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# Source analysis of fine and coarse particulate matter from livestock houses

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**Abstract.** The analyses of the different sources which can contribute to particulate matter (PM) emissions from livestock houses are essential to develop adequate reduction techniques. The aim of this study was to morphologically and chemically characterize several sources of PM from livestock houses. We collected known sources of PM from different housing systems for poultry and pigs, which were later aerosolized in a customized laboratory dust generator to collect fine and coarse PM samples. These samples were morphologically and chemically characterized using scanning electron microscopy with X-ray microanalysis to develop comprehensive morphological and chemical source profiles. Moreover, source particle-size distribution was determined. Results showed distinct and unique particle morphologies in collected sources from different housing systems for poultry and pigs. Although presence of N, Na, Mg, Al, Si, P, S, Cl, K, and Ca were identified in all sources, their relative element concentrations varied amongst sources and could be used to discriminate amongst them. Particle size and size distribution also varied amongst sources (size ranged from 2.1 µm to 18.1 µm projected area diameter), and mainly depended on its mineral or organic origin. The results from this work can be useful information for source identification and quantification in PM from livestock houses, improving the understanding of how PM is generated in such environments, and developing strategies for its reduction.

**Keywords:** Characterization, Dust sources, Livestock Housing, Source profile, SEM-EDX.

# 1. Introduction

High concentrations of particulate matter (PM) can threaten the environment as well as the health and welfare of humans and animals. A close relation between PM air pollution, respiratory and cardiovascular disease, and mortality has been reported (Pope et al., 2002). Particulate matter air pollution can also cause reduced visibility, vegetation stress, and ecosystems alteration (Grantz et al., 2003). Furthermore, small PM can have a direct radiative effect because they scatter and absorb solar and infrared radiation in the atmosphere (IPCC, 2001).

Livestock houses are important contributors to ambient fine (PM2.5) and coarse (PM10-2.5) PM emissions (Takai et al., 1998). In livestock houses, PM has a high organic content, because it is mainly composed of primary coarse particles which originate from feed, manure, bedding, and animal’s skin, feathers, and hair (Donham et al., 1986; Heber et al., 1988). Inside livestock houses, numerous studies have reported higher prevalence of respiratory diseases in livestock farmers compared with other occupations (Bongers et al., 1987; Donham et al., 1984). Furthermore, animal’s respiratory health may also be compromised by PM (Donham and Leininger, 1984).

The best approach to reduce PM in and from livestock houses seems to be to prevent it from being generated. Improved knowledge on where PM comes from in livestock houses and the identification of the major sources of PM, can help develop efficient and practical source-specific reduction techniques to comply with European threshold limits set in air quality regulations, and to protect the environment, and human and animal health and welfare.

Moreover, the characterization of particle properties offers the potential to specifically identify and quantify sources of PM (Casuccio et al., 2004); but to date, there is lack of detailed characterization of particle size, morphology, and chemical composition from sources in livestock houses. With comprehensive particle characterization and detailed source profiles, better estimates of contributions to more specific sources would be possible (Watson et al., 2002). Therefore, the development of specific, accurate, and detailed source profiles for known sources from livestock houses is encouraged.

The aim of this study was to morphologically and chemically characterize individual fine and coarse PM from known sources collected from different housing systems for poultry and pigs, and to develop comprehensive morphological and chemical source profiles. More specifically, the objectives of this study were (i) to identify unique source-specific particle morphologies and define homogeneous morphological types of particles; (ii) to identify element source compositions and compare them amongst sources; and (iii) to determine particle size, and size distribution in each source. The results from this work can be useful information for source identification and quantification in livestock houses, improving the understanding of how PM is generated in such environments, and developing strategies for its reduction.

# 2. Material and methods

## 2.1. Livestock houses and source types

A total of 48 samples from known sources of PM were collected at 14 different livestock locations in The Netherlands, including seven different housing systems for poultry and pigs. Two farms were sampled for each livestock housing system. Table 1 describes the surveyed livestock houses for the different livestock species, and the collected PM sources at each farm. All farms were sampled for manure and concentrate feed. The rest of collected PM source types depended on the housing system.

## 2.2. Known source sample collection and preparation

Sampling was conducted during morning (from 09:00 to 12:00) at each livestock farm. A representative sample from each PM source was obtained by randomly sampling different locations in the livestock house. A total of 200 to 500 grams of feed, clean bedding, and fresh manure samples were collected at each location from the flooring surfaces. A total of 10 to 50 grams of hair, feathers, and skin, were directly collected from clean animals. Samples were stored in clean sealable polyethylene bags, and transported to the laboratory and stored under refrigeration. Each sample was then mixed to achieve a uniform sample and the samples were dried in the oven for 12 h at 70ºC. Dried samples were crushed in a ball mill during 1.5 min at 250 rpm. Dried and milled samples were stored at room temperature.

A representative sample of ambient outdoor fine and coarse PM was also collected upwind, at each location on each sampling day. These PM samples were collected using a virtual cascade impactor (RespiCon, Wetzlar, Germany). This impactor simultaneously sampled PM2.5 and PM10-2.5 particles. A portable pump (Genie VSS5, Buck Inc, U.S.) was used to draw air through the impactor at constant flow of 3.11 L $min^{-1}$. Particles were collected on polycarbonate filters (37 mm Ø, 5 µm pore size), and stored before analysis. Sampling time varied from 30 min to 60 min, aiming at particle loads appropriate for single-particle analysis of 5 to 20 µg particles $cm^{-2}$ filter (Willis et al., 2002).

## 2.3. Size-segregated PM generation and measurements

To obtain size-segregated PM samples from the different known sources, a mechanical agitation system was used. Each milled source was aerosolized by a customized laboratory stainless steel dust generator (Figure 1). The amount of sample and the dust generation time were adjusted to obtain particle loads of 5 to 20 µg particles $cm^{-2}$ filter (Willis et al., 2002). Approximately 0.2 grams of milled feathers and skin, 2 to 3 grams of milled manure, hair and wood shavings, and 40 grams of milled feed were used in the dust generator, rotated at 200 rpm. Sampling time varied from 1 min (feathers), 2 min (manure), 4 min (skin), 20 min (hair), 3 h (wood shavings), and 7 h (feed). The PM2.5 and PM10-2.5 generated particles during agitation were collected using a virtual cascade impactor (RespiCon, Wetzlar, Germany) and a portable pump, using polycarbonate filters. Loaded filter samples were stored in sealed filter cassettes at room temperature (20-25ºC) before analysis.

At the same time, an optical particle counter (OPC, model 1.109, Grimm Aerosol Technik GmbH & Co., Ainring, Germany) was used during the dust generation process to monitor particle-size distribution (PSD) per source. The inlet of the device was connected to the dust generation chamber. Air was sampled through the inlet at 1.2 L $min^{-1}$. The optical particle counter sampled and counted particles in 31 size ranges, from 0.25 µm to 32 µm in diameter using light scattering principle. Recorded values were stored every 6 s. Sampling time was 7 min per sample. This instrument was also used to determine PSD of outdoor particles, outside farm locations.

## 2.4. Scanning electron microscopy analysis

All samples collected on polycarbonate filters were analyzed using high-resolution scanning electron microscopy (SEM) (JEOL, JSM-5410) combined with energy-dispersive X-ray analysis (EDX) (Link Tetra Oxford Analyzer). A small section (approximately 1 $cm^2$) of the as-collected polycarbonate filter from fine and coarse fractions was cut and mounted on a 12-mm carbon stub, and coated with carbon to make it conductive to the SEM electron beam.

The SEM-EDX was conducted manually, operated under the same conditions throughout the study in the secondary electron mode: accelerating voltage 10 keV, working distance 15 mm, electron probe current of 3 nA, magnifications 1000x for coarse PM, and 1800x for fine PM, and X-ray acquisition time 60 s per particle.

Uniformity of particle deposition on the filter was verified examining the filter prior to analysis at low magnification (300x). Then, at least three fields of view per filter sample were analyzed. On each analyzed field, both an image (photomicrograph at 1000x or 1800x) and single particle X-ray spectra of every particle found in that field were obtained and stored. Within each field, the minimum projected area diameter for the coarse particles was set at 1 µm. The minimum projected area diameter for the fine particles was set at 0.1 µm (Conner et al., 2001). These limits were set because otherwise the detection and analysis of smaller particles was not reliable at the used magnifications. A total of 25 to 50 individual particles were analyzed in each sample. All spectra were normalized to 100% and checked manually to correct for the contribution of the filter material (composed of carbon and oxygen).

Photomicrographs (images) of each field of view were acquired at normal gray and saved in tif format (1024x768 resolution). These images were further analyzed using the Object

Based Image Analysis (OBIA) approach (Blaschke, 2010) using FETEX 2.0 software (Ruiz et al., 2010). This image analysis and processing system automatically detected each particle object and calculated the particle projected area. From the particle area, the projected area diameter ($D_p$) was calculated, defined as the diameter of a perfect circle fitted to the measured area of the particle (equation 1).

$$D_p = 2 \times \sqrt{\frac{Area}{\pi}} \qquad (1)$$

## 2.5. Data analyses

Particle types and morphologies were qualitatively analyzed based on the SEM images. These particle types were morphologically described in terms of shape (rounded, spherical, fibrous, flake, angular, aggregate, irregular, flattened, long-thin), surface (layered, smoothed, cracked), edges and borders (sharpness), texture (smooth, grape-like, and rough), and opacity, amongst others (McCrone, 1992; NIST, 2010). In this way, different types of particles were determined in each source, in fine and coarse PM. More than 300 images were qualitatively analyzed.

Particle chemical compositions were summarized to obtain the average relative element concentrations per source in fine and coarse PM, pooled by livestock category. The relative element composition of the PM in the different sources and in each fraction was compared using analysis of variance with SAS software (SAS, 2001). To test multivariate differences between sources, and identify which elements (variables) discriminated best amongst sources per fraction, we performed a stepwise discriminant analysis using SAS software (SAS, 2001). Hierarchical cluster analysis was used to provide evidence of similarities and differences within and amongst sources from different livestock categories, using the average relative element concentrations per source in fine and coarse PM, for each livestock category and housing system. We used Ward’s minimum-variance method for clustering and the squared Euclidean distance as a measure of similarity between clusters using SAS software (SAS, 2001).

Data on size were summarized to obtain the average $D_p$ per source in fine and coarse PM, pooled by livestock category. The average $D_p$ of the PM in the different sources and in each fraction was compared using analysis of variance with SAS software (SAS, 2001).

To determine the PSD per source, we calculated the standardized number fraction ($\Delta f_i$) from the frequency of particles ($F_i$) within a size range ($\Delta d_i$) in each source. The standardized number fraction of particles for the $i^{th}$ size range was calculated with equation 2:

$$\Delta f_i = \frac{\left(\frac{F_i}{\Delta d_i}\right)}{N} \qquad (2)$$

where: $\Delta f_i$= Standardized fraction in units of $\mu m^{-1}$ for the $i^{th}$ size range, $F_i$= Frequency of particles within a size range, $\Delta d_i$= Particle size range, calculated as the difference between the upper and lower limit of the sampling interval (size range measured by the instrument) within each group of particles, N= Total number of particles measured by the instrument (sum of all size ranges).

We also calculated the standardized mass fraction by multiplying the particle number concentrations by an estimated particle mass per source, assuming all particles were spherical, and assuming a value for particle density. Density values of 1.2 g $cm^{-3}$ (feathers), 2.6 g $cm^{-3}$ (feed), 1.3 g $cm^{-3}$ (hair), 1.5 g $cm^{-3}$ (manure and wood shavings), 1.4 g $cm^{-3}$ (skin), and 2.1 g $cm^{-3}$ (outside) were used (McCrone, 1992). The calculation of particle mass from particle numbers per source was done following equation 3:

$$m_i = n_i \times \rho_p \times v_{pi} = n_i \times \rho_p \times \left[\frac{4}{3} \times \pi \times r_i^{\,3}\right] = n_i \times \frac{\rho_p \times \pi \times \left(d_{g_i}\right)^3}{6} \qquad (3)$$

where: $m_i$= particle mass for the $i^{th}$ size range of particles, $n_i$= number of particles measured by the instrument for the $i^{th}$ size range, $\rho_p$= particle density per source, $v_{pi}$= particle spherical volume for the $i^{th}$ size range, $r_i$= equivalent radius of a spherical particle for the $i^{th}$ size range, $d_{gi}$= mean geometric particle diameter measured by the instrument in the $i^{th}$ size range.

This size distribution was also standardized and divided by the total mass of particles to obtain the standardized mass fraction in the same way as for standardized number fraction (equation 2).

# 3. Results

## 3.1. Particle types and morphology (fine and coarse)

Different types of particles were identified per source and thoroughly described below.

### 3.1.1. Feathers

Feathers showed a mixture of irregular, mostly flattened particles in fine and coarse PM. Three morphological types were identified: soft and “fluffy” particles, sometimes bent (Figure 2a and b); rounded, flake-like flattened, sometimes aggregate particles with rough texture (Figure 2c and d); and stiff, elongated, and pointed particles (Figure 2e and f). Each type generally coincided with different livestock categories. In broilers, small soft and “fluffy” particles were dominant in fine and coarse PM. In laying hens, besides showing some soft and “fluffy” structures, also flake-like flattened particles and elongated particles were dominant in fine and coarse PM. Turkeys showed mostly soft and “fluffy” particles in the fine fraction (Figure 2g); whereas flake-like flattened and elongated particles were abundant in coarse PM (Figure 2h).

### 3.1.2. Feed

Four general morphological types of feed particles were identified: rounded and deposited particles, sometimes fragmented (mainly seen in broilers and turkeys) (Figure 3a and b); geometric quadrangular, cubic (Figure 3c and d) or bar-shaped particles (Figure 3e and f); and angular, cracked, fragmented particles (Figure 3g and h). All types were randomly found in fine and coarse PM amongst all livestock categories.

### 3.1.3. Hair

Pig's hair showed long-thin particles. Two types of hair particles were identified in fine and coarse PM: thin pointed particles (Figure 4a and b); and striated tubular particles (Figure 4c and d).

### 3.1.4. Manure

Manure particles showed two morphological types: rounded, spherical, and smooth particles; and fragmented, rough, and angular particles. Rounded spheres were only identified in poultry excreta, in fine and coarse PM. Apart from rounded spheres, irregular and angular particles were also identified in poultry excreta. Rounded spheres were sometimes present as individual particles (Figure 5a), and agglomerated with fragmented angular particles (Figure 5b), or highly agglomerated forming grape-like structures (Figure 5c and d). Rough and ciliated rounded spheres were identified in turkeys and laying hens manure (Figure 5e and f). Fragmented, layered, angular particles were the dominant particles in pigs manure in fine (Figure 6a and b) and coarse PM (Figure 6c and d).

### 3.1.5. Skin

Sow's skin particles were morphologically homogeneous and showed a single type, as big, rounded, thin, flattened, flake-like, transparent particles in fine (Figure 7a and c) and coarse PM (Figure 7b and d). These flake-like particles presented a smooth surface (Figure 7a and c), although some of them presented rough surfaces caused by deposited particles on top (Figure 7b and d).

### 3.1.6. Wood shavings

Wood shaving particles showed two types of particles: flattened, round with irregular borders, others elongated and bent in fine PM (Figure 8a and c); and mostly fibrous particles with sharp edges identified in coarse PM (Figure 8b and d).

### 3.1.7. Outside source

Particles from outside farm sources showed heterogeneous morphologies. Dominant particles were generally small, irregular angular, cracked fragmented particles (sometimes aggregate) (Figure 9a and b); and geometric quadrangular, bar-shaped or cubic particles (Figure 9c and d).

## 3.2. Chemical composition (fine and coarse)

Average relative element concentrations were calculated per source in fine and coarse PM, pooled by livestock category. Figure 10 (fine PM) and Figure 11 (coarse PM) present average particle element relative concentration per source, together with significant differences in average values of element concentrations amongst sources. Hair was not included in the analysis because it showed very high carbon and oxygen peak in the SEM-EDX which was confused with the background filter composition. Presence of N, Na, Mg, Al, Si, P, S, Cl, K, and Ca were identified in all sources, in fine and coarse PM. Generally, differences in these elements amongst sources were obtained between feed, outside, wood, skin, and the rest of sources; or between manure and the rest of sources. Manure showed the highest relative levels of N, Mg, P, and K; skin showed the highest S levels; wood shavings showed the highest levels of Cl and Na; feed showed the highest levels of Si and Ca; and outside source showed the highest levels of Al in fine PM. Traces of heavy elements (metals), with atomic numbers greater than 20 (such as Fe, Ni, Cu, Zn, Ag, Pb, Sn, Ba, and Cu) were mainly identified in feed and outside, and to a smaller extent in wood shavings. Other elements not shown in Figure 10 and Figure 11, were detected in some particles in fine and coarse PM (Co in feed, manure, and outside), and others only in coarse PM (Br, Ti, V, and Sb in feed, wood shavings, and outside), in relative concentrations below 0.2%, and showing no statistical significant differences amongst sources.

Results from the discriminant analysis confirmed the differences in relative element concentrations amongst sources presented in Figure 10 and Figure 11. The first five common variables that best discriminated amongst sources were P, N, Cl, S, and K. Table 2 and Table 3 show the summary of the stepwise discriminant analysis for each variable considered. In fine PM, order of entrance into the discriminant process was: P, N, Cl, S, K, Si, Na, Al, Ca, Mg, and Sn (Table 2). In coarse PM, the order of entrance into the discriminant process was: P, N, K, S, Cl, Al, Ca, Cr, Na, Mg, Ba, and Fe (Table 3).

Cluster analysis revealed three major source groups in fine and coarse PM: one including mainly feed and outside source, another including mainly manure source, and the third one including feathers and skin; being wood shavings either grouped together with feathers and skin or feed and outside. Figure 12 and Figure 13 present the groupings which result from cluster analysis. The horizontal distance between each group is a representation of their dissimilarity. When data were joined into three groups or clusters, the proportion of variance accounted for by the clusters was 46% for fine and 54% for coarse PM; but when data were joined into nine (fine PM) or eight (coarse PM) clusters, this variance reached 80%. Cluster groupings showed similarities and dissimilarities between sources amongst livestock categories, especially within and amongst poultry categories (being for instance broiler's and turkey's manure sources closely related between them, and more closely related to laying hens manure than to pig's manure) and mostly between poultry and pigs (being associations generally made accounting for animal species).

## 3.3. Size and size distributions

In each source, particle size, expressed as $D_p$, was determined from SEM images using image analysis software. Particle-size distribution was determined by the light scattering principle during aerosolization in the dust generator.

### 3.3.1. Particle size

For all sources (except for hair) average $D_p$ in fine PM was from 35% to 46% lower ($P < 0.005$) compared with coarse PM. Skin and hair showed the largest particle sizes ($D_p$ equal to 13 µm in fine PM, and 18 µm in coarse PM); whereas feed and outside particles showed the lowest sizes ($D_p$ equal to 2 µm in fine PM, and 3 µm in coarse PM). Average $D_p$ (standard deviation, SD) for the different sources in fine and coarse PM are shown in Table 4.

### 3.3.2. Particle-size distribution

Figure 14 shows the average particle number-size distribution per source in log-scale, calculated from the average number of particles per size range measured for each source. All sources showed the highest number of particles in the lowest size ranges and the lowest number of particles in the highest size ranges. Particles in the size range from 0.25 µm to 0.28 µm were the most abundant in all sources, being this the minimum size range measured by the instrument. From approximately 0.6 µm, differences amongst size distributions from sources became evident. From this size range onwards, two different size distributions were observed: size distribution from feed and outside which decreased more or less linearly; and size distribution from the rest of sources which showed two peaks, one at 0.8 µm to 0.9 µm, and another at 4 to 5 µm. All sources showed a peak in the last size range (particles bigger than 32 µm), indicating a relatively high number of very big particles.

Figure 15 shows the average particle mass-size distributions per source in log-scale, calculated from the average mass of particles per size range for each source. Particle mass-size distributions showed high masses in the lowest size ranges, in the middle size ranges, but also in the highest size ranges. High mass for feed and outside was observed in the minimum size range measured by the instrument (size range from 0.25 µm to 0.28 µm). For the rest of sources, high masses were found at 4 to 5 µm, where feed and outside showed their minimum mass. Above 5 µm, the mass of feathers and hair decreased more sharply, showing lower masses compared with manure, skin, and wood shavings. Above 5 µm, feed and outside masses increased. Manure's mass distribution showed four very clear peaks at 0.25 µm, 0.4 µm, 0.8 µm, and 4 µm. Again, all sources showed a peak in the last size range, corresponding to particles bigger than 32 µm.

## 4. Discussion

The application of SEM-EDX to individual particles from collected sources in different livestock housing systems for poultry and pigs demonstrated that sources of PM differed in particle morphology, element composition, and size. This study gives a detailed and complete analysis of potential sources of PM from livestock houses including different housing systems for poultry and pigs in size-segregated PM.

Qualitative results revealed different particle morphological types and unique morphological features related to each source. Some of the identified particle types coincided and could be related to a specific livestock category (e.g. type of feathers and manure), although others were generally randomly found in all livestock categories (e.g. types of feed particles). The main differences amongst sources were found between hair and skin and the rest of sources, because these presented the most well defined and homogeneous particle types and morphologies. Furthermore, the use of digital image analysis software could be useful to extract morphological characteristics and quantify further differences.

The different morphological types of particles identified in the SEM analysis could be partly explained by the different livestock production systems. Particle types from feathers could be explained by the feather structure and development process, related to different poultry production systems. In our study, farms with 3 to 4 week-old broilers were sampled. Therefore, broiler's feathers were seen as fine feathers (plumules or down feathers) with "fluffy" structure to provide a high level of insulation to young birds, easily airborne as broiler chicks loose their fluff (scurf). In laying hen houses, hens are generally older than 20 weeks. Therefore, laying hen's feathers have more mass than and differ from down feathers. Laying hen's feathers and also turkey's feathers were more similar to contour feathers than to down feathers. Contour feathers consist of a shaft onto which a feather vane is attached (Leeson and Walsh, 2004). The feather vane, moreover, is composed of filaments, called barbs, which have rows of interlocking barbules that give the feather its shape and rigidity (Leeson and Walsh, 2004). Barbules (also named hooklets after their pointed structure) are also fine structures, easily airborne, which were abundant in samples from laying hens feathers, and clearly identifiable by their pointed and elongated morphology.

The existence of two very distinctive morphological types of manure particles between poultry and pigs could be explained by the particular poultry excretory system, where urea is converted chemically to uric acid. Birds excrete uric acid as encapsulated uric acid crystals through bird's cloaca. Encapsulated uric acid crystals appear as round smooth spheres of varying sizes as those identified in our study, surrounded by a protein material. In the case of pigs, this type of excretion does not exist, and so manure particles were found as fragmented, rough, and angular particles. Feddes et al. (1992) described crystals of uric acid from turkey housing, as round spheres from 3 µm to 8 µm in diameter, and other fecal particles as similar to feed particles with varying sizes from 3 µm to 7 µm in diameter.

The three types of feed particles dominant in the feed source samples were probably related to different feed components: mineral particles (geometric salt-like), and more grain-like organic particles (angular, cracked, fragmented particles) could be found. Outside particles were mainly constituted of salt-like crystals and crustal fragmented particles. Fragmented particles were comparable to soil erosion and dust particles (Skogstad et al., 1999) typical from agricultural environments where livestock houses are located. The rest of the described particle types (hair, skin, and wood shavings) were generally consistent with the known standards (McCrone, 1992) and coherent amongst livestock categories and PM fractions.

A clear difference between mineral particles (rich in Al, Si, and Ca) and organic particles (rich in N, Na, S, Cl, and Ca) could be seen in the chemical (element) composition of the different sources. This difference could be made between feed and outside particles (mineral) and the rest of sources (organic). Differences in element concentrations amongst sources could be used by the discriminant and cluster analysis to distinguish amongst them. In fact, Aarnink et al. (2004) in pigs and Cambra-López et al. (2008) in rabbits reported similar elements present in PM from livestock houses. As regards mineral particles, high levels of Al and Si have also been reported in crustal material (Shi et al., 2003). The presence of metallic trace elements could be explained by the use of some of these elements as feed supplements to improve health and feed efficiency (Bolan et al., 2004). Using variations in element concentrations, discriminant analysis indicated major variables useful to distinguish amongst sources, providing elements which could discriminate well amongst different sources without accounting for livestock categories. Cluster analysis indicated inter-relationships between sources belonging to different livestock categories, providing an initial estimate of source profiles per livestock category.

Particle size varied amongst sources, and mainly depended on its mineral or organic origin. Generally disintegration particles from feed and outside source showed smaller sizes, compared with biological structures (feathers, hair, skin, and wood shavings), which were mainly larger than 4 µm in diameter. Using SEM, the $D_p$ of the particles calculated from the particle area, resulted in $D_p$ higher than 2.5 µm in fine PM. This high figure could be explained by two facts: the first related to the $D_p$ being the diameter in the two-dimensional view, parallel to the plane of the filter; and the second related to the differences between geometric diameter and aerodynamic diameter. As most particles showed irregular shapes, particles would impact on the filter in their most stable orientation, generally exposing the biggest dimension on the filter plane, thus possibly explaining these high figures in $D_p$ (Conner et al., 2001). The geometric diameter of particles is related to its aerodynamic diameter through a dynamic shape factor, which varies with the resistance force of the particle to a fluid motion (Davies, 1979). Therefore, elongated particles (fibrous-like) which can show their longest axis in the direction of the flow, or large and thin (flake-like) particles with low densities, could place small resistance to it, and they could be aerodynamically separated into a smaller diameter during sampling than they would if they were separated by their geometric diameter. Consequently, the accuracy of sizing particles using SEM can be reduced, as particles deviate from spheres (Willis et al., 2002).

All sources showed the highest particle counts in the lowest size ranges. This differed when expressed in mass. Heber et al. (1988) determined more than 50% of particles from pig houses were smaller than 2.7 µm, and found higher particle counts in the smallest size ranges for grain meal than for starch, where most particles were found to be greater than 5.4 µm. Our results suggest that most of the generated particles from our feed samples could come from grain meal rather than from starch. Furthermore, starch agglomerates, which present a specific and identifiable morphology in the SEM (viewed as polyhedral or sub-spherical agglomerate grains) according to McCrone (1992), were rarely seen in the analyzed particles from feed in our study. Measured number PSD in the air of livestock houses have been described elsewhere and have been identified as bi-modal (Lammel et al., 2004). Our results on size distributions could be furthermore useful to identify similarities and differences between on-farm PSD and those from known sources, taking into account differences in the measurement instruments used.

During the experimental dust generation process, an insight of the dust potential (Miller and Woodbury, 2003) of the different sources was achieved. The variable amount of sample and the dust generation time needed to maximize number of particles collected on the filter suggested feathers and manure were readily aerosolized, and thus showed higher dust potentials compared with the rest of sources. Our results suggest that dried manure and feathers could easily become airborne on-farm conditions, when exposed to air movement. This aspect should be confirmed with specific source-apportionment studies in livestock houses, or by comparison of on-farm samples to particle source morphologies and chemical compositions presented in this study.

## 5. Conclusions

1. Distinct particle morphologies were identified in collected sources from different housing systems for poultry and pigs. Detailed source profiles (morphological and chemical) for known sources were developed.

2. Qualitative description of particle types revealed unique morphological features related to each source and different particle morphological types related to livestock production

systems. Digital image analysis software could be useful to extract such characteristics and quantify further differences.

3. Although presence of N, Na, Mg, Al, Si, P, S, Cl, K, and Ca were identified in all sources, their relative element concentrations varies amongst sources and can be used to discriminate amongst them.

4. With the average element concentrations presented in this study, the relative concentrations of P, N, Cl, S, K, Si, Na, Al, Ca, Mg, and Sn are useful for discriminating amongst sources in fine PM. The relative concentrations of P, N, K, S, Cl, Al, Ca, Cr, Na, Mg, Ba, and Fe are useful for discriminating amongst sources in coarse PM.

5. Particle size varies amongst sources (from 2.1 µm to 18.1 µm projected area diameter), and mainly depends on its mineral or organic origin. Generally disintegration particles from feed and outside show smaller sizes, compared with biological structures (feathers, hair, skin, and wood shavings), which are mainly coarse.

6. The described source specific particle-size distributions can be useful to identify similarities and differences between on-farm PSD and those from known sources.

7. Comprehensive particle characterization and complete source analysis was achieved including different housing systems for poultry and pigs in size-fractioned PM. The data presented herein and the developed source profiles will be useful to assign airborne PM samples and individual particles to known sources and to improve source identification and quantification in livestock houses, a preliminary step to develop specific strategies for its reduction.

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## 7. References

Aarnink, A.J.A., Stockhofe-Zurwieden, N., Wagemans, M.J.M., 2004. Dust in different housing systems for growing-finishing pigs. Proceedings of Engineering the Future. AgEng 2004. Leuven, Belgium.

Blaschke, T., 2010. Object based image analysis for remote sensing. ISPRS Journal of Photogrammetry and Remote Sensing 65, 2-16.

Bolan, N.S., Adriano, D.C., Santiago, M., 2004. Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Critical Reviews in Environmental Science and Technology 34, 291-338.

Bongers, P., Houthuijs, D., Remijn, B., Brouwer, R., Biersteker, K., 1987. Lung function and respiratory symptoms in pig farmers. British Journal Industrial Medicine 44, 819-823.

Cambra-López, M., Torres, A.G., 2008. An approach to source apportionment of dust in animal houses: The case of rabbit rearing facilities. Proceedings of International Symposium Livestock and Environment. ILES VIII. Iguassu, Brazil.

Casuccio, G.S., Schlaegle, S.F., Lersch, T.L., Huffman, G.P., Chen, Y.Z., Shah, N., 2004. Measurement of fine particulate matter using electron microscopy techniques. Fuel Processing Technology 85, 763-779.

Conner, T.L., Norris, G.A., Landis, M.S., Williams, R.W., 2001. Individual particle analysis of indoor, outdoor and, community samples from the 1998 Baltimore particulate matter study. Atmospheric Environment 35, 3935-3946.

Davies, C.N., 1979. Particle-fluid interaction. Journal of Aerosol Science 10, 477-513.

Donham, K.J., Leininger, J.R., 1984. Animal studies of potential chronic lung-disease of workers in swine confinement buildings. American Journal of Veterinary Research 45, 926-931.

Donham, K.J., Popendorf, W., Palmgren, U., Larsson, L., 1986. Characterization of dusts collected from swine confinement buildings. American Journal of Industrial Medicine 10, 294-297.

Donham, K.J., Zavala, D.C., Merchant, J.A., 1984. Respiratory symptoms and lung function among workers in swine confinement buildings: a cross-sectional epidemiological study. Archives of Environmental Health 39, 96-101.

Feddes, J.J.R., Cook, H., Zuidhof, M.J., 1992. Characterization of airborne dust particles in turkey housing. Canadian Agricultural Engineering 34, 273-280.

Grantz, D.A., Garner, J.H.B., Johnson, D.W., 2003. Ecological effects of particulate matter. Environment International 29, 213-239.

Heber, A.J., Stroik, M., Faubion, J.M., Willard, L.H., 1988. Size distribution and identification of aerial dust particles in swine finishing buildings. Transactions of the ASAE 31, 882-887.

IPCC, 2001. Climate Change 2001: The Scientific Basis. Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Xiaousu, D. (Eds.), Intergovernmental Panel on Climate Change. Cambridge University Press. Geneve, Switzerland, 944 pp.

Lammel, G., Schneider, F., Brüggemann, E., Gnauk, T., Röhrl, A., Wieser, P., 2004. Aerosols emitted from a livestock farm in southern Germany. Water Air and Soil Pollution 154, 313-330.

Leeson, S., Walsh, T., 2004. Feathering in commercial poultry. I. Feather growth and composition. World's Poultry Science Journal 60, 42-51.

McCrone, W. C. (1992). The Particle Atlas Electronic Edition (PAE2) on CD-ROM.

Miller, D.M., Woodbury, B.L., 2003. Simple protocols to determine dust potentials from cattle feedlot soil and surface samples. Journal of Environmental Quality 32, 1634-1640.

NIST., 2010. Particle morphology glossary. Glossary of morphology terms. U.S. National Institute of Standards and Technology. http://www.nist.gov/lispix/doc/particle-form/part-morph-gloss.htm. Accessed on 21st April, 2010.

Pope, C.A., Burnett, R.T., Thun, M.J., Calle, E., Krewski, D., Ito, K., Thurston, G.D., 2002. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA: The Journal of the American Medical Association 287, 1132-1141.

Ruiz, L.A., Recio, J.A., Fernández-Sarriá, A., Hermosilla, T., 2010. A tool for object descriptive feature extraction: Application to image classification and map updating. Vol. XXXVIII-4/C7. The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences.

SAS., 2001. SAS User's Guide: Statistics. SAS Institute Inc.

Shi, Z., Shao, L., Jones, T.P., Whittaker, A.G., Lu, S., Bérubé, K.A., He, T., Richards, R.J., 2003. Characterization of airborne individual particles collected in an urban area, a satellite city and a clean air area in Beijing, 2001. Atmospheric Environment 37, 4097-4108.

Skogstad, A., Madso, L., Eduard, W., 1999. Classification of particles from the farm environment by automated sizing, counting and chemical characterization with scanning electron microscopy-energy dispersive spectroscopy. Journal of Environmental Monitoring, 379-382.

Takai, H., Pedersen, S., Johnsen, J.O., Metz, J.H.M., Groot Koerkamp, P.W.G., Uenk, G.H., Phillips, V.R., Holden, M.R., Sneath, R.W., Short, J.L., White, R.P., Hartung, J., Seedorf, J., Schroder, M., Linkert, K.H., Wathes, C.M., 1998. Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 70, 59-77.

Watson, J.G., Zhu, T., Chow, J.C., Engelbrecht, J., Fujita, E.M., Wilson, W.E., 2002. Receptor modeling application framework for particle source apportionment. Chemosphere 49, 1093-1136.

Willis, R.D., Blanchard, F.T., Conner, T.L., 2002. Guidelines for the application of SEM/EDX analytical techniques to particulate matter samples. EPA, Washington, U.S., 88 pp.

Table 1. Description of surveyed livestock houses and collected PM sources.

| Livestock species | Housing system | Farm location | Ventilation | Number of animals | Age (weeks) | Collected PM source types |
|---|---|---|---|---|---|---|
| Poultry | Broilers - bedding | 1 | Tunnel | 50 400 | 4 | Fresh excreta<br>Feed (crumbles and pellets)<br>Feathers<br>Wood shavings |
| | | 2 | Roof | 2675 | 3 | |
| | Turkeys - bedding | 1 | Ridge | 5000 | 12 | |
| | | 2 | Ridge | 4040 | 10 | |
| | Laying hens - floor | 1 | Tunnel | 3850 | 71 | Fresh excreta<br>Feed (crumbles and pellets)<br>Feathers |
| | | 2 | Tunnel | 16 500 | 22 | |
| | Laying hens - aviary | 1 | Tunnel | 24 712 | 71 | |
| | | 2 | Tunnel | 35 000 | 50 | |
| Pigs | Piglets- slatted floor | 1 | Roof | 125 | 8 | Fresh feces<br>Feed (pellets)<br>Hair |
| | | 2 | Roof | 75 | 9 | |
| | Growing-finishing pigs - partially slatted floor | 1 | Roof | 120 | 16 | |
| | | 2 | Roof | 60 | 20 | |
| | Dry and pregnant sows - group housing | 1 | Roof | 39 | - | Fresh feces<br>Feed (pellets)<br>Hair<br>Skin |
| | | 2 | Roof | 46 | - | |

Table 2. Summary of the stepwise discriminant analysis showing the squared partial correlation (Partial R-Square), the F-statistic (F-value), and the probability level (Pr > F), from the one-way analysis of covariance in fine PM.

| Order of entrance in the model | Element | Partial R-Square | F-value | Pr > F |
|---|---|---|---|---|
| 1 | P | 0.2576 | 113.04 | < 0.0001 |
| 2 | N | 0.2446 | 105.43 | < 0.0001 |
| 3 | Cl | 0.2392 | 102.31 | < 0.0001 |
| 4 | S | 0.1456 | 55.41 | < 0.0001 |
| 5 | K | 0.1161 | 42.67 | < 0.0001 |
| 6 | Si | 0.0475 | 16.2 | < 0.0001 |
| 7 | Na | 0.0406 | 13.74 | < 0.0001 |
| 8 | Al | 0.0318 | 10.64 | < 0.0001 |
| 9 | Ca | 0.0151 | 4.96 | 0.0002 |
| 10 | Mg | 0.0125 | 4.09 | 0.0011 |
| 11 | Sn | 0.0083 | 2.71 | 0.0190 |

Table 3. Summary of the stepwise discriminant analysis showing the squared partial correlation (Partial R-Square), the F-statistic (F-value), and the probability level (Pr > F), from the one-way analysis of covariance in coarse PM.

| Order of entrance in the model | Element | Partial R-Square | F-value | Pr > F |
|---|---|---|---|---|
| 1 | P | 0.2963 | 139.97 | < 0.0001 |
| 2 | N | 0.2629 | 118.46 | < 0.0001 |
| 3 | K | 0.1772 | 71.51 | < 0.0001 |
| 4 | S | 0.1371 | 52.72 | < 0.0001 |
| 5 | Cl | 0.1181 | 44.43 | < 0.0001 |
| 6 | Al | 0.0372 | 12.82 | < 0.0001 |
| 7 | Ca | 0.0208 | 7.04 | < 0.0001 |
| 8 | Cr | 0.0137 | 4.59 | 0.0004 |
| 9 | Na | 0.0132 | 4.42 | 0.0005 |
| 10 | Mg | 0.0108 | 3.61 | 0.0030 |
| 11 | Ba | 0.0073 | 2.42 | 0.0340 |
| 12 | Fe | 0.0071 | 2.37 | 0.0375 |

Table 4. Average estimated projected area diameter ($D_p$, in µm ) from particle areas from SEM images and standard deviation (SD), for different sources in fine and coarse PM fractions. (N.S. stands for non significant differences).

| Source | n | Fraction | Average $D_p$ (µm) | SD | P-value |
|---|---|---|---|---|---|
| Feathers | 398 | PM2.5 | 3.9 | 2.9 | < 0.0001 |
| | 431 | PM10-2.5 | 5.6 | 5.4 | |
| Feed | 416 | PM2.5 | 2.1 | 2.2 | < 0.0001 |
| | 405 | PM10-2.5 | 3.0 | 2.7 | |
| Hair | 34 | PM2.5 | 11.7 | 5.2 | N.S. |
| | 36 | PM10-2.5 | 10.8 | 5.8 | |
| Manure | 644 | PM2.5 | 4.0 | 2.3 | < 0.0001 |
| | 942 | PM10-2.5 | 5.5 | 2.8 | |
| Skin | 27 | PM2.5 | 13.4 | 8.0 | < 0.05 |
| | 42 | PM10-2.5 | 18.1 | 8.0 | |
| Wood shavings | 130 | PM2.5 | 4.1 | 3.3 | < 0.0001 |
| | 212 | PM10-2.5 | 5.9 | 5.2 | |
| Outside | 350 | PM2.5 | 2.1 | 1.9 | < 0.0001 |
| | 246 | PM10-2.5 | 3.0 | 2.9 | |

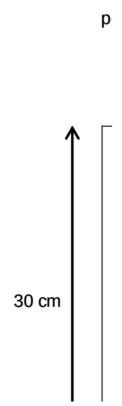
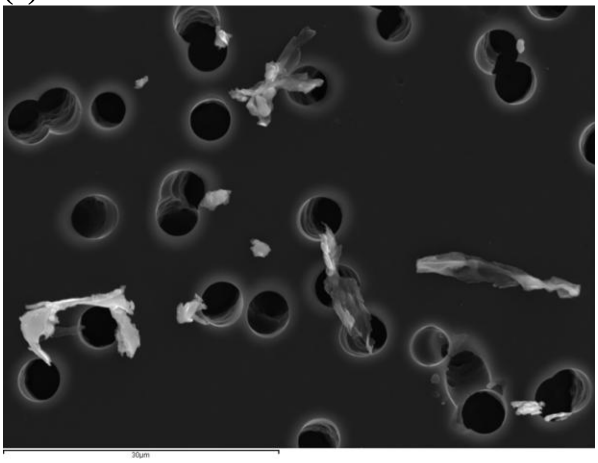
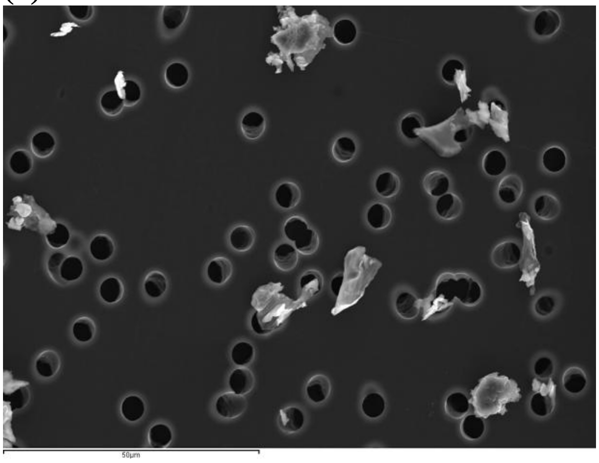


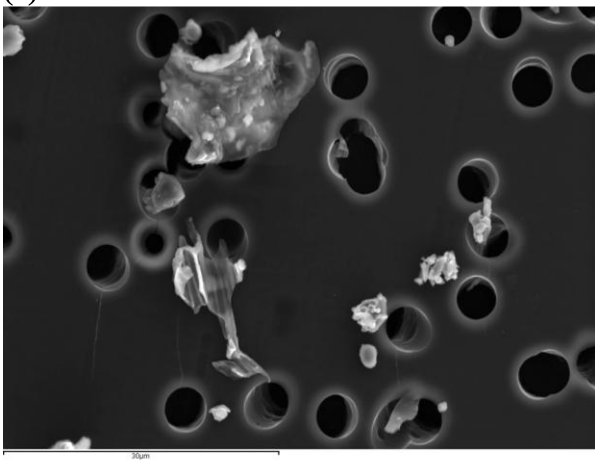

Figure 1. Schematic layout of dust generation process, measurements and position.

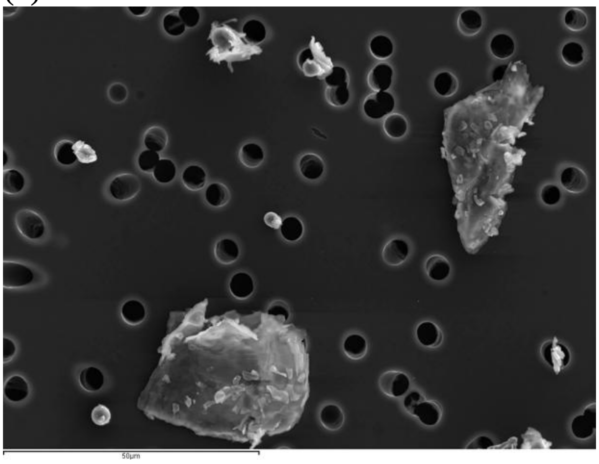
(a) (b)



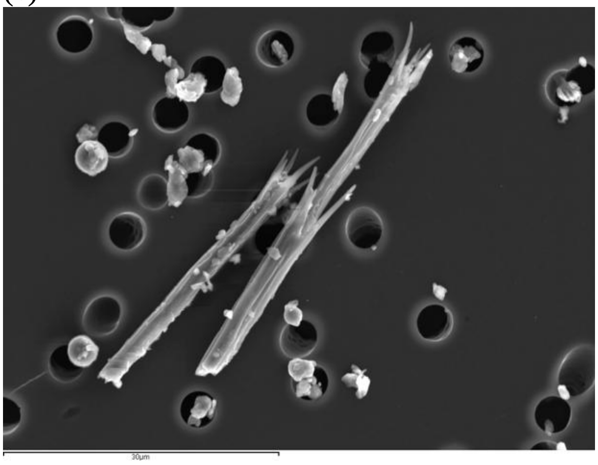


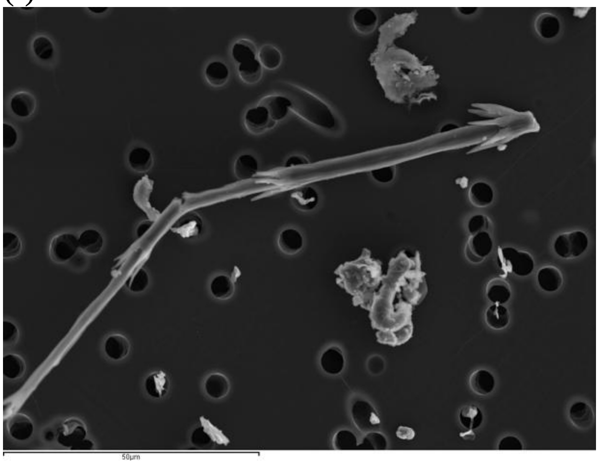
(c) (d)





(e) (f)





(g) (h)

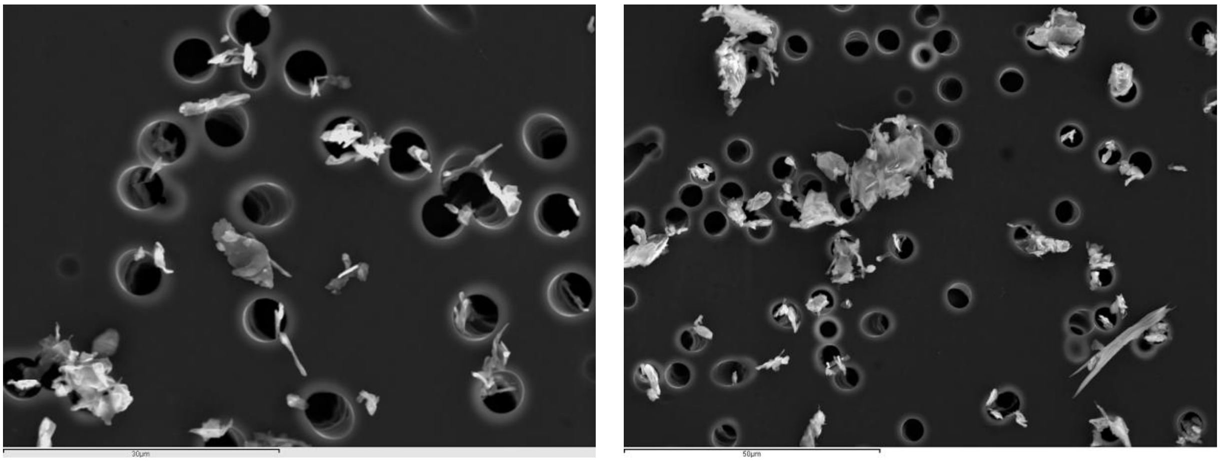


Figure 2. Particles from feathers. (a) Long and "fluffy" particles from broilers in fine PM. (b) Mixture of "fluffy" particles showing different silhouettes from broilers coarse PM. (c) Big rounded, flattened particle together with smaller "fluffy" particles from laying hens in floor system fine PM. (d) Rounded and triangular flattened particles from laying hens in floor system coarse PM. (e and f) Stiff, elongated, and pointed particles from laying hens in aviary system fine PM (e) and coarse PM (f). (g) Soft and "fluffy" particles from turkeys in fine PM. (g) Mixture of "fluffy", flake-like, and elongated particles from turkeys in coarse PM. Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

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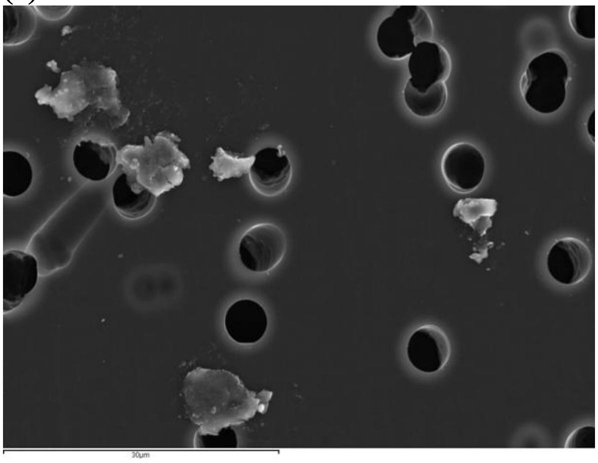
(a)



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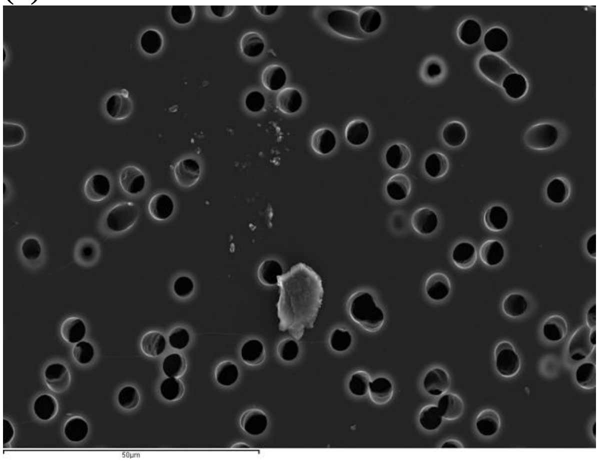

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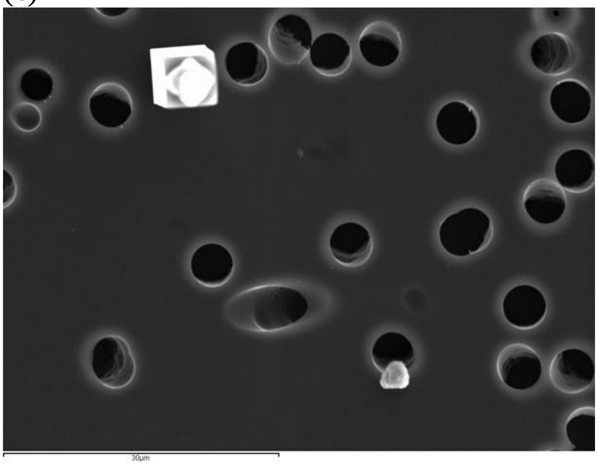
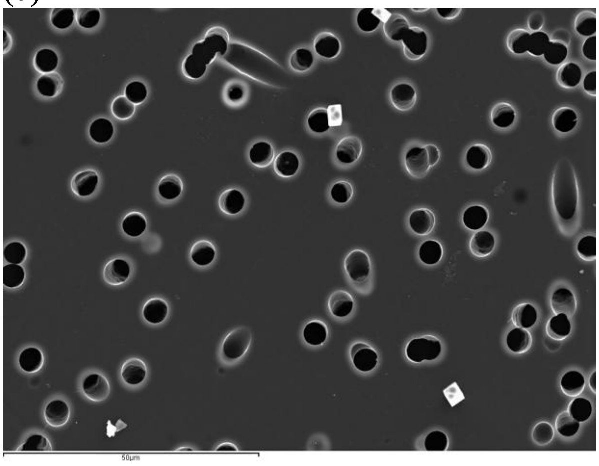
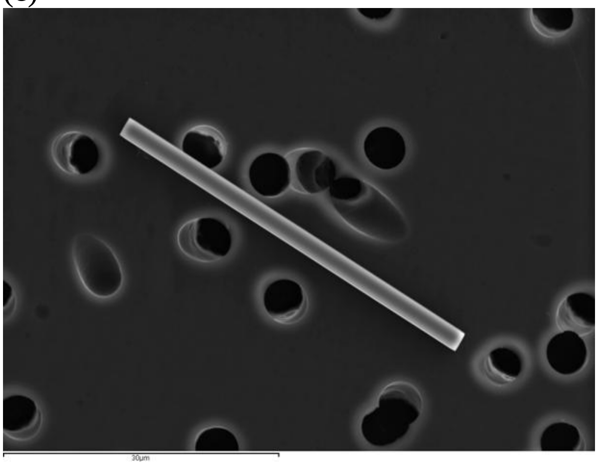


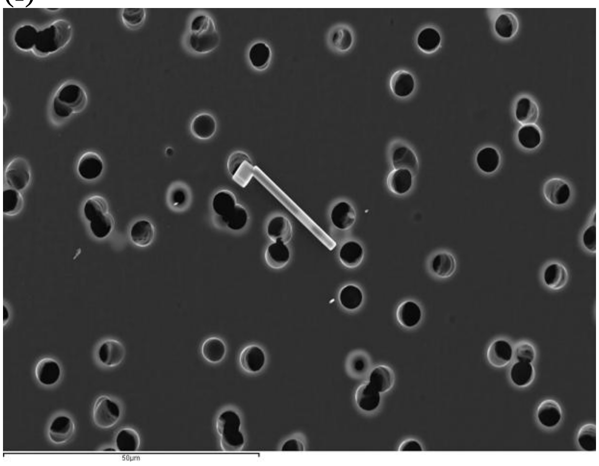
(d)



(e)



(f)



(g)

(h)

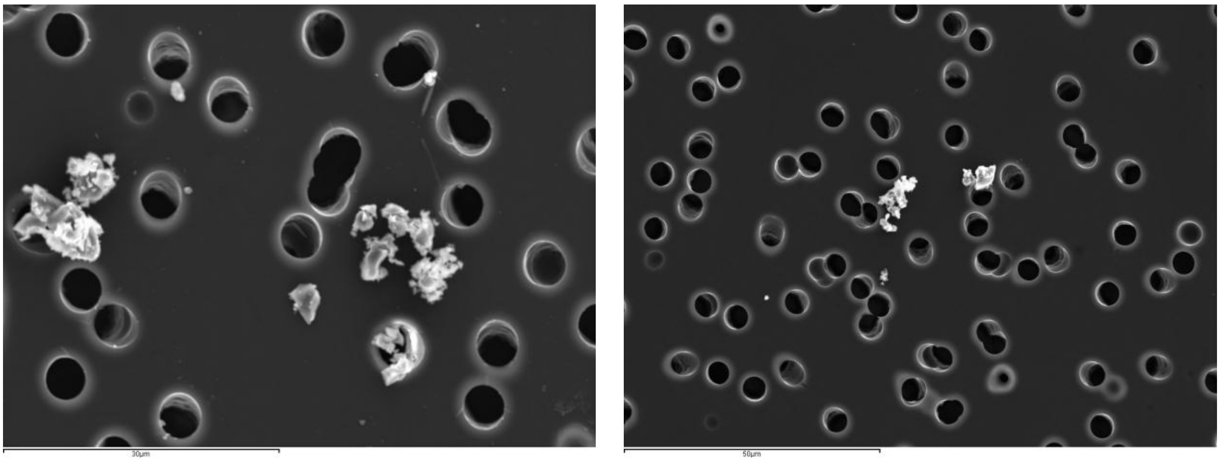


Figure 3. Particles from feed. (a and b) Rounded and deposited particles from broilers fine PM (a) and rests of fragmented particles in coarse PM (b). (c and d) Cubic bright particles from laying hens aviary system fine PM (c) and from sows coarse PM (d). (e and f) Single bar-shaped particles from sows fine PM (e) and laying hens floor system coarse PM (f). (g and h) Several angular, cracked, fragmented particles from laying hens aviary fine PM (g) and growing-finishing pigs coarse PM (h). Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

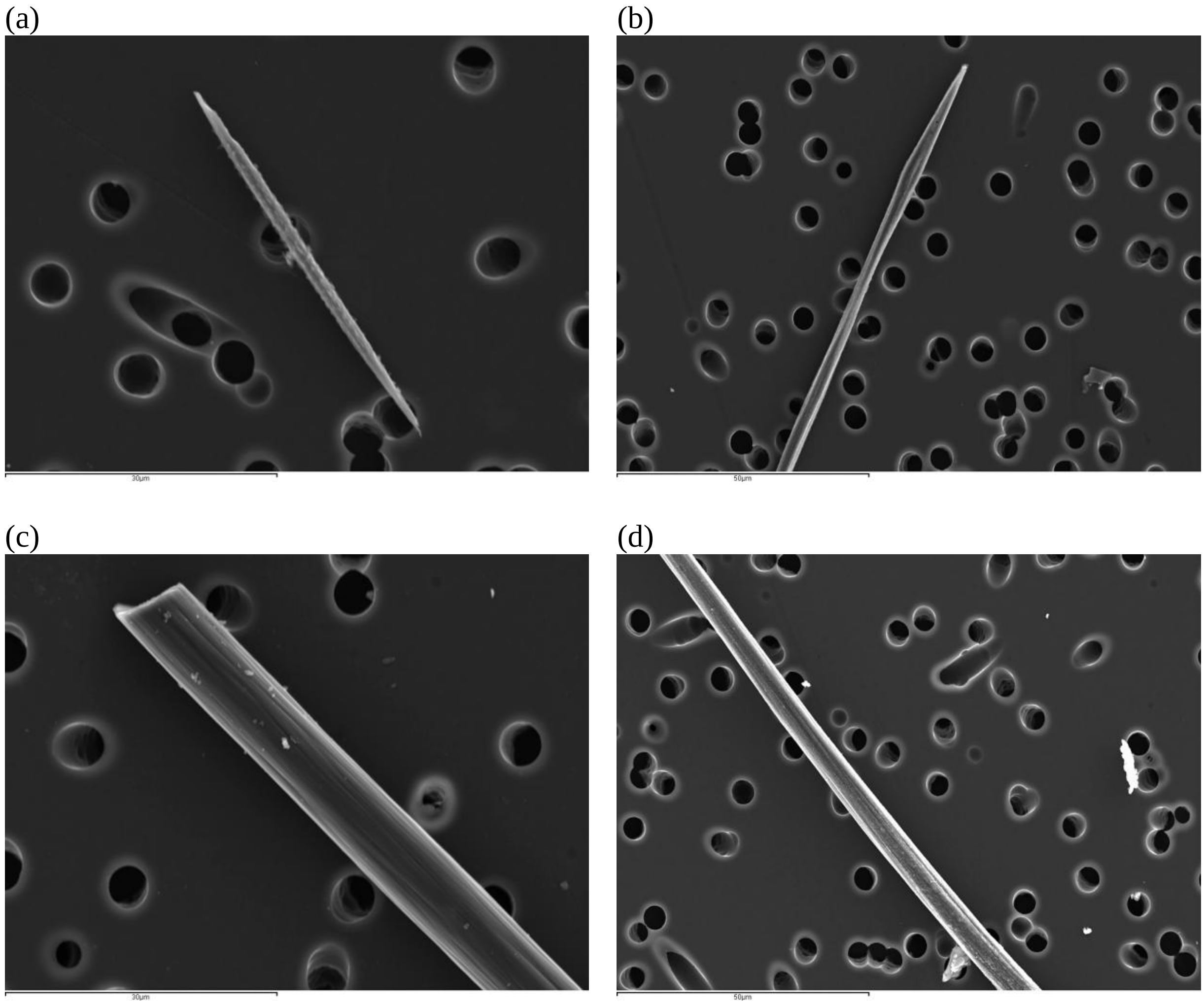



Figure 4. Particles from hair. (a and b) Long-thin pointed particles from growing-finishing pigs fine PM (a) and from piglets coarse PM (b). (c and d) Thick and striated tubular particles from growing-finishing pigs fine PM (c) and from sows coarse PM (d). Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

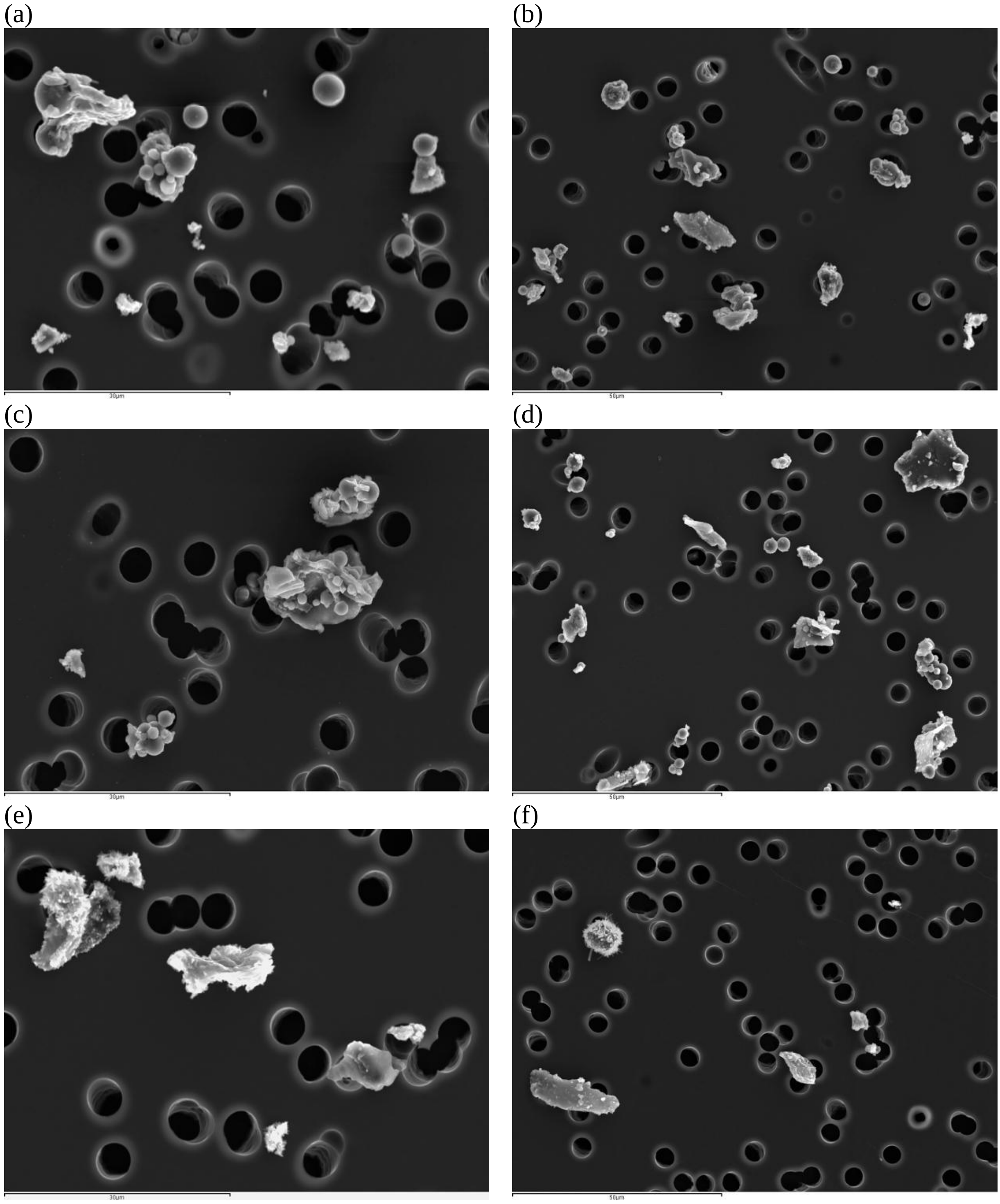


Figure 5. Manure particles from poultry. (a) Mixture of single rounded spherical and irregular particles from laying hens aviary system fine PM. (b) Few single rounded spherical and more abundant fragmented angular particles from laying hens aviary system coarse PM. (c) Agglomerated grape-like particles from broilers fine PM. (d) Some grape-like agglomerated particles and fragmented angular particles from turkeys coarse PM. (e and f) Mixture of rough,

fragmented, angular and ciliated rounded particles from turkeys fine PM (f) and from laying hens floor system coarse PM (f). Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

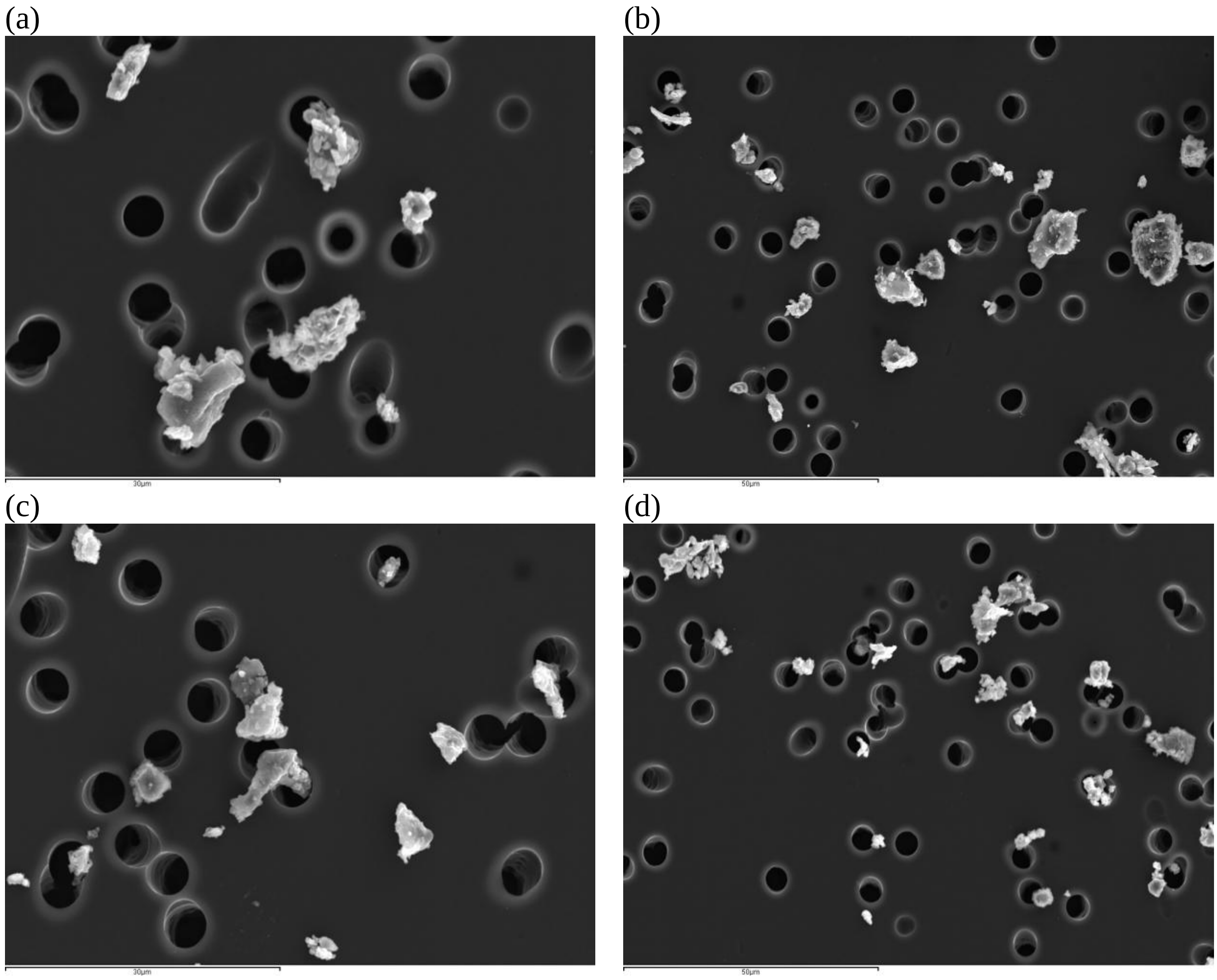



Figure 6. Manure particles from pigs. (a) Fragmented angular particles from piglets fine PM. (b and c) Mixture of fragmented, layered, angular and more rounded particles from growing-finishing coarse PM (b) and from growing-finishing fine PM (c). (d) Abundant layered and angular particles from sows coarse PM. Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

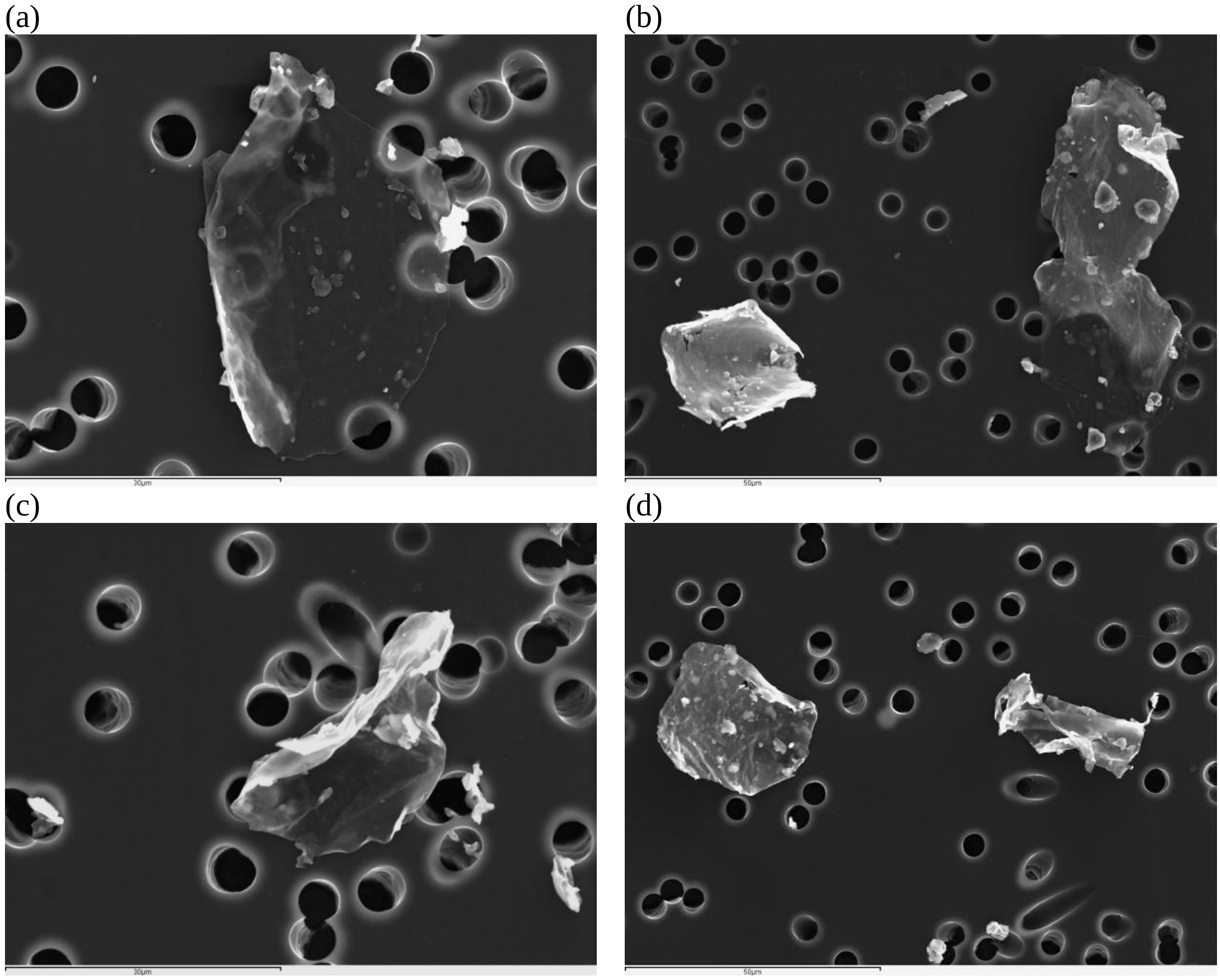


Figure 7. Particles from skin. All particles from sows. (a) Big, transparent, smooth, and flat particle in fine PM. (b) Rounded flake-like particles from coarse PM. (c) Folded and thin particle from fine PM. (d) Rough surfaces caused by deposited particles on top of flattened particles from coarse PM. Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

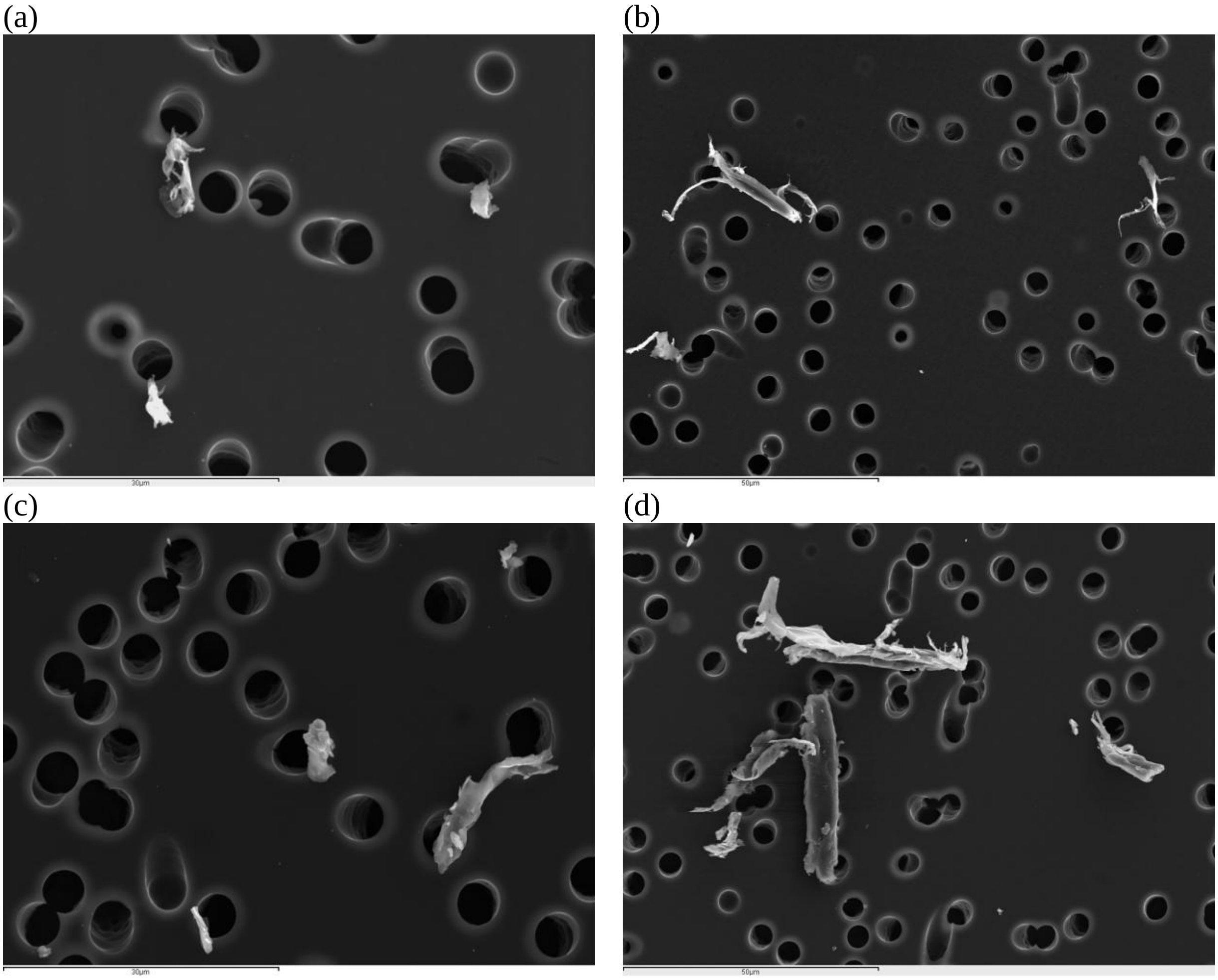



Figure 8. Particles from wood shavings. (a) Rounded flattened particles from broilers fine PM. round with irregular borders in fine PM. (b) Fibers from broilers in coarse PM. (c) Rounded and elongated, bent particle from turkeys fine PM. (d) Fibrous particles with very sharp edges from broilers in coarse PM. Images on the left: fine PM, scale bar 30 µm. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

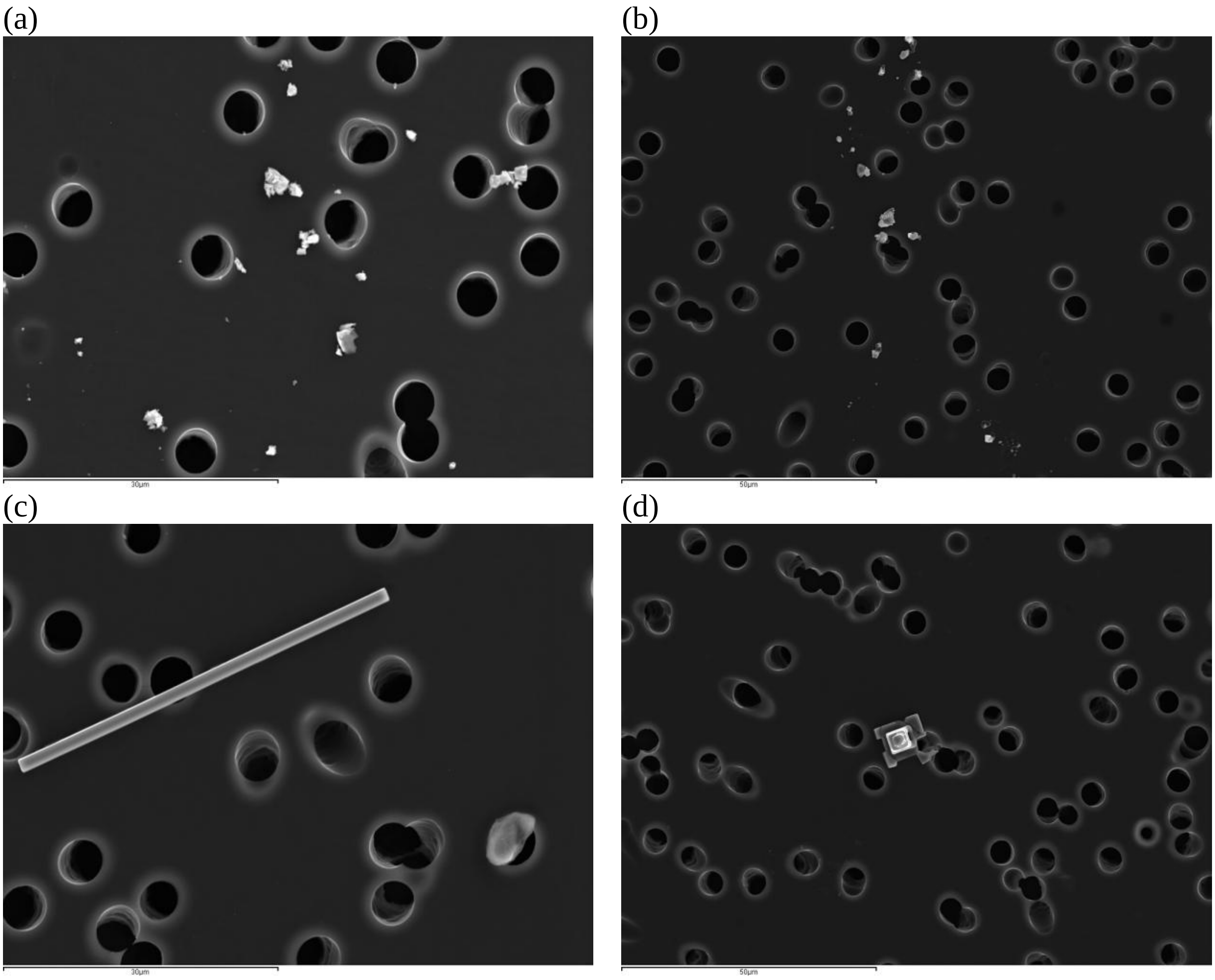



Figure 9. Particles from outside livestock houses. (a and b) Irregular angular, cracked, and fragmented particles in fine PM (a) and coarse PM (b). (c) Bar-shaped particle in fine PM. (d) Cubic particle in coarse PM. Images on the right: coarse PM, scale bar 50 µm. Note 5 µm diameter filter pores, shown as round dark holes.

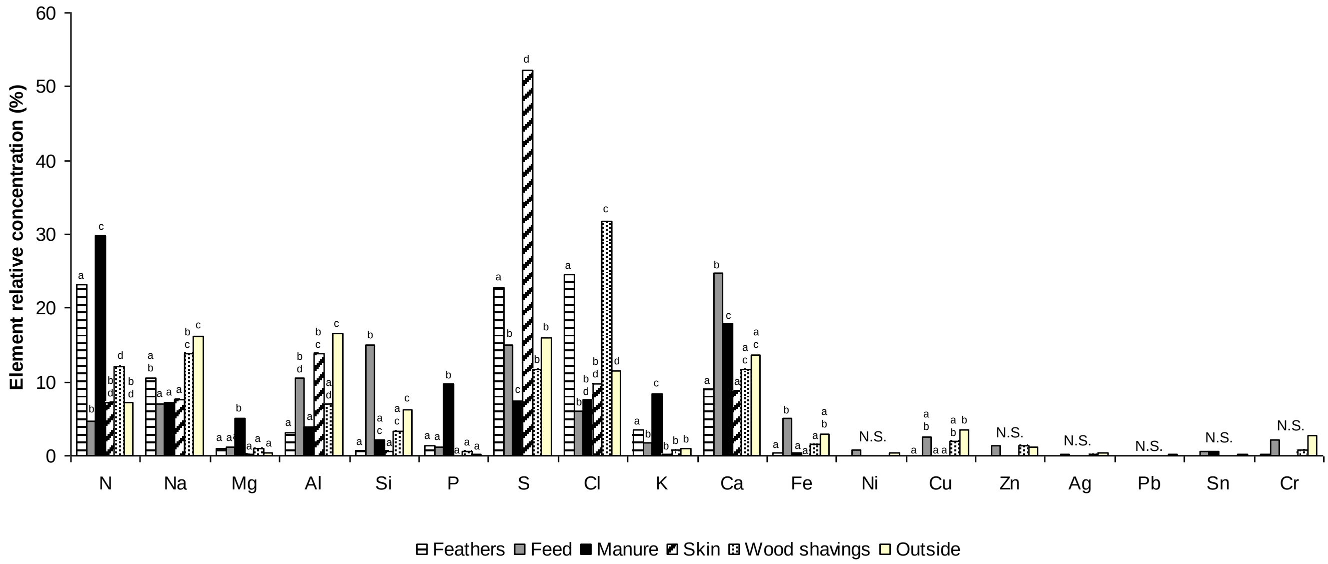



Figure 10. Average element relative concentration (%) for particles from different sources in fine PM2.5. Averages within an element lacking common superscript letter are significantly different ($P < 0.05$). (N.S. stands for non significant differences).

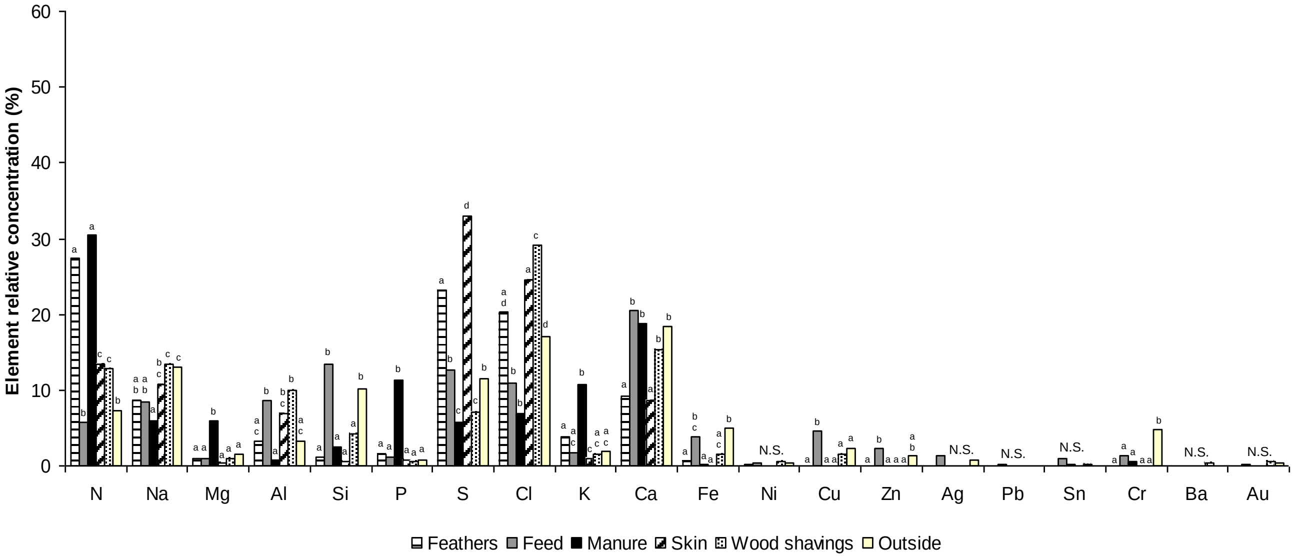



Figure 11. Average element relative concentration (%) for particles from different sources in coarse PM10-2.5. Averages within an element lacking common superscript letter are significantly different ($P < 0.05$). (N.S. stands for non significant differences).

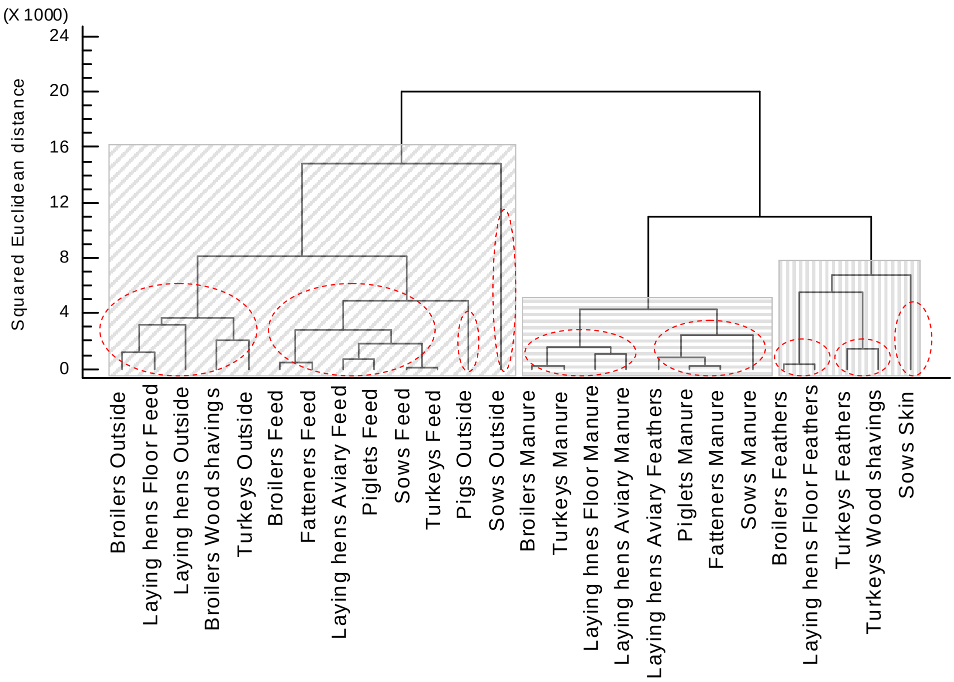



Figure 12. Hierarchical cluster analysis of elemental chemical concentrations of sources in different livestock categories in fine PM. Ward minimum-variance method. Stripped blocks represent three clusters and account for 46% variance explained by the clusters; whereas dotted circles represent nine clusters and account for 82% variance.

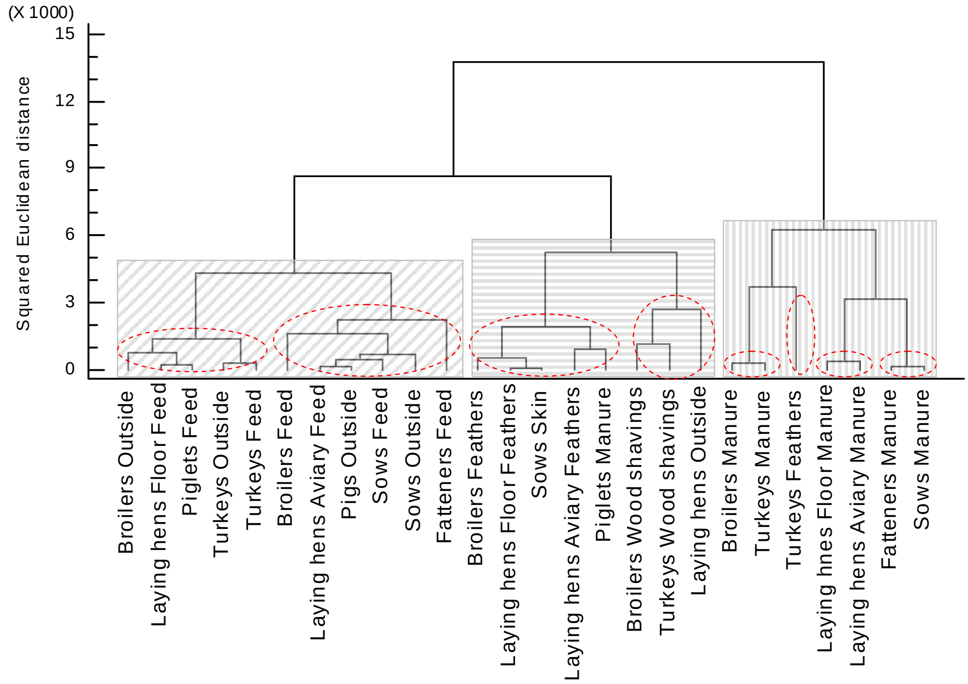



Figure 13. Hierarchical cluster analysis of elemental chemical concentrations of sources in different livestock categories in coarse PM. Ward minimum-variance method. Stripped blocks represent three clusters and account for 54% variance explained by the clusters; whereas dotted circles represent eight clusters and account for 81% variance.

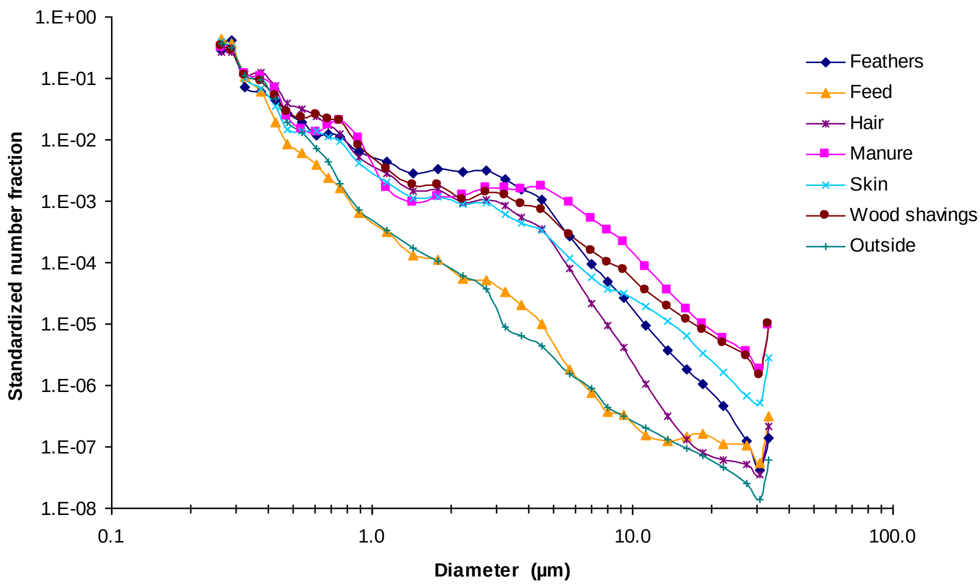



Figure 14. Standardized number fraction size distribution for particles from different sources (log-scale).

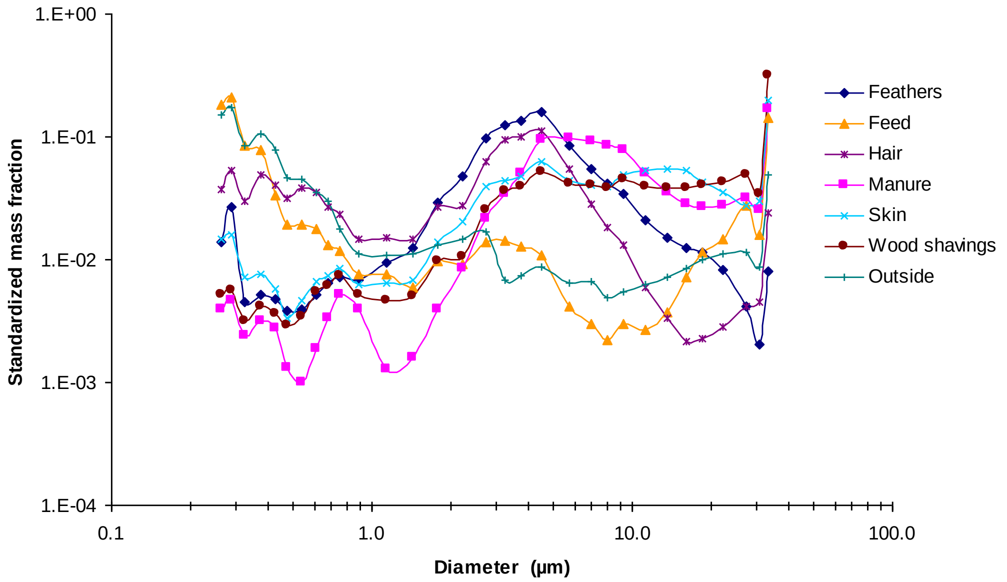



Figure 15. Standardized mass fraction size distribution for particles from different sources (log-scale).

***Research Highlights**

Research highlights

- Individual particles in collected sources from different housing systems for poultry and pigs show distinct and unique particle morphologies.
- Similar elements are present in all sources, but their relative element concentrations vary amongst sources and can be used to discriminate amongst them.
- Particle size and size distribution varies amongst sources and mainly depends on its mineral or organic origin.
- This work provides useful information for source identification and quantification in PM from livestock houses, improving the understanding of how PM is generated in such environments, and developing strategies for its reduction.