Particle size distribution of airborne micro-organisms in the environment – a review

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Abstract

To obtain insight into the particle size distributions of airborne micro-organisms in different environments, a literature search was conducted. More than 190 publications containing relevant data including sampling systems, sampling sites, measuring parameters, sample size and concentrations were included. The size distribution of airborne particles carrying micro-organisms is a well-investigated subject in the range of aerodynamic diameters (AD) of 0.65 µm to 12 µm for many micro-organism groups and environments. It depends primarily on the sampling location and the type of source as well as the method of aerosolisation. Highest median shares of large bacteria-laden particles were found in livestock husbandry and in waste management. Sampling height above ground, air humidity, temperature and solar radiation may also influence particle size. For moulds, the median size distributions in air largely represent the size ranges of their spores. There is little knowledge about particles > 12 µm AD and the actual number of micro-organisms in different particle size classes. Few studies suggest that most micro-organisms are in particle size fractions > 10 µm AD. Future investigations should use sampling systems with high inlet efficiencies for particles > 20 µm AD, and allow sampling in a liquid to separate micro-organisms from aggregates. These systems should rather sample the health and environmentally relevant particle size fractions PM 2.5, PM 4, PM 10 and the total dust to allow for a more precise derivation of health and environmental effects.

Keywords: *bio-aerosols, particle size distribution, size-selective bio-aerosol sampler*

Zusammenfassung

Partikelgrößenverteilung von luftgetragenen Mikroorganismen in der Umwelt – Ein Review

Der vorliegende Beitrag gibt einen Überblick über den Wissensstand zur Partikelgrößenverteilung von luftgetragenen Mikroorganismen in der Umwelt. Dazu wurden mehr als 190 Publikationen, die relevante Daten zu eingesetzten Sammelsystemen, Sammelort, Messparametern, Probenanzahl und gefundenen Konzentrationen beinhalteten, in die Auswertung mit einbezogen. Die Größenverteilung von Mikroorganismen-tragenden Partikeln ist im Bereich von 0,65 bis 12 µm aerodynamischer Durchmesser für viele Umweltbereiche und Mikroorganismengruppen gut untersucht. Sie scheint primär abhängig vom Umweltbereich (Sammelort) zu sein und hier vermutlich von der Art der Quellen der luftgetragenen Partikel sowie der Art und Weise der Aerosolisierung. Besonders bei den Schimmelpilzen repräsentieren die gefundenen Verhältnisse auch die Größenverteilungen der Sporen der untersuchten Arten wieder, da Schimmelpilzsporen im Gegensatz zu Bakterien in der Luft weitgehend vereinzelt vorkommen. Wissensdefizite gibt es aufgrund der bisher eingesetzten Sammelsysteme im Bereich > 12 µm AD. Einige Studien deuten darauf hin, dass sich, abhängig vom Umweltbereich, ein Großteil der Mikroorganismen in der Partikelfraktionen > 10 µm befindet. In Zukunft sollten daher verstärkt Sammelsysteme eingesetzt werden, mit denen nicht nur die Anzahl Mikroorganismen-tragender Partikel, sondern die Anzahl aller Mikroorgansimen in den gesundheitlich relevanten Partikelgrößenfraktionen PM2,5, PM4, PM10 und Gesamtstaub erfasst werden kann.

Schlüsselwörter: *Bioaerosole, Partikelgrößenverteilung, größenselektive Bioaerosolsammler*

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1 Introduction

The exposure to airborne micro-organisms can affect health negatively (Gregory, 1961; Fernstrom and Goldblatt, 2013). This is also generally depending on the particle size (Cheng, 2003; Cho et al., 2005; Miller et al., 1988; Ogden and Birkett, 1975; Sturm, 2012; Thomas et al., 2008). When inhaled, for example, penetration depth is highly depending on particle size: large bio-aerosol particles get already stuck in the nose or mouth whereas small particles can get deep into the lungs. Especially waste management industries or livestock production facilities can be a source of huge amounts of different airborne micro-organisms which are emitting also into the environment (VDI 4250/3, 2014). Therefore approval processes for construction or expansion of such facilities often include the assessment of environmental and health effects (e. g. for Germany: VDI 4250/1, 2014; TA-Luft, 2002). In this context the dispersal of airborne micro-organisms as well as their immission in residential areas is often calculated and predicted with computer models (VDI 4251/3, 2015). For these calculations it is commonly assumed that the size of the microbial particles is below 2.5 µm (Burrows et al., 2009; VDI 4251/3, 2015; TA-Luft, 2002). However, calculated and mea-sured concentrations in agriculture can differ considerably (Seedorf et al., 2005; Springorum et al., 2014). One reason for such disagreements can be the underlying theoretical par-ticle size.

To improve the prediction of dispersion models and the environmental health assessment on the one hand and to get an insight on the particle size distribution of airborne micro-organisms in other relevant environments, e. g. living spaces, public buildings, offices, hospitals or outdoor air, on the other hand, a literature search to this topic was conducted. The results may help regional authorities, environmental auditors and engineering consultants to assess possible risks and to identify lacks of knowledge and need for further investigations.

1.1 Airborne micro-organisms and their aerosolization

Above the land surface in a natural environment, airborne dust consists of up to about 25 % of biological particles (Matthias-Maser and Jaenicke, 1994; Matthias-Maser and Jaenicke, 2000; Jones and Harrison, 2004). In urban and agriculturally-dominated areas the percentage is usually higher (Matthias-Maser and Jaenicke, 1995). Shares of up to 90 % could be found in waste management industries or livestock production, (Aengst, 1984). Airborne biological particles as a whole are also denoted as bio-aerosols. They are a complex mixture consisting of different components, from simple organic molecules with dimensions in the nanometer range, through to viruses, bacteria, bacteria spores, mould spores and hyphae and pollen with diameters of 100 µm and more, as well as animal and plant debris of different sizes. These components can get into the airborne state as single particles or in aggregates. In 1884, Hesse already revealed experimentally that airborne bacteria occur mainly in "colonies", whereas mould spores could be found detached (Hesse, 1884; Hesse, 1888). He also even discussed whether it was expedient to determine the count of bacteria in a given volume of air, or just the number of bacteria-laden particles. Both approaches have been applied to different extents in the studies in the following 130 years.

The fact that airborne bacteria occur mainly in aggregates, and, in contrast, mould spores rather as single cells, can be explained by their mode of life. Natural habitats of most micro-organisms are soil, water, plants and animals and their residues. In these habitats they often form large colonies in complex communities consisting of many different species. Bacteria may rather accidentally get into the airborne state, mainly as large fragments of these colonies together with surrounding matrix. Aerosolization takes place, e. g., through wind (Fulton, 1966; Jones and Harrison, 2004), by excretion of faeces, loss of skin scales (Lewis et al., 1969; Clauß et al., 2013a), breathing, speech, coughing and sneezing (Duguid, 1946; Louden and Roberts, 1967; Papineni and Rosenthal, 1997; Nicas et al., 2005; Yang et al., 2007; Gralton et al., 2011) or by spray (Blanchard and Syzdek, 1972). The dissemination strategies of most of the streptomycetes and actinomycetes, as well as moulds, include aerial distribution. Some species even have mechanisms for an active release of spores into the air (Ingold, 1984; Meredith, 1973). This also includes the strategy to produce large amounts of single spores to increase the chance for successful dissemination. Pasanen et al. (1989) and Heikkilä et al. (1988) found ratios of 2:1 for single spores and small spore aggregates in the airborne state of different moulds and even 5:1 for actinomycetes.

1.2 Particle size definitions

The shape and size of most of bacteria, yeasts and mould spores are well known from several microscopic studies. An overview is given by, e. g., De Hoog et al. (2000), Bergey et al. (1974), and Winkle et al. (1979). However natural bio-aerosol particles often consist of different components and are assembled irregularly. Therefore the specification of particle dimensions, such as length, height, and width as well as density, are more difficult than for an accurately definable geometric body like a spherical mould spore or bacterial rod. Some approximations are used in practice such as the geometric equivalent diameter which is obtained by determining the diameter of a sphere having the same geometric properties (surface, volume or projected area) as the irregularly-shaped particle. The terms "petri ratio size" (Bourdillon et al., 1948; Kethley et al., 1963) and "settling velocity" (Kethley et al., 1963; Wells, 1955) still can be found in earlier literature. Both measurements refer to the number of micro-organisms that settle on a petri dish in a given time. Here, the behavior of particles in the air, for which size and shape and also the density are relevant, is indirectly included. The density for mould spores varies between 0.56 to 1.44 g/cm^3 (Gregory, 1961) and for bacteria it can be assumed to be in the same range. The density of a particle is also included in the measurement "aerodynamic diameter" (AD).

The AD of an irregular particle is defined as the diameter of the spherical particle with a density of (1 g/cm³) and the same settling velocity in air of standard pressure and temperature as the irregular particle (Hinds, 1999). The AD affects sedimentation and deposition in the environment and thereby the distance of transport via air (Hinds, 1999); the probability and location of deposition in the respiratory tract, and therefore potential health effects (Cheng, 2003; Cho et al., 2005; Miller et al., 1988; Ogden and Birkett, 1975; Sturm, 2012; Thomas et al., 2008); resuspension (Lighthart et al., 1993); the efficiency of air cleaning systems (Batel, 1972), and the tenacity of airborne micro-organisms within the particles (Kundsin, 1968; Lighthart and Shaffer, 1997; May and Druett, 1968).

The number of micro-organisms per volume of air is normally given as concentration number in units such as cells/m³. Environmental science and health scientists often use mass concentration for the characterization of airborne particles, defined as the mass of particulate matter per volume with units such as µg/m³ (Hinds, 1999). A reference of micro-organisms-to-mass is not common. But whenever airborne micro-organisms are separated from an air stream by mass inertia, e. g., in an impactor, this also refers to a mass-based cut-off curve. A median cut-off diameter (d50) derives from the progression of the cut-off curve, at which exactly half of the particles of this size incorporate into the weighting. In practice that means that particles with a larger aerodynamic diameter than the d_{50} are deposited with an efficiency of more than 50 % in this stage. The cut-off curves of the different size-selective sampling systems vary depending on when and for which field of application they were developed. In occupational health, the cut-off characteristic of the human respiratory tract is commonly used as the basis for size-selective sampling systems for airborne particles, whereas in environmental science a definite cut is made between the particle size fractions (steep cut-off curve).

For the characterization of airborne dust and in the field of occupational health, e.g., in the DIN EN 481 (1993), the particle size fractions are defined as follows: The "Total Airborne Particles" are all particles surrounded by air in a given volume of air; the "Inhalable Fraction" (d50 = 100 µm) is the mass fraction of total airborne particles which is inhaled through the nose and mouth; the "Thoracic Fraction" (d50 = 10 µm) is the mass fraction of inhaled particles penetrating beyond the larynx, and the "Respirable Fraction" (d50 = 4 µm) is the mass fraction of inhaled particles penetrating to the unciliated airways. The "Respirable Fraction" was formerly denoted as "Fine Particles" or "Fine Dust" (Orenstein, 1960), with a different progression of the cut-off curve and a d50 of 5 µm. Today this term is uncommon and not defined any more. In addition the DIN ISO 7708 (1996) gives a "Respirable Fraction" referred to "Risk Groups" with a d50 of 2.5 µm. In the field of environmental science the definition of the "Total Suspended Particles" or "Suspended Particulate Matter" respectively, is nearly equal to the one used in occupational health, according to, e.g., VDI 2463/1 (1999) only with an upper particle size of about 30 µm without a rigid upper separation limit. The particle mass (PM) fractions PM10 and PM2.5 each have their names from the cut-off diameter and are defined as particles that pass through a size-selective inlet with a 50 % efficiency cut-off at 10 µm or 2.5 µm diameter respectively (DIN EN 12341, 1999; US EPA, 2009). PM10 and PM2.5 roughly correspond to the "Thoracic Fraction" and the "Respirable Fraction" referred to "Risk Groups".

Despite the high importance of airborne micro-organisms for occupational health the progression of the cut-off curves of size-selective sampling systems for micro-organisms is mainly oriented to the environmental sciences. However, only a few systems have appropriate cut-off diameters according to the referred definitions (see also Table 1). Therefore information about the number of micro-organisms in these defined particle classes is rare in literature.

1.3 Factors influencing particle size

Irrespective of the kind of source and the method of aerosolization of airborne micro-organisms some other factors may directly and indirectly influence the ascertainable particle size. Neither the AD nor the mass of a biological particle in the airborne state are fix values. Size, form and density are subject to fluctuations depending directly on air humidity. A significant increase of the size of some airborne bacteria and mould spores was found when the relative humidity (RH) increased, especially between 90 % RH and 100 % RH (Ko et al., 2000; Madelin and Johnson, 1992; Reponen et al., 1996). In contrast, dry conditions may lead to disintegration of airborne particles by decreasing bonding forces and increasing tensions (Jones and Harrison, 2004). Also the size of freshly aerosolized liquid droplets decreases within seconds due to evaporation (Xie et al., 2007). The influence of the season on the size distribution of airborne micro-organisms is not clear but there are some indications for an indirect correlation. Wang et al. (2010), Awad et al. (2013) and Lin and Li (1996) could not find any influence of the season but later authors found an influence of the time of day. The mean size of the particles seemed to be larger at night, possibly due to the higher RH at night. Che et al. (1992), who conducted measurements distributed over 4 years, found influences of the time of day as well as of the season. Especially at noon and in summer, more micro-organisms were found in the larger particle size fractions (> 7 µm). The reason for this finding may be the solar ultraviolet radiation and its direct influence on the tenacity of the micro-organisms. Micro-organisms which become airborne as single cells or in small aggregates are harmed much more by radiation than micro-organisms within large particles or cell aggregates. Therefore, at noon in summer only those micro-organisms which were protected against unfavourable environmental conditions in the larger particles were still detectable by cultivation methods. This assumption is confirmed by the finding that mould spores, which are much more robust against ultraviolet radiation than bacteria (Clauß, 2006), show even distributions in the particle size classes in summer and winter and during the day and at night (Che et al., 1992). Also the height above ground at which the sampling takes place has an influence on the particle size distribution due to sedimentation, especially of the larger particles. Wright et al. (1969) investigated

the particle size distribution of airborne bacteria at heights from 10 to 150 m and the higher the sampling point was the fewer larger particles were found. It should be highlighted at this point that besides these exceptions mentioned, most bio-aerosol samplings were understandably conducted in the daytime, and the measurement systems were placed between 0.75 m and the mean human breathing height at 1.5 m. The choice of the size-selective sampling system, as well as the subsequent analysis, always has an influence on the results.

1.4 Size selective bio-aerosol samplers

Since the 1940s, an increasing number of systems have been developed for the size selective sampling of airborne micro-organisms in different stages (e.g., May, 1945; Wells, 1947). As already mentioned in Chapter 3, each of these stages has a defined d_{50} and particles with a greater AD are deposited with an efficiency of more than 50 % in this stage. The cut-off diameters and cut-off curves of the stages of the different systems are generally well validated. However, comparatively little is known about the inlet efficiencies of most of the

Table 1
Size selective sampling systems that were used for the sampling of airborne micro-organisms in the environment

Sampler	No. of Stages [n]	Flow rate [L/min]	Inlet d_{50} [µm]	Cutpoint of the Single Stages d_{50} [µm]	Reference
Impaction on Nutrient Plates					
Andersen Sampler	2	28.3	12	8.0; 0.95	Turner and Hill, 1975
Custom-designed Particle-sizing Slit Sampler	2	20	n/a	3.0; n/a	Dutkiewicz and Kwapiszewski, 1975
Size-grading Slit Sampler	4	566	28	18.2; 9.6; 4.2; 0.9	Lidwell, 1959
Andersen Sampler	6	28.3	12	7.2; 4.8; 3.2; 2.1; 1.0; 0.6	Andersen, 1958
Modified Andersen Sampler	7	28.3	19	11.2; 7.5; 5.4; 3.5; 2.0; 0.97; 0.6	May, 1964
Andersen Sampler	8	28.3	n/a	11.0; 7.0; 4.7; 3.3; 2.1; 1.0; 0.7; 0.4	Curtis et al., 1975
Impaction On Filter Or Solid Surfaces					
Personal Spectrometer (PERSPEC)	1	2	n/a	*omitted*	Prodi et al., 1988
Membrane Filter + Cyclon Pre-impactor	2	2	n/a	4.0; n/a	Predicala et al., 2002
Static Size-selective Bioaerosol Sampler (SSBAS)	2	18.5	14	7.2; 2.4	Kauppinen et al., 1989
Free Wing Impactor + Two-stage Impactor	1 + 2	- n/a	> 150 n/a	9.0 1.9; 0.11	Jaenicke and Junge, 1967 Jaenicke and Blifford, 1974
Two-stage Bio-aerosol Cyclone (BC)	2 + 1	3.5	n/a	1.8; 1.0; *depending on afterfilter*	Lee and Liao, 2014
Model BC 221	2 + 1	2	n/a	2.6; 1.6; *depending on afterfilter*	Lindsley et al., 2006
Model BC 251	2 + 1	10	n/a	2.1; 0.41; *depending on afterfilter*	Lecours et al., 2012
Personal Size-selective Bioaerosol Sampler	3	2	n/a	10.0; 4.5; 0.8	Mark and Vincent, 1986 Kenny et al., 1999
Modified High Volume Cascade Impactor (HVCI)	4	850	n/a	10.0; 2.4; 0.9; 0.2	Demokritou et al., 2002 Sillanpää et al., 2003 Sippula et al., 2013
May-Casella-impactor Modified May-Casella-impactor	4 4 4	17.5 11.9 11.9	50 n/a n/a	14.5; 4.0; 2.5; n/a 13.0; 4.0; 1.7; n/a 6.4; 2.0; 0.9; 0.4	May, 1945 Lippmann, 1959 Fisar et al., 1990
Marple Personal Cascade Impactor	8	2	n/a	20.0; 15.0; 10.0; 6.0; 3.5; 2.0; 1.0; 0.61	Macher and Hansson, 1987
Andersen Sampler MK I	8	28.3	20	11.0; 7.0; 4.7; 3.3; 2.1; 1.1; 0.7; 0.4	Vaughan, 1989
Andersen Sampler MK II *(with Pre-impactor)	8 + 1*	28.3	20	10.0*; 9.0; 5.8; 4.7; 3.3; 2.1; 1.1; 0.7; 0.4	Vaughan, 1989
Micro-orifice Uniform Deposit Impactor (MOUDI)	10	30	18	10; 5.6; 3.2; 1.8; 1.0; 0.56; 0.32; 0.18; 0.1; 0.056	Marple et al., 1991
Sampling In Liquids					
Multi-stage Liquid Impinger	3	50	>20	6.0; 3.0; 0.8	May, 1966

samplers. This is especially true for particles > 10 µm AD and for the sampling at unfavourable flow conditions or wind regimes (Vaughan, 1989; Yao and Mainelis, 2006). In this regard, the six-stage Andersen sampler is thoroughly evaluated. McFarland (1977) found 0 % inlet efficiency for particles with an AD of 15 µm for an upright impactor g and a wind speed of 4.6 m/s. The inlet efficiency increase with lower wind speeds was negligible. Wedding et al. (1977) found efficiencies between 2 % (15 µm AD) and 67 % (5 µm AD) with internal wall losses of 41 % and 10 % respectively. Excluding some exceptions, it can be assumed that most of the systems are not capable of collecting particles > 20 µm. This limitation has not posed a problem so far because an upper size limit for natural aerosols of 20 to 30 µm AD is commonly agreed upon due to particle diffusion and sedimentation. This cannot be confirmed though. Rather Jaenicke and Junge (1967) found particles up to 150 µm in natural ambient air with their "Free Wing Impactor". Also in ambient air as well as in the exhaust air of pig houses, Fisar et al. (1990) and Clauß et al. (2011a, b) found bio-aerosol particles with sizes up to 100 µm equivalent diameter that furthermore carried hundreds of micro-organisms.

The most frequently used sampling systems are those impacting airborne micro-organisms directly on nutrient media. Sampling of airborne micro-organisms on solid surfaces or filter or sampling in a liquid is only rarely conducted. An overview of the different size-selective sampling systems, which were used for the sampling of airborne micro-organisms, is given in Table 1.

1.4.1 Impaction on nutrient plates

The two-stage Andersen sampler (Turner and Hill, 1975) is one of the most frequently used sampling systems impacting airborne micro-organisms directly on nutrient media. There are 200 round nozzles in both stages. The nozzles in the second stage have a smaller diameter which account for size separation. The airborne particles are deposited on nutrient media in static petri dishes. In contrast, the "custom-designed particle-sizing slit sampler" uses two parallel systems both with slit nozzles for the impaction of airborne particles onto rotating nutrient plates (Dutkiewicz and Kwapiszewski, 1975). One system is equipped with a pre-impactor for the collection of the small particle fraction. The four-stage "size-grading slit sampler" (Lidwell, 1959) has two more stages. At every stage a circular slit nozzle is positioned off-center above a rotating glass petri dish. The air passes into the next stage through a hole in the middle of the petri dish. The standard for size-selective sampling systems for airborne micro-organisms and the most commonly used worldwide is the six-stage Andersen sampler (Andersen, 1958). In its original version every single stage had 400 round nozzles. May (1964) recommended a modified version with 200 nozzles for the first and second stage to reduce particle losses. To increase the inlet efficiency from d_{50} = 12 µm AD to d_{50} = 19 µm AD Lidwell (1965) recommended an additional stage connected upstream. This modified system is not well-established though. There were several other technical and procedural modifications. To increase the inlet efficiency at unfavourable wind regimes, Burge et al. (1977) mounted a vane on the sampler to align the inlet to wind direction. Some authors used the six-stage Andersen sampler and pooled different stages in the results (e.g., Butera et al., 1991; Lembke et al., 1981; Lis et al., 2008; Predicala et al., 2002). Sometimes only single stages of the sampler were loaded with nutrient plates (Solomon, 1970). King and McFarland (2012) covered one half of the nutrient media with a filter to obtain the number of particles carrying micro-organisms and additionally the total number of micro-organisms in the different particle size fractions. Moschandreas et al. (1996) filled the petri dishes with water instead of nutrient media to count collected cells under a fluorescence microscope after staining with acridine orange. In its current version, the six-stage Andersen sampler has a higher collection efficiency compared to many other sampling systems (Gillespie et al., 1981; Jensen et al., 1992). There is also an eight-stage version of the Andersen sampler available (Curtis et al., 1978).

1.4.2 Impaction on filter or solid surfaces

In contrast to the direct impaction on nutrient plates, sampling of airborne micro-organisms on solid surfaces or filters is only rarely conducted, probably due to the risk of dehydration of the micro-organisms on these surfaces and the resulting lower biological sampling efficiency. Therefore, this sampling method is mainly used in combination with molecular biological or microscopic methods. Most of these sampling systems were originally developed for the collection of dust. There are many systems available using different techniques for size separation and particle collection. Relatively simply constructed is the "Personal Spectrometer" (PERSPEC) (Prodi et al., 1988; Prodi et al., 1991; Prodi et al., 1992). In only one stage is re-circulating particle-free air flanked by the sample air sucked through a round nozzle onto a membrane filter. Size separation takes place by deposition of larger particles in the central region of the filter and smaller particles in the boundary areas. Predicala et al. (2002) sampled airborne micro-organisms on membrane filters, too. For size-separation they used a cyclone as pre-impactor. The "Static Size Selective Bio-aerosol Sampler" (SSBAS) developed by Kauppinen et al. (1989) and tested by Rantio-Lehtimäki (1989) consists of a pre-impactor to retain water droplets and insects and a two-stage virtual impactor for size separation. The "Personal Size Selective Bio-aerosol Sampler" is based upon an IOM-f dust sampling head (Kenny et al., 1998; Kenny et al., 1999; Mark and Vincent, 1986) and separates airborne particles by means of two size-selective polyurethane foams in front of a polycarbonate after-filter. A remarkable system is the "Free Wing Impactor" (Jaenicke and Junge, 1967). Instead of sucking the probe air through the sampling system, an impactor plate attached to a rotating cantilever moves through the probe air. With this technique even particles with AD > 150 µm can be sampled. Some authors (Matthias-Maser and Jaenicke, 1994; Matthias-Maser and Jaenicke, 1995; Matthias-Maser and Jaenicke, 2000) used this system in combination with a two-stage impactor

(Jaenicke and Blifford, 1974; Marple, 1970) for outdoor sampling. The two-stage bio-aerosol cyclone developed at the "National Institute for Occupational Safety and Health (NIOSH)", consists mainly of two centrifuge tubes acting as parts of two in-line cyclones, as well as a back-up filter. Different designs and modifications of the system exist (Blachere et al., 2009; Lee und Liao, 2014; Lindsley et al., 2006). To date has mainly been used for the sampling of airborne viruses (Blachere et al., 2007; Blachere et al., 2009; Blachere et al., 2011; Cao et al., 2011; Noti et al., 2012; Verreault et al., 2008), but some authors also used it for the sampling of micro-organisms (Chen et al., 2004; Lecours et al., 2012; Yamamoto et al., 2011). Another system is the modified "High Volume Cascade Impactor" (HVCI), a four-stage slit impactor that collects airborne micro-organisms on polyurethane foams and in the last stage on a filter (Demokritou et al., 2002). The "May-Casella-Impactor" developed by May (1945) and distributed by Casella, is a four-stage system for collection of micro-organisms on glass slides in which the impactor stages are displaced by 90° each. Since 1959, a revised version is also available (Lippmann, 1959). The "Marple Personal Cascade Impactor" is an eight-stage system modified by Macher and Hansson (1987) in such a manner that a thin layer of gelatine can be used as sampling medium. There is also an eight-stage Andersen sampler in the MKI version available,for the sampling of particles onto solid surfaces and in the version MKII with additional pre-impactor (d_{50} = 10 µm AD). At least the "Micro-orifice Uniform Deposit Impactor" (MOUDI) (Marple et al., 1991) is a system with a variable number of stages. With up to 2000 micro-nozzles per stage especially small particles are impacted uniformly onto rotating sampling media. The system is mainly used for the collection of nano-particles and organic carbon compounds (e. g., Chen et al., 2011; Eiguren-Fernandez et al., 2003; McMurry and Zhang, 1989), but a ten-stage system was also used for the collection of endotoxins and bacteria (Kujundzic et al., 2006).

1.4.3 Sampling in liquids

The sampling of airborne micro-organisms in a liquid is preferable to impaction on solid surfaces or deposition on filters because of the higher biological sampling efficiency. There are only a few size-selective sampling systems using this method. May and Druett (1953) developed a pre-impinger serving as pre-separator for a standard impinger. May (1966) has further developed the system to a multi-stage impinger. Originally it was intended for the sampling of airborne micro-organisms, but could not become established for this purpose, probably due to its complex design. However, the multi-stage impinger is now the reference system for the evaluation of medical inhalers (Asking and Olsson, 1997; Mitchell and Nagel, 2003) and has also been used for the sampling of airborne viruses (Donaldson et al., 1977; Verrault et al., 2008).

1.5 Micro-organism analysis methods

The quantitative and qualitative analysis of airborne micro-organisms is conducted mostly by cultivation. If airborne particles carrying micro-organisms are impacted directly on nutrient plates, each particle gives rise to a single colony irrespective of the number of viable units it may have carried. Therefore, the method gives the number of cultivable micro-organism laden particles (MLP) in a selected size fraction. On the nutrient medium directly below the single nozzles the impacted micro-organisms often lie closely side by side. The single colonies often grow into each other and merge together so that they cannot be discriminated when counting. However, this error can be minimized by the "Positive-Hole Correction" (Andersen, 1958; Macher, 1989). If micro-organisms are sampled on solid surfaces or filters and are eluted in a liquid afterwards, the collected cell aggregates may disintegrate to a large extent within the liquid separating the cells. Also by sampling into a liquid medium directly, followed by plating out of the whole or part of the fluid, bacterial aggregates are supposed to break up, partially or completely, and give rise to a higher count than that obtained by sampling directly on to a solid medium. Hence with this method, giving the number of colony forming units (cfu) after cultivation, the count of all micro-organisms in a selected particle size fraction can be obtained theoretically.

In recent years the application of molecular methods, which give the number of more or less specific gene copies in a selected particle size fraction (Lecours et al., 2012; Lee and Liao, 2014; Quian et al., 2012; Schafer et al., 2003; Sippula et al., 2013; Yamamoto et al., 2011), increased. It has to be considered that the number of gene copies may not equal the number of micro-organisms because genes may also occur disengaged in the dust or attached to cell debris or exist in several copies in the same cell. Scanning electron microscope analysis (Heikkilä et al., 1988; Tyrell et al., 2009), light microscopy (Fisar et al., 1990; Kujundzic et al., 2006; Tilley et al., 2001) or fluorescence microscopy (Clauß et al., 2011a; Clauß et al., 2011b; Hara et al., 2011) were also conducted to measure the size of airborne particles and to count the cells of bacteria, yeasts and moulds that are included in the particles. With these methods neither the density of the particles nor the capability for cultivation of the micro-organisms are taken into consideration. However these studies give insight in the internal structure of bio-aerosols and the distribution of micro-organisms on airborne particles themselves, as well as the distribution in selected particle size classes.

2 Material and Method

An extensive literature search was conducted on the size distribution of airborne micro-organisms in the environment. The online database Medline (PubMed) and the search engine Google Scholar were searched for publications containing the keywords *bio-aerosols*, *particle size distribution*, *airborne micro-organisms* using the Boolean operators AND or OR. The found publications were screened for supporting additional keywords and search terms, e. g., the different sampling systems, for an extended enquiry on the used search engines. Search terms and keywords were also translated to German, French and Spanish. Additionally an author

search in PubMed for other publications from the found authors as well as a control of the cited literature for further studies was conducted. After an abstract screening of the found studies laboratory experiments and studies investigating only the size distributions of biological particles by bioaerosol fluorescence spectrometers were excluded. The remaining 197 publications available were summarized to the relevant data such as sampling system, measuring parameter, sampling site, sampling height above ground, concentrations and sample size. Not considered were the season and the time of day because of the differing conclusions of some studies (see Chapter 4). In the publications the size distribution data were presented mainly in figures or tables as median or arithmetic mean of concentrations or percentages of micro-organisms in different size classes. To compare the data they were converted to the median percentage of micro-organisms in the different particle size classes for each sampling system. Distributions that were normalized to the different widths of the particle size classes were back-calculated (TSI, 2012). Despite different sample sizes, every data row presented in the publications was weighted equally because it was assumed that every dataset was representative itself. In this regard one publication was excluded subsequently due to its congruency with another publication of the same author, based on an identical dataset. For the analysis it was generally distinguished between studies investigating the number of micro-organism-laden particles or the number of micro-organisms (cfu, cell count or gene copy) in a selected particle size fraction.

3 Results

3.1 Size distribution of airborne particles carrying micro-organisms

The size distribution of micro-organism-laden particles in the environment was investigated worldwide, mostly in ambient air and in living spaces (e. g., Bovallius et al., 1978b; Chen et al., 2008; Hu et al., 1994a; Fang et al., 2005; Hu et al., 1994b). Despite the environmental and (occupational) medical relevance fewer studies were conducted in waste management sites (e. g. Heo et al., 2010), sewage works or wastewater spray irrigation sites (e. g. Brandi et al., 2000; Bausum et al., 1982) or in hospitals (Noble et al., 1963a). Some measurements took also place in such exotic places as a war vessel (Wright et al., 1968), a research ship (Pósfai et al., 2003) or in a subterranean sanatorium (Frączek and Grzyb, 2010). In majority of investigations the six-stage Andersen sampler was used and therefore most data is available for this sampling system.

Figure 1 shows the size distribution of airborne particles carrying cultivable mesophilic bacteria in different environments obtained with the six-stage Andersen sampler. The box and whiskers plots represent the summarized results of different studies and include different numbers of medians or arithmetic means. Attention should be paid to the unequal widths of the size classes of the six-stage Andersen sampler and to the fact that, due to its inlet efficiency, only particles < 12 µm AD were sampled.

Although large variations can be found there are clear differences among the investigated environments. In ambient air only 15 % of the bacteria-laden particles are < 2.1 µm AD and more than 25 % are > 7.2 µm AD (medians). Lighthart (1997) presented similar results in his review article but with 40 % particles > 7 µm. In livestock husbandry and in waste management more than 90 % of the particles are > 2.1 µm AD, in the latter even 45 % are > 7.2 µm AD. The reason could be the combination of large area sources for airborne micro-organisms (soil, high animal numbers, waste) together with a generally high activity (wind, animal activity, compost shifting) leading to aerosolisation of a higher percentage of larger particles. A generally high activity is also found in public buildings as well as in public areas of hospitals. However, there is a lack of sources for airborne micro-organisms because these areas normally have easy to clean surfaces and effective air cleaning systems. Probably for this reason most of the bacteria-laden particles were found between 1.1 µm AD and 2.1 µm AD in these environments. Clauß et al. (2013a) found only a slight increase of the concentration of particles carrying bacteria in the air during the opening hours of an international trade fair, depending on the number of visitors and mainly by skin scales and small liquid droplets. The investigated exhibition hall also had large air filter systems. In the food and feedstuff industry the size distribution is similar to the one for public buildings and offices, probably for the same reasons. In contrast, in the median of the living spaces 25 % of the bacteria-laden particles are > 7.2 µm AD, probably due to additional sources for airborne micro-organisms like carpets, plants, domestic animals or damp walls and mouldy wallpapers. Reponen et al. (1992) found a short-period increase in size of airborne particles carrying micro-organisms caused by vacuum cleaning and potting plants, accompanied by increasing concentrations of *Penicillium* species. In sewage works, the size of most of the bacteria-laden particles is between 2.1 µm AD and 3.3 µm AD. Probably the wastewater processing generates many small droplets carrying bacteria. At least the size distribution of bacteria-laden particles in operating theatres follows no clear pattern. The concentration found in this area is so low that no clear trend can be deduced. These low concentrations are probably due to complex air ventilation and filter systems and high hygienic standards.

In general it should be also considered that there may be differences regarding the size distributions of particles carrying micro-organisms within a type of environment and even within the same facility. For example Bovallius et al. (1978a) investigated ambient air and found different size distributions of bacteria-laden particles above the Swedish mainland (37.8 % > 7.2 µm AD) compared to the coast (48.9 % > 7.2 µm AD). Brandi et al. (2000) examined the size distribution of bacteria-laden particles in a newly build sewage plant and found that 35.4 % of the particles were between 0.65 µm AD and 2.1 µm AD at the beginning but only 20.2 % after 25 days. This shows that due to various circumstances such as progressing biofilm formation or differing air humidity (s. a. chapter 4) particle size distribution can be influenced considerably within one environment.

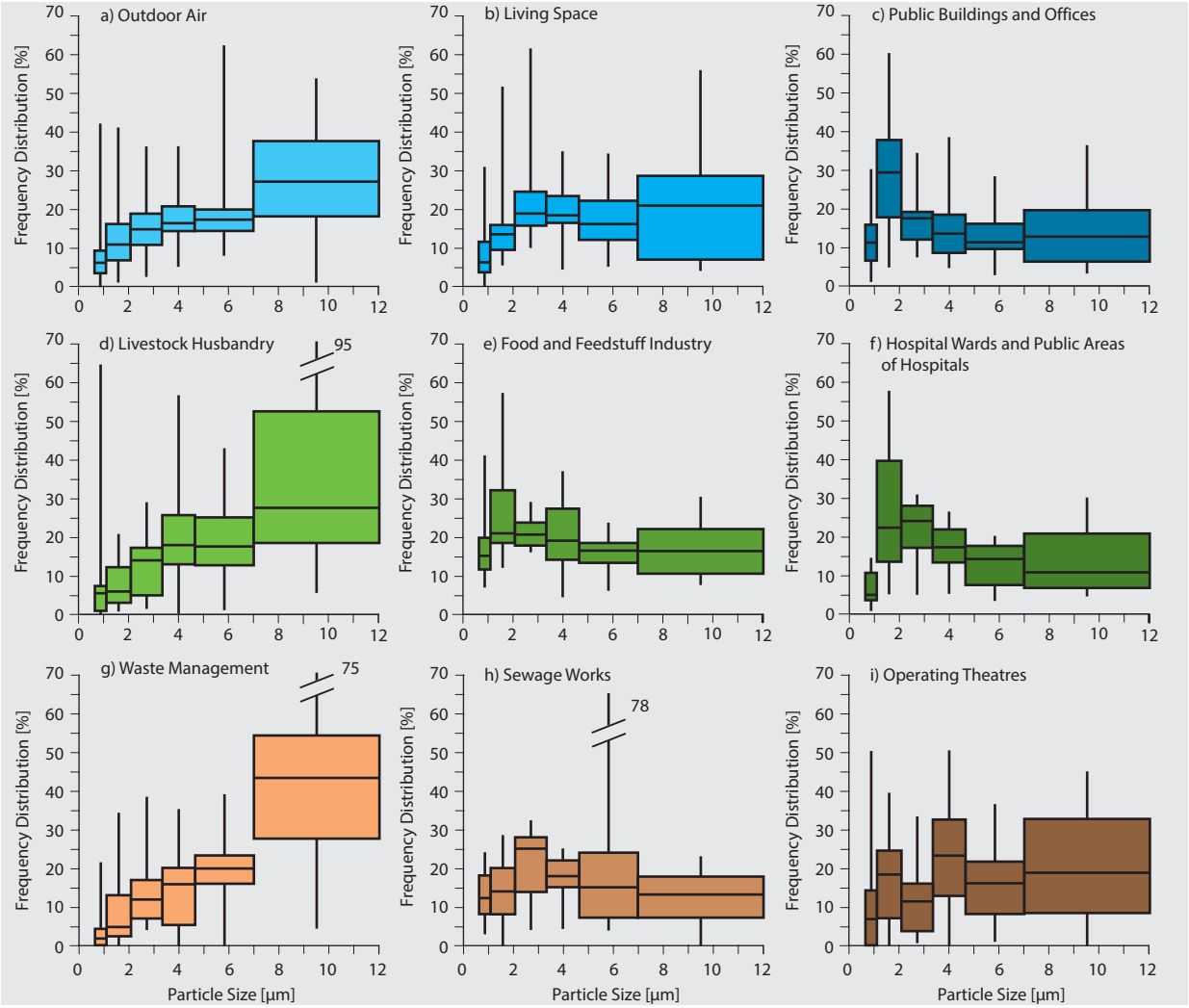

Figure 1

Size distribution of airborne particles carrying culturable mesophilic bacteria in different environments (a-i). Data basis [n = number of data rows, number of individual measurements]: a) Bovallius et al., 1978a; Chen et al., 2012; Fang et al., 2008; Glysson et al., 1974; Gołofit-Szymczak and Górny, 2010; Kim et al., 2009; Li et al., 2011; Lighthart and Shaffer, 1995; Moschandreas et al., 2003; Nasir et al., 2012, 2013; Nasir and Colbeck, 2012; Raisi et al., 2010, 2013; Rajasekar and Balasubramanian, 2011; Roobsuaydee et al., 2010; Rosas et al., 1994; Shilpa et al., 2013; Tsai and Liu, 2009; Wright et al., 1969; Wu and Yao, 2011; Xu and Yao, 2013 [n = 69, 4368]. b) Colbeck and Nasir, 2009; Fang et al., 2013; Moschandreas et al., 2003; Nasir et al., 2012; Nasir and Colbeck, 2010, 2012; Simard et al., 1983; Wu and Yao, 2011; Xu et al., 2013; Xu and Yao, 2013 [n = 37, 1753]. c) Grigorevski-Lima et al., 2006; Gołofit-Szymczak and Górny, 2010; King and McFarland, 2012; Meklin et al., 2002; Roobsuaydee et al., 2010; Rajasekar and Balasubramanian, 2011; Shilpa et al., 2013; Wang et al., 2010; Wu and Yao, 2011; Xu and Yao, 2013 [n = 22, 1183]. d) Aarnink et al., 2012; Adell et al., 2011a; b; Chai et al., 2001; Chinivasagam and Blackall, 2005; Lenhart et al., 1982; Liu and Ma, 2010; Sowiak et al., 2011; Siggers et al., 2011; Zhao, 2011; Zheng et al., 2013 [n = 26, 155]. e) Kim et al., 2009; Tsai and Liu, 2009 [n = 3, 15]. f) Coggins et al., 2012; Nasir et al., 2013; Pastuszka et al., 2005 [n = 6, 67]. g) Byeon et al., 2008; Glysson et al., 1974; Rahkonen et al., 1990; Zhang et al., 2009, 2012 [n = 43, 385]. h) Kim et al., 2012; Laitinen et al., 1994; Li et al., 2013; Zhao, 2011 [n = 13, 109]. i) Nasir et al., 2013; Pankhurst et al., 2012; Pastuszka et al., 2005 [n = 11, 75].

The size distributions of airborne particles carrying fungi are totally different from those carrying bacteria (Figure 2). In almost all areas most of the particles are between 1.0 µm AD and 3.2 µm AD. Probably the particle size distributions are representing the size distribution of the predominant mould species at the sampling location, because mould spores are occurring as single spores in more than 65 % (Heikkilä et al., 1988; Pasanen et al., 1989). However, according to Kanaani et al. (2009), the particle size distributions also depend on the wind, the method of aerosolisation, and on the environment. Vijay et al. (1999) stated that the size of mould spores in ambient air is mostly between 2 µm and 20 µm, Reponen et al. (1994) found spore sizes up to 10 µm in indoor air. However, in this review the calculated median size for fungi-laden particles in living spaces is between 3.2 µm AD and 4.8 µm AD and for that higher than in ambient air. A possible reason

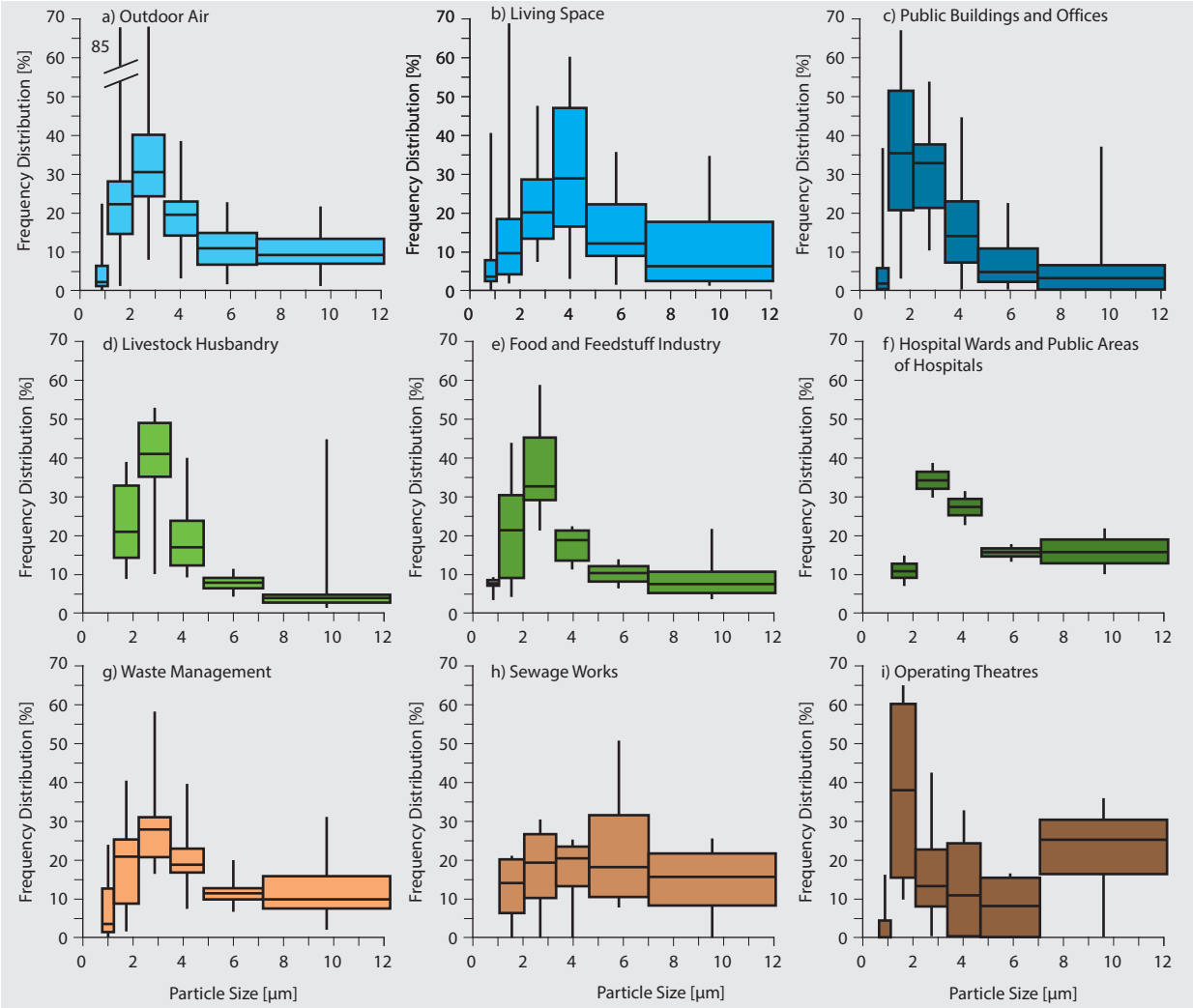

Figure 2

Size distribution of airborne particles carrying culturable mesophilic fungi in different environments (a to i). Data basis [n = number of data rows, number of individual measurements]: a) Fang et al., 2008; Gołofit-Szymczak and Górny, 2010; Kim et al., 2009; Lin and Li, 1996; Li et al., 2011; Nasir et al., 2012, 2013; Nasir and Colbeck, 2012; Rajasekar and Balasubramanian, 2011; Raisi et al., 2013; Roobsuaydee et al., 2010; Rozej et al., 2011; Shilpa et al., 2013; Tsai and Liu, 2009; Wang et al., 2010; Wu and Yao, 2011; Xu et al., 2013; Xu and Yao, 2013 [n = 47, 1406]. b) Fang et al., 2013; Hyvärinen et al., 2001; Nasir et al., 2012; Nasir and Colbeck, 2010, 2012; Reponen et al., 1992; Xu and Yao, 2013 [n = 32, 219]. c) Gołofit-Szymczak and Górny, 2010; Grigorevski-Lima et al., 2006; Meklin et al., 2002; Rahkonen et al., 1990; Rajasekar and Balasubramanian, 2011; Roobsuaydee et al., 2010; Rozej et al., 2011; Shilpa et al., 2013; Wang et al., 2010; Wu and Yao, 2011; Xu and Yao, 2013 [n = 32, 1520]. d) Chien et al., 2011; Liu and Ma, 2010; Siggers et al., 2011 [n = 10, 23]. e) Abdel Hameed et al., 2007; Kim et al., 2009; Tsai und Liu, 2009 [n = 6, 24]. f) Coggins et al., 2012; Nasir et al., 2013 [n = 2, 55]. g) Reinthaler et al., 1997; Zhang et al., 2009, 2012 [n = 12, 248]. h) Kim et al., 2012; Li et al., 2013 [n = 4, 77]. i) Nasir et al., 2013 [n = 4, 64].

could be that many of the studies that investigated the size distribution of fungi indoors were conducted in buildings with obvious mould problems. In this regard Reponen et al. (1994) found larger mould-laden particles in mouldy houses than in houses without such a problem. The distribution of particles carrying fungi is comparatively even in waste management. Especially the different biological materials as sources for airborne fungi and the high activity in this environment could lead to aerosolisation of many different species with different spore sizes. Similarly the even more equal distribution in sewage plants is still unexplained; presumably the data basis is too low. For comprehensible reasons in operating theatres the concentrations of fungi-laden particles are also very low but with a peak between 1.0 µm AD and 2.1 µm AD. So it can be supposed that especially the large fungi particles were eliminated from the air by the filter systems.

In summary differences can be found in the median size distributions of airborne particles carrying bacteria or fungi among the different environments. Between 0.65 µm AD and 12 µm AD the size of bacteria-laden particles mainly seems to be dependent on the kind of source and the mechanism

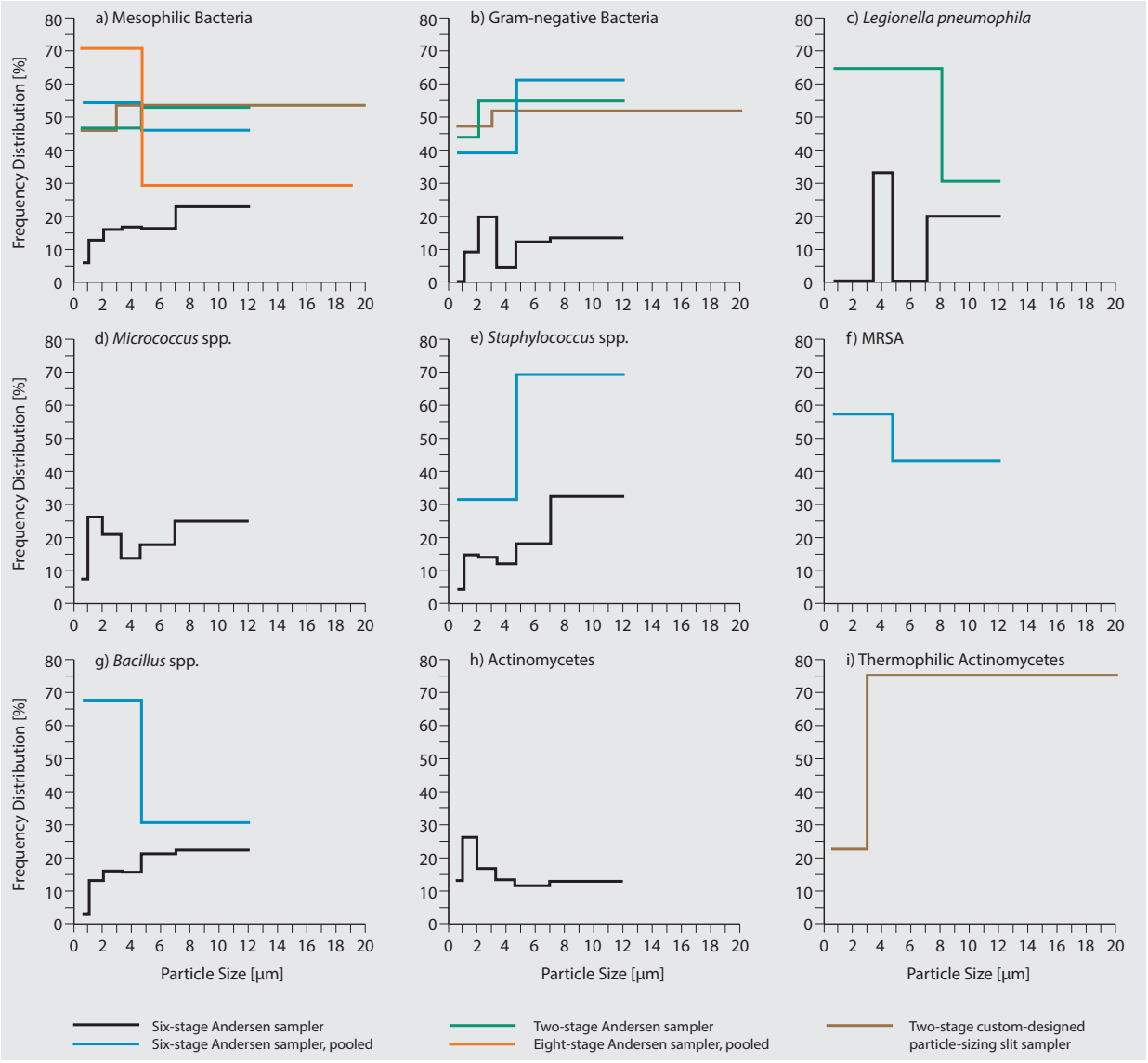

Figure 3

Size distribution of airborne particles carrying selected groups of bacteria (a to i). Data basis [n = number of data rows, number of individual measurements]: Six-stage Andersen sampler: a) see figure 1 [n = 242, 8164]. b) Lenhart et al., 1982; Lundholm, 1982; Nasir et al., 2012, 2013 [n = 17, 83]. c) Bollin et al., 1985 [n = 3, 3]. d) Górny et al., 1999; Kim et al., 2006, 2010; Kim and Kim, 2007 [n = 12, 193]. e) Coggins et al., 2012; Górny et al., 1999; Kim et al., 2006, 2010; Kim and Kim, 2007; Moschandreas et al., 2003 [n = 18, 2080]. g) Górny et al., 1999; Kim et al., 2006, 2010; Kim and Kim, 2007 [n = 12, 193]. h) Fang et al., 2008; Grigorevski-Lima et al., 2006; Li et al., 2012, 2013; Raisi et al., 2013; Zhang et al., 2009 [n = 13, 1060]. Six-stage Andersen sampler, pooled: a) Butera et al., 1991; Cormier et al., 1990; Ferguson, 2012; Kim and Kim, 2007; Lembke et al., 1981; Lis et al., 2008; Predicala et al., 2002 [n = 89, 399]. b) Chen et al., 2012; Clark et al., 1983; Cormier et al., 1990; Rosas et al., 2001 [n = 13, 92]. e) Chen et al., 2012 [n = 3,3]. f) Ferguson, 2012 [n = 8, 24]. g) Chen et al., 2012 [n = 3, 3]. Two-stage slit sampler: a) Dutkiewitcz et al., 1994, 2000, 2001a, b, 2002; Krysinska-Traczyk et al., 2002, 2004; Prazmo et al., 2003a, b [n = 103, 1290]. b) Dutkiewitcz et al., 1994, 2002; Krysinska-Traczyk et al., 2004; Prazmo et al., 2003a [n = 50, 404]. i) Dutkiewitcz et al., 1994, 2001a, b, 2002; Krysinska-Traczyk et al., 2004; Prazmo et al., 2003a, b [n = 79, 1114]. Two-stage Andersen sampler: a) Alvarado et al., 2009; Awad et al., 2013; Curtis et al., 1978; Jones and Cookson, 1983; Lester, 2008; Mota et al., 2008a; Zhu et al., 2003a, b [n = 65, 1095]. b) Lester, 2008 [n = 4, 67]. c) Bollin et al., 1985 [n = 5, 5]. Eight-stage Andersen sampler, pooled: a) Curtis et al., 1975; 1978 [n = 56, 112].

of aerosolisation, whereas the size of fungi-laden particles mainly seems to be dependent on the cell or spore size of the predominant species. There is a lack of information for particles > 12 µm AD, due to limitations of the size selective sampling systems that were used.

3.2 Size distribution of airborne particles carrying selected groups of micro-organisms

This chapter subsumes the study results for the size distribution of airborne particles carrying selected micro-organisms independent from the environment or the source. Especially

groups or species that are of environmental or hygienic relevance, or for which a lot of data are available, were chosen for the compilation. Figure 3 shows the size distribution of airborne particles carrying selected groups of bacteria. Here, also attention should be paid to the unequal size class widths of the different sampling systems.

There are differences regarding the median particle size distributions between the different bacteria as well as among the sampling systems. Some of these results seem to be contradictory. For example with the pooled two- or eight-stage Andersen sampler more particles carrying mesophilic bacteria were found with < 4.8 µm AD, whereas with the two- and six-stage Andersen sampler and with the two-stage slit sampler more particles were found in the larger particle classes. Also for Gram-negative bacteria the results obtained with three two-stage systems differ from the results obtained with the six-stage Andersen sampler. With the latter a peak at 1.0 µm AD and 2.1 µm AD was detected whereas with the other systems most Gram-negative bacteria were found in the larger particle size classes. The size distributions presented for *Legionella pneumophila* are not reliable due to a lack of data. The median size distribution of particles carrying *Micrococcus luteus* is in accordance with the finding that these species often form small aggregates of only a few cells. *Staphylococcus spp.* seems to appear mainly in larger aggregates, whereas MRSA were found on particles of < 4.8 µm AD. The curves presented for particles carrying *Bacillus spp.* are inconsistent. In the air especially the resistant endospores of this group should be expected. The size of such endospores normally ranges between 0.8 µm to 1.0 µm, which at least is in accordance with the results of Chen et al. (2012). However, the median size distribution obtained with the six-stage Andersen sampler indicates that spores of *Bacillus spp.* may also exist in larger aggregates in the airborne state. The size distribution of particles carrying the likewise spore-forming actinomycetes shows a peak between 1.0 µm AD and 2.1 µm AD. This could be an indication for single airborne spores of this size range. In contrast 75 % of particles carrying thermophilic actinomycetes found with the two-stage slit sampler were in the size fraction > 3.0 µm AD.

In summary, most of the presented median size distributions of particles carrying different selected bacteria groups or species have to be scrutinized. The differences among the different groups as well as among the results of the different sampling systems may be due to the different sampling locations. Most bacteria occur in aggregates in the airborne state and their sizes are presumably dependent on the source and the method of aerosolisation and not on the group or species itself.

Table 2 and 3 specify further investigations of the size distribution of airborne particles carrying selected bacteria groups or species that were not mentioned before, in which different sampling systems were used. There is also a trend that the size distribution is mainly dependent on the sampling location. Similar to Figure 1, higher median percentages of bacteria-laden particles were found in livestock husbandry, ambient air and waste management followed by public buildings and offices than were found in the other areas.

Figure 4 shows the median size distribution of airborne particles carrying a selected fungi group or species. Here there are also differences regarding the median particle size distributions among the different fungi as well as among the

Table 2

Study results on the size distribution of airborne particles carrying other selected groups of bacteria when the six-stage Andersen sampler was used for sampling.

Microorganism	Sampling Location	Median % per Stage						Reference
Six-stage Andersen Sampler, Stage:		**6**	**5**	**4**	**3**	**2**	**1**	
Particle Sizes In Each Stage [µm]:		**0.6 - 1.0**	**1.0 - 2.1**	**2.1 - 3.2**	**3.2 - 4.8**	**4.8 - 7.2**	**7.2 - 12.0**	
Aeromonas spp.	Living Space	17	17	16	21	18	20	Górny et al., 1999
α-Hemolytic Bacteria	Waste Incinerator Plant	7	7	18	12	13	44	Glysson et al., 1974
β-Hemolytic Bacteria	Waste Incinerator Plant	31	31	12	9	5	0	Glysson et al., 1974
Coliform Bacteria	Outdoor Air	1	1	2	5	36	55	Rosas et al., 1994
Corynebacterium spp.	Nursing	25	25	13	11	21	23	Kim and Kim, 2007
Enterobacteriaceae	Pig House	6	6	9	12	13	49	Siggers et al., 2011
Enterobacteriaceae	Poultry Slaughterhouse	0	0	4	5	14	75	Lenhart et al., 1982
Escherichia coli	Waste Incinerator Plant	0	0	0	100	0	0	Glysson et al., 1974
Facultative Anaerobic Bacteria	Living Space	8	8	13	18	24	36	Hambraeus and Benediktsdottir, 1980
Marine Bacteria	Coastal Outdoor Air	11	11	17	20	18	33	Li et al., 2011
Nocardia spp.	Living Space	53	53	20	0	13	0	Górny et al., 1999
Pseudomonas spp.	Living Space	15	15	19	21	18	28	Górny et al., 1999
Staphylococcus aureus	Hen House	35	23	37	4	2	0	Chai et al., 2001
Strictly Anaerobic Bacteria	Living Space	7	7	6	25	32	25	Hambraeus and Benediktsdottir, 1980

Table 3
Study results on the size distribution of airborne particles carrying other selected groups of bacteria when systems other than the six-stage Andersen sampler were used for sampling.

Microorganism	Sampling Location	Median % per Stage				Reference
Custom-designed Particle-sizing Slit Sampler, Stage:		**2**	**1**			
Particle Sizes In Each Stage [µm]:		**< 3.0**	**> 3.0**			
Lactobacillus spp.	Herb Processing Plant	0	100			Dutkiewitcz et al., 2001b
Size-grading Slit Sampler, Stage:		**1**	**2**	**3**	**4**	
Particle Sizes In Each Stage [µm]:		**0.9 - 4.2**	**4.2 - 9.6**	**9.,6 - 18.2**	**18.2 - 28**	
Streptococcus spp.	Office	Median ø 10.0 - 12.4 µm				Noble et al., 1963a
Streptococcus salivarius	Office	Median ø 11.0 - 14.4 µm				Noble et al., 1963a
β-Hemolytic Streptococci	Office	Median ø 11.7 - 12.5 µm				Noble et al., 1963a
Enterococcus spp.	Office	Median ø 10.8 - 11.0 µm				Noble et al., 1963a
Staphylococcus aureus	Hospital Ward	Median ø 13.3 - 15.7 µm				Noble et al., 1963a
Bacillus spp.	Outdoor Air	Median ø 3.0 µm				Noble et al., 1963a
Clostridium welchii	Outdoor Air/Hospital	Median ø 11.0 - 17.2 µm				Noble et al., 1963a
Clostridium welchii	Outdoor Air/Hospital	14	19	30	36	Noble, 1961
Six-stage Andersen Sampler, Pooled, Stages:		**6 – 3**	**2,1**			
Particle Sizes In Each Stage [µm]:		**0.6 - 4.8.**	**4.8 -12**			
Bacillus cereus	Outdoor Air	23	77			Chen et al., 2012
Bacillus subtilis	Outdoor Air	95	5			Chen et al., 2012
Enterobacter cloacae	Outdoor Air	91	9			Chen et al., 2012
Faenia rectivirgula	Pig Houses	0	100			Cormier et al., 1990
Klebsiella pneumonia	Outdoor Air	83	17			Chen et al., 2012
Micrococcus luteus	Outdoor Air	100	0			Chen et al., 2012
Pseudomonas aeruginosa	Outdoor Air	100	0			Chen et al., 2012
Pseudomonas putida	Outdoor Air	57	43			Chen et al., 2012
Serratia marcescens	Outdoor Air	92	8			Chen et al., 2012
Staphylococcus capitis	Outdoor Air	100	0			Chen et al., 2012
Staphylococcus epidermidis	Outdoor Air	87	13			Chen et al., 2012
Staphylococcus hominis	Outdoor Air	17	83			Chen et al., 2012
Staphylococcus lugdunensis	Outdoor Air	74	26			Chen et al., 2012
Staphylococcus saprophyticus	Outdoor Air	83	17			Chen et al., 2012
Staphylococcus simulans	Outdoor Air	65	35			Chen et al., 2012
Staphylococcus warneri	Outdoor Air	90	10			Chen et al., 2012
Streptococcus mitis	Outdoor Air					Chen et al., 2012
Eight-stage Andersen Sampler, Pooled, Stages:		**7 - 3**	**2 - 0**			
Particle Sizes In Each Stage [µm]:		**0.4 - 4.7**	**> 4.7**			
Coliform Bacteria	Pig Houses	91	9			Curtis et al., 1975
Staphylococcus spp.	Pig Houses	79	21			Curtis et al., 1975
Streptococcus spp.	Pig Houses	79	21			Curtis et al., 1975

sampling systems. In contrast to bacteria-laden particles, the size distribution of particles carrying different selected moulds mainly describes the size distribution of their spores. For example, the average diameter of *Aspergillus fumigatus* spores is 2.5 µm to 3.0 µm (Madsen et al., 2009). With the different sampling systems the highest percentages were found exactly in this range. This is also true for *Penicillium spp.*, *Cladosporium spp.* and *Cryptococcus neoformans*. Sometimes there are also apparent differences among the sampling systems. The median particle size distribution of *Aspergillus spp.* shows a peak at 2.1 µm AD to 3.2 µm AD for measurements in different environments with the six-stage Andersen sampler, analogue to the average spore size of *Aspergillus* species. With the pooled six-stage Andersen sampler higher median percentages were found for *Aspergillus*-laden particles > 4.8 µm AD. However, this distribution

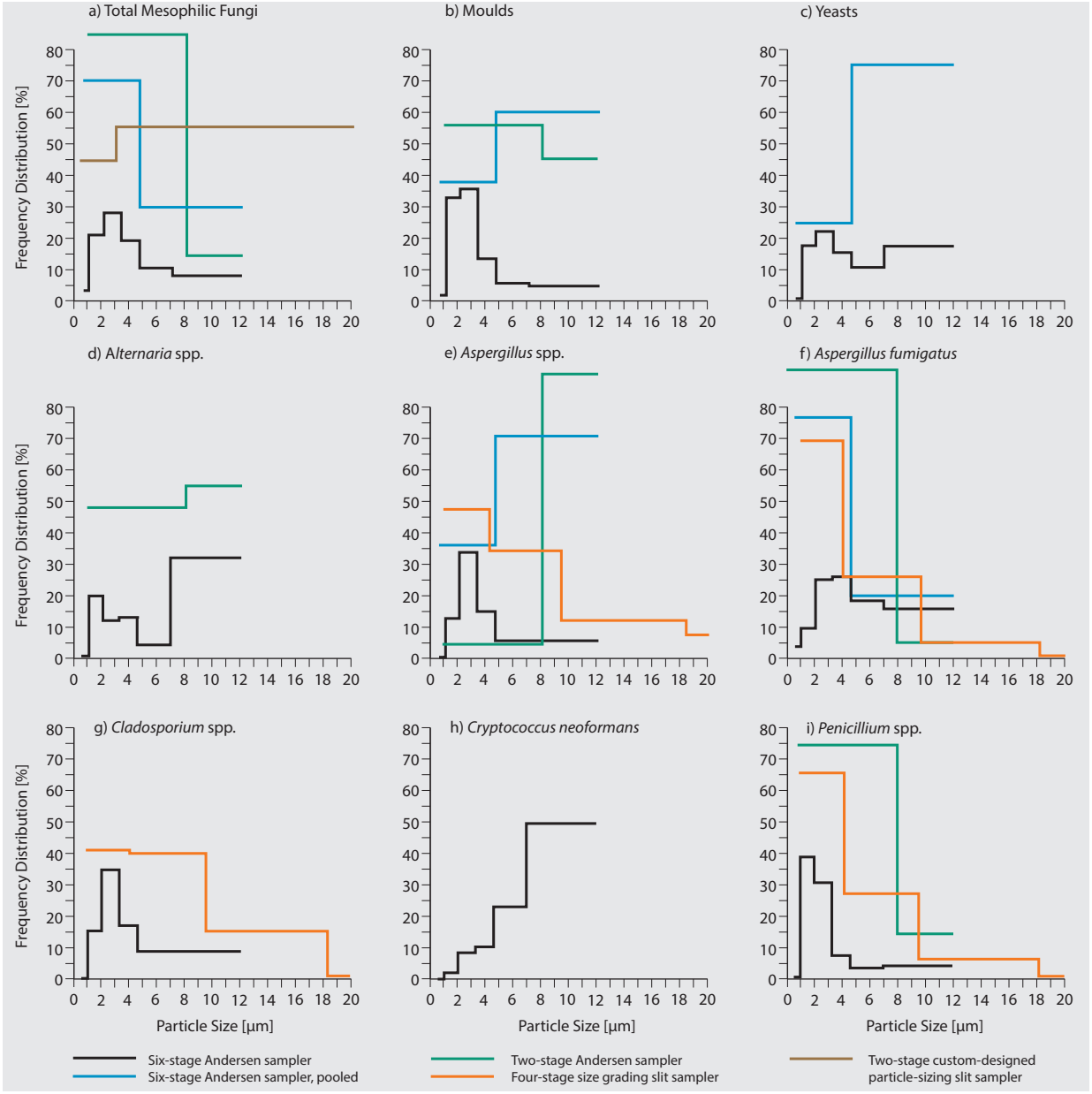

Figure 4

Size distribution of airborne particles carrying selected groups of fungi (a-i). Data basis [n = number of data rows, number of individual measurements]: Six-stage Andersen Sampler: a) see figure 2 [n = 43, 1324]. b) Colbeck and Nasir, 2009; Raisi et al., 2010; Reponen et al., 1994; Sowiak et al., 2011; Yu et al., 2013; Zuraimi et al., 2009 [n = 38, 2526]. c) Górny et al., 1999; Lin and Li, 1996; Reponen, 1995 [n = 17, 132]. d) Fang et al., 2008, 2013; Kim et al., 2006, 2010; Lin and Li, 1996; Sayer et al., 1969 [n = 18, 1128]. e) Abdel Hameed et al., 2007; Deacon et al., 2009; Fang et al., 2008, 2013; Górny et al., 1999; Kim et al. 2006, 2010; Kim and Kim, 2007; Lin and Li, 1996; Marchisio et al., 1989; Millner et al., 1980; Reponen, 1995; Sayer et al., 1969; Zuraimi et al., 2009 [n = 41, 2583]. f) Deacon et al., 2009; Millner et al., 1980 [n = 2, 33]. g) Fang et al., 2008, 2013; Kim et al., 2006, 2010; Kim and Kim, 2007; Lin and Li, 1996; Marchisio et al., 1989; Reponen, 1995; Zuraimi et al., 2009 [n = 31, 2394]. h) Powell et al., 1972; Ruiz and Bulmer, 1981 [n = 7, 12]. i) Fang et al., 2008, 2013; Górny et al., 1999; Kim and Kim, 2007; Kim et al., 2006, 2010; Lin and Li, 1996; Marchisio et al., 1989; Reponen, 1995; Sayer et al., 1969; Zuraimi et al., 2009 [n = 40, 2584]. Six-stage Andersen sampler, pooled: a) Chen et al., 2012; Kim and Kim, 2007; Lis et al., 2008 [n = 11, 93]. b) Cormier et al., 1990; Rosas et al., 2001 [n = 6, 26]. c) Cormier et al., 1990 [n = 4, 24]. e) Cormier et al., 1990 [n = 4, 24]. f) Clark et al., 1983 [n = 7, 68]. Two-stage custom-designed particle-sizing slit sampler: a) Dutkiewitcz et al., 1994, 2001a, b, 2002; Krysinska-Traczyk et al., 2002, 2004; Prazmo et al., 2003a, b [n = 93, 1254]; Two-stage Andersen sampler: a) Alvardo et al., 2009; Awad et al., 2013; Mota et al., 2008a, b [n = 23, 924]. b) Lester, 2008 [n = 4, 67]. d) Mota et al., 2008b; Rosas et al., 1997 [n = 10, 509]. e) Jones and Cookson, 1983; Mota et al., 2008b; Rosas et al., 1997 [n = 11, 541]; f) Jones and Cookson, 1983 [n = 1, 94]. i) Lacey, 1973; Rosas et al., 1997 [n = 6, 328]. Four-stage particle-sizing slit sampler: e, f, g, i) Noble et al., 1963b n = 1, 7].

Table 4
Study results on the size distribution of airborne particles carrying other selected groups of fungi when the six-stage Andersen sampler was used for sampling.

Microorganism	Sampling Location	Median % per Stage						Reference
Six-stage Andersen Sampler, Stage:		**6**	**5**	**4**	**3**	**2**	**1**	
Particle Sizes In Each Stage [µm]:		**0.6 - 1.0**	**1.0 - 2.1**	**2.1 - 3.2**	**3.2 - 4.8**	**4.8 - 7.2**	**7.2 - 12.0**	
Alternaria alternata	Outdoor Air	0	0	0	11	33	56	Marchisio et al., 1989
Aspergillus niger	Outdoor Air	0	0	33	67	0	0	Marchisio et al., 1989
Aspergillus versicolor	Outdoor Air	0	0	43	57	0	0	Marchisio et al., 1989
Aspergillus flavus	Corn Dust	0	2	24	38	21	15	Hill et al., 1984
Balcomycetidae	Outdoor Air	19	15	15	7	9	34	Marchisio et al., 1989
Bothrytis cinerea	Outdoor Air	38	38	13	13	0	0	Marchisio et al., 1989
Blastomycetidae	Outdoor Air	19	15	15	8	9	34	Marchisio et al., 1989
Candida albicans	Indoor and Outdoor Air	0	20	0	20	40	20	Sayer et al., 1969
Chaetonium indicum	Outdoor Air	0	0	0	7	14	79	Marchisio et al., 1989
Cladosporium cladosporoides	Outdoor Air	4	17	34	25	19	1	Marchisio et al., 1989
Cladosporium herbarum	Outdoor Air	17	5	78	0	0	0	Marchisio et al., 1989
Diplospora spp.	Indoor and Outdoor Air	0	0	9	4	44	44	Sayer et al.,1969
Emericelle nidulans	Outdoor Air	57	0	22	22	0	0	Marchisio et al., 1989
Epicoccum spp.	Indoor and Outdoor Air	0	0	0	0	8	92	Sayer et al., 1969
Eurotium amstelodami	Outdoor Air	15	23	31	15	0	15	Marchisio et al., 1989
Fusarium monoliforme	Corn Field	18	2	16	22	12	30	Ooka and Kommendahl, 1977
Fusarium spp.	Outdoor Air	0	13	16	29	20	13	Lin and Li, 1996
Geotrichum spp.	Kindergarden	0	46	34	8	3	1	Zuraimi et al., 2009
Gliocladium spp.	Indoor and Outdoor Air	5	3	17	60	15	0	Sayer et al., 1969
Hemispora spp.	Indoor and Outdoor Air	1	30	47	11	6	5	Sayer et al,. 1969
Hormonema spp.	Outdoor Air	30	20	10	0	20	20	Marchisio et al., 1989
Hormodendrum spp.	Outdoor Air	0	0	6	26	37	29	Sayer et al., 1969
Marine Fungi	Coastal Outdoor Air	1	14	43	22	11	9	Li et al., 2011
Monilia sitophilia	Indoor and Outdoor Air	0	0	0	0	100	0	Sayer et al., 1969
Monotospora spp.	Indoor and Outdoor Air	0	0	0	4	53	43	Sayer et al., 1969
Nigrospora spp.	Indoor and Outdoor Air	0	0	0	0	0	100	Sayer et al., 1969
Oospora spp.	Indoor and Outdoor Air	0	40	0	60	0	0	Sayer et al., 1969
Paecilomyces spp.	Indoor and Outdoor Air	0	0	9	26	65	0	Sayer et al., 1969
Paecilomyces varioti	Outdoor Air	13	13	20	53	0	0	Marchisio et al., 1989
Penicillium italicum	Outdoor Air	0	36	43	21	0	0	Marchisio et al., 1989
Penicillium purpurogenum	Outdoor Air	0	25	0	12	25	37	Marchisio et al., 1989
Penicillium verrucosum	Outdoor Air	0	5	14	73	0	9	Marchisio et al., 1989
Pullularia spp.	Indoor and Outdoor Air	0	0	28	27	22	23	Sayer et al., 1969
Rhinocladiella mansonii	Outdoor Air	50	14	5	9	14	9	Marchisio et al., 1989
Rhizopus spp.	Indoor and Outdoor Air	1,7	0	1,7	42	43	12	Sayer et al., 1969
Rhodoturula spp.	Indoor and Outdoor Air	0	0	22	33	22	22	Sayer et al., 1969
Sacharomyces spp.	Indoor and Outdoor Air	0	9	6	10	19	57	Sayer et al., 1969
Scytalidium spp.	Outdoor Air	66	24	7	2	0	0	Marchisio et al., 1989
Sepedonium spp.	Indoor and Outdoor Air	0	0	25	0	25	50	Sayer et al., 1969
Stemphilium spp.	Indoor and Outdoor Air	0	0	1	4	18	78	Sayer et al., 1969
Streptomyces spp.	Indoor and Outdoor Air	25	0	0	0	50	25	Sayer et al., 1969
Trichophyton spp	Outdoor Air	0	0	0	17	39	40	Lin and Li, 1996
Ustilago zeae	Indoor and Outdoor Air	0	84	12	0	4	0	Sayer et al., 1969

Table 5
Study results on the size distribution of airborne particles carrying other selected groups of fungi when systems other than the six-stage Andersen sampler were used for sampling.

Microorganism	Sampling Location	Median % per Stage				Reference
Two-stage Andersen Sampler, Stage:		**2**	**1**			
Particle Sizes In Each Stage [µm]:		**0.95 - 8.0**	**8.0 - 12**			
Aphanocladium spp.	Cork Factory	95	5			Lacey, 1973
Bipolaris spp.	Indoor and Outdoor Air	48	52			Mota et al., 2008b
Cercospora spp.	Indoor and Outdoor Air	50	50			Mota et al., 2008b
Monila spp.	Cork Factory	10	90			Lacey, 1973
Mucor spp.	Cork Factory	16	84			Lacey, 1973
Phoma spp.	Indoor and Outdoor Air	36	64			Mota et al., 2008b
Rhizopus spp.	Indoor and Outdoor Air	43	57			Mota et al., 2008b
Stachybothrys spp.	Indoor and Outdoor Air	50	50			Mota et al., 2008b
Stemphylium spp.	Indoor and Outdoor Air	49	51			Mota et al., 2008b
Thermophilic moulds	Outdoor Air	91	9			Jones and Cookson, 1983
Size-grading Slit Sampler, Stage:		**1**	**2**	**3**	**4**	
Particle Sizes In Each Stage [µm]:		**0.9 - 4.2**	**4.2 - 9.6**	**9.6 - 18.2**	**18.2 - 28**	
Aspergillus niger	Hospital Ward	33	47	16	4	Noble et al., 1963b
Didymocladium spp.	Hospital Ward	5	32	49	15	Noble et al., 1963b
Monilia sitophila	Hospital Ward	3	50	39	8	Noble et al., 1963b
Paecilomyces spp.	Hospital Ward	55	35	6	4	Noble et al., 1963b
Rhizopus spp.	Hospital Ward	26	39	31	4	Noble et al., 1963b
Rhodoturula spp.	Hospital Ward	57	38	5	0	Noble et al., 1963b
Syncephalastrum spp.	Hospital Ward	24	47	26	3	Noble et al., 1963b

represents only few measurements in pig houses conducted by Cormier et al. (1990). Because of the high dust concentrations in pig houses there is a higher probability that mould spores are attached to larger particles. This finding shows that, as for bacteria, the sampling location also has an influence on the particle size distribution of mould spores.

Tables 4 and 5 specify further investigations of the size distribution of airborne particles carrying different groups or species of fungi that were not mentioned before, in which different sampling systems were used. Here, as before, the size distribution of particles carrying different fungi mainly describes by trend the size distribution of their spores. Also an influence of the sampling location or rather of the source or the method of aerosolisation is shown. For example in Table 4 the median size of airborne particles carrying different *Aspergillus* species with similar spore size (Marchisio et al., 1989) are differently distributed in ambient air compared to corn dust (Hill et al., 1984).

3.3 Number distribution of airborne micro-organisms in different particle size fractions

The previous two chapters deal only with the size distribution of airborne particles that carry different micro-organisms, independent of the actual number of micro-organisms on such a particle. King und McFarland (2012) showed that there may be large differences in this regard. In each stage of a six-stage Andersen sampler they covered half of the nutrient plates with a filter to get the number of all bacteria corresponding to the number of bacteria-laden particles by eluting the filter after sampling in a liquid followed by cultivation. With this method they found ten times more bacteria than bacteria-laden particles in the air of classrooms. Assuming the densest sphere packing, and a cell size of 1 µm, a bacteria aggregate of 5 µm diameter may theoretically consist of 100 bacteria cells, a 10 µm aggregate even of 650 cells.

In contrast to the large number of studies dealing with the size distribution of airborne particles carrying micro-organisms, studies on the number distribution of airborne micro-organisms in different particle size fractions are rare. Table 6 shows the mean percentages of colony forming units of different airborne micro-organisms in different particle size fractions according to Predicala et al. (2002). In a pig house most airborne bacteria were found in the particle size fraction > 4.0 µm AD, especially even about 80 % to 90 % for bacteria groups that include pathogens such as staphylococci or *Listeria*.

Clauß et al. (2011a) used a fluorescence microscopic method to investigatethe number and size of bacteria-laden

Table 6
Mean percentages of colony forming units of different airborne micro-organisms in selected particle size fractions

Microorganism	Sampling Location	Median % per Stage		Reference
Membrane Filter + Cyclon Pre-impactor, Stage:		**2**	**1**	
Particle Sizes In Each Stage [µm]:		**< 4.0**	**> 4.0**	
Mesophilic Bacteria	Pig Houses	16	84	Predicala et al., 2002
Staphylococcus spp.	Pig Houses	12	88	Predicala et al., 2002
Pseudomonas spp.	Pig Houses	35	65	Predicala et al., 2002
Bacillus spp.	Pig Houses	13	87	Predicala et al., 2002
Listeria spp.	Pig Houses	18	82	Predicala et al., 2002
Enterococcus spp.	Pig Houses	7	93	Predicala et al., 2002
Nocardia spp.	Pig Houses	26	74	Predicala et al., 2002
Lactobacillus spp.	Pig Houses	54	46	Predicala et al., 2002
Penicillium spp.	Pig Houses	73	27	Predicala et al., 2002

particles as well as the number of cells on each of these particles in raw gas and clean gas of a three-stage biological air cleaning system in pig houses (Table 7). Of about 2000 investigated bacteria-laden particles in raw gas, only 40 % were < 10 µm. Most bacteria cells were found on particles between 80 µm and 100 µm. In clean gas more than 90 % of bacteria-laden particles were < 10 µm and none > 40 µm. Most bacteria cells were found on particles between 10 µm and 20 µm. Also in ambient air (urban, rural and forest areas) most bacteria cells can be found between 10 µm and 40 µm (Clauß et al., 2013b). Fisar et al. (1990) investigated the size distribution of cells of bacteria and yeasts and mould spores in urban ambient air by size-selective sampling and cell count analysis in the different impactor stages by light microscopy. Most bacteria cells could be found in the size class < 0.9 µm AD, most fungi between 0.9 µm AD and 2.0 µm AD. No information was given for size classes > 6.4 µm AD. Vestlund (2009) investigated the size distribution of micro-organisms in composting facilities by sampling on filters and particle size analysis by scanning electron microscopy. He distinguished between "large cells" (fungi) and "small cells" (bacteria) and found that the small cells existed to 1 % to 70 % in aggregates with sizes of 1 µm to 5 µm subject to the sampling location, and most of the large cells in aggregates of 4 µm to 5 µm.

Recently an increasing number of studies investigated the distribution of airborne gene copies specific for diverse groups of micro-organisms in different particle size classes (Table 8). Lee and Liao (2014), Lecours et al. (2012) and Yamamoto et al. (2011) often found more than 90 % of the gene copies of different micro-organisms in the size range > 2 µm AD in different environments. Sippula et al. (2013) found 52 % to 93 % of gene copies in the size fraction > 2.4 µm AD in indoor and outdoor air. Quian et al. (2012) and Yamamoto et al. (2012) used an eight-stage Andersen sampler and found most gene copies of bacteria and moulds in particles

Table 7
Mean percentages of cell counts of different airborne bacteria, yeasts and moulds in selected particle size fractions

Microorganism	Sampling Location	Median % per Stage								Reference
Fluorescence Microscopic Method Particle Size [µm]:		**0 - 5**	**6 - 10**	**11 - 20**	**21 - 40**	**41 - 60**	**61 - 80**	**81 - 100**	**101 - 200**	
Bacteria	Pig House Raw Gas	1	2	9	23	19	12	27	7	Clauß et al., 2011a
Bacteria	Pig House Clean Gas	6	34	59	1	0	0	0	0	Clauß et al., 2011a
Bacteria	Outdoor Air	13	16	22	27	13	2	7	n/a	Clauß et al., 2013b
May-Casella Impactor, Stage:		**4**		**3**		**2**		**1**		
Particle Sizes In Each Stage [µm]:		**0.4 - 0.9**		**0.9 - 2.0**		**2.0 - 6.4**		**> 6.4**		
Bacteria	Outdoor Air	54		28		18		n/a		Fisar et al., 1990
Yeasts	Outdoor Air	33		56		11		n/a		Fisar et al., 1990
Moulds	Outdoor Air	22		43		35		n/a		Fisar et al., 1990
Ten-stage MOUDI, Stages:		**10 - 7**	**6**	**5**	**4**	**3 - 1**				
Particle Sizes In Each Stage [µm]:		**0.056 - 0.56**	**0.56 - 1.0**	**1.0 - 1.8**	**1.8 - 3.2**	**3.2 - 18.0**				
Bacteria	Living Space	35	19	28	13	n/a				Kujundzic et al., 2006

Table 8
Study results on the number distribution of specific gene copies of different airborne micro-organisms in selected particle size fractions

Micro-organism	Sampling Location	Median % per Stage							Reference
Two-Stage Bio-Aerosol Cyclone, Stage:		**3***	**2**	**1**					
Particle Sizes In Each Stage [µm]: *(afterfilter)		**< 1.0**	**1.0 - 1.8**	**> 1.8**					
Moulds	Agriculture	0	10	89					Lee and Liao, 2014
Two-Stage Bio-Aerosol Cyclone Modell BC251, Stage:		**3***	**2**	**1**					
Particle Sizes In Each Stage [µm]: *(afterfilter)		**0.4 - 0.41**	**0.41 - 2.1**	**> 2.1**					
Bacteria	Cattle Farming	0	9	91					Lecours et al,. 2012
Archaebacteria	Cattle Farming	0	2	98					Lecours et al., 2012
Two-Stage Bio-Aerosol Cyclone Model BC221, Stage:		**3***	**2**	**1**					
Particle Sizes In Each Stage [µm]: *(afterfilter)		**< 1.6**	**1.6 - 2.6**	**> 2.6**					
Alternaria alternata	Outdoor Air	0	1	99					Yamamoto et al., 2011
Cladosporium cladosporoides	Outdoor Air	1	0	99					Yamamoto et al., 2011
Epicoccum nigrum	Outdoor Air	0	1	99					Yamamoto et al., 2011
Penicillium chrysogenum	Outdoor Air	0	1	99					Yamamoto et al., 2011
Harvard High-Volume Cascade Impactor, Stages:		**4**	**3**	**2+1**					
Particle Sizes In Each Stage [µm]:		**0.2 - 0.9**	**0.9 - 2.4**	**> 2.4**					
Total Bacteria	Indoor and Outdoor Air	1	23	77					Sippula et al., 2013
Cladosporium cladosporoides	Indoor and Outdoor Air	0	7	93					Sippula et al., 2013
Mycobacterium spp.	Indoor and Outdoor Air	0	9	90					Sippula et al., 2013
Penicillium/Aspergillus spp.	Indoor and Outdoor Air	0	25	75					Sippula et al., 2013
Streptomyces spp.	Indoor and Outdoor Air	0	45	52					Sippula et al., 2013
Eight-Stage Andersen-Sampler MKII, Stages:		**7+6**	**5**	**4**	**3**	**2**	**1**	**0**	
Particle Sizes In Each Stage [µm]: *(pre-separator Cut-off)		**0.4 - 1.1**	**1.1 - 2.1**	**2.1 - 3.3**	**3.3 - 4.7**	**4.7 - 5.6**	**5.6 - 9.0**	**9.0 -10***	
Bacteria	Indoor and Outdoor Air	2	5	25	25	25	10		Quian et al., 2012
Fungi	Indoor and Outdoor Air	2	5	23	37	23	13		Quian et al., 2012
Aspergillus fumigatus/ Neosartorya fischeri	Outdoor Air (20 m)	n/a	n/a	7	22	12	6	2	Yamamoto et al., 2012
Penicillium spp	Outdoor Air (20 m)	n/a	n/a	15	62	8	15	0	Yamamoto et al., 2012
Aspergillus/Penicillium	Outdoor Air (20 m)	n/a	n/a	18	44	25	11	2	Yamamoto et al., 2012
Cladosporium cladosporoides	Outdoor Air (20 m)	n/a	n/a	11	42	21	21	5	Yamamoto et al., 2012
Alternaria alternata	Outdoor Air (20 m)	n/a	n/a	0	0	5	30	66	Yamamoto et al., 2012
Epicoccum nigrum	Outdoor Air (20 m)	n/a	n/a	0	0	6	32	61	Yamamoto et al., 2012
Ten-Stage MOUDI, Stages:		**10 – 1**							
Particle Sizes In Each Stage [µm]:		**0.056 - 18.0**							
Mycobacterium tuberculosis	Whirlpools	All Stages Positive							Schafer et al., 2003

measuring between 3.3 µm AD and 10 µm AD. Once more this is for the moulds in the range of their spore sizes. Schafer et al. (2003) found in the air above whirlpools gene copies of *Mycobacterium tuberculosis* in the size range of 0.056 µm AD to 10 µm AD. This is an indication for gene copies existing in the airborne state independent from intact cells because the cell size of the rod-shaped bacterium is about 0.5 µm x 2.0 µm.

Finally two general points should be kept in mind: The different stages of all size selective sampling systems with their defined cut-points do not mean an insuperable obstacle for larger particles. Depending on the mass-based cut-off curves, also larger particles reach the final stages of the sampling systems and may influence the results. For example Madsen et al. (2009) found considerable amounts of culturable moulds in the PM 1 dust fraction sampled by a triplex-cyclone. The second point, and important in regard to the possible health effects of biological particles, is that besides pathogenic micro-organisms with cell sizes of rarely < 0.5 µm, also other harmful cell components such as allergens from moulds (Cho et al., 2005; Górny et al., 2002; Madsen et al., 2009; Reponen et al., 2007) or endotoxins

(Attwood et al., 1986; Kujundzic et al., 2006; Monn und Becker, 1999; Olenchock et al., 1982) can be found, especially in smaller particle size classes.

4 Conclusion and outlook

The size distribution of airborne particles carrying culturable micro-organisms in the range of 0.65 µm AD to 12 µm AD has been well investigated for many micro-organism groups and environments depending on the available size selective sampling systems. It depends primarily on the sampling location, or rather the environment, and here presumably on the kind of source for airborne micro-organisms and the method of aerosolisation. Also sampling height above ground, air humidity, temperature and solar radiation may have an influence. For moulds the found median size distributions in air largely represent the size ranges of spores of the detected groups or species. There is a lack of information for particles > 12 µm AD and especially > 20 µm AD, due to limitations of the size selective sampling systems that were used. There is also little knowledge concerning the actual number of micro-organisms (cfu and cell count) in the different particles size classes. A few studies suggest that depending on the environment most micro-organisms are in the particle size fraction > 10 µm. In future investigations preferably size selective sampling systems should be used that have high inlet efficiencies for particles > 20 µm AD and that allow sampling in a liquid to separate micro-organisms from aggregates. In addition, these systems should sample rather the medical and environmental relevant particle size fractions PM 2.5, PM 4, PM 10 and the total dust.

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