A methodology to select particle morpho-chemical characteristics to use in source apportionment of particulate matter from livestock houses

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Abstract. Intensive poultry and pig houses are major point sources of particulate matter (PM). The knowledge on the contribution of individual sources to PM in different fractions is essential to improve PM reduction from livestock houses. We developed a methodology to investigate which input data (particle chemical, morphological or combined characteristics) were best to distinguish amongst specific sources of airborne PM in livestock houses. We used a validation procedure with classification rules based on decision trees and analyzed misclassification errors. The PM from two livestock species (poultry and pigs), and in two different fractions (fine and coarse) was studied. Results showed the selection of the best input data varied with the sources, which depend on livestock species. Using only particle chemical characteristics resulted in higher overall classification accuracies (62 to 68%) than using only morphological characteristics (40 to 64%) in poultry and pigs. Particle morphological characteristics can add value when sources show distinctive and well defined morphologies or differ in size. Using combined chemical and morphological resulted in the highest overall classification accuracies (average of 69% of particles correctly assigned to their source) and lowest misclassification errors. This study provides a

methodological approach to assess input data and identifies the most effective characteristics to apportion PM in livestock houses. These data are promising to determine the contribution of different sources to PM in livestock houses and give insight in under and overestimation errors in the source apportionment.

Keywords: Animal housing, Atmospheric pollution, Dust, Expert systems, Image analysis.

1. Introduction

Livestock production systems are major point sources of particulate matter (PM). In certain European regions such as in the Netherlands, Flanders, North Italy, or North-East Spain where background PM concentrations due to other sources (traffic and industrial activities) are already high, PM emitted from livestock houses can cause exceedance of the limits established by the European air quality regulations (Directive 1999/30/EC and Directive 2008/50/EC).

To protect the environment and to ensure health and welfare of humans and animals in and around livestock houses, the concentrations and emissions of PM within such buildings must be reduced. One of the main challenges to reduce PM in livestock houses is to identify which sources to tackle. Sources of PM in livestock houses can be very variable, including: manure, feed, feathers, skin, bedding material, and micro-organisms (germs, fungi, viruses, bacteria, toxins and allergens) (Donham et al., 1986; Heber et al., 1988; Feddes et al., 1992; Qi et al., 1992; Cambra-López et al., 2011a). The knowledge on the contribution of each individual source to airborne PM (source apportionment) in different fractions would be useful to improve PM reduction in this field.

Additionally, information on size, morphology and chemical composition of individual particles offers the potential to specifically identify and quantify PM sources (Casuccio et al., 2004). Single-particle analysis with scanning electron microscopy (SEM) can provide chemical and morphological descriptive characteristics from hundreds of individual particles which can be further used to classify particles into distinct classes which resemble sources (Kim and Hopke, 1988; Willis et al., 2002; Coz et al., 2010). To do this, each source must have distinctive morphological and/or chemical features, which can be used to discriminate amongst them. When this is not the case or very specific sources need to be apportioned and distinguished, detailed morpho-chemical source profiles are necessary. Acquiring a detailed morpho-chemical source profile, however, is both expensive and time-consuming. Therefore, adequate methods which can select the best variables to discriminate amongst sources are required to improve the selection of particle characteristics to use in source apportionment of PM.

In livestock husbandry, as PM is mainly composed of primary particles of biological origin, most particles have a similar element composition, rich in nitrogen, sodium, magnesium, aluminium, silicon, chlorine, potassium, and calcium (Cambra-López et al., 2011b). However, Cambra-López et al. (2011b) reported that, although similar elements could be present in all sources, their relative element concentrations vary amongst sources and this can be used to discriminate amongst them. Furthermore, individual particles from different sources can show unique morphological features. The use of an automated system to extract such features can be useful to identify similarities and differences amongst sources. Consequently, to quantify the contribution of sources of PM in livestock houses, an assessment of input data to differentiate effectively amongst sources, and the selection of the morpho-chemical characteristics to be used in source apportionment of PM is necessary.

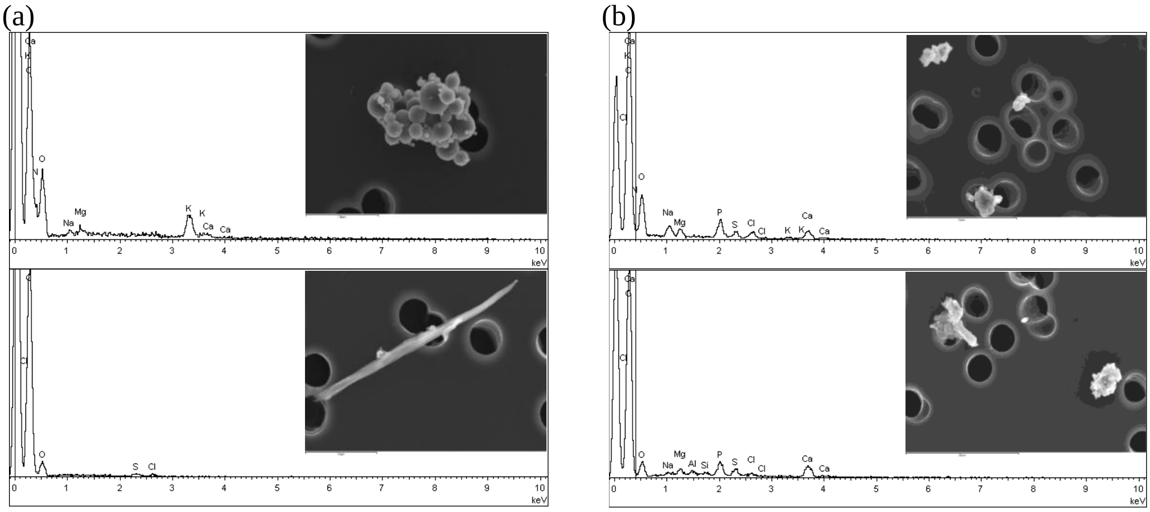
The aim of this work was to develop a methodology to investigate which input data (particle chemical, morphological or combined characteristics) were best to distinguish amongst specific sources of airborne PM in livestock houses. The PM from two livestock species (poultry and pigs), and in two different fractions (fine PM2.5 and coarse PM10-2.5) was studied. The convenience of using each input data was analyzed using a validation procedure with classification rules based on decision trees. The overall accuracy of the classification, and the underestimation and overestimation errors were calculated for each source. Its implications for use in source apportionment studies are discussed. This study provides a methodological approach to assess input data and identifies the most effective characteristics to apportion PM in livestock houses. With this information, individual apportionment to specific sources of PM in livestock houses will be improved, contributing to reduce this pollutant.

2. Material and methods

Fine (PM2.5) and coarse (PM10-2.5) PM source samples from poultry and pig houses were used in the assessment. We tested three scenarios to select the best input data to distinguish between specific sources of airborne PM in poultry and pig houses: firstly, classification using only particle chemical characteristics; secondly, classification using only particle morphological characteristics; and thirdly, the combination of both data sets.

Figure 1 shows examples of apportioning of particles to certain sources, chemically or morphologically. Examples: (a) particles from manure (top) and long-thin particle from feathers (bottom) in poultry showing different elemental composition and morphology; (b) particles showing very similar elemental composition and morphology but belonging to different sources in pigs, manure (top) and feed (bottom); (c) particles showing very similar morphologies but different elemental composition, feathers (top) and wood shavings (bottom); and (d) particles showing very similar elemental compositions (rich in sodium, Na; and chlorine, Cl) but different morphology belonging to different sources in pig feed (top) and outside pig houses (bottom).

(a) (b)



(c) (d)

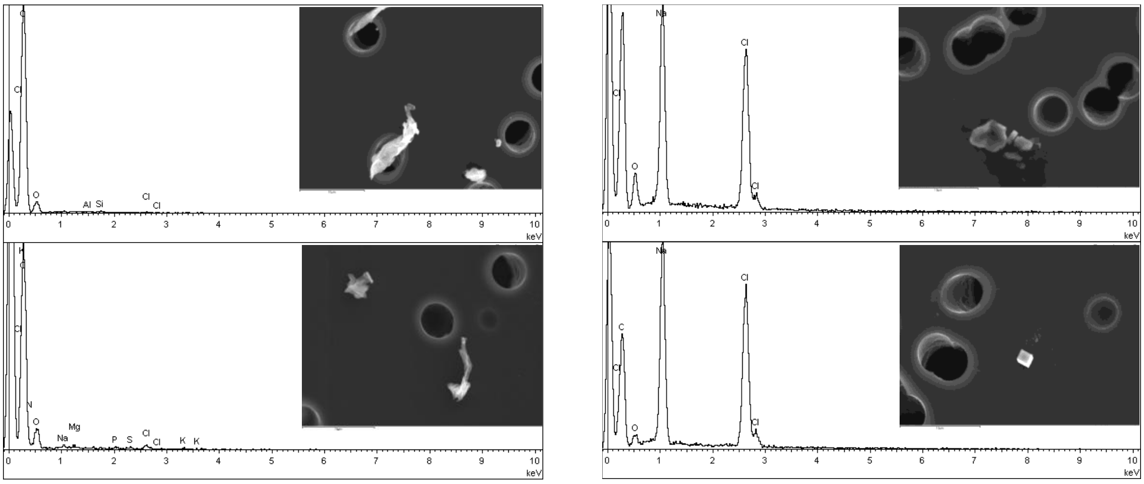


Figure 1. Examples of scanning electron microscopy photomicrographs of particles and X-ray elemental spectra showing chemical and morphological similarities and differences amongst sources of PM from poultry and pig houses. (a) Particle from poultry manure (top) and one long-thin particle from feathers (bottom); (b) particle from pig manure (top) and from pig feed (bottom); (c) particle from turkey feathers (top) and from wood shavings (bottom); and (d) particle from pig feed (top) and from outside source (bottom). Magnifications from 3000 to 3500x. Note 5 µm diameter filter pores, shown as round dark holes.

Single particle chemical and morphological characteristics were obtained using scanning electron microscopy (SEM) combined with energy-dispersive X-ray analysis (EDX). Single particle chemical and morphological data were obtained from particles from homogeneous known source samples. These data were used separately to develop a set of rules. The same particle data used to develop the set of rules were then used to test them following a validation procedure. In this procedure, each particle (from a known reference source) was assigned to one of the sources applying the classification rules. The accuracy of the particle source assignment (correct particle classification) was evaluated through error matrices. A scheme showing the procedure used in this study is shown in Figure 2.

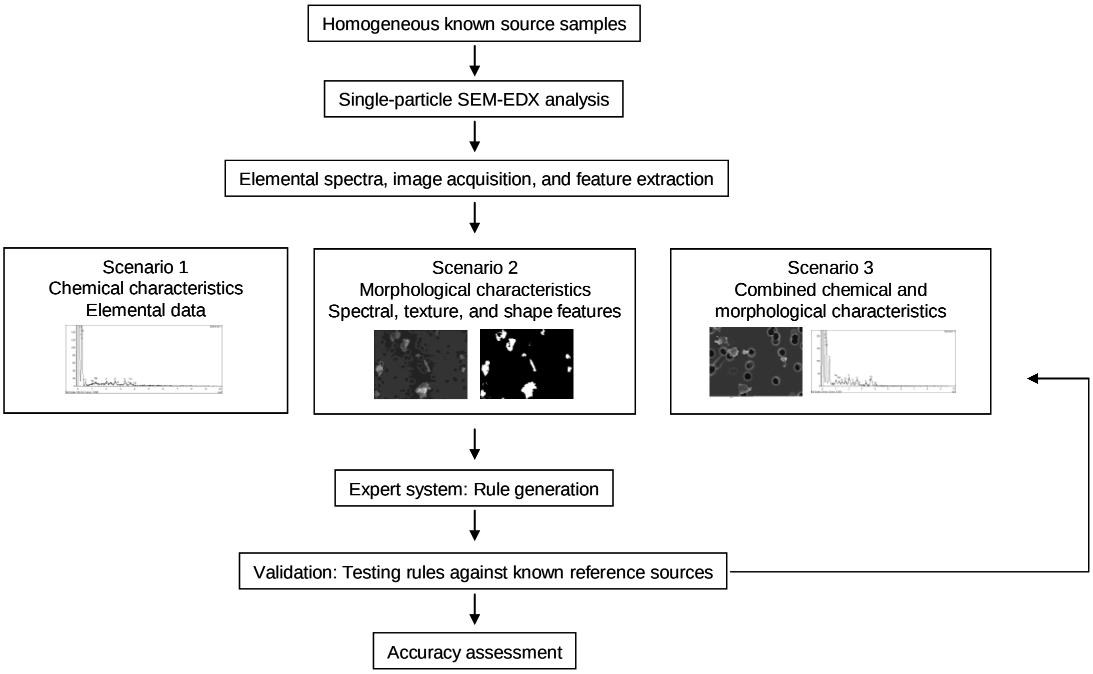


Figure 2. Flow diagram with the process used in this study.

2.1. Input data: single-particle SEM-EDX analysis

Known source samples, collected at 14 different farm locations for poultry (including broilers, laying hens in floor and aviary system, and turkeys) and pigs (including piglets, growing-finishing pigs, and dry-pregnant sows) were used in the assessment (Table 1). Two farms per housing system were sampled. Source samples were collected from feathers, feed, manure, skin, and wood shavings at each farm location, identified as major sources of PM in the study by Cambra-López et al. (2011a). Composite samples of potential PM sources were collected per source and farm by randomly sampling different locations in the livestock house. Skin samples were collected only from sows because it was impractical to collect such samples from younger animals (piglets and growing-finishing pigs) whose skin was not as loose as a sow's dandruff (Table 1).

Each source sample per farm was dried for 12 h at 70°C and then crushed in a ball mill for 1.5 min at 250 rpm. Dried and milled samples were stored at room temperature, and then airborne PM was generated in a laboratory dust generator to collect airborne fine and coarse PM samples from each source. The dust generator consisted of a stainless steel cylinder of 20 cm diameter and 30 cm height with an airtight lid, which had a mechanical agitation system with rotary blades. A varying quantity, from 0.2 g (feathers) to 40 g (feed), of each milled source per farm was introduced in the dust generator and agitated at 200 rpm. The generated PM was collected using a virtual cascade impactor (RespiCon, Wetzlar, Germany) which was placed inside the generator. This device sampled airborne fine and coarse PM onto separate polycarbonate filters (37 mm dia., 5 μm pore size). It is a two-stage virtual impactor that follows the convention of the European Standard (CEN, 1993) with a 50% cutoff at an aerodynamic diameter of 2.5 μm (for fine PM) and 10 μm (for coarse PM). A portable pump (Genie VSS5, Buck Inc, U.S.) was used to draw air through the impactor from the dust generator, at constant a flow of 3.11 L min^{-1}. A detailed description of the dust generation process and setup can be found in Cambra-López et al.(2011b). Sampling time during dust generation varied from 1 min to 7 h, depending on the amount of particles generated, aiming at particle loads of 5 to 20 μg particles cm^{-2} filter, to avoid particle agglomeration and perform individual particle SEM analysis (Willis et al., 2002). The generation procedure simulated the process by which PM can be generated in the livestock houses. According to Gill et al. (2006), generating, collecting, and measuring PM in a controlled laboratory setting are useful tools to determine emission potential per mass of source, and its physical, morphological, and chemical characteristics. The laboratory dust generation procedure used in our study worked by generating a large cloud of particles and then collecting a small amount of them.

Additionally, a representative sample of ambient outdoor fine and coarse PM was collected on each sampling day, at each location at a distance of about 10 to 15 m upwind using a virtual cascade impactor, same as for laboratory generated samples. Sampling time outside varied from 30 to 60 min. Table 1 summarizes the origin of the data used in the assessment and the sources used in the analysis.

Table 1. Summary of sources types for each livestock species and housing system used in the assessment (n= filter samples same for fine and coarse PM).

Livestock species	Housing system	Source types	n
Poultry	Broilers - bedding	Feed	8
	Turkeys - bedding	Feathers	8
	Laying hens - floor	Manure	8
	Laying hens - aviary	Wood shavings	4

		Outside	8
Pigs	Piglets- slatted floor Growing-finishing pigs - partially slatted floor Dry and pregnant sows - group housing	Feed	6
		Manure	6
		Skin	2
		Outside	6

High-resolution SEM (JEOL, JSM-5410) combined with EDX (Link Tetra Oxford Analyzer) was used to obtain single particle-by-particle chemical and morphological data. 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 with a double-sided carbon adhesive tape. Samples were then coated with carbon using a vacuum evaporator, to provide electrical conductivity and create a conductive coating for exposure to the SEM electron beam.

The SEM-EDX was conducted manually, operated under the same conditions throughout the study: 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. Secondary electron mode was used for particle location, measurement, analysis, and image acquisition. At least three fields of view (spots) per filter sample were analyzed. On each analyzed field, both an image (photomicrograph at 1000x or 1800x, saved in tif format 1024x768 resolution) and single particle X-ray spectra of every particle 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). The projected area was calculated from the two-dimensional projection of each particle. From the particle area, the projected area diameter was calculated. These size limits were set to minimize the amount of data acquired for non-particle features (e.g., filter substrate) at the magnifications used. All x-ray spectra were processed with INCA software (Oxford Instruments, Abingdon, U.K.), confirmed manually to correct for element omission or confusion, and checked to eliminate the contribution of the filter material (carbon and oxygen).

A total of 25 to 50 individual particles per sample were analyzed in each sample. Therefore, a total of 618 particles were analyzed in sources from poultry houses for PM2.5, and 805 for PM10-2.5 (including feed, feathers, manure, wood shavings, and outside source). A total of 317 particles were analyzed in sources from pigs for PM2.5, and 337 for PM10-2.5 (including feed, manure, skin, and outside source).

2.1.1. Feature extraction

Particle chemical characteristics: Elemental data

Elements with atomic number ≥ 6 (carbon) were obtained from elemental x-ray spectra for each particle in each source. All spectra were confirmed and checked manually to correct for the contribution of the filter material (carbon and oxygen). Based on chemistry, each particle was characterized by 25 elements: nitrogen (N), sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), silver (Ag), lead (Pb), tin (Sn), chromium (Cr), cobalt (Co), barium (Ba), bromide (Br), titanium (Ti), vanadium (V), antimony (Sb), and gold (Au). All elements were introduced in the expert system at once, because the decision tree approach can take into account correlation between variables, before applying rules.

Particle morphological characteristics: Spectral, texture, and shape features

The stored images (SEM photomicrographs of each field of view) were analyzed using the Object Based Image Analysis (OBIA) approach (Blaschke, 2010) using FETEX 2.0 Software (Ruiz et al., 2011). All images were radiometrically corrected by background values to avoid spectral differences due to acquisition conditions and to equalize the background value to compare intensity values between images. Individual particles were defined by means of segmentation using thresholding. The OBIA software extracted both image and shape based features for each detected particle (object): spectral and texture features (image based), and morphological features (shape based).

Spectral features provided information about the spectral response of particles through their grey level (intensity) properties. Texture features provided information about the spatial distribution of the intensity values in the image, giving information about heterogeneity, contrast, and rugosity of particles. These features were uniquely referred to an object, extracted from the group of pixels that constituted a particle (Balaguer et al., 2010). Histogram-based (kurtosis and skewness) features and seven of the most commonly used texture features based on the grey level co-occurrence matrix proposed by Haralick et al. (1973) were extracted: contrast, uniformity, entropy, variance, covariance or product moment, inverse difference moment, and correlation. Entropy was used as a measure of information content, defined as the randomness of intensity distribution. Finally, also as texture features, the mean and the standard deviation of the edgeness factor, representing the density of edges present in the neighborhood of each pixel (Laws, 1985) were extracted. Morphological features provided information about the complexity in the shape of the particles. Particle projected area, perimeter, and ellipse semi-axis values were extracted. Based on ratios between the area and the perimeter of the particles, compactness (C) (equation 1) (Bogaert et al., 2000), shape index (SI) (equation 2), and fractal dimension (FD) (equation 3) (Krummel et al., 1987; McGarigal and Marks, 1995) were calculated. Based on morphological characteristics, each particle was characterized by 23 variables, summarized in Table 2.

$$C = \frac{4 \times \pi \times Area}{Perimeter^2} \tag{1}$$

$$SI = \frac{Perimeter}{4 \times \sqrt{Area}} \tag{2}$$

$$FD = 2 \times \frac{\log\left(\frac{Perimeter}{4}\right)}{\log(Area)} \tag{3}$$

where:

Perimeter is the length of the outline of a particle surrounding the area.

Area is the surface of the particle.

The most meaningful morphological descriptive features were selected before being introduced in the expert system to avoid redundancy and obtain an efficient object description. Correlation analysis was used to group and interpret the redundancies in the information provided by the analyzed morphological variables using SAS Software (2001). Correlation between the complete set of variables was computed and analyzed. With this information, non-explanatory variables could be removed from the analysis.

Table 2. List and description of morphological particle characteristics based on spectral, texture and shape features.

Morphological feature	Basis and description	Variables
Spectral	Grey level intensity properties of particles	Mean, standard deviation, minimum, maximum, and range of intensity
Texture	Histogram-based characteristics	Skewness and kurtosis
	Based on the grey level co-occurrence matrix	Contrast, uniformity, entropy, variance, covariance or product moment, inverse difference moment, and correlation
	Density of edges present in the neighbourhood of each pixel	Mean and the standard deviation of the edgeness factor
Shape	Particle length and size	Area, perimeter, and ellipse semi-axis (axis A and B)
	Ratios between the area and the perimeter of the particles	Compactness, shape index, and fractal dimension

2.2. Expert system: User-defined classification rules

We used a rule-generator expert system to create classification rules based on decision trees from the single-particle data from homogeneous known source samples. An expert system is software that simulates the judgment and behaviour of a human with expert knowledge and experience in a particular field (Jensen, 2005). For each livestock species (poultry and pigs) and in each scenario, chemical, morphological or combined characteristics were introduced in the system to generate rules.

2.2.1. Rule generation based on decision trees

The process of building a set of rules in the form of a decision tree worked by dividing data using mutually exclusive conditions until the newly generated subgroups were homogeneous, i.e. all the elements in a subgroup belonged to the same source or a stopping condition was fulfilled. Decision trees used a hierarchical structure to develop the set of rules for each particle belonging to a known reference source, using organized conditions such as greater than, less than, equal to, addition, and subtraction to search the variables and conditions for which it could best separate particles from one source from the others with the given input data. Decision trees were built using See 5

Software, using the C5.0 classification algorithm. The C5.0 algorithm manages several data types, such as continuous or discrete, thus it is the most widely used to deduce decision trees for classifying images (Zhang and Liu, 2004). To improve accuracy, the boosting multi-classifier method was used, where the final classification rule results from the weighed average of ten decision trees, where the next decision tree corrects from the errors of the previous one (Freund, 1995).

Classification rules based on decision trees were generated for each group of sources in a given livestock species (see sources in Table 1). Classification rules were generated separately for the different input data in each scenario, separately for poultry and pig sources, and separately for fine and coarse PM. Figure 3 shows an example of a decision tree. It is the first decision tree generated using chemical and morphological particle characteristics in pig sources for fine PM, using See 5 Software.

```
See5 [Release 2.03]           -------------------
    Options:
          Test requires 2 branches with >= 4 cases
Read 317 cases

Decision tree:
P > 2.44:
:...Mg > 0.35: Manure (120/6)
:   Mg <= 0.35:
:   :...Si <= 1.98: Manure (4)
:       Si > 1.98: Feed (4)
P <= 2.44:
:...Al > 61.59: Outside (39/4)
    Al <= 61.59:
    :...MAX INTENSITY > 251:
        :...MIN INTENSITY <= 70: Skin (11/4)
        :   MIN INTENSITY > 70: Outside (17/3)
        MAX INTENSITY <= 251:
        :...CORRELATION > 0.96: Skin (12/3)
            CORRELATION <= 0.96:
            :...MEAN INTENSITY <= 137.85:
                :...Ca <= 96.78: Feed (19/3)
                :   Ca > 96.78:
                :   :...ELLIPSE B <= 0.21: Feed (5)
                :       ELLIPSE B > 0.21: Manure (5)
                MEAN INTENSITY > 137.85:
                :...N > 30.27: Manure (6/2)
                    N <= 30.27:
                    :...ENTROPY > 2.45: Feed (27/1)
                        ENTROPY <= 2.45:
                        :...S > 39.72: Outside (5/2)
                            S <= 39.72:
                            :...INVERSE DIFFERENCE MOMENT <= 0.29: Outside (5/1)
                                INVERSE DIFFERENCE MOMENT > 0.29: Feed (38/5)
```

Figure 3. Example of a set of rules in the form of a decision tree generated using chemical and morphological particle characteristics in pig sources for fine PM. Chemical and morphological variables are indicated on the left, whereas classes are indicated on the right. Each line represents a

condition (greater than, less than or equal to) within a rule. Each rule includes the conditions to be fulfilled by each class (i.e. manure, feed, outside, or skin). Numbers in parentheses next to each class (m/n) represent: m, the number of cases that fulfil the conditions within each rule; n (where indicated) the number of cases that do not fulfil the conditions within the rule.

2.2.2. Validation of classification rules against known reference sources

We used the jackknifing procedure (a form of leave-one-out-cross validation statistical method) to assess the accuracy of the classification rules and validate them against reference source data in each scenario. This method involves re-sampling data, by repeatedly applying the generated rules to the same sampled set of data used to create them. The jackkniffing procedure worked by leaving out a single observation at a time (one particle), generating rules for the rest of the particles, and then validating those rules against the left out particle observation. This was done for all observations. As a result from this validation, the accuracy of the classification and the degree of misclassification among sources was analyzed using error matrices or contingency tables (Aronoff, 1982; Story and Congalton, 1986; Congalton, 1991).

The error matrix was built by comparing the source assigned to each particle observation after the validation process with its reference source; and it presented the number of times a correct particle source assignment was made. These steps were essential to assess how well the classification rules fitted to the reference source data. Error matrices were also used to analyze the degree and direction of the most frequent misclassifications and to understand better and predict how the future classification of airborne on-farm samples would work when applying these classification rules to a mixture of unknown particles.

As an example, the construction of the error matrix in a given scenario, for a given number of particles (N observations) from two sources (source 1 and 2), worked by classifying each observation into one of the sources, corresponding to one of the four cells in the error matrix (Table 3). The classification rules would assign each particle observation into source 1 or 2 depending on its characteristics (input data), which vary depending on the scenario. In the example below, a, b, c, and d are the observed particle frequencies of source 1 and 2. They add up to the sample size (N).

The sum of reference particles, the row total (n_x), equals the frequency (total number of particles) actually belonging to each source. The sum of all classified particles, the column total (m_x), equals the frequency (total number of particles) classified into each source after validation process. On the one hand, ‘a’ equals the number of times a particle belonging to source 1 was correctly classified into source 1; ‘b’ equals the number of times a particle from source 1 was misclassified into source 2; analogously, ‘c’ equals the number of times a particle belonging to source 2 was misclassified into source 1; and finally ‘d’ equals the number of times a particle belonging to source 2 was correctly classified into source 2. In other words, the number of particles ‘b’ should have been assigned to source 1; and the number of particles ‘c’ should have been assigned to source 2. Cell ‘b’ and ‘c’ are related in the way that ‘b’ represents the underestimation of source 1, as the number of particles omitted from source 1 and incorrectly assigned to source 2. Cell ‘c’ represents the overestimation of source 1, as the number of particles from source 2 incorrectly assigned to source 1.

Table 3. Example of error matrix or contingency table for N observation and two sources.

	Classified as		
Reference	Source 1	Source 2	Row total (n_x)
Source 1	a	b	n_1
Source 2	c	d	n_2
Column total (m_x)	m_1	m_2	N=(a+b+c+d)

Overall measure of accuracy was obtained by dividing the total correct validations in each source (diagonal cells in Table 3) by the total number of classified particles (N) (equation 4). Misclassifications were calculated as measures of underestimate and overestimate error, as the complementary function of accuracies. One minus the sum of the number of particles that have been incorrectly assigned to the reference source divided by the row total represented the underestimate error for each source the row represented (equation 5 and 6). One minus the sum of the number of particles that have been incorrectly assigned to the classified source divided by the column total represented the overestimate error for each source the column represented (equation 7 and 8). To compare results and analyze under and over estimations, error matrices were standardized by the reference number of particles in each source (n_x). This means that after standardization n_2 equals n_1. The prediction accuracy of source apportionment was finally calculated dividing the column total (m_x) by the row total (n_x) for each source (equation 9 and 10).

$$Overall\ accuracy = \frac{(a+d)}{N} \tag{4}$$

$$Underestimate\ error\ source\ 1 = 1 - \left(\frac{b}{n_1}\right) \tag{5}$$

$$Underestimate\ error\ source\ 2 = 1 - \left(\frac{c}{n_2}\right) \tag{6}$$

$$Overestimate\ error\ source\ 1 = 1 - \left(\frac{c}{m_1}\right) \tag{7}$$

$$Overestimate\ error\ source\ 2 = 1 - \left(\frac{b}{m_2}\right) \tag{8}$$

$$Prediction\ accuracy\ source\ 1 = \frac{m_1}{n_1} \tag{9}$$

$$Prediction\ accuracy\ source\ 2 = \frac{m_2}{n_2} \tag{10}$$

We also estimated error matrices and overall accuracies based on particle mass instead of particle numbers (frequency). We calculated the particle mass in each source, in each livestock species and PM fraction using the particle-by-particle masses. The overall accuracy was then obtained by dividing the mass from each correct validation in each source by the total mass of all classified particles. Misclassification errors (underestimate and overestimate) were also calculated in the same way as for particle numbers. The mass for each particle was calculated from the area and diameter provided by the SEM images, assuming a value for particle density. Density values of 1.2 g cm^{-3} (feathers), 2.6 g cm^{-3} (feed), 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). Calculations in numbers and in mass were performed because as particles from each source can have different sizes and consequently different masses, the effect of correct classifications and misclassifications could differ.

3. Results

3.1. Scenario 1: Particle classification based only on chemical composition

Overall accuracies of the generated rules using particle chemical characteristics were slightly higher in pigs compared with poultry. Overall accuracies varied from 57 to 62% in poultry and from 64 to 68% in pigs, for PM2.5 and PM10-2.5.

In poultry (Table 4), average misclassification errors ranged from 38 to 55%. In number of particles, manure source showed the lowest misclassification errors, being underestimate errors (from 9 to 15%) lower than overestimate errors (from 27 to 30%). Wood shavings source showed the highest misclassification errors, being underestimate errors (from 63 to 77%) higher than overestimate errors (from 37 to 44%). This means that 63 to 77% of particles from wood shavings were omitted from its reference source (underestimate error) and incorrectly assigned to other sources, but only 37 to 44% of particles from other sources were incorrectly assigned to wood shavings (overestimate error). The other sources presented similar underestimate and overestimate errors. Overall, misclassification errors were comparable in PM2.5 and PM10-2.5. Expressed in particle mass, outside source presented much higher underestimate and overestimate errors (ranging from 65 to 95%) than when expressed in number of particles, especially in PM10-2.5. In feed, overestimate errors also increased when expressed in mass, whereas in wood shavings, overestimate errors sharply decreased, especially in PM10-2.5. The rest of sources presented relatively similar figures when expressed in numbers compared with mass, showing a similar distribution between over and underestimate errors.

In pigs (Table 5), average misclassification errors ranged from 24 to 51%. In particle numbers, all sources showed lower misclassification errors (ranging from 9 to 50%) compared with poultry, except for outside source in PM10-2.5 which presented a high underestimate error (83%). Manure showed the lowest misclassification errors. Both feed and manure sources showed higher overestimate than underestimate errors; whereas skin and outside sources showed higher underestimate than overestimate errors for PM2.5 and PM10-2.5. Expressed in particle mass, manure presented no difference in over and underestimate errors. The other sources, however, presented differences in the distribution between over and underestimate errors, especially between PM2.5 and PM10-2.5. This is the case of feed, which presented higher over and underestimate errors in particle mass compared with particle numbers only in PM10-2.5; and also the case of skin, which presented higher overestimate errors when expressed in mass in PM10-2.5; and outside source which presented lower overestimate errors in both fractions when expressed in particle mass compared with particle numbers.

Table 4. Underestimate error (UE) and overestimate error (OE) per source and average, in percentage (%) per particle number and mass, for poultry, for PM2.5 and PM10-2.5, using only particle chemical composition.

Reference source	PM2.5				PM10-2.5			
	Number		Mass		Number		Mass	
	UE	OE	UE	OE	UE	OE	UE	OE
Feathers	30.8	54.6	23.7	48.8	25.7	42.3	29.4	32.0
Feed	55.3	41.3	77.1	74.4	45.1	45.7	49.2	67.0
Manure	14.7	29.9	8.2	38.7	8.6	26.6	6.0	40.7
Wood shavings	76.6	44.0	86.5	48.9	62.8	37.1	52.3	12.1
Outside	37.7	42.4	70.3	65.2	45.8	37.4	95.0	83.2
Average	43.0	42.4	53.2	55.2	37.6	37.8	46.4	47.0

Table 5. Underestimate error (UE) and overestimate error (OE) per source and average, in percentage (%) per particle number and mass, for pigs, for PM2.5 and PM10-2.5, using only particle chemical composition.

Reference source	PM2.5				PM10-2.5			
	Number		Mass		Number		Mass	
	UE	OE	UE	OE	UE	OE	UE	OE
Feed	31.0	48.4	37.2	43.5	20.6	50.4	81.4	80.8
Manure	8.9	20.6	9.8	30.7	9.6	30.2	8.6	41.6
Skin	47.4	12.1	36.5	2.7	32.3	20.0	43.0	51.2
Outside	39.7	34.1	24.5	19.1	83.0	34.0	72.0	6.5
Average	31.7	28.8	27.0	24.0	36.3	33.6	51.3	45.0

3.2. Scenario 2: Particle classification based only on morphological characteristics

Overall accuracies of the generated rules using particle morphological characteristics were higher in pigs compared with poultry, and lower than in scenario 1, especially in poultry. Overall accuracies varied from 40 to 59% in poultry and from 63 to 64% in pigs, for PM2.5 and PM10-2.5.

In poultry (Table 6), average misclassification errors ranged from 37 to 61%. In number of particles, all sources showed similarly high errors, which were only remarkably lower for manure in PM10-2.5 (only underestimate error), and for wood shavings and outside source also in PM10-2.5 (overestimate errors). Feed showed higher misclassification errors in PM2.5 (from 72 to 86%) than in PM10-2.5. Expressed in particle mass, outside source showed higher underestimate errors than in number of particles. Particle mass from feed and outside sources showed especially high underestimate errors in PM2.5 (86 to 93%), but also high overestimate error (96%) in outside source in PM10-25.

In pigs (Table 7), average misclassification errors ranged from 33 to 57%. In number of particles in PM2.5 and PM10-2.5, misclassification errors were lower than in poultry. Manure source showed the lowest underestimate errors (from 13 to 15%) but presented high overestimate errors (from 42 to 48%), consequently showing more particles from other sources incorrectly assigned to manure source. On the contrary, skin source showed the lowest overestimate errors (2 to 5%). Overall, feed and outside sources showed the highest misclassification errors. In particle mass, feed and outside source showed generally higher misclassification errors than in number of particles. Underestimate errors of feed and outside were much higher (from 77 to 97%) compared with overestimate errors (from 20 to 61%), being these remarkably high (82%) in outside source in PM10-2.5. Skin source showed totally different results in mass compared with numbers, showing higher overestimate (37 to 46%) than underestimate errors (0.5 to 2%) in mass.

Table 6. Underestimate error (UE) and overestimate error (OE) per source and average, in percentage (%) per particle number and mass, for poultry, for PM2.5 and PM10-2.5, using only morphological characteristics.

Reference source	PM2.5				PM10-2.5			
	Number		Mass		Number		Mass	
	UE	OE	UE	OE	UE	OE	UE	OE
Feathers	53.5	67.4	35.6	68.0	34.9	54.5	12.9	50.5
Feed	85.9	72.3	85.8	30.3	47.4	36.5	54.5	65.3
Manure	36.5	56.9	36.8	58.5	15.6	43.4	16.3	36.6
Wood shavings	68.8	43.5	53.9	58.6	59.0	23.0	61.5	31.0
Outside	54.4	56.0	92.6	50.4	48.6	28.1	96.3	28.6
Average	59.8	59.2	60.9	53.1	41.1	37.1	48.3	42.4

Table 7. Underestimate error (UE) and overestimate error (OE) per source and average, in percentage (%) per particle number and mass, for pigs, for PM2.5 and PM10-2.5, using only morphological characteristics.

Reference source	PM2.5				PM10-2.5			
	Number		Mass		Number		Mass	
	UE	OE	UE	OE	UE	OE	UE	OE
Feed	46.0	48.0	87.1	60.9	41.2	43.9	85.8	56.4
Manure	14.8	41.9	16.8	46.3	13.4	48.2	9.9	53.6
Skin	21.1	5.4	1.6	46.4	19.4	2.4	0.5	36.8
Outside	63.5	44.6	77.3	20.3	72.3	39.2	97.1	81.6
Average	36.3	35.0	45.7	43.5	36.6	33.4	48.3	57.1

3.3. Scenario 3: Particle classification using combined data set (both chemical and morphological characteristics)

Overall accuracies of the generated rules using both chemical and morphological characteristics were higher in pigs compared with poultry, and higher than in scenario 2. Overall accuracies varied from 58 to 68% in poultry and from 72 to 78% in pigs, for PM2.5 and PM10-2.5.

In poultry (Table 8), average misclassification errors ranged from 30 to 42%. In number of particles, most sources showed misclassification errors varying from 25 to 60% in PM2.5 and PM10-2.5, except for manure source. Manure source showed the lowest misclassifications, and presented higher overestimation errors (from 23 to 26%) than underestimate errors (from 6 to 15%). Wood shavings source showed the highest misclassification errors showing much higher underestimate errors (from 60 to 77%) than overestimate errors (from 18 to 44%). In particle mass, misclassification errors for wood shavings source in PM10-2.5 were lower compared with number of particles. In particle mass, outside source presented very high underestimate error (96%) in PM10-2.5. For the rest of sources, misclassifications results were generally comparable in particle mass and in number.

In pigs (Table 9), average misclassification errors ranged from 21 to 30%. In number of particles, all sources except for outside source in PM10-2.5 showed low misclassifications expressed as low underestimate and overestimate errors (ranging from 7 to 45%) in PM2.5 and PM10-2.5. In particle mass, skin source showed much higher overestimate errors (from 23 to 31%) than underestimate errors (1%). Mass of skin followed the same trend as in scenario 2, presenting opposite results in number of particles compared with mass as regards over and underestimation. For other sources, results were generally comparable in particle mass and in number.

Table 8. Underestimate error (UE) and overestimate error (OE) per source and average, in percentage (%) per particle number and mass, for poultry, for PM2.5 and PM10-2.5, using combined chemical and morphological characteristics.

Reference source	PM2.5				PM10-2.5			
	Number		Mass		Number		Mass	
	UE	OE	UE	OE	UE	OE	UE	OE
Feathers	29.1	53.2	18.0	58.7	24.8	44.5	11.2	32.1
Feed	49.4	39.3	49.2	13.1	27.1	34.7	43.4	60.1
Manure	15.3	26.0	10.9	23.5	5.9	23.4	5.3	22.3
Wood shavings	76.6	43.7	71.3	40.5	60.3	17.9	23.7	20.7
Outside	38.6	43.7	30.4	11.2	43.0	30.1	95.7	53.4
Average	41.8	41.2	36.0	29.4	32.2	30.1	35.9	37.7

Table 9. Underestimate error (UE) and overestimate error (OE) per source and average, in percentage (%) per particle number and mass, for pigs, for PM2.5 and PM10-2.5, using combined chemical and morphological characteristics.

Reference source	PM2.5				PM10-2.5			
	Number		Mass		Number		Mass	
	UE	OE	UE	OE	UE	OE	UE	OE
Feed	25.0	35.4	45.3	32.6	10.8	45.3	65.8	49.2
Manure	8.9	19.0	11.1	34.1	7.0	18.5	6.4	13.4
Skin	21.1	6.5	0.5	22.9	22.6	6.9	0.5	30.7
Outside	33.3	23.3	52.6	13.7	70.2	24.7	39.0	24.8
Average	22.1	21.1	27.4	25.8	27.7	23.9	27.9	29.5

4. Discussion

Our results showed that overall accuracies ranged from 40% to 79%. Overall accuracies were higher when using only particle chemical characteristics (scenario 1) compared with scenario 2 (morphological characteristics); whereas the highest accuracies were obtained using scenario 3 (combined chemical and morphological characteristics). This indicates that PM from livestock houses comprises a wide range of particle types not only between but also within sources, which makes it difficult to find a single feature (based on chemical or morphological characteristics only) that can distinguish one source from the rest as a rule of thumb. Results in scenario 3 showed higher overall accuracies and lower misclassification errors compared with the other scenarios. In this scenario, the classification rules could search for the best criteria for classification from a wider range of options, using chemical characteristics when sources were more similar morphologically, and morphological characteristics when sources were more similar chemically. Therefore, the selection of the best input data can vary depending on the sources, which depend on livestock species. Our results showed each scenario performed differently in poultry compared with pigs, suggesting livestock species can be a variation factor in the selection of particle characteristics. In our study, only feed, manure, and outside source were common in poultry and pig tests.

In poultry, higher accuracy and lower misclassifications were observed in scenario 1 compared with scenario 2, while in pigs scenario 1 and 2 performed more similarly. These results indicate that most sources in poultry houses are best differentiated by their chemical composition instead of by their morphological characteristics. This could be influenced by the strong presence of P and K in particles from manure in poultry compared with other sources (Schneider et al., 2001; Cambra-López et al., 2011b). This results in a more homogeneous element composition of manure from poultry, compared with its diverse and complex morphology. The higher misclassification errors in scenario 2 compared with scenario 1 for the manure source in poultry, could be explained by the existence of two types of manure particles from poultry's excreta. Feddes et al. (1992) reported the presence of these two morphological types of particles in poultry excreta: rounded spheres from 3 to 8 μm in diameter, and other less rounded and more irregular fecal particles in turkeys.

Furthermore, particle size could also explain the high misclassification errors in scenario 2 in poultry compared with scenario 1. Cambra-López et al. (2011b) reported a smaller range for particle size (expressed as projected area diameter) in particles from poultry sources than from pig sources. For instance, average particle's diameter of feathers, feed, manure, wood shavings, and outside was shown to vary between 2.1 and 5.9 μm; whereas particles from skin and hair (only present in pigs) can show diameters two-fold to three-fold higher. This could also be the reason why feed and outside sources generally presented higher misclassification errors in scenario 2 compared with scenario 1 (especially in poultry), and higher in PM2.5 than in PM10-2.5. These two sources have been reported to show irregular and angular morphologies and similar size and size distributions (Cambra-López et al., 2011b). Moreover, our results show that size-only is not a recommendable variable to distinguish amongst most sources in livestock houses, because particles from different sources can be found in the same size ranges. Size can only be useful to distinguish amongst sources when one source with large particles (e.g. skin) with distinctive and well defined individual particle morphology, wants to be distinguished from the rest. Nevertheless, the accuracy of sizing particles using SEM can be reduced, as particles deviate from spheres (Willis et al., 2002). In our study, most particles showed irregular shapes, particles would impact on the filter in their most stable orientation, generally exposing the largest dimension on the filter plane. Moreover, the projected area diameter calculated from the particle area in this study, could be influenced by the projected area diameter being the diameter in the two-dimensional view, parallel to the plane of the filter; and the differences between geometric diameter and aerodynamic diameter.

Despite these limitations, the observed differences in misclassification errors between particle numbers and particle mass indicate two facts: (i) in sources showing small particles (e.g. feed and outside), large particles are more frequently misclassified into other sources than small particles; and (ii) in sources showing large particles (e.g. skin), small particles are more frequently misclassified into other sources than large particles. This could be seen in the higher underestimate errors in mass compared with numbers for sources showing generally small particles (feed and outside). Furthermore, our results indicate that these misclassified particles (from feed and outside) were incorrectly assigned to sources showing large particles (such as skin), suggested by the higher overestimate errors in mass compared with numbers for skin source. Sources showing large particle masses (such as feathers and wood shavings in poultry, and especially skin in pigs) presented higher overestimate than underestimate errors in mass compared with numbers suggesting it was probably small particles which had little influence on the mass which were misclassified. In mass, the effect of one single misclassification of a large particle could have more effect than a misclassification of a small particle, expressed in number. Nevertheless, to improve the understanding of misclassification and their influence in particle mass, the selection of particles should have been focused on coarse particles, and not on the whole size range as in this study.

The main objective of this study was to develop a methodology to investigate which input data (particle chemical, morphological or combined characteristics) were more appropriate to distinguish amongst specific sources of airborne PM in livestock houses. This can help to improve the knowledge on the most cost-effective input data to use. Our results suggest that this can depend on which source to apportion. When identification and quantification of the contribution of all individual sources to PM concentrations and emissions in livestock houses is the objective, a combination of chemical and morphological characteristics give high accuracies. However, obtaining complete particle characterization is time consuming and manual SEM-EDX single-particle analysis is laborious and expensive. Our results suggest that when only few sources want to be distinguished from the rest, the use of particle chemical or morphological particle characteristics as separate input data could yield acceptable results. However, this can only be applied in specific cases. For instance, if particles from manure want to be distinguished from the rest of sources, the use of only chemical particle characteristics would result in 70 to 91% of manure particles being correctly classified. If skin wants to be distinguished from the rest of sources as in pig houses, then the use of only morphological particle characteristics would result in 79 to 98% of skin particles being correctly classified. To distinguish feed from the rest of sources, which might be of interest when evaluating the effect of certain reduction techniques which focus on “low-dust” feeding systems (Dawson, 1990; Nannen et al., 2005; Costa et al., 2007), according to our results, either using particle chemical characteristics or combined combination of particle chemical and morphological characteristics would result in 45 to 89% of particles from feed being correctly classified. To make a general recommendation for future studies, Table 10 presents a list of the sources analyzed in this study and the recommended scenario (lowest misclassification errors) according to our results. When misclassification errors differ between scenarios, recommendations are straightforward. However, when misclassification errors are similar (less than 5% difference) amongst scenarios for a given source (for instance in feathers, manure or skin source), more than one scenario can be recommended.

Nevertheless, based on our results, to apportion all individual sources to PM concentrations and emissions in livestock houses, we would recommend the use of combined chemical and morphological particle characteristics (scenario 3). In this scenario, an average overall accuracy of 69% (standard deviation of 6%) for particle number and mass in PM2.5 and PM10-2.5 was obtained. In other words, on average 69% of particles belonging to a mixture of sources were correctly assigned to their reference source based on their chemical and morphological characteristics. This accuracy can be considered reasonable because it implies that only about 30% of the particles would be misclassified and incorrectly apportioned. The implications for source apportionment in livestock houses of this misclassification value are low, because the main aim of source apportionment in livestock houses is to provide knowledge on most important sources which can be used to develop new PM reduction techniques and optimize the existing ones. Therefore, this level of accuracy would be sufficiently high and would allow obtaining the overall picture of the major or dominant sources of PM in livestock houses.

Table 10. Check list of recommended scenario for particle identification from different sources.

Source	Scenario 1 Particle chemical characteristics	Scenario 2 Particle morphological characteristics	Scenario 3 Combined chemical and morphological particle characteristics
Feathers	X		X
Feed			X
Manure	X		X
Skin		X	X
Wood shavings			X
Outside			X

Error matrices in this study were used to analyze the degree and direction of the most frequent misclassifications. Our results indicate that when applying classification rules to airborne on-farm samples, certain sources could be systematically under or overestimated. Table 11 and Table 12 summarize the estimated under or overestimation for each source in poultry and pigs for the recommended scenario 3, derived from Table 8 and Table 9. Although errors are inherent to all calculations, the results presented in this study can be used in such a way that under and overestimation errors can be better understood and corrected using these figures, taking into account, that in real conditions, the final under or over estimation will depend on the contribution of each source to the airborne PM sample.

Table 11. Prediction accuracy of source apportionment for poultry based on underestimate and overestimate errors when using scenario 3.

Reference source	PM2.5		PM10-2.5	
	Number	Mass	Number	Mass
Feathers	1.5	2.0	1.4	1.3
Feed	0.8	0.6	1.1	1.4
Manure	1.1	1.2	1.2	1.2
Wood shavings	0.4	0.5	0.5	1.0
Outside	1.1	0.8	0.8	0.1

Table 12. Prediction accuracy of source apportionment for pigs based on underestimate and overestimate errors when using scenario 3.

Reference source	PM2.5		PM10-2.5	
	Number	Mass	Number	Mass
Feed	1.2	0.8	1.6	0.7
Manure	1.1	1.4	1.1	1.1
Skin	0.8	1.3	0.8	1.4
Outside	0.9	0.6	0.4	0.8

5. Conclusions

From our work using feathers, feed, manure, wood shavings, and outside PM sources in poultry, and feed, manure, skin, and outside PM sources in pigs, we can conclude that:

- The selection of the most appropriate particle characteristics (chemical, morphological or combined morpho-chemical characteristics) to distinguish amongst particles from different sources in livestock houses depends on the sources, which depend on livestock species.
- Using only particle chemical characteristics results in overall classification accuracies varying from 57 to 62% in poultry and from 64 to 68% in pigs; it can be useful to apportion specific sources such as manure from the rest. In this case, the use of only chemical particle characteristics would result in 70 to 91% of manure particles being correctly classified.

- Using only particle morphological characteristics results in overall accuracies varying from 40 to 59% in poultry and from 63 to 64% in pigs; it can add value to using only chemical characteristics when sources show distinctive and well defined individual particle morphology or differ in size.
- Using combined chemical and morphological particle characteristics results in overall accuracies varying from 58 to 68% in poultry and from 72 to 78% in pigs (average 69%); it is the recommended approach to apportion all individual sources to PM concentrations and emissions in livestock houses.
- This study provides a methodological approach to assess input data and identifies the most effective characteristics to apportion PM in livestock houses. These data are promising to determine the contribution of different sources to PM in livestock houses. Results in this study also give insight in under and overestimation errors in the source apportionment.

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