Characterization of Maturity Level in Laying Hen Manure by Chemical and Thermogravimetric Analysis

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Abstract: This study aims at investigating maturity levels in manure from laying hens in order to encourage its agronomic re-utilization. In fact the use of unstable/insufficiently mature manure could potentially damage both soils and crops. Effective, easy to reproduce methods are needed in order to assess bio-stabilisation and maturity levels, particularly for biomass that has not undergone conventional composting. This study compares samples of caged laying hen manure, an organic matter rich in nutrients, N and P and devoid of litter or bulking agents, at different levels of maturation. Both chemical (dry matter, ashes, carbon and its fractioning, total and ammoniacal nitrogen) and physical methods, such as thermogravimetry, were used to characterize them. Such physical methods do introduce any sample modification and shorten the analysis time. From a statistical point of view, chemical methods are effective only in distinguishing among different drying methods connected with manure management systems. Only thermogravimetric analysis can identify mature samples by means of total mass loss in the range RT- 900°C, mass loss in the range 350-425°C and energy release at 500°C. In addition, thermogravimetric profiles could be used to define a fingerprint for this kind of biomass.

Key words: Poultry manure, thermogravimetry, mass loss, calcite

INTRODUCTION

Re-utilization of animal and organic waste by manufacturing bio-fertilizer or spreading manure on soils requires the development of appropriate techniques for final characterization of organic fertilizers. Such techniques would allow assessing their level of stabilization and maturity, in order to improve agronomic performance and reduce environmental impact. In fact, the application of unstable organic materials on soil could damage both crops and the soil itself because of (they introduce) phyto-toxic compounds and could sometimes lead to competition for N between microbial biomass and crops1,2. Poultry manures (PM), both the litter and caged laying hen varieties, need bio-stabilisation and maturation in order to be used safely. Caged laying hen manure (LHM) in particular is an organic matter rich in nutrients, N and P, without litter or bulking agents, with a low dry matter content at origin (DM<25%)3,4. Most of N (approximately 60-70%) excreted in PM is in the form of uric acid and urea5, which undergoes rapid mineralization in wet conditions. Furthermore LHM has a high phosphate content, due also to specific feed supplements (calcium phosphate or carbonate) added to enhance egg production6,7. With 450 millions hens in Europe7 and a 4,000,000 DM ton potentiality4, LHM could be an important source of organic carbon to fertilize Southern European soils, which are known to be suffering organic matter depletion8. However, in order to be used safely, LHM requires biostabilisation, maturing and hygienization, as recommended by EU standards9 as well.

Methods for detecting biomass stabilization or maturity are still under development. Stability indices have been developed for composting processes and matter, based on physical, chemical and biological methods, such as respiration index for composted matter10. Commercial maturity test kits based on respiration intensity or enzyme activity, such as Solvita° compost maturity test and Apyzim°, are
also available on the market. Some of these methods can be applied to composted PM\textsuperscript{15}. Physical techniques such as thermogravimetric analysis have also been applied to assess compost biostabilisation; for this technique a small sample is sufficient, it does not require chemical extraction and is easy to reproduce\textsuperscript{1,16}.

Fewer studies are available on methods to assess PM stabilization or maturity, particularly of caged laying hen manure which has not undergone “conventional” composting processes. In the US, laying hen droppings are often stored and spread in semi-liquid phase rather than being dried and re-utilized to manufacture commercial bio-fertilizers\textsuperscript{16}.

This study focuses on the characterization of caged laying hen manure (LHM). Six samples with different maturity levels were analysed by means of chemical (DM, VS, C, fractioning, humification indices, TKN, P) and physical methods (thermogravimetry and X-ray spectrometry). Samples were obtained with LHM management systems representative of EU industry standards in the 21st century and referred to as best available technique (BAT)\textsuperscript{17}.

This study aims at achieving the following goals: (i) LHM characterization at the end of usual managing system by means of chemical and physical methods, (ii) verification that physical methods, such as thermogravimetric analysis, could effectively measure LHM levels of maturity.

### MATERIALS AND METHODS

**Sample collection:** Six LHM samples, PM1-PM6, were collected directly from commercial poultry units in Italy during the period from May to September 2005. All units, with a 100,000 animal capacity, belonged to a single organization that has a centralized feed preparation and distribution systems, but different LHM management systems resulting in different levels of maturation. All these systems fall within the Best Available Techniques (BAT) for intensive poultry rearing in Mediterranean countries\textsuperscript{17}.

PM1 was collected from a unit equipped with vertical tiered cages, a manure belt and manure drying system (MDS) outside the housing, manure removed from housing after 24-36 h and dried in the subsequent 72 h in a tunnel\textsuperscript{18}; in this case the tunnel is located outside the facility and not over the cages. PM2 was collected from a unit equipped with vertical tiered cages, manure belts and forced air drying, where the manure was moved at least once a week to a covered storage\textsuperscript{19}. PM1 and PM2 were samples of fresh LHM, with a few days aging. PM3 sample was collected from a non-caged housing for laying hens that had a deep litter system with forced air manure drying\textsuperscript{20} and lower capacity. PM4-6 samples were collected from units equipped with a cage battery system and aerated open manure storage (aerated deep pit system\textsuperscript{21}). PM4 was collected from dried poultry droppings in an aerated deep pit, removed from pit after 12 months and stored in a covered stack/pile/heap on a solid impermeable floor for an additional 12 months; it was a mature LHM sample. An enzyme blend was added to the PM windrows at the beginning of the cycle production according to a patent pending process\textsuperscript{22}. PM5 and PM6 were taken from open storage under cages, after 12 months collection in static windrows, therefore they were a mixture of fresh and mature manure with a high DM content; PM6 received an additional enzyme blend as in the case of PM4. PM4-6 samples are similar to unpaved deep pit stored manure, typical of the USA system, except for the presence of ventilation spanning from the area where birds live to the manure storage area, to improve and speed drying.

For PM1 and PM2, fresh manure sub-samples were collected at 6 different times of day at the end of the drying pathway. In the case of the aerated deep pit, 24 sub-samples were taken, 4 sub-samples for each of the 6 windrows. PM4 was composed of 8 sub-samples, collected from the heap/pile. The total sample, consisting of about 10-12 kg, was poured into a clean, dry polythene bag and mixed thoroughly, then about 3 kg of material were extracted and used as sample for analysis. The composite manure samples were stored in a cool environment (4°C) prior to analysis. A summary listing of PM samples is reported in Table 1, with management systems specified as well.

**Chemical analysis:** Sub-samples of “wet manure” were taken and analysed for dry matter (DM) content, pH, nitrogen as TKN and NH\textsubscript{3}+ N. Dry matter was ground and analysed for total phosphorus.

| Samples | PM type | PM management system |
|---------|---------|----------------------|
| PM1     | Fresh   | Manure belts and MDS (Manure Drying System) |
| PM2     | Fresh   | Manure belts and forced air drying |
| PM3     | Mixed: mature and fresh | Non-cage housing/Deep litter with forced drying |
| PM4     | Mature  | Aerated deep pit+12 mon heap |
| PM5     | Mixed: Mature and fresh | Aerated deep pit |
| PM6     | Mixed: Mature and fresh | Aerated deep pit |

Table 1: List of LHM samples analyzed together with managing system used
Ashes were determined on ignition ashing DM in an oven at 550°C for 2 h and determining the residual weight. Additional wet manure sub-samples were taken and oven dried at 60°C for more than 12 h (until constant weight) and ground to a micro-mill down to <0, 2 mm and analysed for organic carbon (C) and its fractioning, thermal analysis.

With reference to organic carbon fractioning, total extracted carbon (TEC) and humic-like and fulvic-like fraction (HA+FA) were identified; the degree of humification (DH) and the rate of humification (RH) were calculated:

\[
DH(\%) = \frac{HA + FA}{TEC} \times 100
\]

\[
RH(\%) = \frac{HA + FA}{C} \times 100
\]

DH and RH represent the percentage of humified carbon in the extract with reference to extracted and total C in the sample. All analyses were performed in accordance with officially recognized methods for compost analysis\[^{23}\]; NH\textsubscript{4}\textsuperscript{+}-N analysis were performed according to officially recognized methods for fertilizer\[^{24}\]. All analysis performed on wet samples were repeated at least once to check the homogeneity of sub-samples and analysis methods.

**Thermal analysis:** TG and DTA methods are based on programmed heating of the sample in a controlled atmosphere. Thermal tests were performed for each sample using an STA 409C simultaneous analyzer (Netzsch, Selb, Germany) equipped with TG/DTA sample carrier supporting a type B thermocouple. During thermogravimetric analysis (TGA), the weight change in a sample is measured during the thermal program. The first derivative of the TG trace represents the weight loss rate DTG (expressed as % min\(^{-1}\)). The samples were analysed after manual grinding in an agate mortar. The conditions were: heating rate of 10°C min\(^{-1}\) from 20 to 1000°C under dynamic air atmosphere (100 ml min\(^{-1}\)), alumina crucible, calcined alumina as reference, sample weight about 10-20 mg. Data processing was performed with the Netzsch TA window software.

**Statistical analysis:** The results of analytical determination were verified with a software program used for analytical method validation\[^{25}\]. First, the software program checks if results of analytical determination fall within a normal distribution pattern (Shapiro-Wilks test, 5%). In addition, anomalous data checks are performed with Huber (median) 5% test.

For each parameter, these tests could check whether the six LHM samples belonged to a single statistical population and if they contained anomalous data, that is to say if significant differences for sample properties were found.

**RESULTS**

**Chemical characterization:** The results of LHM chemical characterization are reported in Table 2 and 3. All samples had very high DM values, except PM2 with DM<50%, value significantly different. The dry LHM samples had a high ash content, ranging from 27% (PM1) to 45% (PM4, mature sample) and high C content, ranging from 37 to 27% for PM1 and PM4 respectively. The effect of maturing is a loss of C because of mineralization. Organic fractioning (TEC, HA+FA) seemed to display a structured behaviour. The TEC represented about 53-59% of C, independently of maturation level and carbon concentration. Humic fraction values expressed by (HA+FA) are lower for fresh samples, while mature ones display higher values. Consistently with the above, the humification indices, DH and RH, are low in fresh poultry manures (22 and 13% respectively) and increases with maturing time (poultry manure age) up to 58 and 33%, as expected.

Table 2: Chemico-physical characterization of LHM samples

| Parameters | DM\(^{1}\) (%) | Ashes (%DM) | C\(^{2}\) (%DM) | TEC\(^{3}\) (%DM) | HA+FA\(^{4}\) (% DM) | TKN\(^{5}\) (%) | N-NH\textsubscript{3}\(^{6}\) (%) |
|------------|----------------|-------------|-----------------|-----------------|-----------------------|-----------------|----------------|
| PM1        | 92.6           | 27.4        | 37.5            | 22.4            | 5.0                   | 8.8\(^{*}\)     | 0.7             |
| PM2        | 48.2\(^{*}\)   | 34.7        | 35.8            | 21.0            | 4.7                   | 2.8             | 0.8             |
| PM3        | 82.0           | 34.6        | 30.8            | 16.4            | 6.1                   | 5.3             | 1.0             |
| PM4        | 76.6           | 45.1        | 27.2            | 15.4            | 9.0                   | 3.8             | 0.8             |
| PM5        | 88.4           | 26.7        | 36.1            | 21.4            | 8.7                   | 4.8             | 0.4             |
| PM6        | 82.0           | 36.6        | 31.1            | 17.7            | 6.1                   | 4.8             | 0.4             |
| Average    | 78.3           | 36.6        | 33.1            | 19.0            | 6.6                   | 5.0             | 0.7             |

*Note:* \(^{1}\): DM Dry Matter (100–DM = moisture or water content), \(^{2}\): Organic Carbon, \(^{3}\): TEC Total Extracted Carbon, \(^{4}\): HA+FA humic-like and fulvic-like carbon, \(^{5}\): TKN sum organic and ammonia (um) nitrogen as measured by the laboratory Kjeldhal procedure, \(^{6}\): Ammonia (um) nitrogen as measured by the laboratory Kjeldhal procedure, \(^{*}\): Represent 5% significant level
Table 3: Indices from chemico-physical characterization of LHM samples

| Parameters | DH (%) | RH (%) | C/TKN (%) | NH₄⁻N/TKN (%) |
|------------|--------|--------|-----------|--------------|
| PM1        | 22.1   | 13.2   | 3.9       | 29.9         |
| PM2        | 22.2   | 13.0   | 6.2       | 29.9         |
| PM3        | 37.1   | 19.7   | 4.8       | 21.3         |
| PM4        | 58.2   | 32.9   | 5.5       | 21.3         |
| PM5        | 40.6   | 24.0   | 6.6       | 7.7          |
| PM6        | 34.6   | 19.7   | 5.9       | 9.1          |
| Average    | 35.8   | 20.4   | 5.5       | 15.8         |

Note: 1°: DH degree of humification, defined as humic-like and fulvic-like carbon fraction to total extracted carbon (HA+FA)/TEC, 2°: RH rate of humification, defined as humic-like and fulvic-like carbon fraction to total carbon (HA+FA)/C.

In terms of TKN content, the highest value was found in PM1 and the lowest N concentration (about 3% on wet basis) in PM2, the fresh samples. In addition, statistical analysis showed that TKN content in PM1 had a higher N content, statistically different from the others. Ammonia’s (NH₄⁻ N) contribution to TKN varied from less than 10 % (PM1) to 30 % for PM2, for the wet sample. P content was not affected by drying methods, with values ranging from 3.2 to 5.5%, for dry samples. The highest concentration was in PM4, because of the concentration effect due to carbon and nitrogen loss during mineralization (maturing). The C/N ratio varied from 4 to 7, with the lowest value in the case of PM1.

In terms of statistical analysis, Shapiro-Wilks tests verified that all parameters reported in Table 2 and indices in Table 3 fell within a normal distribution, i.e., they are a single population. According to Huber test 5%, the only anomalous data were TKN (8.80 % on wet samples) value for PM1 and DM (48%) value for PM2.

**Thermal analysis:** The thermogravimetric analyses (TGA) were performed both in air and argon. The TG losses in air of the PM1 sample, together with the DTG, is reported in Fig. 1 and the deduced thermal parameters of all the samples are summarized in Table 4 and 5.

TG losses in air analysis shows that there are five mass transitions located in the RT-180, 180-350, 350-425, 425-600 and 600-850°C regions respectively. These five regions were identified taking the local maxima (arrows in Fig. 1a) of the first derivative of the thermogravimetric analysis (DTG, Fig 1). All these thermal transitions can be ascribed to different groups of molecules with different behaviour. The first transition, at approx. 124°C, is attributed to residual water removal (free and bound water) from the samples.[1,26] The three transitions in the range 180-600°C, approx. 275, 381 and 475°C, are due to different organic pools. Mass loss at 731°C is due to the CaCO₃ decarboxylation.[1,16] The presence of CaCO₃ is typical of these samples because it is a part of animal

![Fig. 1: TG, DTG and DTA curves registered for the PM1 sample in air flow (100 mL min⁻¹). The arrows indicates the five transition regions](image-url)

Table 4: TG in air flow (100 ml/min): main thermogravimetric weight loss (% of initial sample) and temperature ranges in which they occurred

| T range (°C) | ΔM_average (%) | ΔM_P1 (%) | ΔM_P2 (%) | ΔM_P3 (%) | ΔM_P4 (%) | ΔM_P5 (%) | ΔM_P6 (%) | ΔM_P7 (%) | ΔM_P8 (%) |
|--------------|----------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 0-180        | -11.6±2.8      | -11.4      | -15.5      | -10.8      | -13.1      | -7.0       | -11.5      |
| 180-350      | -31.4±3.2      | -32.7      | -31.1      | -30.7      | -26.6      | -36.3*     | -30.6      |
| 350-425      | -10.6±1.4      | -10.6      | -12.5      | -11.0      | -8.2*      | -10.2      | -11.2      |
| 425-600      | -21.4±2.4      | -23.0      | -18.8      | -21.2      | -18.6      | -24.7      | -22.4      |
| 600-850      | -8.0±1.5       | -6.6       | -8.0       | -8.6       | -10.1      | -5.8       | -8.7       |
| Total Mass Loss | -83.0±3.3      | -84.3      | -85.9      | -82.3      | -76.6*     | -84.0      | -84.4      |

Note: °: ΔM mass variation as % of initial sample mass, *: Represent 5% significant level

Table 5: DTA in air flow (100 ml/min): mean values of peak temperature; the first and second thermal transitions are exothermic, the third is endothermic

| T range (°C) | T_average (°C) | T_P1 (°C) | T_P2 (°C) | T_P3 (°C) | T_P4 (°C) | T_P5 (°C) | T_P6 (°C) | T_P7 (°C) | T_P8 (°C) | T_P9 (°C) |
|--------------|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 180-350 (1*) | 320±18.7       | 326.1      | 342.7      | 321.1      | 321.3      | 326.2      | 285.9      |
| 425-600 (2*) | 502±12.3       | 501.0      | 506.0      | 514.5      | 481.3      | 497.3      | 513.7      |
| 600-850 (3*) | 751±5.5        | 750.9      | 749.5      | 763.5      | 751.7      | 748.2      | 755.4      |
feed. Considering the total mass loss (TML) shown in Table 4, the first evidence is that TML in the mature sample (PM4) was lower than in fresh ones (PM1, PM2) and in partially matured samples (PM3, PM5, PM6), that showed similar mass decreases; this difference was statistically significant. Mass loss tests for each transition indicated that this difference could be ascribed to the transitions at 275 and 381°C; also mass loss for the transition at 275°C is statistically significantly lower for PM4 than for the others. Inorganic ashes represented 15 to 23% of initial sample (Table 4). The simultaneous analysis of thermal transitions in air showed a similar pattern for all LHM samples: there were two exothermic peaks and an endothermic one associated with mass transitions (DTA curves in Fig. 1). The temperatures of these transitions are reported in Table 5. The thermal transitions at 320, 502 and 751°C are associated with mass transitions at 275, 475 and 732°C respectively (the thermal transitions occurred at temperatures slightly above their respective mass transitions because of the inertia in the measurement system). The peak area at around 500°C for PM4 sample showed a value of -790.5 μVs mg⁻¹, statistically lower (Huber median 5% test) than the others (-1271, -1218, -1240, -1356 and -1449 μVs mg⁻¹ of PM1, PM2, PM3, PM5 and PM6 respectively).

**DISCUSSION**

Chemical analysis showed that LHM could be utilized as a very effective source of organic matter for soils, with an organic carbon concentration ranging from 27 to 37% on dry basis, values are comparable with those typical of trade composts[27]. Fresh LHM is very rich in C, C lowers during maturing, as expected, but concentration remains high; furthermore LHM has high ash content, ranging from 28 to 45% on dry basis, where calcite represents the main component. There is a correspondence between ash values from chemical and termogravimetric analysis. The degree of humification (DH) and the rate of humification (RH) could be used as a parameter to study maturing, because they show a progression with maturing time (from 22 to almost 60%). Organic carbon had humic fraction values expressed by (HA+FA) consistent with data reported in earlier literature[28] and humification indices values are lower than those typical of composts[1,33]. All the samples showed low C/N values, in the range of 4 to 7, not apparently connected to maturing process; however all values were lower than 10-12 which is considered optimal for soil application to avoid microbial immobilization of N[1].

According to statistical analysis performed for this data set, the only anomalous data were DM for PM2, with a low value and TKN for PM1, with a very high content. Chemical data evidenced the different drying performance of manure managing systems: all manure management systems had a high performance in drying except manure belts with forced air drying system, producing PM2 with DM<50%, a value that is significantly different. DM<50% is a threshold value to avoid large amount of N losses[29]. Therefore PM2 was the sample with the lowest TKN concentration and the higher ammonia percentage (30% TKN), just as a result of N mineralization process with ammonia losses[30]. On the other end, PM1 had the highest value (>8% on wet manure). Ammonia was less than 10%, as described in excreted droppings[4]. This can be explained considering the fast drying method with respect to excretion: all nitrogen in droppings was kept “frozen” by means of speed drying. In the other cases, humidity conditions had allowed mineralization process with ammonia losses. Moisture content and drying speed are more important than (maturity) age for TKN content. Dry matter (DM) content is an important parameter, because it has a direct effect on nutrient content of wet sample (as fertilizer contribution) but also on nitrogen behaviour. MDS allowed to “freeze” the fermentation process, leading to the highest values for C and TKN (PM1); TKN values are similar to organic-mineral fertilizers in the case of PM1[27].

The thermogravimetric analysis in air flow shows the presence of five mass transitions defined in the regions between the RT-180, 180-350, 350-425, 425-600 and 600-850°C. According to TG data (table 4), the total mass loss (TML) in the ripened sample (PM4) was lower than the others, which showed similar mass decrease; this difference is statistically significant. Upon examination, mass loss for each transitions indicates that this difference could be ascribed to the transitions at 275 and 381°C, suggesting that the aging process mainly affects the degradation of organic not aromatic compounds (see below). Furthermore, for this thermal data set, the statistical analysis identified mature sample as anomalous, among fresh ones or fresh and mature manure mixes.

The first and the last mass loss are due to water elimination and calcite decomposition respectively. The other three transitions are due to thermal degradation of different organic pools which composed volatile substances (Table 4 and 5). The first organic pool was subjected to thermal decomposition and volatilization in the range of 180-350°C (mass transition centred at 275°C), independently from aging and represented...
almost half of volatile substances. This mass loss was also connected to an exothermic process centred at 320°C. A second pool decomposes in the range 350-425°C (average temperature at 381°C) and the third pool decomposes at about 475°C, connected to a second exothermic process (centred at 501°C). According to earlier studies, the first mass (thermal) transition at 275°C can be ascribed to aliphatics, while the mass (thermal) transition, in the range 425-600°C (475°C), to the thermal breakdown of more aromatic moieties, either naturally occurring in the sample or resulting from molecular rearrangements[16]. It is important to underscore that the enthalpy associated to this transition (502°C, table 5) for the PM4 sample (the mature one) is statistically lower than the other ones. This fact is rather important because this parameter can be used as a means to distinguish fresh or mature LHM. Therefore the mass transition at 275 and 475°C are similar to compost transitions[1,16], due to organic non aromatic and aromatic pool thermal degradation respectively.

The mass loss centred at 381°C is probably due to another unknown group of molecules, typical of poultry manure. It could be connected with characteristic N-ureic compounds. This was significantly lower (statistically significant) for mature sample.

The mass transition centred at 731°C and associated to a thermal transition at 751°C was related with CaCO₃ decarboxylation; this peak is much more evident for LHM than compost matter[1,16]. In fact calcite represented up to 25% of initial DM sample.

CONCLUSIONS

This study was meant as a first approach to the characterization of caged Laying Hen Manure (LHM), with chemical and physical methods. Samples from different management system were analysed and compared with a mature sample (one year heap storage). The chemical and physical analysis of PM samples obtained from different management system displays that is possible to distinguish between fresh and mature samples. In particular the chemical analysis shows that, the trends of C, DH and RH, are good indices of the maturity level, even if there is not any statistical significance.

The thermogravimetric analysis indicates also that the total mass loss in the range RT-900°C, the mass loss in the region included between the 350-425°C and the enthalpy at 500°C are the three parameters able to identify the mature samples. These three parameters are much more indicative with respect to the chemical ones, because their values are statistically different. This study shows that thermal analysis can be used as a method to distinguish between fresh and mature LHM and poultry manure. It may be useful to apply such physical methods to characterize LHM maturing degree and evolution during maturing. These methods have proved to be fast, with good reproducibility and require limited sample preparation. Hence, they are of great interest because of the time savings they afford and their effectiveness in assessing manure maturity.

Finally this study shows that the LHM characteristics depend on manure managing system, particularly on drying performance and speed. For example, the different manure drying systems in fresh samples (PM1 and PM2) lead to significant changes in DM and TKN content and therefore in agronomic use of final fertilizer.

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