



RAPID COMPOSITION AND SOURCE SCREENING OF HETEROGENEOUS POULTRY LITTER BY X-RAY FLUORESCENCE SPECTROMETRY

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Received May 14, 2007; in final form July 9, 2007;
Accepted July 19, 2007

ABSTRACT

Variability in composition has limited the predictability of manure nutrient availability and crop responses, reducing the value of poultry litter as a fertilizer in production agriculture. Current analytical methods are not conducive to rapid analysis of a large number of replicates of such heterogeneous materials that is needed for improving accuracy of land application rates and optimal allocation of manure-borne constituents on the farm. Seventy-one litter samples were collected across poultry producing regions of Arkansas, Oklahoma and Maryland to evaluate the application of energy dispersive x-ray fluorescence (EDXRF) spectrometry for fast and accurate multi-elemental analysis and screening of manure types. The total phosphorus (P) results by the phosphomolybdate-ascorbic acid, and EDXRF methods were correlated, with a root mean square error (RMSE) of 1.4 g kg⁻¹ between methods. Energy dispersive XRF and inductively coupled plasma atomic emission spectrometric results for P, sulfur (S), potassium (K), calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn), and arsenic (As) also were well correlated ($P < 0.001$). A successful differentiation was made between layer and broiler litter, based on Ca and As concentrations. The results suggest that the EDXRF pressed disc method is an accurate alternative to individual wet chemistry methods due to its simplicity, while for instance providing corollary

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knowledge of other components that affect speciation and bioavailability of P. Timely knowledge of litter mineral composition may result in more frequent manure analysis to assist in efforts to maintain optimal manure nutrient balance on the fields. A high throughput allows more extensive measurements of nutrient accumulation on regional and larger spatial scales.

Keywords: phosphorus, metals, poultry litter, animal manure, multi-elemental analysis, energy-dispersive x-ray fluorescence

1. INTRODUCTION

The possibility of rapid, simultaneous, and accurate measurements of multiple elements in animal manure is crucial to the implementation of a balanced approach to natural resource management and the mitigation of environmental contamination risks caused by organic nutrient inputs and production practices. Animal manure is re-used beneficially for producing food and fiber crops and improving the physical properties and fertility of soils, particularly in organic farming systems [1-4]. Many studies employing wet chemistry and spectrometric procedures have been conducted to determine the value of animal manure as an organic source of nitrogen and phosphorus, or a carbon source for increasing organic matter in agricultural soils and improving their physical and biochemical properties [5-7]. However, animal manure is highly variable in composition over time and space within an animal production facility and between farms and regions of the country [8, 9]. This variability, along with the time and labor requirements and high cost of wet chemical analyses, diminishes the value of manure to a farmer as a reliable source of plant nutrients. A source of inconsistent composition reduces the predictability in nutrient mineralization and crop responses to manure when compared to commercial fertilizers.

Because of the variability, a large number of samples and frequent analysis are necessary to quantify accurately and characterize the accumulation of manure nutrients on a whole farm basis or at the watershed level. Deficiencies or over-applications of nutrients can result in undesirable consequences to either crop yields or the quality of nearby water resources [10, 11]. Agricultural soils have become phosphorus-enriched as repeated manure applications were made over the years in regions where there is a high density of confined animal feeding operations (CAFO) [12-16]. In addition to phosphorus (P),

dietary trace elements such as copper (Cu), zinc (Zn), or arsenic (As) may potentially accumulate in soil and reach phytotoxic levels [17, 18]. These metallic elements also control a nutrient's physical state and the speciation of environmentally sensitive nutrients such as P or As [19-21].

Methods of manure analysis have been extensively examined in recent reviews [22, 23]. Analytical procedures for extractable- and total nutrient concentrations are often based on single or sequential extractions, and elemental concentrations have been determined by a wide range of methods, including colorimetry, atomic-, and molecular spectroscopic techniques [6, 24, 25]. For example, measurement of phosphate is based on the widely used phosphomolybdate-ascorbic acid method, with color development and detection calibrated to $\text{PO}_4\text{-P}$ concentrations [24, 26]. Detection of extracted $\text{NH}_4\text{-N}$ or acid-digest total N (i.e., Kjeldahl N) is based on the salicylic acid modification of the indophenol blue method [27]. Nuclear-resonance spectroscopy, high performance liquid chromatography, or x-ray absorption near-edge structure (XANES) spectrometry have been used to identify specific inorganic and organic P [20, 28, 29]. Atomic absorption and emission spectroscopic methods both are routinely used for the determination of soil mineral nutrients that are essential to optimal crop growth [30]. Multi-element extraction solutions such as the Mehlich III extractant have been developed to obtain as much information on nutrient composition and availability in a single extract of soil and plant samples [31]. However, elaborate sample preparation, wet chemistry and spectroscopic analyses require a large investment of time and labor that contribute to the eventual high cost of an analysis.

X-ray fluorescence spectroscopy may present an opportunity to rapidly determine mineral nutrient composition of manure solids such as poultry litter, as well as many other CAFO by-products that are generated at rates exceeding the storage and assimilation capacities of many regions of intensive animal production agriculture [32, 33]. Compositional information on other inorganic constituents of the manure may provide insights into the potential types of interactions that exist between environmentally sensitive anions such as phosphate or arsenate and their counterions. For example, the types, and molar and charge concentrations of the counterions, were observed to control the solubility and dephosphorylation of inositol phosphates in manures and in soils [14, 20], or the exchangeability between phosphate and arsenate or phosphate and other polydentate, anions [21, 34].

Energy dispersive X-ray fluorescence (EDXRF)

methods have been used non-destructively to characterize diverse media, ranging from additives and trace elements in oil and lubricants to rocks and ores, soil and consolidated geologic materials, and eroded sediments [35-37]. Energy dispersive XRF methods have been applied to the determination of macronutrients and trace elements in plant materials [38-40]. Nonetheless, many concerns relating to sample preparation and presentation of samples to x-ray irradiation have been raised. The required reproducibility and accuracy of analysis may be major challenges because the samples can be mixtures of components of widely different particle density and particle sizes. The heterogeneity results in irregular surface properties presented for x-ray irradiation, which leads to artifacts and imprecise results.

These factors may be amplified in heterogeneous materials such as poultry manure that is composed of animal excreta, partially digested feed, feathers, and bedding materials that include substances as diverse as wood chips or small grain hulls. Therefore, the objective of the study was to evaluate the conditions of measurement and use of EDXRF with polarized x-rays in determining manure-borne mineral nutrients, in particular P along with elements such as calcium (Ca), iron (Fe), or aluminum (Al) that control P solubility and environmental behavior. We report on whether the compositional data can be used to distinguish the type of poultry litter in a mixed group of field samples, thereby reinforcing the technique's utility in screening large quantities of heterogeneous specimens.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Poultry Litter, Bedding and Feed Samples

Bulk samples (ca 2 kg) of manure cake, litter scraped from the floor of poultry production houses during periodic clean up, samples of clean wood chips used as bedding materials, and samples of feed were collected from farms located on the Eastern Shore of Maryland. Manure cake was a mixture of excreta and feed that was wetted and fused together upon drying, forming an irregularly shaped platelet. Additional bulk samples of litter were collected throughout the eastern Oklahoma-western Arkansas poultry producing areas ($n = 67$). The samples were air-dried and subsamples (10 g) were used to determine litter water content following drying at 65°C and expression of concentration results on a dry weight basis.

Compositionally representative samples of bedding wood chip, feed, or poultry litter (100 g) were crushed and preliminarily pulverized by hand with an agate mortar and pestle to pass through a polyethylene screen with 4.75-mm openings. A 30-g subsample was

then used to grind it to a fine powder (< 63 μm) using a shaker ball mill. The sample container and balls were made of agate. The ball-milled powdered samples were stored in closed plastic containers at 4°C until analysis by selected analytical techniques.

Table 1 Energy-dispersive X-ray fluorescence spectrometric conditions

Sample type	Polarizer or secondary target	Applied Voltage (kV)	Applied current (mA)	Measurement time (sec)	Measured Element Z number
Loose powder	Molybdenum	40	3.5	150	24-39
	Al ₂ O ₃	50	6	300	40-57
	HOPG	15	13	300	11-23
Pressed disc	Molybdenum	40	3	400	26-38 and 72-83
	Al ₂ O ₃	50	5.6	600	47-60
	Palladium	40	1.9	600	39-42 and 90-92
	Cobalt	35	4	400	19-25
	HOPG	15	13	300	11-17

2.2. Methods

2.2.1 Energy-Dispersive X-ray Fluorescence Spectrometric Conditions.

An EDXRF spectrometer with polarized radiation (Model X-Lab 2000, Spectro Analytical Instruments, Fitchburg, MA, USA)[†] was used in this study. The spectrometer was equipped with a 400-watt palladium (Pd) end window x-ray tube that can be focused on various crystal targets to produce polarized and near monochromatic x-rays. The primary x-rays were polarized by Barkla scatter with an Al₂O₃ target and by Bragg diffraction with a highly oriented pyrolytic graphite (HOPG) target. They also were used to irradiate secondary targets, i.e., molybdenum, cobalt, palladium and aluminum crystals, to produce near monochromatic x-rays to excite the sample. The x-ray tube had a 75 μm thick beryllium window. A lithium-drifted silicon Si(Li) detector attained a spectral resolution of 154 eV at the Mn K α -line at 10000 counts s⁻¹. Duplicate counts were obtained for each sample as measurements of x-ray intensities are always associated with a counting statistical fluctuation [41]. Specific measurement conditions are summarized in Table 1.

[†] The mention of a trade or manufacturer names is made for informational purposes only and does not imply an endorsement, recommendation, or exclusion by the USDA-Agricultural Research Service.

2.2.2 Sample Preparation

A comparison of EDXRF measurements was made between two forms of the pulverized sample, i.e., a loose powder and a pressed disc to evaluate sample homogeneity, packing density, and the effects of a simplified sample handling process on the precision and accuracy of the compositional analysis of poultry litter. Triplicate subsamples of litter samples obtained from different farms, coded anonymously MD-N1, MD-O2, MD-N3, MD-O4 were used for this comparison. Procedural parameters that were evaluated included sample grinding (i.e., hand vs. ball milling), form of the sample, sample size, and precision of measurements of Ca, K, P, and dietary trace metallic additives. The complete set of 71 litters was used to determine how well correlated the EDXRF results of pressed discs were with those of conventional techniques, e.g., the inductively-coupled plasma atomic emission spectroscopic (ICP-AES) and colorimetric phosphomolybdate-ascorbic acid methods for determining P.

Subsamples of the loose powder (4 g) were enclosed in a polyethylene cylindrical cell that was 32 mm in diameter and 22 mm in height. The cell window was covered with an XRF-grade thin polycarbonate film (4 μm in thickness). Another powdered subsample was pressed into a disc (diameter = 31 mm) with a custom-made hydraulic press. A die and tungsten carbide pellet set was used to press the powdered samples, using a range of sample weight (1

to 4 g) and compression pressure (140 to 275 MPa). The pellets in contact with the samples were covered with an XRF-grade thin film prior to compression to minimize any transfer of metallic contaminants.

2.2.3 Additional Calibration Standards of Poultry Litter

In addition to the library of selected standards of mineral and organic materials, additional multi-element calibration standards were prepared for direct analysis of loose powders and for analysis of manure solids by the pressed disc method. These standards were prepared in exactly the same manner as unknown samples of poultry litter, utilizing the same sample preparation tools and experimental conditions. The base material was made from wood chips that were used as bedding matter in the chicken rearing houses. Bulk samples of wood chips (20 g) were ball-milled and the powdered samples were amended with stock solutions of Ca, Al, Fe, Mn, Cu, Zn, As and P. Reagent grade chemicals, i.e., $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, AlCl_3 , FeCl_3 , MnCl_2 , CuSO_4 , ZnSO_4 , NaH_2AsO_4 and Na_3PO_4 were dissolved and added to attain concentrations of between 10 and 5000 mg kg^{-1} for Al, Ca and P, and 0.1 and 1000 mg kg^{-1} for Fe, Mn, Cu, and Zn. The sulfur (S) concentration, resulting from the use of the sulfate salts of Cu and Zn was 990 mg kg^{-1} . Spiked powdered samples were air-dried and thoroughly mixed before preparing loose-powdered samples and pressed discs to extend the intensity-concentration calibration curves with these additional standards [42]. Calibration parameters were developed for the new library of original and additional standards to correct intensity data for background and inter-element matrix effects. The latter interferences result from fluorescence radiation being absorbed by co-existing elements and thereby reducing fluorescence from the element of interest and enhancement of fluorescence radiation intensity due to secondary excitation by co-existing elements [41, 43, 44].

Elemental composition of the powdered litter, feeds, and wood chip bedding samples was also determined following digestion in a $\text{HCl-H}_2\text{SO}_4$ acid mixture at 180°C by inductively coupled plasma emission spectroscopy (ICP-AES) [25]. Calcium, Mg, Al, Fe, Mn, Cu and P contents were quantified according to Method 3120A on a Spectro ICP-CIROS^{CCD} (Spectro-USA, Fitchburg, MA). The P and N concentrations of acid-digests, using a concentrated H_2SO_4 -persulfate mixture were also determined using the phosphomolybdate-ascorbic acid and the indophenol blue methods, respectively [24, 27, 45].

The effects of sample form, size, and pressure of compaction were evaluated following statistical

analysis of the data of experiments that were based on a completely randomized design. Elemental analyses were made on triplicate litter samples. Significance of the differences between and within treatment was detected at the 0.05 level of probability. In addition, binary regressions between elemental concentration results of the various analytical methods were made using Proc GLM of the Statistical Analysis System [46]. Individual and common regression analyses were calculated: 1) to evaluate the homogeneity of residual variances and slopes of regression lines, and 2) to detect significant differences in slope and intercept of the regressions of paired analytical methods at the 0.05 level of probability.

3. RESULTS AND DISCUSSION

3.1. Optimization of EDXRF Measurement Methods

Table 2 summarizes elemental concentrations as estimated by direct measurement of loose powdered samples of a subset of bedding, feed, and poultry litter. The results showed a good precision of measurements of most of the selected elements, as a relatively uniform surface of the ball-milled samples was presented for irradiation behind a thin film window. Coefficients of variation (CV) of 78% of all measurements were less than 4.3%, while CVs of two analyses of all measurements exceeded 8.8%. However, the standard deviations of selected measurements were large; for example, the mean P concentration of fresh litter MD-N1, was found to vary by $\pm 930 \text{ mg kg}^{-1}$. Differences in the packing of a loose powder into XRF sample cups likely existed at the micrometer-scale as heterogeneous samples were pulverized, packed, and irradiated. The litter samples were varied in proportion of manure to bedding wood chips before ball-milling. This suggested that there was inhomogeneity in the particle density and packing density. The finer particles may tend to settle at the window during filling and packing of the XRF sample cups, hence irregular packing at the surface was presented for irradiation. However, the simplicity and low coefficients of variation of loose powder measurements may be appropriate for a rapid screening procedure to identify samples that clearly exceed a certain elemental concentration threshold.

The penetration of incident and fluorescence radiation occurs in a shallow layer from which spectral information is obtained. Hence, this requires a homogenous sample. Table 3 shows the EDXRF results for the same litter samples when analyzed as pressed discs. There was a clear improvement in the reproducibility of the analytical results as radiation

intensity measurements were made under a vacuum, with no intervening thin film positioned between sample disc and detector. Foremost, the increased bulk density of the pressed discs increased the absorption of incident x-ray radiation, causing different mass attenuation coefficients and resulting in equal or greater count rates registered for all elements when compared to those of the loose powders (Fig. 1).

Increased count rates were observed for most elements and ranged from 5% to 13.1% for silicon (Si), P, S, Cl, K and Ca, or for elements that are most relevant to the chemistry of poultry litter P (e.g., Ca and Al). The simultaneous detection and corollary knowledge of the latter elements are most useful in understanding the environmental behavior of P in animal manure or in soil [20, 34].

Table 2 Concentrations of selected elements in samples of poultry manure and litter, feed, and bedding material samples from Maryland Eastern Shore broiler feeding operations by a direct analysis of loose powdered samples

Sample	Al	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	As
	g kg ⁻¹						mg kg ⁻¹				
Litter MD-N1 [†]	< 0.02 [‡]	13.1	7.5	12.4	38.9	23.3	616	898	720	524	82
	0.02 [§]	0.9	0.3	0.8	1.7	0.1	26	62	43	23	5
Litter MD-O2	8.4	14.2	6.9	11.8	39.3	24.4	567	4260	520	420	62
	0.3	0.1	0.1	0.1	0.2	0.5	4	2	8	4	1
Litter MD-N3	< 0.02	12.9	7.0	11.2	35.4	21.5	574	812	612	481	73
	0.02	0.4	0.3	0.3	1.5	0.2	11	8	6	14	0.4
Litter MD-O4	1.9	13.5	6.6	10.2	36.4	22.7	516	2230	479	400	58
	0.17	0.4	0.5	0.3	1.2	0.4	1	68	10	4	0.1
Feed sample	< 0.01	4.1	2.7	3.5	10.3	5.2	114	138	13	109	18
	0.01	0.2	0.1	0.1	0.4	0.3	4	5	1	4	0.1
Bedding wood chip	< 0.01	0.5	0.3	0.2	1.9	1.5	76	126	10	18	2
	0.01	0.01	0.00	0.00	0.01	0.00	5	11	3	1	0.4

[†] Anonymous litter codes; [‡] Mean; and [§] standard deviation of 3 observations.

Table 3 Concentrations of selected elements in samples of poultry manure and litter, feed, and bedding material samples from Maryland Eastern Shore broiler feeding operations by the pressed disc method

Sample	Al	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	As
	g kg ⁻¹						mg kg ⁻¹				
Litter MD-N1 [†]	< 0.01 [‡]	13.5	6.0	11.5	32.5	23.0	532	789	643	515	82
	0.01 [§]	0.04	0.02	0.02	0.04	0.03	2	6	3	2	1
Litter MD-O2	1.6	13.9	5.1	10.3	33.8	23.1	483	3425	444	401	62
	0.03	0.05	0.02	0.02	0.05	0.03	2	11	2	2	1
Litter MD-N3	< 0.01	11.5	5.2	9.5	26.3	19.3	463	677	469	416	68
	0.01	0.04	0.01	0.01	0.04	0.03	1	5	2	2	1
Litter MD-O4	< 0.01	12.7	4.9	9.0	29.0	21.3	441	1696	405	372	57
	0.01	0.04	0.01	0.01	0.04	0.03	1	8	2	2	1
Feed sample	< 0.01	3.6	1.6	3.0	7.0	4.7	76	129	9	94	15
	0.01	0.01	0.01	0.01	0.01	0.01	0.4	1.7	0.3	0.7	0.2
Bedding wood chip	< 0.01	< 0.03	< 0.02	0.0	0.5	0.8	41	96	5	11	1
	0.01	0.03	0.02	0.00	0.00	0.00	0.2	1.4	0.2	0.2	0.1

[†] Anonymous litter codes; [‡] Mean; and [§] standard deviation of 3 observations.

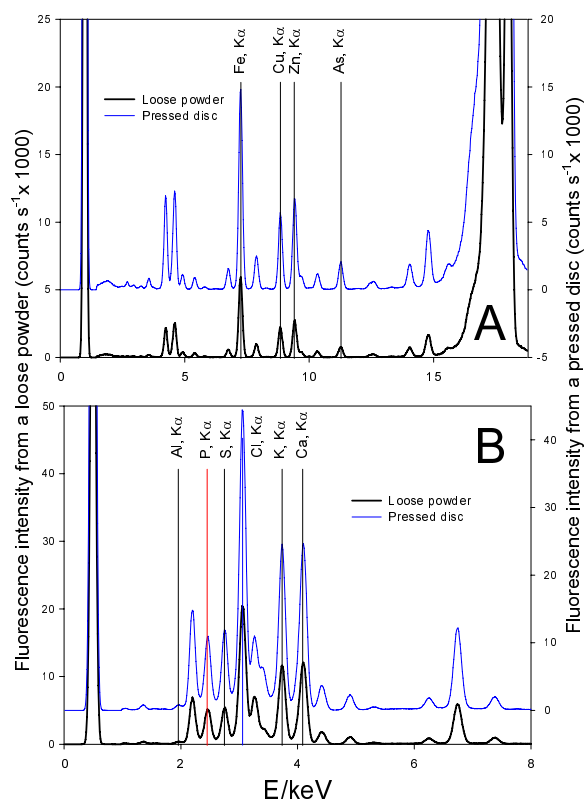


Figure 1 Effect of two powdered forms on the energy-dispersive x-ray fluorescence spectra of a sample of broiler litter excited with a molybdenum secondary target (A) and with a highly oriented pyrolytic graphite Barkla scatterer (B).

Solubility and bioactivity of insoluble inorganic and organic phosphates are controlled by the types and concentrations of polyvalent counterions. As strong ligands, phosphate and organic forms such as inositol phosphates (IP) have a high affinity for Al^{3+} , Fe^{3+} , or Ca^{2+} , Mg^{2+} , or Zn^{2+} , etc. In previous studies, the release and dephosphorylation of phytic acid (IP6), a commonly found form of IP have been shown to be progressively inhibited when cation to IP6-P molar ratios approach a ratio of 1 or higher [20]. Intermolecular bridging by the polyvalent cations increases the concentrations of insoluble Al-, Fe-, or Ca-IP6, limiting the hydrolytic activity of phosphohydrolases.

Localized variability of mass densities within the pressed disc and in the x-ray penetration zone near the cell window was minimized. These conditions resulted in reduced measurement errors that were one to two orders of magnitude for elements of $Z < 20$ when compared to those of the loose powder samples (Tables 2 and 3). Therefore, the experimental results

suggest that the direct analysis of the loose powdered samples is not accurate for quantitative characterization of highly variable poultry litter. Although a direct measurement of loose powders was a less laborious process, future improvements of the measurement conditions and reproducibility for a loose powder of poultry litter might include improved homogenization and standardized protocols for sample packing in XRF cups and more standards of known concentrations of the elements with the least accuracy, e.g., Al, S, K and Cu.

3.2. Poultry Litter Characteristics

Table 4 summarizes elemental composition of all 71 samples of litter as measured by the pressed disc method. The set of poultry manure cake and litter samples contained major and trace element concentrations that were in general agreement with reported characteristics of poultry litter [8, 18, 47]. For example, Cu and Zn were reported present in poultry litter at about 748 and 718 mg kg^{-1} , respectively, while As varied between 0 - 77 mg kg^{-1} . In this study, the data showed a wide range in concentrations of each element and reflected varied conditions across the farms used in the study. Arsenic, Fe and Ca were among the most variable elements, exhibiting CVs that ranged from 61 to 89%. The heterogeneity may be attributed partly to the variability in feed composition across a wide geographical distribution of source farms, the stage-feeding regimes, and production strategies and goals. In addition, manure collection, storage and age of the litter piles differed widely. Therefore, EDXRF's high throughput, versatility, and multi-element analysis capability proved well-suited to detection of changing nutrient-management needs at the farm level.

Results of the EDXRF analyses were correlated with those of the colorimetric method for P in acid-digest of the litter and feed samples ($P < 0.001$) (Fig. 2). The EDXRF and the phosphomolybdate-ascorbic acid results for litter P had a slope not significantly different from unity and a root mean squared error (RMSE) of 1.4 g kg^{-1} for a set of geographically diverse samples. In addition, the EDXRF results were significantly related to ICP-AES results for P (Fig. 3). In general, EDXRF yielded slightly lower P concentrations that averaged 7.5% less in magnitude than ICP-AES measurements in acid digests. Nonetheless, this linear correspondence reinforced the agreement found between EDXRF and the phosphomolybdate-ascorbic acid results. The correlation between EDXRF and ICP-AES results was also high for Ca and K concentrations of all litter samples.

Table 4 Concentrations of selected elements in 71 manure and litter samples collected from poultry feeding operations across Arkansas, Oklahoma, and Maryland Eastern Shore by the pressed disc method

Statistic	N [†]	g kg ⁻¹					mg kg ⁻¹				
		P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	As
All litter samples											
Average	22.1	17.2	7.2	8.8	29.0	34.0	621	1759	694	549	32
Standard error	0.8	0.5	0.2	0.3	0.6	2.5	19	191	31	13	2.3
Maximum	42.0	31.4	12.9	14.3	41.7	106	1101	11810	1292	919	101
Minimum	4.6	10.2	2.6	2.6	13.0	16	347	607	54	316	0.9
Median	22.4	16.8	6.9	9.6	28.5	26	572	1393	655	537	32
CV (%)	30	22	27	31	18	61	25	89	37	19	59
Broiler litter (n = 64)											
Average	22.3	16.9	7.2	9.3	28.8	27.4	609	1776	708	541	35.8
Standard error	0.8	0.4	0.2	0.3	0.6	0.9	19	202	31	13	1.9
Layer litter (n = 7)											
Average	19.8	15.0	3.3	2.4	27.2	90.5	715	1615	571	612	1.9
Standard error	0.9	0.4	0.4	0.2	0.6	1.1	17	41	32	12	0.1

[†] Acid digest total N by the indophenol blue method [28].

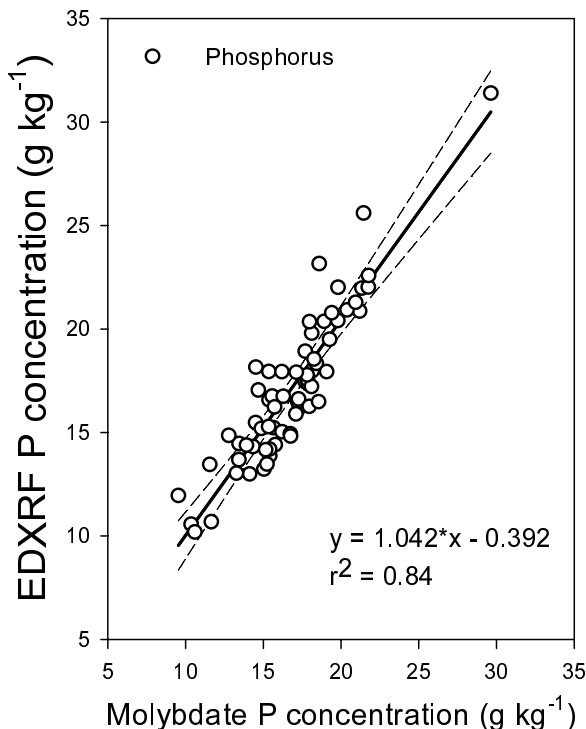


Figure 2 Relationship between energy-dispersive x-ray fluorescence (EDXRF) and the phospho-molybdate-ascorbic acid results for total phosphorus concentrations in 71 poultry litter samples collected across three US poultry-producing regions.

For S, Mn, Cu, Zn and As, the agreement between the two spectroscopic methods was even stronger and there was less scattering about the regression line than in the results for elements lighter than Ca (i.e., $Z < 20$). For example, the RMSEs were 52, 33, 49 and 2.4 mg kg⁻¹ for Cu, Zn, Mn and As regression lines, respectively (Fig. 3). Although the use of polarized x-rays has significantly reduced background noise, it appears that the lower correlation found between the latter spectroscopic methods may be in part attributed to the relatively lower precision of measurements of low-Z elements by EDXRF.

3.3. Litter Source Screening

The simultaneous multi-element analytical capability of EDXRF proved beneficial to categorizing or typing of the litter samples. The experimental results suggest that some of the samples ($N = 7$ samples) likely were collected from farms producing laying hens. Calcium concentrations were notably higher (90.5 ± 9.3 g kg⁻¹) in laying hen litter than other poultry litter samples because of Ca importance in eggshell quality [48]. The remaining 64 samples were from broiler feeding operations, and had Ca concentrations that averaged 27.4 g kg⁻¹. Broiler litter also contained a higher concentration of As (35.8 mg kg⁻¹) than layer litter (1.9 mg kg⁻¹). It was noted that 15 samples of this group had As concentrations ≥ 40 mg kg⁻¹ that exceed the threshold limit for land application of As in biosolids under 40CFR 503 [49].

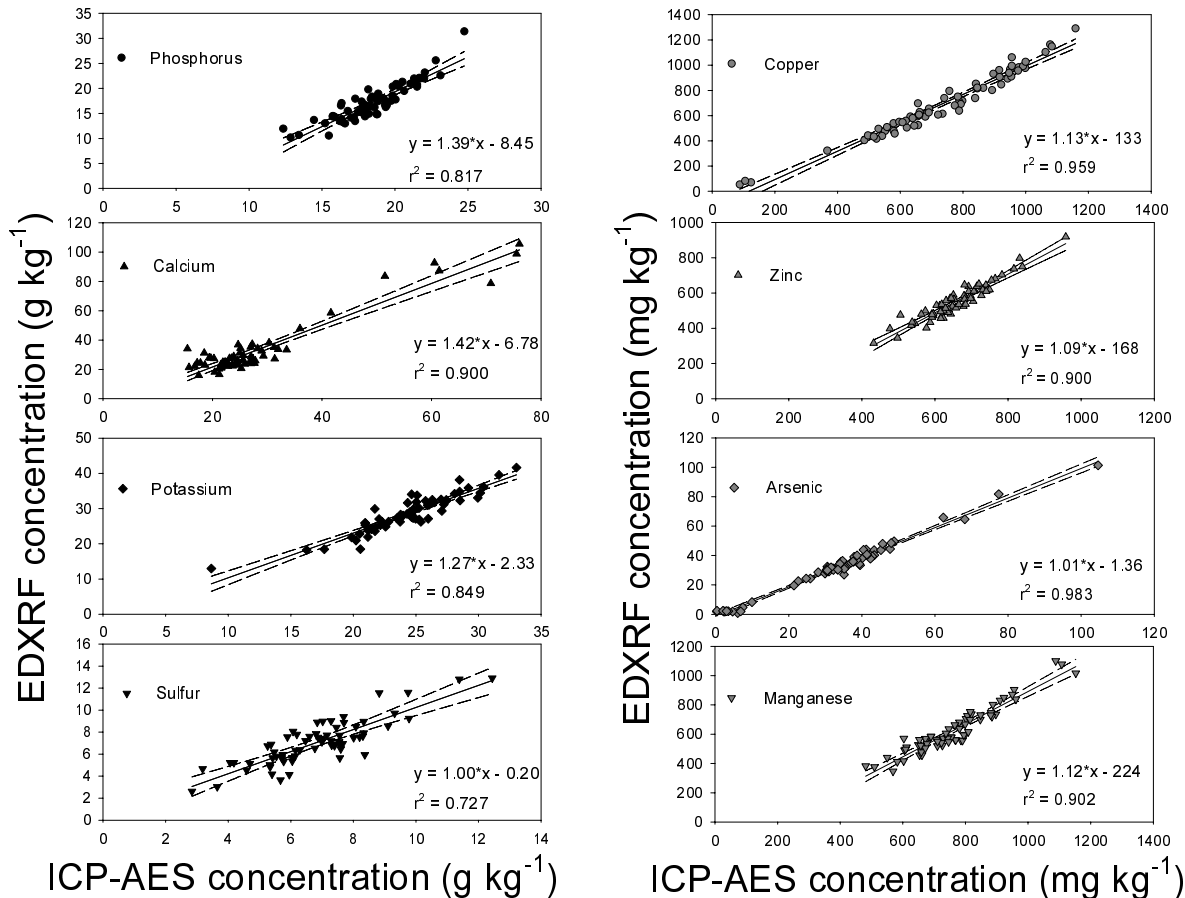


Figure 3 Relationships between energy-dispersive x-ray fluorescence (EDXRF) and inductively-coupled plasma (ICP-AES) spectrometric results for selected elements in 71 samples of poultry litter collected across three US poultry-producing regions.

In addition, a chlorine-based indexing of the litter samples helped in distinguishing the two groups of poultry litter. The group with a chlorine-to-P ratio of <2.5 (previously identified as broiler litter) also showed a significant linear relationship between P and Ca ($P < 0.001$; data not shown) whereas the layer litter group did not. Therefore, corollary knowledge of Cl and As concentrations proved valuable in identifying the litter type and confirmed a relationship that was known to exist between Ca and P in broiler litter samples.

Elements P, K, Fe, Mn, Cu and Zn were not significantly different in the litter from the two types of poultry (Table 4). Copper and Zn were present in poultry litter at concentrations averaging 694 and 549 mg kg⁻¹, respectively, with the EDXRF results agreeing with those of ICP-AES analyses (Table 4 and Fig. 3). Therefore, the experimental results suggest that the

EDXRF is a relatively fast technique for simultaneous analysis of elements of agronomic importance such as P, Ca, K, S and trace elements that are essential in plant nutrition.

The EDXRF technique can be used in exploratory analyses of potential environmental contaminants in poultry litter or other similar livestock manure solids, as rapid simultaneous analysis can yield accurate data on P, Cu, Zn and As, along with other metallic elements such as chromium (Cr) or selenium (Se) [data not shown]. It is, however, well beyond the scope of this study to address which other trace element should be of interest and, therefore the accuracy of its determination. From an environmental point of view, the flexibility and robustness of EDXRF spectrometry thus might enhance routine manure testing that is critical to comprehensive nutrient management plans on the farm and avoidance

of nutrient surpluses such as P-enrichment in animal manure and agricultural soils. In addition, on-farm elemental analysis of manure solids may be feasible with the development and refinement of field portable EDXRF instruments. Whether similar accuracy may be achieved with hand held spectrometers that are currently available on the market remains to be determined. Improvements in the measurement of elements of low Z number that include major plant macronutrients would permit *in situ* field measurements and real-time knowledge of mineral nutrient loading on specific fields or in hydrologically active areas of particular watersheds.

4. CONCLUSIONS

Simultaneous measurement capability and timely knowledge of mineral nutrients such as P and trace metallic elements such Cu, Zn, Mn, As and Se in poultry litter and other animal manure solids is vital to attaining and maintaining a balance between renewable resource conservation and environmental protection in today's strict regulatory atmosphere. Litter samples collected on farms located across three US states were highly heterogeneous mixtures of excreta, bedding material, intact and partially digested feed and shed feathers. The simplicity in sample preparation for multi-element analyses and high throughput of EDXRF was beneficial in elemental characterization of such types of environmental specimens. Although direct analysis of the loose powders by EDXRF was attractively simple and quick, the pressed powder disc method yielded more reproducible analytical results. Strong correlation was observed between analytical results of a widely-used colorimetric method and ICP-AES and EDXRF spectrometric methods for important nutrients (i.e., P, Ca, Mg, Fe, S, K). Dietary mineral additives (i.e., Zn, Cu, As or Se) were also accurately determined, and provided corollary information that enhance understanding of macronutrient behavior in complex environmental media.

Potential cross-reaction between environmentally sensitive anions (e.g. inositol phosphates) and their counterions would provide insights into their speciation pattern and bioavailability [16, 50]. Although we have reported the direct analysis of solid specimens, liquid extracts also can be analyzed by EDXRF (Dao, 2007, unpublished data). While EDXRF yielded an extensive list of mineral components, further development of the instrumentation must be made to detect N and C. At this point, an analysis for N must be made separately to have a more complete picture of the equivalent fertilizer value of

poultry litter. Nevertheless, the simplicity of the EDXRF method can be advantageous in revealing differences in efficiency of nutrient absorption and assimilation in animal nutrition studies, nutrient excretion and release from manure, providing timely information for optimal manure macronutrient and trace element management in agronomic production systems, and enhancing the understanding of their chemical behavior and eventual fate in the environment.

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