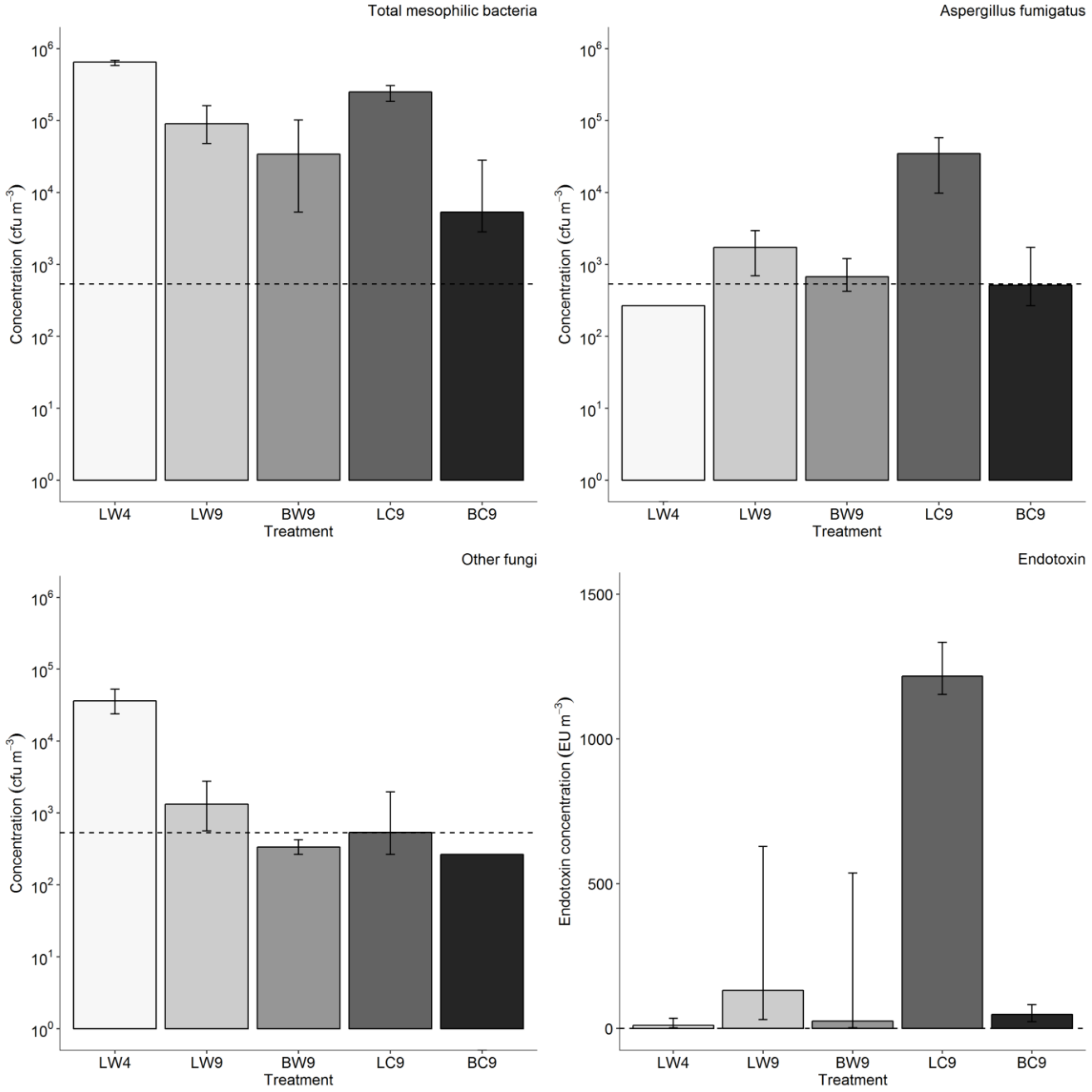


Figure 3: Endotoxin concentrations from the bioaerosols emitted from each bin treatment during the 'simulated waste disposal' scenario. BW= bagged waste at 23°C, LW = loose waste at 23°C, BC = bagged waste at 7 °C & LC = loose waste at 7 °C. Values represent the geometric mean of six replicates 95% confidence interval estimated from 5000 bootstrap samples. The dashed line represents proposed exposure limits for endotoxin proposed by the Health Council of the Netherlands (2012).


Total mesophilic bacteria
Aspergillus fumigatus
Other fungi
Endotoxin
Concentration (cfu m^{-3})
Endotoxin concentration (EU m^{-3})
10^0
10^1
10^2
10^3
10^4
10^5
10^6
0
500
1000
1500
LW4
LW9
BW9
LC9
BC9
Treatment

290

291 **Figure 4:** The concentrations of microorganisms and endotoxin emitted as bioaerosols during the ‘tipping’ scenario. LW4 = loose waste at 23

292 °C, week 4; LW9 = loose waste at 23 °C, week 9; BW9 = bagged waste at 23 °C, week 9; LC9 = loose waste at 7 °C, week 9; BC9 = bagged

293 waste at 7 °C, week 9. Values represent the geometric mean of six replicates 95% confidence interval estimated from 5000 bootstrap samples.

294 The dashed line represents the detection limit.

295 *3.2.2. Tipping*

296 At week four, LW4 bins were tipped to assess potential bioaerosol emissions for waste
297 collection operators emptying bins (Figure 4). In this scenario, the highest mesophilic
298 bacteria and other fungi counts were found in LW4 (6.51 x10^5 cfu m^{-3} and 3.64 x10^4 cfu m^{-3}
299 respectively). Whereas, the highest *A. fumigatus* counts were recorded in LC9 (3.47 x10^4 cfu
300 m^{-3}). As data only exists for week 4 in LW bins, statistical comparisons were only made for
301 the treatments LW4 and LW9. According to Mann Whitney comparisons, mesophilic bacteria
302 and other fungi counts were significantly higher in LW4 than LW9 ($P < 0.01$ for both) whilst
303 the opposite held true for *A. fumigatus* ($P < 0.01$).

304 Endotoxin emissions during tipping are illustrated in Figure 4. Mean endotoxin
305 emissions of 11 EU m^{-3} at week four were recorded for warm loose tipped material (LW4).
306 By week nine, levels had increased 12-fold to 132 EU m^{-3} (LW9). Higher levels of 537 EU m^{-3}
307 and 1217 EU m^{-3} were recorded in BW9 and LC9 material respectively. At week 9, the
308 lowest endotoxin levels were recorded in BC9 (48 EU m^{-3}). No significant difference was
309 observed between endotoxin concentrations in loose, warm waste at week 4 and 9 (LW4 &
310 LW9; $P > 0.05$). The sometimes large variability among replicate bins is probably the reason
311 for the lack of statistical significance. The highest (1→3)-β-D-glucan concentration was
312 recorded in LW4 (10 ng m^{-3}) which equalled the proposed 10 ng m^{-3} exposure limit
313 (Rylander, 1997). All other treatments were recorded at 6 ng m^{-3} or lower (data not shown).
314 In this instance, (1→3)-β-D-glucan concentration was significantly higher in the LW4
315 treatment than the LW9 ($P < 0.05$). This suggests that emissions could be slightly different
316 between week 4 and week 9 with (1→3)-β-D-glucan and fungi being more of an issue at
317 week 4, and endotoxin may be more of an issue at week 9. This could have important
318 implications for a missed bin collection that might be left for longer than four weeks.
319

4. Discussion

The process of breakdown of waste material begins when the material is deposited within a container ready for collection. The behaviour of organic and biodegradable materials within residual waste in storage containers are of particular interest when considering prolonged storage intervals. During this study, in the period of the trial when a fresh residual waste mix was regularly added simulating an extended waste collection, (weeks 1-4), the bins were dominated by bacterial growth from mesophilic bacteria. Although concentrations regularly exceeded the Environment Agency's precautionary 1000 cfu m^{-3} guideline in the bins, these measured concentrations do not necessarily equate to exposure and it should be noted all bioaerosols were sealed within the bin straight after waste deposition and concentrated to create a 'worst case scenario'. Searl & Crawford (2012) reported that adverse health effects in waste workers was linked to concentration exceeding 10^5 cfu m^{-3} of total viable bacteria. These higher concentrations were not reached in the bins in this part of the study, but were exceeded during 'tipping' which was intended to mimic an occupational activity linked to bin emptying. For the subsequent 'missed collection' (weeks 5-8), bacterial levels in the bins stored at 7°C (BC & LC) became undetectable. However, bacteria in the warmer bins (BW & LW) took slightly longer to reduce to below the detection limit of the method. This domination by bacteria over fungi in the bins is seen in similar studies such as Choi et al., (1998) whom on investigating food waste and found early heavy colonisation by yeasts, with subsequent growth of thermophilic bacteria after 2 days, and also reported that the activity of fungi was not significant (related to 'in container' growth). Ryckeboer et al., (2003) also reported that bins of organic waste are first colonised by bacteria and Mayrhofer et al., (2006), using organic material within a small bin, also found that bacteria were one to three orders of magnitude higher than fungal colonies. Hence it is

344 concluded that bacteria are the main initial colonisers of residual material and in longer term
345 storage beyond one week.

346 It is of interest that Gram-negative bacteria were not detected on the selective media,
347 which contained mainly *Streptomyces* or *Staphylococcus* spp. Previous studies in containers,
348 such as Ryckeboer *et al* (2003), did identify gram-negative bacteria, but most collection
349 studies have concentrated on measuring endotoxin (Nielsen et al., (2000), Lavoie et al.,
350 (2002), Neumann et al., (2002) and Wouters et al., (2002)). Since endotoxin was detected in
351 significant concentrations it is concluded that gram-negative bacteria were present, but were
352 not culturable by this method. The possibilities are that they did not survive the sampling
353 procedure or were outcompeted on the selective media by other species. One of the outcomes
354 of this study is that measurement of gram-negative bacteria are recommended to be removed
355 from the UK Protocol going forward as selective media are not reliable for reporting these
356 microorganisms.

357 In terms of concentrations of endotoxin this increased and peaked during the 'missed
358 collection' period at week 7, most likely because Gram-negative bacteria cells died after
359 growth on material already added to the bins. Indeed, a negative correlation was recorded for
360 endotoxin concentration and total mesophilic bacteria in both the treatments containing loose
361 material ($R = -0.64$, $P < 0.001$ and $R = -0.56$ & $P < 0.001$ for LW & LC respectively). It is
362 possible that lower oxygen levels, in addition to lack of fresh waste input, contributed to
363 aerobic microorganisms dying out, thus releasing endotoxin. Endotoxin has previously been
364 measured in waste containers, for example in the 'percolate' liquid from containers, (Nielsen
365 et al., 1998) which found no difference between one to two weeks of storage and none in the
366 bioaerosol, this is perhaps not surprising as the differences in this study were only apparent
367 after 4-6 weeks peaking at week 7. Endotoxin as a bioaerosol has more often been measured
368 in waste collection. Nielsen et al., (2000), Lavoie et al., (2002), Neumann et al., (2002) and

Wouters et al., (2002) all measured endotoxin in waste collections (of organic and mixed waste) and found concentrations ranging between 16-100 EU m^{-3}, and hence some were above and others were below the 90 EU m^{-3} suggested as an occupational exposure limit, (Health Council of the Netherlands, 2012). However, these collections were either weekly or fortnightly. There is a lack of studies on collectors who collect residual waste after a longer storage interval, which would be interesting after the results of this study.

It is also interesting to note that internal bin temperature did not vary much in either the warm (23 °C) or cold bins (7 °C), unlike Ryckeboer et al., (2003) who saw distinct heating and cooling phases in containers of organic waste during 30 days of storage. It is possible the organic content was not sufficient to generate heating within residual wastes, or that in this study the regulation of the ambient storage temperature prevented such variations. As a result conditions which might have enabled certain species of thermophilic fungi to thrive and take over from bacteria which has been seen in studies such as that by Ryckeboer et al., (2003) did not occur in this study and concentrations of fungi were not as prevalent as bacteria. Temperature was measured in this study as previous research has shown higher concentrations of bioaerosols in waste collection during summer conditions (Nielsen et al., 2000, Lavoie et al., 2002). However, no strong statistical associations were seen with temperature in this study.

This study favoured the growth of *Aspergillus fumigatus* due to the use of the AfOR Protocol (2009) and hence the growth of fungi at 40°C. *A. fumigatus* causes invasive aspergillosis in immunocompromised people (Latge, 1999, O'Gorman, 2011) and is often found in high numbers in bioaerosols from composting sites (Gutarowska et al., 2015) and during organic or food waste collection (Nielsen et al., 1997, Nielsen et al., 2000). Indeed Poole & Wong (2013) recommend that garden waste collectors are screened for asthma and *Aspergillus* sensitivity, cystic fibrosis, bronchiectasis and immunodeficiency if *Aspergillus*

spp. cannot be controlled sufficiently. However, in this study *A. fumigatus* appeared only sporadically in significant numbers (above the limit of detection) in weeks three and four in BW & LW, and in LC material. In a review of the literature, Kuijer et al. (2010) reported general fungal concentrations emitted from waste collections in the range of 5.9 $x10^3$ and 6.3 $x10^4$ cfu m^{-3}. Combined *A. fumigatus* and other thermotolerant fungi counts were consistently lower than these figures within the bins. Eduard et al. (2012) proposed a guideline limit of 1.0 $x10^5$ cfu m^{-3} for spores, concentrations in this study were below this but it should be noted that the growth temperature in this study may not reflect the full range of fungi that may have grown at lower temperatures. It should also be noted that existing garden waste collections have shown to have higher concentrations of total fungi, and *A. fumigatus* in particular, (Nielsen et al., 1997) than those found in this study.

With regards to the reference guidelines on *A. fumigatus* suggested by the Environment Agency to protect the public downwind of composting facilities, counts in this study exceeded the reference guideline of 500 cfu m^{-3} on only one occasion each for LC, BC and BW (week 3, 2.79 $x10^4$ cfu m^{-3}; week 3, 1.63 $x10^3$ cfu m^{-3}; and week 4, 6.10 $x10^2$ cfu m^{-3} respectively), and twice for LW (week 3, 5.33 $x10^3$ cfu m^{-3} & week 4, 1.14 $x10^3$ cfu m^{-3}); and therefore shows that *A. fumigatus* counts were consistently below this within individual bins. It is possible that fungi, including *A. fumigatus*, were not becoming airborne in undisturbed waste due to the damp atmosphere within the bins. It is also possible however, that *A. fumigatus* is not the primary fungi of concern in material with mixed organic and residual waste, particularly if most households keep their green waste separate, or this may be a limitation of the growth temperature required by the AfOR Protocol (2009). Residual waste may have a differing emission profile from purely organic materials such as food and garden waste. Therefore, future studies should consider identifying a wider range of fungi to determine the community structure within the bins and the emissions emanating from them.

In terms of limitations to the study methodology it is important to note that the concentrations measured in this study were monitored directly from sealed bins. These concentrations should therefore be considered a 'worst-case scenario' from the mix generated in the laboratory but should also not be directly related to exposure, hence the results are considered illustrative. It should also be noted that temporary exposure from opening a non-sealed bin to throw out waste will not necessarily lead to the same level of exposure. It is noted that there is a lack of exposure-response data to determine the impact of different exposure times. However, based on available information, the concentrations of bacteria and fungi measured should not present an issue to the average healthy adult. Opening a lid outside could also lead to faster dispersal and dissipation of waste emissions, compared to the controlled conditions of the laboratory.

In summary, the primary issue for householders with extended four-weekly collections appears to be elevated airborne bacteria up to the four-week point, and elevated endotoxin thereafter should the extended collection be missed. This reinforces the importance of managing missed collections, such as those beyond four weeks. However, there was no significant difference in concentrations of mesophilic bacteria, *A. fumigatus*, other fungi, endotoxin and (1→3)-β-D-glucan ($P > 0.05$ for all) in bins associated with fortnightly collections (weeks 2) and the extended four-weekly collection (week 4). The only two exceptions being a significantly higher concentration of *A. fumigatus* in LW at week 4 than week 2 ($P < 0.05$), and a significantly higher concentration of (1→3)-β-D-glucan in BW in week 2 than week 4 ($P < 0.05$). It is also important to note this study only examined bioaerosols, and handling of waste materials may lead to hand-to-mouth transmission, but this was not within the scope of this study and should be explored further in subsequent work.

For waste collectors, the movement and disturbance of waste material presents the greatest issues and concerns. 'Tipping' tests were designed to determine whether disturbance

may cause waste components to become airborne. Based on the concentrations measured, the study suggests there may be some risks associated with the potential inhalation of endotoxin during the 'disturbance' of bins that have been stored for up to eight weeks, i.e. a missed extended collections, with concentrations at week 4 peaking at 35 EU m^{-3} and at week 9 at 533 EU m^{-3}. The potential risk of exposure to bioaerosols is an issue that waste collectors should have included in COSHH (Control of Substances Hazardous to Health) risk assessments that should be undertaken prior to collection of such wastes which should specifically mention the issues associated with bioaerosols and mitigation measures, e.g. handling measures and any protective equipment.

5. Conclusions

When introducing an extended collection programme, local authorities should provide advice for householders that will help to minimise exposure to emissions; for example, to start bagging waste in warmer conditions. Missed collections on an extended cycle should also be minimised as much as possible; the importance of frequency is demonstrated by endotoxin concentrations which increase after four weeks. As a range of microorganisms were found aerosolised from enclosed waste containers, it is recommended householders should also receive advice regarding hand-to-mouth transmission.

COSHH risk assessments need to be carried out for collectors of residual waste materials on extended collection cycles, for microorganisms and particularly endotoxin which has a known dose-response relationship. Because of the presence of endotoxin in significant concentrations within the waste and hence potential for aerosolisation during processing at a facility, it is also advised that waste operatives, including collectors and processing staff, undergo evidence-based worker health surveillance for early signs of occupational illness.

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