

Morphological Classification of Bioaerosols from Composting using Scanning Electron Microscopy

A. Tamer Vestlund^{a,b}, R. Al-Ashaab^a, S.F. Tyrrel^a, P.J. Longhurst^a, S.J.T. Pollard^a,
G.H. Drew^{a,1}

^a*Centre for Energy and Resource Technology, Environmental Science and
Technology Department, School of Applied Sciences, Building 40, Cranfield
University, Bedfordshire MK43 0AL, UK*

^b*FIRA International Ltd. Maxwell Road, Stevenage, Herts., SG1 2EW, UK*

Abstract:

This research classifies the physical morphology (form and structure) of bioaerosols emitted from open windrow composting. Aggregation state, shape and size of the particles captured are reported alongside the implications for bioaerosol dispersal after release. Bioaerosol sampling took place at a composting facility using personal air filter samplers. Samples were analysed using scanning electron microscopy. Particles were released mainly as small ($< 1 \mu\text{m}$) single, spherical cells, followed by larger ($> 1 \mu\text{m}$) single cells, with aggregates occurring in smaller proportions. Most aggregates consisted of clusters of 2-3 particles as opposed to chains, and were $< 10 \mu\text{m}$ in size. No cells were attached to soil debris or wood particles. These small single cells or small aggregates are more likely to disperse further downwind from source, and cell viability may be reduced due to increased exposure to environmental factors.

Keywords: Bioaerosols, dispersion, SEM, particle size, aggregation

¹ Corresponding author: Gillian Drew; g.h.drew@cranfield.ac.uk; Tel: + 44 (0)1234 750111; Fax: +44(0)1234 75167

25

26 **1. Introduction**

27 Bioaerosols are airborne particles of biological origin (Cox and Wathes, 1995),
28 ranging from 0.02 to 100 µm in size (Dowd and Maier, 2000; Ariya and Amyot,
29 2004), including living microorganisms such as bacteria, fungi, yeasts and
30 protozoans, or fragments and constituents of microorganisms (ADAS/SWICEB,
31 2005). Bioaerosols are released as a consequence of compost agitation activities
32 (shredding, turning and screening), but do also occur naturally in the environment
33 and exposure to bioaerosols is not limited to composting facilities (Dutkiewicz, 1997;
34 Lacey, 1997; Nielsen et al., 1997; Reponen et al., 1998; Sánchez-Monedero and
35 Stentiford, 2003; Seedorf et al., 1998; Swan et al., 2003). Under prolonged or acute
36 exposure conditions, bioaerosols have the potential to pose health risks to immune-
37 compromised or vulnerable humans, particularly where high concentrations are
38 emitted close to residences, schools, hospitals and other public facilities
39 (Environment Agency, 2007).

40

41 The physical and morphological characteristics of bioaerosols are central to
42 understanding emissions and downwind dispersal from composting facilities. The
43 behaviour of bioaerosols after release is governed by physical factors, including
44 gravitational forces and Brownian motion, as well as environmental factors, such as
45 wind speed, relative humidity and temperature (Pillai and Ricke, 2002). Bioaerosol
46 properties such as size, shape, aspect ratio, surface characteristics and their affinity
47 for aggregation, also affect their behaviour and are important factors in predicting
48 their dispersal (Levetin, 1995; Madelin and Johnson, 1992; McCartney, 1994;
49 McCartney et al., 1997). For example, a larger particle or aggregate might be subject

to higher deposition velocities than a smaller one (Wheeler et al., 2001; Swan et al., 2003), with implications for the distance and time particles remain airborne (Pillai and Ricke, 2002).

Research examining bioaerosol size distribution and aggregation from composting emissions is limited. Kanaani et al. (2008) found that deposition rates for bioaerosols and non-biological particles were a function of particle size, not the nature of the particle. Byeon et al. (2008) found that aerodynamic diameters of microorganisms were larger than expected and attributed this to the possibility that they were suspended as aggregates with other bioaerosols and/or with dust particles. Feng et al. (2011) claim that size and shape of bioaerosols can be clarified in real-time environmental monitoring by means of analysing the special distribution of scattered light, although their research is still requires further development.

Our research attempts to improve understanding of bioaerosol transport from source to sensitive receptor. In an attempt to improve characterisation of aggregation and size distribution of compost bioaerosols, experiments were undertaken to:

- a) determine the size distribution of particulates released from compost and composting facilities, and
- b) examine and characterise the nature of bioaerosol aggregates released from compost and composting facilities.

Images of microorganisms and their aggregates have been published before using Scanning Electron Microscopy (SEM) from either pure cultures or from substrates other than composts (Heikkilä et al., 1988; Klich, 2002; Kormendy and Wayman,

1972; Karlsson and Malmberg, 1989; Prescott et al., 1999a, b; Wittmaack et al., 2005). SEM has also been previously used as a technique for characterising morphological properties of small particles and bioaerosols (Friedbacher and Grasserbauer, 1995; Hiranuma *et al.*, 2008; Pasanen *et al.*, 1989; Skujins *et al.*, 1971; Williams, 1970). SEM was therefore chosen as the method to study bioaerosols emitted from compost.

2. Materials and Methods

Bioaerosols were initially sampled under controlled experimental conditions, with samples being analysed using SEM and through traditional culture techniques. These results confirmed the suitability of SEM as an analysis method and the presence of bioaerosols typically sampled from composting facilities, notably *Aspergillus fumigatus* (for further details see Tamer Vestlund, 2009).

2.1. Site sampling techniques

Samples were collected from a composting facility from a windrow (static source) using a wind tunnel and from agitation activities as described below (Jiang and Kaye, 2001; Taha et al., 2005). Particles were sampled in triplicate at a height of 1.8 m for a period of 30 minutes at ten locations around the windrows and screening area (one upwind; three at 10, 50 and 100 m downwind of the windrows respectively; two by the screening area, and two at source). Calibrated (with an SKC Ltd. rotameter) personal SKC (Universal dust and vapour) air filter samplers were connected to IOM sampling heads by a 10 mm internal diameter Tygon tube (Taha et al., 2006; 2007). Particles were collected on polycarbonate filters (SKC Ltd.) with 0.8 µm pore size and 25 mm diameter. Air was drawn through the sampling heads at a flow rate of 2 ±

0.2 L min⁻¹ (SKC, 2002). After sampling the cassettes and filters were placed in a sterile 30 mL Nalgene vial (121 °C, 15 min) and stored in an ice-box at 4 °C for transport. The filters had an effective exposed diameter of 15 mm.

2.2. Scanning electron microscopy protocol

The filters were mounted onto a 25.3 mm (diameter) SEM stub prior to gold coating within 24 hours of sample collection (Polaron Equipment Ltd., SEM gold coating unit ES100). The coated filters were examined with a high-resolution SEM (XL30SFEG, Phillips; 10-12 kV beam size, 3-4 spot size) according to standard SEM practices. Nine pairs of coordinates were selected for analysis (Figure 1) using a systematic sampling design. Initial focus of the microscope was on the upper right edge (x=6000, y=6000) of the filter at a magnification of x30 and then increased to x2000 when particles of interest (0.5 - 10 µm in size) were found. New viewing fields were selected at each of the nine pairs of coordinates until ten fields containing at least one particle were found for each pair of coordinates with 20 viewing fields around the central set of coordinates to account for 100 viewing fields in total (Heikkilä et al., 1988). The magnification was adjusted to ensure the visual properties of the particles were sufficiently clear to analyse and record their number, size, shape, type of particle, and aggregation status. Blank viewing fields, defined as fields with no particles of interest, were also considered and recorded to calculate the total area examined per filter. Blank viewing fields were scanned at magnifications of x500, x1000 and x2000.

Figure 1 here

2.3. Statistical analysis

Description of the data was performed by arithmetic mean values and standard error to measure variability, and a correlation analysis where required. One-factor ANOVA and, where applicable, Fisher tests were used to analyse the differences between independent data groups, using STATISTICA 8 (StatSoft Ltd.).

3. Results and Discussion

All SEM results shown correspond to the total area of 100 viewing fields (0.252 mm²) plus blanks scanned per filter as explained above. Filters (total area 490.8 mm² each) with low numbers of particles had a larger area analysed than those heavily populated, resulting in an average of 0.19% of the filter being analysed.

3.1. Particle size distribution and characterisation

In this study, particles observed were classified as small (0.5 - 1 µm) and large cells (2 - 3 µm). These were further classified into 8 different small cells and two major large cell types according to their physical appearance (Table 1). Particles such as filamentous and pollen-like particles (>10 µm), or those with no structure, were considered to result from structural defects of the filters according to additional analyses of filters that were not exposed to composting emissions (Tamer Vestlund, 2009).

Table 1 here

A wide variety of microorganisms is present in and released from compost. Michel et al. (2002) identified over 94 species of microorganisms in green waste compost.

Similarly, Epstein (1997) listed 16 species of bacteria, 16 of actinomycetes and 35 species of fungi derived from compost. Although the presence of the bioaerosols typically associated with composting (e.g. *Aspergillus fumigatus*) was confirmed by culture (see Tamer Vestlund, 2009), difficulties in identifying particular species could arise as sample preparation for SEM analysis results in the dehydration of the sample that causes collapse and distortion of the image (Heikkilä et al., 1988). Therefore, this research focused on the observable properties of bioaerosols (size, shape and aggregation status), irrespective of the bioaerosol species.

Figure 2 shows the dominant cell types according to sampling position at the composting facility. Cell type A was the most commonly occurring at all distances, with types B and D also found in the samples taken at source. Cell type G was also found in high proportions at 100m downwind of the composting facility. The overall tendency was for small cells to occur in higher frequencies than the large cells in all experiments, with the majority of particles present in the 100 viewing fields examined from compost samples are in the 0.5-1 μm size range. The dominance of smaller particles reflects previous research from compost facilities using Andersen 6 stage samples. Reinthaler et al. (1997) found that 56-73% of all particles were smaller than 3.4 μm . Kamilaki and Stentiford (2001) found that 80% of all the *A. fumigatus* captured on stages 3, 4 and 5 of an Andersen sampler were in the size range of 1.1 to 3.3 μm . Byeon et al. (2008) examined bioaerosols in a municipal composting facility and reported concentrations of 10^8 CFU/m³ total airborne particles sized 0.3 μm , which drastically decreased as the particle diameter increased. While not directly comparable, these studies provide the only other published indications of the size range of bioaerosols emitted from composting.

Figure 2 here

3.2. Aggregate size distribution and characterisation

Airborne microorganisms have been found in aggregates consisting of 2-6 spores in various environments (Bell et al., 2000; Karlsson and Malmberg, 1989; Lacey, 1991; Lacey and Dutkiewicz, 1976a, b; Levetin, 1995; Madelin and Johnson, 1992; Trunov et al., 2001). However, Figure 3 demonstrates that in all cases, single cells dominated over aggregates. The majority of cells observed for all sampling locations were small cells (66-99%); while their aggregates accounted for 1.4-30%. The proportion of single large cells and their aggregates are 1.3-6 % and 0.7-1.4 %, respectively. In addition, no aggregate structures were observed at 100 m downwind from the compost source, suggesting that aggregates drop out from the pollutant plume. Although, with a sampling height of 1.8 m, there is the possibility that the full pollutant plume was not sampled and aggregates may have disintegrated during the sampling process.

Bioaerosol survival rates within aggregates exceed that of single cells due to the protective effect of the outer layer for the inner cells (Carrera et al., 2005; Duncan and Ho, 2008; Lighthart and Schaffer, 1994; Marthi et al., 1990; Thomas et al., 2008; Tong and Lighthart, 1997). As most of the particles studied here consisted of single cells, it is conceivable that even if the particles were dispersed further downwind due to their small size, they will be less protected from environmental factors, and therefore cell viability could be reduced. This suggests that traditional culture

techniques often used for sampling downwind of composting facilities may underestimate the actual concentration of particles in the plume.

Bioaerosols have various release mechanisms. Filamentous structures or mycelia that extend above the growth substrate can become airborne as short chains, single spores or as fractions of mycelium (Gregory, 1973; Jankowska et al., 2000; Kanaani et al. 2008; Lacey, 1997; Madelin and Madelin, 1995; Pillai and Ricke, 2002). These can disintegrate into smaller sections and single spores, either due to release mechanisms or during sampling (Madelin and Johnson, 1992; Trunov et al., 2001). Single particles could also aggregate once airborne to make larger units (Calleja, 1984).

Based on the results, aggregates of cells were classified into clusters and chain-like structures, depending on either width or length. The vast majority of aggregates were clusters indicating that either a larger proportion of non-filamentous microorganism aggregates become airborne, or that cells are clustering into aggregates subsequent to release (Figure 4). Furthermore, small aggregates dominated over large ones regardless of their shape. Approximately 50% of the small aggregates had a diameter of $< 2 \mu\text{m}$ in size, equating to aggregates of 2-3 cells based on the assumption that single cells ranged from 0.5 to $1 \mu\text{m}$. Agitation produced more aggregates than static windrows ($p=0.005$; Figure 4). Aggregates of three or more cells were more abundant in samples from the source than in any downwind sample (Figure 4). No aggregates were identified in upwind samples, suggesting that the composting activities may have an impact on the formation of aggregates. It is also

possible that the sampling technique has impacted on the number and formation of aggregates.

Several studies suggest that particles can be released as single cells, aggregates and as cells attached to other particles such as dust or wood fibres (Swan et al., 2003; ADAS/SWICEB 2005; Wittmaack et al., 2005). The results here do not suggest that the release of bioaerosols is dependent on the release of matter such as dust or wood fibres. However, only a small portion of each filter (maximum of 1.1%) was examined. There is therefore the possibility that these particles could have existed in areas that were not examined or that the filters did not effectively sample or retain wood fibres.

3.3. Particle morphology

The majority of the particles, both single and aggregated cells, were spherical in nature with an aspect ratio of 1 (Figure 5). Gregory (1973) showed that the falling rate of a particle due to gravitational forces is proportional to the square of its radius. Furthermore, non-spherically shaped particles might fall more slowly due to an increased surface drag that would result in a delay in deposition (Lacey, 1991; McCartney, 1994; Levetin, 1995). Therefore, as the majority of particles observed in this study were spherical or almost spherical (aspect ratio 1 to 1.5), the effects of surface drag on bioaerosols is proposed to be minimal.

Figure 5 here

3.4. Limitations of methodology

SEM is able to provide accurate and detailed information on particle surface and physical particle size; however the samples are prepared and scanned under vacuum conditions, which causes dehydration, collapse and distortion of particles that might bias the actual size and surface characteristics of the particle (Heywood, 1969; Skujiņš *et al.*, 1971; Gwaze *et al.*, 2007). Furthermore, due to the fact that only a very small percentage of the overall filter was analysed, the results here are only a representation rather than absolute values of the overall bioaerosol concentrations. The classification of the shape and nature of particles of interest was based on subjective assessment. Similar limitations have been reported due to the tendency of the operator to focus on more interesting particle features (Gwaze *et al.*, 2007; Shekunov *et al.*, 2007)

3.5. Implications

Bioaerosol dispersion modelling could be an invaluable tool to estimate downwind concentrations, particularly for regulatory compliance and in the design of control strategies. Knowledge on the physical attributes of bioaerosols is thus crucial to provide confidence in model outputs for composting facilities. A key decision for modellers is whether to model as a particle or as a gas (Drew *et al.*, 2007). However, there is currently insufficient information available to fully define the particle properties within dispersion models. The results here suggest that modelling as a gas would suffice, as the majority of particles found were small enough for this to be a suitable option.

Studies on the health impact of airborne pollutants have shown that smaller particles (<2.5 µm) are more likely to negatively affect sensitive receptors as they can penetrate deeper into the lungs (Dockery *et al.*, 1993; Levy *et al.*, 2000; Schwartz *et al.*, 1996; Spengler and Wilson, 1996; Sturm, 2011). Thomas *et al.* (2008) argued

that a lower dose of aggregate particulates is required to initiate an adverse health impact compared to non-aggregate particles, because aggregates contain higher number of individual cells. This has important implications in determining a dose-response relationship for bioaerosols.

4. Conclusions

To the authors' knowledge, this is the first study that has classified bioaerosols emitted from compost according to shape and size. The results suggest the following conclusions regarding bioaerosols from composting sites:

- The majority of bioaerosols released in this study were single cells, shaped spherically or almost spherically, suggesting that they may disperse further than heavier aggregate structures.
- Eight types of small (0.5-1 μm) cells and 2 types of large (1-2 μm) cells and their aggregates were released from both static (i.e. compost windrow) and active (i.e. agitation) compost sources.
- The majority of all aggregates consisted of 2-3 cells and were smaller than 10 μm . Again, these are more likely to disperse further downwind, but would not benefit from the protection that larger aggregates would provide from environmental factors.
- Aggregate structures were primarily released in clusters as opposed to chains.
- There were no aggregate structures observed at 100 m downwind from compost source, or upwind, suggesting that composting facilities impact on the formation of aggregates.

Acknowledgements:

298 This work has been jointly funded by the EPSRC, the Scottish Environment
299 Protection Agency (SEPA) and the Scotland and Northern Ireland Forum for
300 Environmental Research (Sniffer). Reyna Al-Ashaab was a Daphne Jackson Trust
301 Fellow supported by NERC. The authors thank the composting site for access to the
302 facility.

303

304 **References:**

305 ADAS/SWICEB (2005) Bioaerosol Monitoring and Dispersion from Composting
306 Sites. SWICEB Report, August 2005.

307

308 AfOR (2008) The State of Composting and Biological Waste Treatment in the UK
309 2006/07. Association for Organics Recycling, Northamptonshire, Wellingborough.

310

311 Ariya, P.A. & Amyot, M. (2004) New Directions: The Role of Bioaerosols in
312 Atmospheric Chemistry and Physics. *Atmospheric Environment*, 38, 1231-1232.

313

314 Bell, E.C., Hale, T.L., Shaw, M.J. & Black, R.S. (2000) Evaluation of a novel
315 bioaerosol generation device for the use in studies requiring airborne microorganism
316 aggregates. *Journal of Aerosol Science*, 31 (Suppl.1), S80-S81.

317

318 Byeon, J.H., Park, C.W., Yoon, K.Y., Park, J.H. & Hwang, J. (2008) Size distributions
319 of total airborne particles and bioaerosols in a municipal composting facility.
320 *Bioresource Technology*, 99, 5150-5154.

321

322 Calleja, G.B. (1984) *Microbial Aggregation*, CRC Press, Boca Raton, FL., U.S.A.

323

324 Carrera, M., Kesavan, J., Zandomeni, R.O. & Sagripanti, J.L. (2005) Method to
325 determine the number of bacterial spores within aerosol particles. *Aerosol Science*,
326 39, 960-965.

327

328 Composting Association (2007) The state of composting and biological waste
329 treatment in the UK 2005/06. Association for Organics Recycling, Northamptonshire,
330 Wellingborough.

331

332 Cox, C.S., & Wathes, C.M. (1995) Bioaerosols in the Environment. In: C.S. Cox &
333 C.M. Wathes (Eds.) *Bioaerosols Handbook*, CRC Press, Boca Raton, U.S.A.

334

335 Dockery, D.W., Pope III, C.A., Xu, X., Spengler, J.D., Ware, J.H., Fay, M.E., Ferris,
336 B.G. & Speizer, F.A. (1993) An association between air pollution and mortality in six
337 U.S. cities. *New England Journal of Medicine*, 329, 1753-1759.

338

339 Dowd, S.E. & Maier, R.M. (2000) Aeromicrobiology. In: R.M. Maier, I.L. Pepper &
340 C.P. Gerba (Eds.) *Environmental Microbiology* (pp. 91-122). Academic Press,
341 Canada.

342

343 Duncan, S. & Ho., J. (2008) Estimation of viable spores in *Bacillus atrophaeus*
344 particles of 1 to 9 µm size range. *Clean*, 36(7), 584-592.

345

346 Dutkiewicz, J. (1997) Bacteria and fungi in organic dust as potential health hazard.
347 *Annals of Agricultural and Environmental Medicine*, 4, 11-16.

348

349 Eduard, W. & Aalen, O. (1988) The effect of aggregation on the counting precision of
350 mould spores on filters. *Annals of Occupational Hygiene*, 32, 471-479.

351

352 Environment Agency (2007) Position on Composting and Health Effects, Bristol.

353 From: http://intranet.ea.gov/ams_document_library/icontent/DocDir07/compo2.doc.

354

355 Epstein, E. (1997) Microbiology. In: E. Epstein (Ed.) *The Science of Composting*, pp.
356 53-74. Technomic Publishing Company, U.S.A.

357

358 Feng, (2011) Theoretical studies on bioaerosol particle size and shape measurement
359 from spatial scattering profiles. *Chinese Optics Letters*, 9 (9), 092901 (pp1 - 4).

360

361 Friedbacher, G. & Grasserbauer, M. (1995) Investigation of environmental aerosol by
362 atomic force microscopy. *Analytical Chemistry*, 67, 1749-1754.

363

364 Gregory, P.H. (1973) *The Microbiology of the Atmosphere*. Edited by P.H. Gregory,
365 2nd Edition, Leonard Hill, UK.

366

367 Gwaze, P., Annegarn, H.J., Huth, J. & Helas, G. (2007) Comparison of particles
368 sizes determined with impactor, AFM and SEM. *Atmospheric Research*, 86, 93-104.

369

370 Heikkilä, P., Kotimaa, M., Tuomi, T., Salmi, T. & Louhelainen, K. (1988) Identification
371 and counting of fungal spores by scanning electron microscope. *Annals of*
372 *Occupational Hygiene*, 32(2), 241- 248.

373

374 Heywood, V.H. (1969) Scanning electron microscopy in the study of plant materials.
375 Micron, 1, 1-14.

376

377 Hiranuma, N., Brooks, S.D., Auvermann, B.W. & Littleton, R. (2008) Using
378 environmental scanning electron microscopy to determine the hygroscopic properties
379 of agricultural aerosols. Atmospheric Environment, 42, 1983-1994.

380

381 Jankowska, A., Reponen, T., Willeke, K., Grinshpun, S.A. & Choi, K-J. (2000)
382 Collection of fungal spores on air filters and spore re-entrainment from filters into air.
383 Journal of Aerosol Science, 31(8), 969-978.

384

385 Jiang, J. & Kaye, R. (2001) Sampling techniques for odour measurement. In: R.
386 Stuetz & F-B. Frechen (Eds.) Odours in Wastewater Treatment Measurement,
387 Modelling and Control, pp. 95-119. IWA Publishing. UK,

388

389 Kamilaki, A. & Stentiford, E. (2001) Assessing compost stability and determining
390 levels of *Aspergillus fumigatus* resulting from different composting operations.

391 Project No. 0651, The University of Leeds, Leeds from:

392 <http://www.mondegreen.org.uk/documents/CompostStabilityandAsperilligusFumigatu>
393 [s.pdf](http://www.mondegreen.org.uk/documents/CompostStabilityandAsperilligusFumigatus.pdf)).

394

395 Kanaani, H., Hargreaves, M., Ristovski, Z. & Morawska, L. (2008) Deposition rate of
396 fungal spores in indoor environments, factors effecting them and comparison with
397 non-biological aerosols. Atmospheric Environment, 42, 7141-7154.

398

399 Karlsson, K. & Malmberg, P. (1989) Characterization of exposure to molds and
400 actinomycetes in agricultural dusts by scanning electron microscopy, fluorescence
401 microscopy and the culture method. *Scandinavian Journal of Work Environmental*
402 *Health*, 15, 353-359.

403

404 Klich, M.A. (2002) Identification of common *Aspergillus* species, 1st edition, Ponsen
405 & Looijen, The Netherlands.

406

407 Kormendy, A.C. & Wayman, M. (1972) Scanning electron microscopy of
408 microorganisms. *Micron*, 3, 33-47.

409

410 Lacey, J. (1989) Thermoactinomycetes. In: William & Wilkins Bergey's Manual of
411 Systemic Bacteriology, Baltimore, 4, 2573-3585.

412

413 Lacey, J. (1991) Aggregation of spores and its effect on aerodynamic behaviour.
414 *Grana*, 30, 437-445.

415

416 Lacey, J. (1997) Actinomycetes in compost. *Annals of Agricultural and*
417 *Environmental Medicine*, 4, 113-121.

418

419 Lacey, J. & Dutkiewicz, J. (1976a) Isolation of actinomycetes and fungi from mouldy
420 hay using a sedimentation chamber. *Journal of Applied Bacteriology*, 41, 315-319.

421

422 Lacey, J. & Dutkiewicz, J. (1976b) Methods for examining the microflora of mouldy
 423 hay. *Journal of Applied Bacteriology*, 41, 13-27.

424

425 Latgé, J.P. (1999) *Aspergillus fumigatus* and aspergillosis. *Clinical Microbiology*
 426 *Reviews*, 12(2), 310-350.

427

428 Levetin, E. (1995) Fungi. In: H. Burge (Ed.) *Bioaerosols Indoor Air Research* (pp. 87-
 429 121).CRC Press, U.S.A.

430

431 Levy, J.I., Hammit, J.K. & Spengler, J.D. (2000) Estimating the mortality impacts of
 432 particulate matter: What can be learned from between-study variability?
 433 *Environmental Health Perspectives*, 108, 109-117.

434

435 Lighthart, B. (1997) The ecology of bacteria in the alfresco atmosphere. *FEMS*
 436 *Microbiology Ecology*, 23, 263-274.

437

438 Lighthart, B., Shaffer, B.T., Marthi, B. & Ganio, L.M. (1993) Artificial wind-gust
 439 liberation of microbial bioaerosols previously deposited on plants. *Aerobiología*, 9(2-
 440 3), 189-196.

441

442 Lighthart, B. & Shaffer, B.T. (1994) Bacterial flux from chaparral into the atmosphere
 443 in midsummer at a high desert location. *Atmospheric Environment*, 28(7), 1267-
 444 1274.

445

446 Madelin, T.M. & Johnson, H.E. (1992) Fungal and actinomycete spore aerosols
 447 measured at different humidities with an aerodynamic particle sizer. *Journal of*
 448 *Applied Bacteriology*, 72(5), 400-409.

449

450 Madelin, T.M. & Madelin, M.F. (1995) Biological analysis of fungi and associated
 451 molds. In: C.S. Cox & C.M. Wathes (Eds.) *Bioaerosols Handbook*, pp. 361-380.
 452 Lewis Publishers/CRC Press, Inc., Boca Raton, Fla.

453

454 Marthi, B., Fieland, V.P., Walter, M. & Seidler, R.J. (1990) Survival of bacteria during
 455 aerosolization. *Applied Environmental Microbiology*, 56, 3463-3467.

456

457 Matthias-Maser, A. & Jaenicke, R. (1995) The size distribution of primary biological
 458 aerosol particle with radii $>0.2 \mu\text{m}$ in an urban/rural influenced region. *Atmospheric*
 459 *Research*, 39, 279-286.

460

461 McCartney, H. A. (1994) Dispersal of spores and pollen from crops. *Grana*, 33, 76-
 462 80.

463

464 McCartney, H.A., Fitt, B.L.D. & Schmechel, D. (1997) Sampling bioaerosols in plant
 465 pathology. *Journal of Aerosol Science*, 28(3), 349-364.

466

467 Menetrez, M.Y., Foarde, K.K., Dean, T.R., Betancourt, D.A. & Moore, S.A. (2007) An
 468 evaluation of protein mass of particulate matter. *Atmospheric Environment*, 41, 8264-
 469 8274.

470

471 Michel, F.C. Jr., Marsh, T.J., & Reddy, C.A. (2002) Bacterial community structure
 472 during yard trimmings composting. In: H. Insam, N. Riddech & S. Klammer (Eds.)
 473 Microbiology of Composting, pp. 25-43. Springer-Verlag Berlin Heidelberg, Germany.
 474

475 Nielsen, E.M., Breum, N.O., Nielsen, B.H., Würtz, H., Poulsen, O.M. & Midtgaard, U.
 476 (1997) Bioaerosol exposure in waste collection: A comparative study on the
 477 significance of collection equipment; type of waste and seasonal variation. *Annals of*
 478 *Occupational Hygiene*, 41, 325-344.
 479

480 Papagianni, M. (2006) Quantification of the fractal nature of mycelial aggregation in
 481 *Aspergillus niger* submerged cultures. *Microbial Cell Factories*, 5(5), 1-13.
 482

483 Pasanen, A.-L., Kalliokoski, P., Pasanen, P., Salmi, T. & Tossavainen, A. (1989)
 484 Fungi carried from farmers' work into farm homes. *American Industrial Hygiene*
 485 *Association Journal*, 50 (12), 631-633.
 486

487 Pillai, S.D. & Ricke, S.C. (2002) Bioaerosols from municipal and animal wastes:
 488 background and contemporary issues. *Canadian Journal Microbiology*, 48(8), 681-
 489 696.
 490

491 Prescott, L.M., Harley, J.P. & Klein, D.A. (1999a) Bacteria: the High G+C Gram
 492 Positives. In: *Microbiology*, pp. 506-522. 4th edition, McGraw-Hill, USA.
 493

494 Prescott, L.M., Harley, J.P. & Klein, D.A. (1999b) The Fungi (Eumycota), Slime
 495 Molds, and Water Molds. In: Microbiology, pp. 522-540. 4th edition, McGraw-Hill,
 496 USA.
 497
 498 Reinthaler, F.F., Haas, D., Frierl, G., Schlacher, R., Pichler-Semmelrock, F.P., Köck,
 499 M., Wüst, G., Feenstra, O. & Marth, E. (1997) Comparative investigation of airborne
 500 culturable microorganisms in selected waste treatment facilities and in neighbouring
 501 residential areas. Zentralblatt für Hygiene und Umweltmedizin, 202, 1-17.
 502
 503 Reponen, T.A., Willeke, K., Ulevicius, V., Reponen, A. & Grinshpun, S.A. (1996)
 504 Effect of relative humidity on the aerodynamic diameter and respiratory deposition of
 505 fungal spores. Atmospheric Environment, 30, 3967-3974.
 506
 507 Reponen, T., Gazenko, S.V., Grinshpun, S.A., Willeke, K. & Cole, E.C. (1998)
 508 Characteristics of airborne actinomycete spores. Applied and Environmental
 509 Microbiology, 64(10), 3807-3812.
 510
 511 Reynolds, K.A. & Pepper, I.L. (2000) Microorganisms in the environment. In: R.N.
 512 Maier, I.L. Pepper and C.P. Gerba (Eds.) Environmental Microbiology, pp. 7- 41.
 513 Academic Press, Canada.
 514
 515 Samson, R.A., Hoekstra, E.S., Frisvad, J.C. & Filteborg, O. (1995) Introduction to
 516 Food-Borne Fungi. Centraalbureau voor Schimmelcultures, AG BAARN, The
 517 Netherlands.
 518

519 Sánchez-Monedero, M.A. & Stentiford, E.I. (2003) Generation and dispersion of
 520 airborne microorganisms from composting facilities. *Process Safety and*
 521 *Environmental Protection*, 81(3), 166-170.

522

523 Schwartz, J., Dockery, D.W. & Neas, L.M. (1996) Is daily mortality associated
 524 specifically with fine particles? *Journal of the Air and Waste Management*
 525 *Association*, 46, 927-939.

526

527 Seedorf, J., Hartung, J., Schröder, M., Linkert, K.H., Phillips, V.R., Holden, M.R.,
 528 Sneath, R.W., Short, J.L., White, R.P., Pedersen, S., Takai, H., Johnsen, J.O., Metz,
 529 J.H.M., Groot Koerkamp, P.W.G., Uenk, G.H. & Wathes, C.M. (1998) Concentrations
 530 and emissions of airborne endotoxins and microorganisms in livestock buildings in
 531 northern Europe. *Journal of Agricultural Engineering Research*, 70, 97-109.

532

533 Shekunov, B.Y., Chattopadhyay, P., Tong, H.H.Y & Chow, A.H.L. (2007) Particle
 534 size analysis in pharmaceuticals: principles, methods and application. *Pharmaceutical*
 535 *Research*, 24(2), 203-227.

536

537 SKC. (2002) Operating instructions Universal Air Sampling Pump for Models 224-44,
 538 225-PCTX4 and 224-PCTX8.

539

540 Skujiņš, J., Puķīte, A. & McLaren, A.D. (1971) Application of scanning and
 541 transmitting electron microscopy in the differentiation of soil *Streptomyces*. *Soil*
 542 *Biology & Biochemistry*, 3, 181-186.

543

544 Spengler, J. & Wilson, R. (1996) Emissions, dispersion and concentration of
 545 particles. In: *Particles in Our Air Concentrations and Health Effects*, Harvard
 546 University Press, U.S.A.

547

548 Sturm R. (2011) Modeling the deposition of bioaerosols with variable size and shape
 549 in the human respiratory tract – A review. *Journal of Advanced Research*,
 550 doi:10.1016/j.jare.2011.08.003.

551

552 Swan, J.R.M., Kelsey, A., Crook, B. & Gilbert, E.J. (2003) Occupational and
 553 environmental exposure to bioaerosols from composts and potential health effects –
 554 A critical review of published data (pp. 15-20). Research Report 130, HSE Books.

555

556 Taha, M.P.M., Pollard, S.J.T., Sarkar, U. & Longhurst, P. (2005) Estimating fugitive
 557 bioaerosol release from static compost windrows: Feasibility of a portable wind
 558 tunnel approach. *Waste Management*, 24, 445-450.

559

560 Taha, M.P.M., Drew, G.H., Longhurst, P.J., Smith, R., & Pollard, S.J.T. (2006)
 561 Bioaerosol releases from compost facilities: Evaluating passive and active source
 562 terms at a green waste facility for improved risk assessments. *Atmospheric*
 563 *Environment*, 40(6), 1159-1169.

564

565 Taha, M.P.M., Drew, G.H., Tamer Vestlund, A., Aldred, D., Longhurst, P.J., &
 566 Pollard, S.J.T. (2007) Enumerating actinomycetes in compost bioaerosols at source
 567 – Use of soil compost agar to address plate ‘masking’. *Atmospheric Environment*,
 568 40(22), 4759-4765.

569

570 Tamer Vestlund, A. (2009) Characterisation and Dispersal of Bioaerosols Emitted
571 from Composting Facilities. PhD Thesis. Cranfield University.

572

573 Thomas, R.J., Webber, D., Sellors, W., Collinge, A., Frost, A., Stagg, A.J., Bailey,
574 S.C., Jayasekera, P.N., Taylor, R.R., Eley, S. & Titball, W. (2008) Characterization
575 and deposition of respirable large- and small-particle bioaerosols. *Applied and*
576 *Environmental Microbiology*, 74(20), 6437-6443.

577

578 Tong, Y. & Lighthart, B. (1997) Solar radiation has a lethal effect on natural
579 populations of culturable outdoor atmospheric bacteria. *Atmospheric Environment*,
580 31(6), 897-900.

581

582 Trunov., M., Trakumas, S., Willeke, K., Grinshpun, S.A. & Reponen, T. (2001)
583 Collection of bioaerosol particles by impaction: Effect of fungal spore agglomeration
584 and bounce. *Aerosol Science and Technology*, 35, 617-624.

585

586 Wheeler, P.A., Stewart, I., Dumitrean, P. & Donovan, B. (2001) Health effects of
587 composting – A study of three compost sites and review of past data. R&D Technical
588 Report P1-315/TR, Environment Agency.

589

590 Wittmaack, K., Wehnes, H., Heinzmann, U. & Agerer, R. (2005) An overview of
591 bioaerosols viewed by scanning electron microscopy. *Science of the Total*
592 *Environment*, 346, 244-255.

593



DSPACE

<https://dspace.org/>

Morphological classification of bioaerosols from composting using scanning electron microscopy

Tamer Vestlund, Asli; Al-Ashaab, R.; Tyrrel, Sean F.; Longhurst, Philip J.; Pollard, Simon J. T. ...

2014-07-31T00:00:00Z

<http://dx.doi.org/10.1016/j.wasman.2014.01.021>

A. Tamer Vestlund, R. Al-Ashaab, S.F. Tyrrel, P.J. Longhurst, S.J.T. Pollard, G.H. Drew. Morphological classification of bioaerosols from composting using scanning electron microscopy. Waste management, 2014, Volume 34, Issue 7, pg1101-1108.