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Research Article

A Chemometric Comparison of Organic Manure from Different Animal Sources using a Principal Component Analysis

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Abstract: This study was conducted to determine the physico-chemical properties of organic manure from different animal sources, namely: farm and village cows a pig and layers chicken droppings. The application of principal component was used to differentiate between these sources. Several physico-chemical parameters including ash content, pH, conductivity, as well as some nutrients were used as variables. The results showed differences between the four animals with the chicken affording the largest variation (PC1 = 62.5%) then the pig (PC2 = 4.5%) while the two cow samples overlapped showing that both animals do not differ significantly. The major contributor to the difference between the samples was conductivity (about 90%), a property that is conferred by the dissolved ions irrespective of their identities, followed by calcium (about 35%), a vital element in layers chicken for egg shell formation, hence a major nutrient in the chicken feed. The major contributors in the second component, ascribed more by the pig, were potassium, copper, zinc and iron, while the other parameters did not confer much difference. This report therefore demonstrates that the chicken manure contains more dissolved ions than the other samples. This may not necessarily be an advantage since the basal level of soil ion content needs to be evaluated before application of any manure. However, the study has demonstrated the application of multivariate analysis in comparison of the organic manure and has differentiated the organic manure by animal source.

Keywords: Animal feed, animal manure, chicken, farm and village cows, physico-chemical properties, pig

INTRODUCTION

Among the factors that affect soil fertility and hence improved crop production is the content of organic matter coupled with the soil structure itself (Tiessen *et al*., 1994). Organic matter content in the soils is believed to, not only affect nutrient content, but it also affects other parameters such as the soil temperature, biota and soil moisture which are necessary to support and promote crops growth (Reeves, 1997). Various elements are necessary in plant metabolism at different levels, macro, micro as well as trace elements, whose presence, while not required, has clear positive or negative effects on plant growth (Ayeni and Adeleye, 2011). The need for these minerals has led to the development of commercial fertilizers (Tisdale *et al*., 1985). Fertilizers are broadly divided into organic fertilizers composed of enriched organic matter whether plant or animal derived while the inorganic fertilizers consists of synthetic chemicals and/or minerals (Chen, 2006). Fertilizers typically provide, in varying proportions, the three major plant nutrients: nitrogen, phosphorus and potassium abbreviated as N-P-K. They may also provide secondary plant nutrients such as calcium, sulphur, magnesium and some micronutrients. Collectively, with the secondary macronutrients, namely, calcium, magnesium and sulphur that are required in roughly similar quantities, their availability is often managed by liming and manure application rather than fertilizers (Abidemi, 2011).

The use of organic manure has been in existence since the ancient times. Lately, this has seen a considerable attention since the dawn of the term "organic farming" (Lampkin *et al*., 2000). These manure come mainly from animal droppings although almost any materials that is decomposable can be used (Svensson *et al*., 2004). While the use of inorganic fertilizers affords an advantage of quick release of the nutrients (Makinde *et al*., 2011), organic manure is believed to hold several advantages to the inorganic fertilizers. These advantages include low to no carbon

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footprint since they are not manufactured, low capital intensive, provide high organic matter, improve the soil structure hence its water retention (Schjønning *et al*., 1994), as well as improving its microbial content beneficial for soil structure and fertility (Zhong *et al*., 2010). There are studies assessing the nutrient content in organic manure from different animal sources. As expected, droppings from different animals will surely afford different nutrients depending on the type of feed (husbandry) and the efficiency of the digestive system of such an animal. For example, the 'wild animals' that rely on wild grass only cannot be comparable to those kept under close monitoring receiving food supplements.

Comparison of different manure from different sources is usually made using simple univariate approach (Zhang *et al*., 2012). However, recent studies have introduced multivariate statistics in analysing even the smallest possible differences between different data sets (Wang *et al*., 2007; Zhong *et al*., 2010). Multivariate statistics is able to identify the differences between data sets and ascribe such differences to responsible parameters (Bro and Smilde, 2014). This approach has been used extensively in the analyses of complex extracts such as plant metabolites, animal and human body fluids in biomedical and animal care studies. Recently this method was applied in a very simple experiment to profile the river pollution Tanor, *et al*., 2014), comparison of food stuffs from different plant sources (George *et al*., 2014; George and Moiloa, 2015).

Herein we report the use of multivariate statistics, specifically principal component analysis in discerning the organic manure from different animal sources: Farm cow, village cow, farm pig and a farm layers chicken.

MATERIALS AND METHODS

Sample collection and storage: Organic manure was collected in Schott bottles from the Faculty of Agriculture farm at the University (farm cow and chicken), as was well from a kraal from a neighbouring village for village cattle samples. The samples were allowed to air-dry to a constant weight. Thereafter they were kept in a fridge below 5°C in airtight Schott® bottles to prevent moisture entering till they were used. Prior to use, the samples were ground using mortar and pestle to fine powder with an average size of about 2 mm since some samples were fibrous hence could not be more finely ground.

Determination of physico-chemical properties: The physico-chemical parameters selected for determination included H^+ activity (pH), electrical conductivity, organic matter, nutrients (nitrates and phosphates, calcium, potassium, iron, copper and zinc) and ash. Most of the analysis followed the New South Wales guidelines for sampling and analysis of water pollutants (Department of Environment and Conservation, 2004). Total nitrogen was determined according to the US-EPA Method 1688 based on Kjeldhal titration (Gomez-Taylor, 2001) while phosphorus was determined as phosphate ion using the EPA Method 365.3 (US EPA, 1978).

Conductivity and pH were determined by suspending about 100-g samples in about 100 mL distilled and allowed to soak overnight (24 h) thereafter the parameters were measured using a conductometer and pHmeter, respectively. For metal content analyses, portions of 100 g samples were weighed and digested using concentrated sulphuric acid (6M H_2SO_4). The digestion was stopped when the initially very dark-coloured medium had cleared to colourless. This solution was kept in the refrigerator below 5°C, for further mineral analyses for its metal ion content. Unless otherwise stated, all the experiments were made in triplicate (n = 3).

Principal component analysis of the data for global comparison of samples: The obtained data were exported into the SIMCA-P® SIMCA 13.0 Software (Umetrics, Umea Sweden) for principal component analysis modeling to decipher the major contributors to the closeness or difference between the samples hence their sources. The corresponding standard deviation was determined for the assessment of the precision of the measurements.

RESULTS AND DISCUSSION

Determination of physico-chemical properties of manure: The percentage content of nitrogen, phosphorus, ash and total acidity of different sources of animals manure are depicted in Fig. 1. The entries for ash content and total acidity were reduced by a factor of 10 in order to fit in the same scale as the other parameters plotted together (Fig. 1). The farm cow manure showed the highest amount of (0.18 g) of nitrogen content followed by the village cow of (0.17 g) per 100 g of sample unlike the chicken and pig manure that showed the lesser amount (0.10 g/100 g) of nitrogen. Poultry and pig manure has low content of N and minerals compared to cow manure. According to Perkins (2001) and Nahm (2005), the low content of N in broilers manure could be due to the less content of undigested protein and uric acid which will be decomposed into ammonia during the anaerobic fermentation process. For practical application, however, due to the low C/N ratio in Chicken Manure (CM), inhibition of ammonia from being volatile has been the main problem faced in farms applying chicken manure.

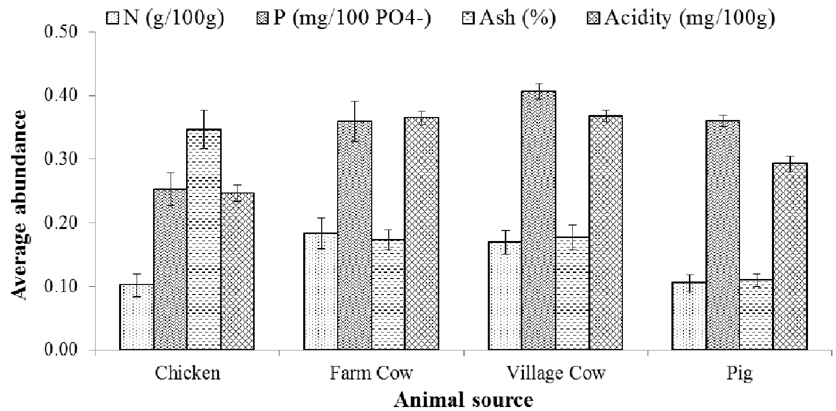

Fig. 1: The amount of nitrogen (nitrates), phosphorus (phosphates), ash content (%) and total acidity of the chicken, cow and pig manure

Thus, the loss of N in volatile forms during its accumulation in the soil, lower its proportion in the soil. These minerals and associated compounds in chicken manure are not available for plant uptake due to high pH and water lodging effect in the soil (Perkins, 2001). The nitrogen present in poultry manure is in the form of ammonia, which bubbles when contacted with water and lower the water activity (A_w) and its acidity in the rhizosphere (Waldrip *et al*., 2011). When applied in the soil, this has been known to impair the activity of Plant Growth Promoting Rhizobacteria (PGPR) around the rhizosphere (Waldrip *et al*., 2011).

The differences of N mineralization between manures were probably related to animal diets and C/N ratio of manures (Calderón *et al*., 2005; Cordovil *et al*., 2007; Azeez and van Aerbeke, 2010). Chickens and pigs fed mainly on grains and cakes with higher protein/ fat content compared with cattle forages with straws resulting in higher C/N ratios. The low C/N organic materials cause mineralization of N as they are decomposed, whereas the high C/N organic materials cause immobilization of mineral N because available N is utilized by soil microbial biomass (Schimel *et al*., 1992; Mary *et al*., 1996). Studies on dairy cattle N mineralization indicated that net mineralization occurred when C/N<16.0 but net immobilization occurred when C/N>19.0 (Calderón *et al*., 2004; Li and Li, 2014).

The variation in phosphorus content of cows' manure could be due to the diet fed to the farm cows relative to the village cattle that feeds on grass only without any supplements. Regarding the phosphorus content, the village cow was found to have the highest concentration of phosphates as 0.419 mg/100 g sample while the chicken sample had the lowest concentration of 0.246 mg/100 g of sample. The difference in the phosphate content between the two cow samples could not be accounted for since the diet for each of the sample sources could not be ascertained.

Literature sources state that on average plants require nitrogen and phosphorus at the level of 15 mg and 10 mg, respectively per gram of plant (Epstein, 1965), while a different source report that generally animal manure contains more phosphorus than plants require, about 8:1 of nitrogen to phosphates is required by plants (He and Shi, 1998). These findings entails therefore that since no single sample would be ideal to supply both minerals, it is important to combine the organic manure from different animal sources. Other factors like organic N fraction or other physical and chemical characteristics of manures might be responsible for the differences (Burger and Venterea, 2008; Fangueiro *et al*., 2010). The data revealed that organic manure are a good alternative to inorganic fertilizers since no foreign chemicals are introduced to the farming processes. Another benefit is that organic manure also helps in improving the humus content and water retention properties to soil (Hillocks, 1998).

Determination of the different metal ions content: The metal ion content of different sources of animal manure is depicted in Fig. 2. The chicken manure found to contain the highest amount of calcium (250 mg/100 g), which must have been fortified in the chicken feed to enhance egg shell formation since the chicken were layers. This was followed by the pig at about 175 mg/100 g and the two cow samples which showed almost equal (150 mg/100 g) but, the list concentration of calcium content. Interestingly iron content seemed to be equal in all the samples averaging about 175 mg/100 g with the pig and chicken samples containing slightly above average amounts. The rest of the parameters, zinc, copper and potassium seemed to be comparable across the sample types with copper registering the lowest amount at about 50 mg/100 g.

Determination of the H^+ activity and electrical conductance: The hydronium (H^+) activity in solution, commonly referred to as pH, is one of the basic

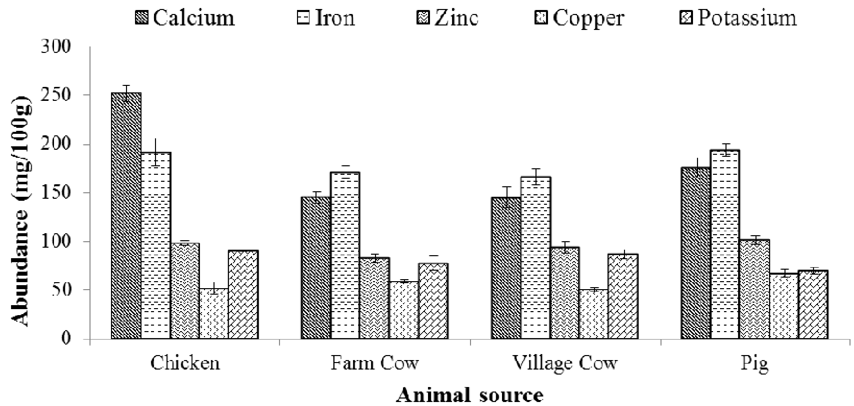

Fig. 2: Determination of the metal ion content in the different samples

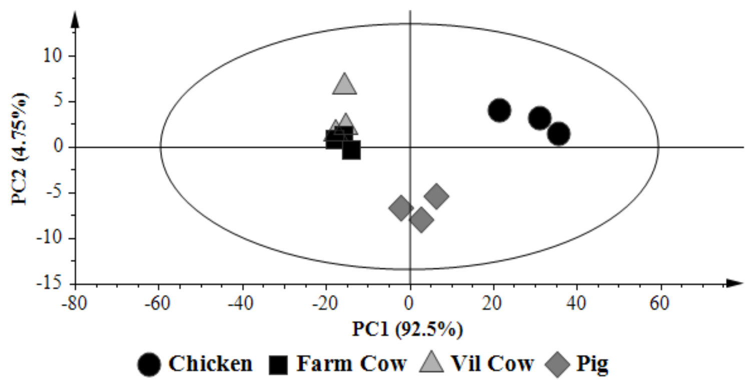

Fig. 3: PCA scores plot: the scores plot (PC1 vs PC2) showing clustering the different samples

Table 1: The pH and electrical conductance of the manure suspensions

Parameters	Chicken	Farm cow	Village cow	Pig
pH	9.34 (5.6)	7.97 (1.5)	8.09 (2.0)	8.19 (0.9)
Conductivity (µS)	1237 (10)	474 (9.8)	461 (4.5)	783 (9.3)

* The values in parentheses depict the associated relative standard deviation (%RSD) for n = 3

parameters that determines the soil's potency in crop production as it plays a role in the translocation of metal ions (Clarholm and Skyllberg, 2013). Due to its high mobility, H^+ ion is responsible for the electrical properties of aqueous solutions. Recently, a study showed a good correlation between pH and the conductivity of the aqueous solutions subjected to a cation exchange adsorbent (George and Ramollo, 2014). As a consequence of this anticipated mutual interdependence, these two parameters were studied together. Table 1 shows the proton activity and electrical conductivity of the suspensions of the manure samples prepared by suspending 10 g of the respective samples in 100 mL of deionised water respectively.

There is a positive correlation between pH and conductivity although it is relatively poor (R^2 = 0.9036). This could be attributable to the presence of the other ions such as Ca^{2+} which was found to be more plentiful in the chicken samples than in the other samples as discussed earlier. These other ions can also increase the solutions conductivity although their molar conductivities are much lower than that of H^+ ions due to its small size hence a higher charge/volume ratio.

Principal component analysis of the data: As it has been argued PCA is able to detect and magnify the hidden differences in the data. The PCA of the data plotted as a two-component model, explaining 97.3% of the variance (with the accuracy of prediction of 77.2%), was computed and presented in Fig. 3. A scores plot (Fig. 3) was constructed from PC1 and PC2 [R^2X(cum) of 0.973, Q^2(cum) of 0.772 and 95% confidence] and shows a differential clustering of the samples.

As can be seen in Fig. 3, the sample of the chicken manure is relatively more different to the rest, while the sample from the two cows are significantly similar as there is a significant overlap between the from the two samples. The sample from the pig lies between the cows and the chicken sample along first component -PC 1 (showing the maximum difference) and seem to be different from the rest of the samples along the second component (PC 2). To decipher the underlying

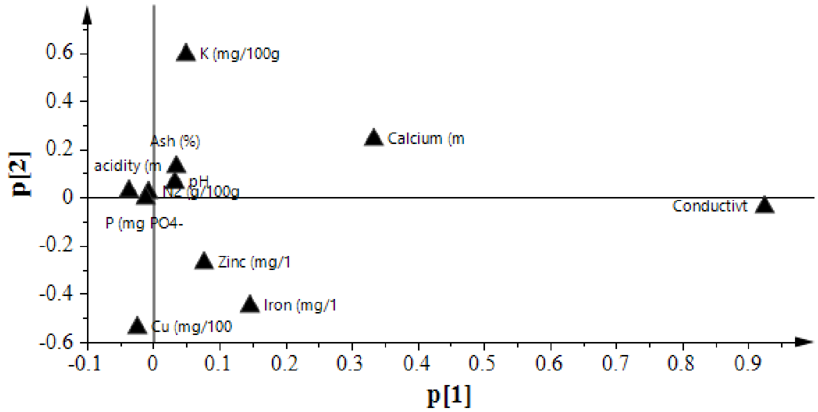

Fig. 4: PCA loadings plot: the scores plot (PC1 vs PC2) showing variables that are responsible for sample clustering seen on scores plot

variables responsible of the clustering observed on scores plot, a loadings plot was computed (Fig. 4).

From the loadings plot, Fig. 4, it could be seen that variable that contribute significantly to the clustering in the first component (PC 1) are mostly conductivity, while Cu and K are responsible for clustering along the second component (PC 2). The other variables seem to contribute more towards the samples grouping other than clustering. These include nitrogen, phosphorus, acidity, ash and phosphates.

CONCLUSION AND RECOMMENDATIONS

This study has revealed some interesting information: Firstly that the different animal sources would results in different physico-chemical properties. This was expected given that even if the animals were subjected to the same feed, they possess different digestive systems hence they would not digest the same feed with the same efficiency hence their excreta would be different. Secondly, the manure from the chicken seems more different from the rest of the animals, mainly due to calcium content ascribed to possibly the high calcium content feed to enable egg shell formation. Thirdly, the two cow samples were almost identical with the village cow exceeding the farm cow only in the phosphates content. However this could not be linked to any possible source. The application of the multivariate statistics showed the overall differences glaringly with the four samples being discerned significantly. The main contributor to the differences explained by the 90% variability was conductivity-a property ascribed to the dissolved ions, in the chicken samples. This could be attributed to the fact that the chicken manure used in this study came from layers that were supposed to be highly productive, laying as many eggs as they can possibly do, it would be befitting that their feed is adequately fortified with the requisite minerals, calcium being one such mineral. And, since all the provided minerals cannot be fully assimilated nor stored, some are excreted as dissolved ions, hence an increase in conductivity. Intra-sample precision was sufficiently high since the replicate analyses from the same samples demonstrated significant overlaps, with those from the village cow almost sitting on top of one another (Fig. 4).

Despite these gratifying results, one cannot make a mistake of recommending any manure blindly without highlighting the importance of an intensive soil analyses to determine what basis level exist before application of any manure. The type of crops being anticipated to be planted also is very critical in determining the type of fertilizer to be applied as alluded to in the literature. Nevertheless, the objective of the study has been met, to use the chemo metric approach to discern between the manure from different animal sources.

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Conflict of Interest: In any matters of this manuscript, there is no conflict of interest among authors.

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