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Phosphorus Determination in Forage and Manure using Portable X-ray Fluorescence Spectroscopy to Support Comprehensive Nutrient Management Planning

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Abstract

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Phosphorus (P) runoff from agricultural fields is a major cause of water quality degradation problems. A P mass balance across the farm could maintain profitability and sustainability of animal-based farms and minimize water quality problems. Comprehensive Nutrient Management Plans (CNMPs) require mineral composition, especially P, data on soil, forage, and manure samples for accurate planning. However, traditional wet chemical methods of P determination are costly, time-consuming, and generate hazardous waste. X-ray fluorescence (XRF) could overcome many of these disadvantages and allow rapid determination of P concentrations. Portable XRF (PXRF) units are Energy Dispersive (ED) systems with low power X-ray tubes (10-40W) in comparison to benchtop units (50-300W). They are light and convenient to use either in benchtop or field-analysis modes. When a sample is scanned, the resulting spectrum identifies the element (peak position or energy); area under the peak (intensity) is proportional to concentration. A few studies have indicated the possible use of PXRF for the analysis of plant tissue and compost samples. However, there is a lack of information for analysis of heterogeneous forage and manure samples, including optimal sample preparation (particle size and moisture content) and instrumental parameters (scan time). The objective of this study was to evaluate the effect of manure moisture content and forage particle size on elemental concentrations determination using PXRF in benchtop mode. Manure samples (n=40) were oven dried at 50 and ground to 0.5 mm size and adjusted to four gravimetric moisture ranges: 10-20%, 20-30%, 40-50%, and 60-70%. Dry hay samples (n=42) were oven dried (60^0C for 3 days) and ground into two particle sizes (0.25-0.5 mm and 1-2 mm). Prepared samples were scanned by PXRF using a vacuum (<10 torr) and without a filter. Samples were placed in a sample cup over a thin proline X-ray film and scanned for 180s. Some forage samples (n=29) were also scanned for 60s and 120s for scan time analysis. Spectra for each analysis, photon counts (intensity) and P concentration were collected using the S1PXRF software. Reference standards were prepared by microwave digesting forage and manure samples in triplicate followed by elemental quantification through Inductively Coupled Plasma Optical Emission Spectrometry (ICP). Regression analysis, two sample t-test, matched paired t-test, and repeated measures ANOVA were used for data analysis. ICP measured P, K, Ca, Mg, Fe and Cu were in close agreement with West Virginia Department of Agriculture-measured concentration in manure samples. Dried and ground manure samples produced a stronger relationship with ICP-determined P, K, Ca, Fe, Cu, Zn (r^2> 0.90) and Mg (r^2=0.59). Presence of moisture negatively affected elemental determination in manure samples. The strength of the relationship decreased with increasing moisture content beyond 20%.

Comparison of PXRF concentration with ICP-determined value indicated that forage P, K, Ca, and Fe ($r^2 > 0.88$) could potentially be determined with portable XRF. The strength of the relationship increased with decreasing particle size in forage samples. The particle size of the sample was significant while the scan time and the interaction between scan time and particle size were not significant. There was no significant difference between ICP and PXRF measured concentration for 0.25-0.5 mm sample but significant difference between ICP and PXRF concentration for 1-2 mm samples. Likewise, there was a significant difference ($p < 0.001$) between PXRF concentration measured with the two particle size of the sample. Use of different scanning time (60s, 120s, and 180s) did not affect the relationship with ICP concentration for the samples of particle size. Thus, P, K, Ca, Fe, Cu and Zn can be accurately be measured in manure samples and P, K, Ca, and Fe in forage samples using PXRF in benchtop mode. Additional work is needed to extend this to in-field analysis. This simplification of the elemental analytical method could expedite CNMP planning process and contribute to better whole-farm P management.

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Chapter 1: Introduction

Comprehensive Nutrient Management Plans

A Comprehensive Nutrient Management Plan (CNMP) is a conservation plan for animal feeding operations (AFO). It is used to help AFO owners manage on-farm nutrients and meet local, state, and federal water quality goals and regulations (USDA-NRCS, 2003). It includes conservation practices and management activities related to livestock and cropping operations. It emphasizes optimal animal feeding practices to minimize mineral nutrients in manure, appropriate use of manure in the field, and the use of conservation practices that address natural resource concerns related to manure and organic by-product effects on water quality. Manure and wastewater handling and storage, land treatment practices, nutrient management, record keeping and feed management are the major components of a CNMP (USDA-NRCS, 2010).

The Conservation Planning Standard for Nutrient Management (Ac. 590) has defined nutrient management as the management of the amount, source, placement, form, and timing of nutrient applications and soil amendments (NRCS-WV, 2010). The major objectives of the 590 standard are to budget and supply plant nutrients, properly utilize manure and organic by-products in plant nutrition, maintain or improve soil health and minimize surface and groundwater pollution due to agricultural nonpoint sources (NRCS-WV, 2010; Evanylo and Beegle, 2006). Soil, manure, forage, and feed analysis are an important part of the planning process that helps to determine application rates of manure and commercial fertilizers in crop fields and optimize animal rations.

Phosphorus (P) is one of the important macronutrients essential for growth and development of a plant. It is a building block of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in plant cells and responsible for energy storage and transfer. Plants acquire all of their P from the soil. Weathering process, external applications of manure, fertilizers, and crop residues add P to soil. In animal farms, manure is a major source of soil P.

Since the nitrogen (N)-to-P ratio in manure is less than what is required by crop plants, N-based manure applications will lead to excess soil P and thus potential losses to water bodies (Maguire, 2014). High soil test P, soil erosion, and runoff are major contributors to P loss from farms. Since there is no gaseous form of P; amounts applied in excess of plant removal could be a potential source to nearby water bodies. Therefore, P mass balance analysis is essential for the sustainability of animal farms. Best management practices could reduce P loss through soil erosion but optimal use of manures and fertilizers based on soil and manure tests are needed to solve problems associated with positive P mass balances (Maguire, 2014).

Mineral composition data, especially P, of soil, forages, feed, and manures are important components of P mass balance evaluations and the CNMP development process. However, getting mineral composition data is time-consuming and costly. Simplification of the mineral analysis process could expedite the planning process at a reduced cost. Conventionally, minerals are quantified with expensive and time-consuming wet-chemical methods. This involves acid digestion of samples followed by spectroscopic techniques (Karla, 1998). Although they provide accurate quantification of minerals, they are not free from serious shortcomings. The handling and disposal of acids may result in serious health and environmental consequences. The sample preparation and preservation is tedious. Likewise, these methods are destructive in nature preventing sample reanalysis and there can be a significant lag-time between when the samples are collected and when the data are available.

Mineral Analysis

Forages

Minerals occupy almost 10% of herbage dry matter (Fleming, 1973). Of the important minerals indispensable for animal growth and development, major elements include P, K, Ca, Mg, S, and Cl and trace elements include Fe, Mn, Co, Cu, Zn, I, and Se (Jones and Thomas, 1987). Mineral composition of forage is influenced by soil mineralogy, moisture content, pH, and external inputs. External inputs of fertilizers into the soil mainly influence N, P, and K level in forage while the trace element level is mainly influenced by soil mineralogy (Fleming, 1973). Nitrogen fertilization increases the concentrations of K, Ca, and Mg while decreasing the concentration of P in plants (Reid et al., 1970). In addition, species, plant maturity stage, growing season, and temperature influence mineral composition (Whitehead, 1966; Reid et al., 1970; Fleming, 1973). For example, concentrations of Ca and Mg are higher in legumes compared to grasses (Reid et al., 1970; Baker and Reid, 1977). With advancing maturity, N, P, and K concentrations decrease while micronutrient concentrations are unaffected (Baker and Reid, 1977). In addition, maximum concentrations of P and K are found in early spring growth but Ca and Mg levels are low during spring growth and increase through summer and fall (Reid et al., 1970).

Forage is the major feed and source of minerals for ruminant farm animals. The performance and health of the animal are directly influenced by the mineral content of the forages (Minson, 1990; Van Soest, 1994). The deficiency or excess of specific mineral elements in forage results in poor animal performance and economic return (Reid et al., 1970). The quantity of forage essential to each animal type is determined by the expected level of animal performance, quality of the forage, and its mineral content. Thus, knowing elemental composition (Mg, Ca, P, K, Fe, Cu, and Zn) in forage sources is crucial for farmers and/or nutritionists in formulating feeding plans for animals. In addition, large herds require enormous amounts of feed and forage for daily consumption indicating the need to know the nutritional status of the feed and forages frequently for appropriate feeding planning. Since the nutritional status of the forage changes over time, regular sampling and analysis are crucial for ration balancing. However, this increases the monetary burden to farmers (Berzaghi et al., 2005).

Manure and Compost

Animal manure is rich in minerals essential for plant growth. When available, manure can enhance the production and quality of forage crops. Inadequate use may cause a nutrient deficiency, however, excessive use results in nitrate leaching and P runoff to water bodies causing eutrophication and associated environmental hazards (Rosen and Bierman, 2005). Having knowledge of the mineral composition of manure either produced within a farm or imported from elsewhere is of tremendous importance for farm nutrient management. Several factors like dietary inputs, the addition of bedding material, water, and storage conditions affect mineral composition of farm manure (Shepherd et. al., 2002).

The availability of minerals to plants depends on the decomposition of manure and soil organic matter and release of minerals into the soil solution. Generally, 70 to 80% of the P and 80 to 90% of the K from manure will be available to plants in the first year after application (Rosen et al., 2005). Residual amounts will be available in subsequent years. However, higher residual amounts may result in runoff to water bodies. Mineral concentrations in manure can be predicted from ‘book values’ (Isleib, 2016), however, the actual concentration varies from farm to farm depending on the feed, type and amount of bedding, amount of water used, manure handling and storage. Thus, they are used for prediction of manure composition in the absence of manure tests and do not give exact concentrations (Isleib, 2016). Research from our group has indicated that ‘book values’ are not always accurate for P (Brackenrich et al., 2016). Thus, only appropriate sampling and testing can provide accurate mineral concentrations and in turn, realistic manure management plans (Martin, 2009; Peters and Combs, 2003).

Forage samples are dried, ground, digested either in a muffle furnace or microwave and elemental concentrations are determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Near-infrared spectroscopy can be used for forage quality analysis along with some elemental concentrations like N, P, K, Ca, Mg, S, and Cl. The liquid manure samples are well mixed, subsampled, and digested either in a muffle furnace or microwave and determined by ICP. Solid manure samples can be processed as received, however, the homogenization is quite difficult. They can be chopped and rotated to establish homogeneity. Solid manure samples can also be dried and ground before acid digestion for the determination of non-volatile elements like P (Peters and Combs, 2003).

Most laboratories still rely on conventional acid digestion methods for elemental quantification. However, modern spectroscopic techniques have the potential to minimize or eliminate wet-chemical methods.

X-ray fluorescence (XRF) spectroscopy

Principle

X-rays are electromagnetic radiation with a wavelength from 0.01 – 10 nm. X-ray photons are emitted from an X-ray tube by the interaction of electrons with a metal anode. When the energy of the incident X-ray is greater than the binding energy of electrons in the shell, inner shell electrons are ejected, leaving a vacant space. In order to fill this vacant space the electron from a higher shell moves to the inner shell emitting secondary X-ray radiation of energy/wavelength characteristic to each element. The emitted radiation is then detected. X-ray energy is inversely proportional to wavelength and is expressed as keV (kilo-electron volt) (Kalnicky and Singhvi, 2001). XRF possesses a unique ability to measure several elements simultaneously in an extensive concentration range with an accuracy of 1% (Piorek, 1997).

Based on the way in which the X-ray spectrum is dispersed before it reaches the detector, XRF spectroscopy can be either wavelength dispersive (WD) or energy dispersive (ED).

In WDXRF, an analyzing crystal disperses or splits secondary X-rays such that a detector can measure each wavelength separately (Willis and Duncan, 2008). It has superior resolution and minimal spectral overlaps allowing easy, accurate identification of peaks. Working with higher X-ray intensities it produces better detection limits than EDXRF. However, they are large, complex systems, with higher power X-ray tubes and are thus more expensive.

In EDXRF, secondary X-ray photons are detected directly without being dispersed. The dispersion of the entire spectrum occurs directly in the detector in the energy domain (Piorek, 1997). The energy and intensity of X-ray fluorescence are detected in the form of a spectrum. The peak position in the spectrum is proportional to the energy of X-rays (Willis and Duncan, 2008). Thus, the position of peaks identifies elements present in a sample while the magnitude of a peak is proportional to elemental concentration (Weindorf et. al., 2014). They are less expensive and available in portable form.

Portable XRF

Portable XRF is based on ED principle and has low power X-ray tube (10-40W) in comparison to benchtop units (50-300W). It is light and convenient to use either in benchtop or field analysis modes. Thus, the potential for *in-situ* measurement and immediate availability of the elemental concentrations are the most appealing parts of PXRF (Potts and West, 2008). Although PXRF can measure most elements, air attenuation of low energy X-rays restricts the measurement of light elements in the periodic table, especially below silicon (Potts and West, 2008) except when equipped with a unique chamber capable of working under vacuum, or a helium atmosphere (Bueno Guerra et al., 2014; Brouwer, 2013). Since air absorbs the radiation from light elements, use of a vacuum is crucial for the detection of light elements like Na, Mg, Si, P, S, K, and Ca. If a vacuum results in evaporation problems for liquid and wet samples, a helium atmosphere can be used (Brouwer, 2013).

PXRF may be qualitative, semi-quantitative, or quantitative as an analytical technique. The quantitative data are obtained by calibrating the XRF with reference wet chemical methods (Maarschalkerweerd and Husted, 2015) or standard addition methods (Reidinger et al., 2012).

XRF analysis has been widely used to quantify elements in several disciplines. Recent advances in XRF technology have led to the development of portable instruments that can be carried to desired places and used to establish mobile laboratories for immediate sample analysis. They can even be used in-situ without sample preparation (AMC, 2008). Some portable units are comparable to benchtop XRF in elemental quantification and limits of detection (Bueno Guerra et al., 2014) but superior in terms of portability, cost-effectiveness, simplicity of operation, potential for in-situ measurement, and analysis of large samples (Bueno Guerra et al., 2014; AMC, 2008). In addition, it can be a superior alternative to wet chemistry in terms the cost- and time-effectiveness, and non-destructive analysis of samples (Reidinger et al., 2012). Measurement precision varies from 0.01 to 0.5%, depending on the element of interest, its concentration, and the sample matrix (Piorek, 1997). It quantifies total element concentration in the sample volume. In the case of soil, not all portions of the elements present in soil are plant-available. Hence, it is not typically used to determine available elements in the soil.

While PXRF has been found to be effective in quantifying elements in archeology, geology, mining, metallurgy, soil science, and agronomy (Weindorf et al., 2014), very few works have been conducted on its use in plant, compost and manure analysis. To our knowledge, there are no published studies using PXRF in the elemental quantification of forage crops.

Calibration

Calculation of elemental concentration from the intensity of X-ray photons is done using an appropriate calibration equation. Calibration can be with either fundamental parameters or empirical. Fundamental parameter calibration is a standard-less calibration based on the geometry of XRF instruments and assumption of about sample matrix to produce semi-quantitative estimates of elemental concentration (Towett et al., 2016). Only one sample-containing element of interest is used as a reference standard (Smagunova et al., 2012). It assumes all samples are flat, smooth, homogenous, and infinitely thick for all analyte wavelengths (Willis and Duncan, 2008). This calibration is mostly used in commercial and industrial instruments but not reliable for research purposes (Towett et al., 2016).

Reference standards of known concentration are used to develop empirical calibration models for elemental quantification from XRF intensity. Specific empirical calibrations could be developed for each sample matrix using standard reference materials of the same matrix. Matrix effects arise due to elemental interactions (absorption and enhancement) and physical characteristics of particle size, surface, and chemical state of each matrix (Willis and Duncan, 2008). The measured intensity of the target element is influenced by variation in concentration of interfering elements in the matrix (Kalnicky and Singhvi, 2001), indicating the need for matrix-specific calibration. This can be done using the Lucas-Tooth and Price empirical calibration equation (Towet et al., 2016).

$$C_i = r_0 + I_i(r_i + \sum r_{in} + I_n)$$

Where,

C_i = concentration of element,

r_0 = intercept/empirical constant for element i,

r_i = slope/empirical coefficient for intensity of element i,

r_{in} = slope/empirical constant for effect of element n on element i,

I_i = net intensity of element i,

I_n = net intensity of element n.

Empirical calibration considers the variable depth of measurement, uneven voltage distribution, and peak overlap. The reliability of calibration depends on the variability of the reference standards and resemblance to the sample matrix (Towett et al., 2016).

Enhancement effects

X-ray fluorescence can either be primary or secondary. Primary X-ray fluorescence is produced by the action of incoming X-rays with the atom of an element. Secondary X-ray fluorescence is produced by the action of the emitted primary fluorescence on the atom of another element. The primary fluorescence produced by higher energy elements in a composite sample causes enhancement of the lower energy elements through the emission of secondary fluorescence (Salesh, 1988). The fluorescence that reaches to the detector is the combination of primary and secondary fluorescence. It is possible to have tertiary or even higher fluorescence, however, it is almost negligible in practice (Brouwer, 2013). In some cases, 10-30% of the measured intensity can be attributed to enhancement effect (Salesh, 1988).

Dyck et al. (1986), using EDXRF, in an experiment with environmental samples of medium thickness found that enhancement effect can only be neglected for very thin samples (geological samples: <0.5mg/ cm^2, biological samples: <20mg/ cm^2). Thus, the enhancement effect increases with sample thickness until it satisfies infinitely thick criterion.

Some PXRF bears the special facility to control energy, current, and use of a filter for the determination of a specific range of elements. There is a general rule that to get fluorescence from elements, at least 2 KeV more of energy should be released to the sample. For the detection of light element (Mg to Fe) a voltage level of 15 KeV and anode current of 26 µA is used. The greatest benefit associated with this setting is the reduction of enhancement effects due to the fluorescence associated with higher energy elements.

Depth of measurement

The volume of the sample interrogated depends on the energy of emitted X-ray and the critical penetration and escape depths. The critical penetration and escape depths are the depth of the sample beyond which almost all of emitted x-rays are absorbed, which is very low (in µm range) for light elements (Potts and West, 2008). In addition, it depends on the mass attenuation coefficient and density of the matrix (Towett et al., 2016).

The concentration of the analyte, the thickness of the samples, and their matrix characteristics are the major factors influencing radiation intensity. The intensity of radiation increases with sample thickness up to a point called critical thickness (a point where infinite thickness starts). Below the critical thickness (intermediate thickness) point, both sample thickness and matrix affect the intensity, but beyond critical thickness (infinitely thick samples) intensity is affected by sample matrix only. Thus, a thickness correction is essential for samples of intermediate thickness. The matrix effect is negligible for very thin samples (Sitko, 2009).

Heavy elements have a short wavelength, high energy, and thus deeper penetration depth. However, light elements, like P, have a longer wavelength, low energy, and thus low penetration depth. In addition, the composition of plant matrix also affects measurement depth. The estimated analytical measurement depth of P is 80 µm in water, 70 µm in cellulose and 60 µm in fructose (Towett et al., 2016). Thus, P is measured almost at the sample surface that is in contact with the nose of the PXRF reducing the influence of sample thickness.

Analytical parameters

Accurate determination of the composition depends on proper sample preparation, sample introduction and instrumental setup of the XRF (Towett et al., 2016). In addition, the elemental quantification is affected by the energy level of the element, scanning time, the particle size of the sample, and moisture content (McLaren et al., 2012). Likewise, samples should meet the conditions of homogeneity and infinite thickness along with proper calibration.

Scan time effect

Raw peak count and spectral resolution increase with increasing scan time (McLaren et al., 2012, Kalnicky and Singhvi, 2001). Along with element of interest and sample matrix, detection limit depends on scan time. Scanning samples for longer time decreases detection limits to smaller levels, however, the number of samples analyzed will be reduced (Kalnicky and Singhvi, 2001), and radiation damage is possible.

Bueno Geurrea et al. (2014) optimized measurement time as 150 seconds by scanning a pellet of NIST SRM 1515 apple leaves from 10 to 300 seconds. The coefficient of variation ranged from 0.1% for Ca to 3.3% for P at 150s. Reidinger et al. (2012) used 30s for scanning pelletized ground plant samples. Towett et al. (2016) used 180s for fine powdered (< 53 µm) plant samples. McLaren et al. (2012) evaluated scan time (120s and 300s) for cotton leaf powder. The spectral peaks were higher for 300s than 120s scanning time in cotton powder. Even though the two scan times produced similar data quality, regression slopes will be greater for longer scanning time thus increasing the accuracy of measurement (McLaren et al., 2012, Kalnicky and Singhvi, 2001). Paltridge et al. (2012a) used 60s for the determination of Zn and Fe in rice and pearl millet and for the determination of Zn, Fe, and Se in whole grain wheat (Paltridge et al. 2012b).

Particle size effect

The texture of sample surface is extremely important in XRF measurement. The surface of the sample should have mirror-finish, which can only be realized with fine powders or metals (Willis and Duncan, 2008). PXRF intensity increases with a decrease in particle size of the sample and is more sensitive for light elements (Maruyama et al., 2008).

Particle size effect is more pronounced in light elements than heavy elements because of lower penetration depth and measurement surface located on or in the proximity of the surface. In addition, there is shadowing effect if the sample is of heterogeneous particle size. Incoming X-rays will irradiate not all particles (Fig 1.1a) and thus no fluorescence will be received from shadowed particles (Yamada, 2014). The best approach to deal with particle size of the sample is to grind to a particle size less than the measurement depth of the element to be determined (Willis and Duncan, 2008). However, grinding sample to fine power is not always beneficial; there is the possibility of contamination by blades in grinding mill.

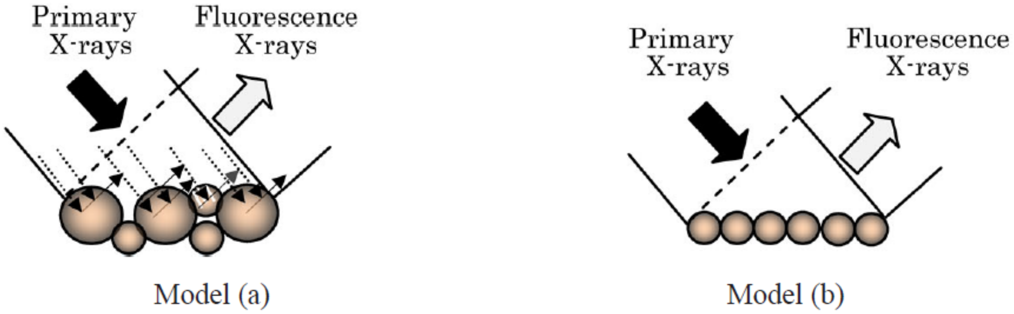


Figure 1.1: Effect of particle size of sample on X-ray Fluorescence (Yamada, 2014)

Imanishi et al. (2010) in a study with soil samples found that particle size of soil samples affected XRF intensity in the determination of light elements. Samples with small particles produced better results for light elements than large particle sizes.

McLaren et al. (2012) found that differences in particle size of cotton (powdered and 2 mm) did not affect slope and intercept of the regression line between PXRF and ICP values and indicated the need for further study of particle size effects.

Moisture level effect

Water molecules scatter primary X-rays and absorb characteristic X-rays of the analyte, thus reducing the photon counts (Ge et al., 2005; Stockmann et al., 2006). Increased moisture content reduces the apparent concentration and fosters a lower precision, poorer detection limit and reduced accuracy (Stockmann et al., 2006).

Kalnicky and Singhvi (2001) mention that presence of moisture, typically above 20%, will attenuate x-rays and dilute the concentration of elements affecting quantification of elements. This effect is larger for light elements (below 5 Kev) and may be negligible for the heavy elements, like Pb.

Imanishi et al. (2010) in a study using energy dispersive XRF with soil samples found that dried soil samples with small particles produced better results for light elements than wet samples. Likewise, Sahraoui and Hachichu (2017) in a study to determine the effect of moisture content of soil in elemental concentration of Ca, Mg, P, Zn, Fe, Mn, Cd, Cr, Cu, Ni, and Pb found that soil moisture content caused significant underestimation of elemental concentration.

McWhirt et al. (2012) in an experiment with the effect of moisture content on the elemental concentration of compost samples using PXRF (Delta Premium DP-4000, Olympus Innov-X, USA) indicated a moderate reduction in predictive ability (r^2 decreased by 5-20%) compared to dry scans.

Plant mineral analysis by portable XRF

Previous studies on different aspects of plant elemental compositional analysis have indicated some avenues to analyze forage samples by portable XRF. Bueno Guerra et al. (2014) in an experiment (using Tracer III-SD; Bruker Elemental, Kennewick, WA) with top visible dewlap leaves of 23 sugarcane varieties found a good correlation (r ranges from 0.91 to 0.99) between acid digestion values and XRF counts for P, K, Ca, P, K, Ca, S, Fe, Mn, and Si. Reidinger et al. (2012), using PXRF (Niton XL3t900 GOLDD Analyzer, Thermo Scientific, Winchester, UK), obtained a linear calibration curve for silicon in Si-spiked methylcellulose between acid digestion and portable XRF counts. Likewise, they found a good correlation (r=0.98) for P determination in certified reference material.

Towett et al. (2016) in an experiment with analytical parameters of PXRF (Tracer III-SD; Bruker Elemental, Kennewick, WA) taking diverse plant samples found that direct contact on the surface of a portable XRF with the aid of vacuum provided highest sensitivity and accuracy (r^2>0.90) for light elements (Mg to P) instead of using prolene sample cups. However, compromising some lower detection limits, elements like S, K, and Ca be analyzed without vacuum. The use of sample cups negatively affected the measurement of nutrients indicating the potential for the in-situ analysis of plant samples.

Kalcsits (2016) studied the suitability of PXRF (Tracer III-V; Bruker Elemental, Kennewick, WA) in measuring Ca and K concentration in apple and pear fruits. There was a significant correlation (r= 0.73 to 0.97) between PXRF measurement and wet chemical analysis results.

McLaren et al. (2012) using PXRF (Tracer III-V; Bruker Elemental, Kennewick, WA) studied the effect of scanning time and particle size on data quality using four plant species: corn tops (2 mm), wheat tops (2 mm and powder), cotton leaves (powder), and soybean grains (powder). They found similar r^2 values for the same sample at different scan times. Likewise, they obtained similar r^2 for corn and cotton at 120 seconds indicating potentiality of using a single calibration curve for different plant species. They found a significant linear relationship between the acid digest and portable XRF readings for Ca, Co, Cr, Fe, K, Mn, Ni, P, S, Si, and Zn in three plant species (corn, cotton, and soybean).

Paltridge et al. (2012a) in an experiment using PXRF (X-Supreme 8000, Oxford Instruments, Abingdon, UK) to determine Zn and Fe concentration in rice and pearl millet grain, found that EDXRF results were highly correlated with ICP values (r^2=0.79-0.98). EDXRF predicted Zn and Fe in rice within 1.9 and 1.6 mg kg^{-1} of ICP values, and in pearl millet within 7.6 and 12.5 mg kg^{-1} of ICP values at a 95% confidence level. In a similar experiment with whole wheat grain, Paltridge et al. (2012b) found EDXRF values for Zn, Fe, and Se were highly correlated with ICP-OES values. Standard errors of prediction were ±2.2 mg Zn kg^{-1}, ±2.6 mg Fe kg^{-1}, and ±1.5 mg Se kg^{-1}.

Compost mineral analysis by portable XRF

Elemental quantification of compost has implications in both plant nutrition and environmental quality (Weindorf et al., 2014). McWhirt et al. (2012) in an experiment with compost samples using PXRF (Delta Premium DP-4000, Olympus Innov-X, USA) indicated that the results for Ca, Cr, Cu, Fe, K, Mn, P, and Zn were in close agreement with ICP values. Weindorf et al. (2008) used PXRF (Alpha series, Innov-X, USA) with the single beam for elemental quantification of a single compost feedstock of composted dairy manure. They concluded that PXRF could be a potential tool for quantification of Ni, Cu, Zn, Se, Mo, and Pb. Recent advances in PXRF technology, especially the addition of vacuum, have extended the applicability of XRF for detection of light elements indicating the need for further study in using XRF for manure samples.

PXRF has been tested for homogenous plant materials but their applicability to heterogeneous plant materials like forage is yet to be determined. As a first step to using PXRF in-field for elemental determinations in manure and forage to support CNMP planning, this study starts with samples at the best analytical conditions (dried and ground samples) and then systematically evaluates the effects of moisture content (manure) and particle size (forage). In addition, appropriate scan times were determined. Standardization of analytical parameters in forage and manure samples would open new avenues in forage and manure mineral analysis.

Objectives

The overall goal of this thesis is to evaluate the suitability of PXRF to facilitate the development of CNMPs, specifically, the determination of P in manure and forage to construct whole-farm P-balances. Because other elements are obtained simultaneously, results for K, Ca, Mg, Fe, Cu and Zn are also reported.

To initiate this process we assumed that water content would be the limiting factor for manure analyses and particle size would be the limiting factor for forage analyses. Thus the specific objectives were

1. Quantify the effect of manure moisture content on elemental concentrations (Chapter 2), and
2. Quantify the effect of forage particle size and scan time on elemental concentrations (Chapter 3)

Literature Cited

Analytical Methods Committee (AMC), Royal Society of Chemistry. 2008. Evaluation of analytical instrumentation. Part xxiii. Instrumentation for portable x-ray fluorescence spectrometry. Accred. Qual. Assur. 13:453–464

Baker, B.S and R.L. Reid. 1977. Mineral composition of forages species grown in central West Virginia on various soil series. West Virginia University, Agricultural and Forestry Experiment Station, Morgantown. Bulletin 657.

Berzaghi, P., L. Serva, M. Piombino, M. Mirisola, and F. Benozzo. 2005. Prediction performances of portable near infrared instruments for at farm forage analysis. Ital. J. Anim. Sci. 4(SUPPL. 3): 145–147.

Brackenrich, J., L.M. McDonald, T. Basden and K. Shaffer. 2016. Optimizing feeding strategies to minimize phosphorus losses from roofed winter feeding areas. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America Annual Meeting, Phoenix, AZ. Nov. 6-9, 2016.

Brouwer, P. 2013. Theory of XRF. PANanalytial B.V., Almelo, The Netherlands.

Bueno Guerra, M.B., E. de Almeida, G.G.A. Carvalho, P.F. Souza, L.C. Nunes, D.S. Júnior, and F.J. Krug. 2014. Comparison of analytical performance of benchtop and handheld energy dispersive X-ray fluorescence systems for the direct analysis of plant materials. J. Anal. At. Spectrom. 29(9): 1667.

Dyck , P.M., S. B. Torok, and R.E. Grieken. 1986. Enhancement effect. Anal. Chem. 58(8): 1761–1766.

Evanylo, G.K. and D. Beegle. Introduction to nutrient management. 2006. In K. C. Haering and G. K. Evanylo. Mid-Atlantic nutrient management handbook. Mid-Atlantic Regional Water Program, USA.

Fleming, G. A. 1973. Mineral composition of herbage. In G.W. Butler and R.W. Bailey (ed.). Chemistry and biochemistry of herbage. Volume I. Academic Press Inc, New York.

Ge, L., W. Lai, and Y. Lin. 2005. Influence of and correction for moisture in rocks, soils and sediments on in situ XRF analysis. X-Ray Spectrom 34(28): 28–34.

Imanishi, Y., A. Bando, S. Komatani, S. I.Wada, K. Tsuji. 2010. Experimental parameters for XRF analysis of soils. Denver X-ray Conference on Applications of X-ray Analysis. International Centre for Diffraction Data. 1–7.

Isleib, J. 2016. Manure provides plant nutrients, but not always at book value. Michigan State University Extension. http://msue.anr.msu.edu/news/manure_provides_plant_nutrients_but_not_always_at_book_value

Jones, D.I.H., and T. A Thomas. 1987. Minerals in pastures and supplements. In R.W. Snaydon (ed.). Ecosystems of the world 17B, Managed grasslands analytical studies. Elsevier, Oxford.

Kalcsits, L.A. 2016. Non-destructive measurement of calcium and potassium in apple and pear using handheld X-ray fluorescence. Front. Plant Sci. 7:442.

Kalnicky, D.J., and R. Singhvi. 2001. Field portable XRF analysis of environmental samples. J. Hazard. Mater. 83(1–2): 93–122.

Karla, Y. P. 1998. Handbook of reference methods for plant analysis. CRC Press Taylor & Francis Group.

Maarschalkerweerd, M., and S. Husted. 2015. Recent developments in fast spectroscopy for plant mineral analysis. Front. Plant Sci. 6(March): 1–14.

Maguire, R.O. 2014. Importance of farm phosphorus mass balance and management options. Virginia Cooperative Extension. Publication No. CSES-98P.

Martin, J. 2009. Manure sampling for nutrient management planning. Agronomy Facts 69. Penn State Extension.

Maruyama, Y., K. Ogawa, T. Okada, and M. Kato. 2008. Laboratory experiments of particle size effect in X-ray fluorescence and implications to remote X-ray spectrometry of lunar regolith surface. Earth Planets Sp. 60: 293–297.

McLaren, T.I., C.N. Guppy, and M.K. Tighe. 2012. A rapid and nondestructive plant nutrient analysis using portable X-ray fluorescence. Soil Sci. Soc. Am. J. 76(4): 1446–1453.

McWhirt, A., D.C. Weindorf, and Y. Zhu. 2012. Rapid analysis of elemental concentrations in compost via portable X-ray fluorescence spectrometry. Compost Sci. Util. 20(3): 185–193.

Minson, D.J. 1990. Forage in ruminant nutrition. Academic Press, San Diego, USA.

NRCS-WV. 2010. Conservation practice standard, Nutrient Management (Ac.) 590. Natural Resources Conservation Service. West Virginia.

Paltridge, N.G., L.J. Palmer, P.J. Milham, G.E. Guild, and J.C.R. Stangoulis. 2012a. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. Plant Soil 361(1–2): 251–260.

Paltridge, N.G., P.J. Milham, J.I. Ortiz-Monasterio, G. Velu, Z. Yasmin, L.J. Palmer, G.E. Guild, and J.C.R. Stangoulis. 2012b. Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat. Plant Soil 361(1–2): 261–269.

Peters, J. and S. Combss. 2003. Sampling livestock waste for analysis. In: J. Peters, S.M. Combss, B. Hoskins, J. Jarman, J.L. Kovar, M. E. Watson, A.M. Wolf and N. Wolf. Recommended methods of Manure Analysis, Cooperative Extension Publishing, University of Wisconsin-Extension.

Piorek, S. 1997. Field-portable X-ray fluorescence spectrometry : past, present, and future. F. Anal. Chem. Technol. 1(6): 317–329.

Potts, P. J., and M. West. 2008. Portable X-ray fluorescence spectrometry: capabilities for in situ analysis. The Royal Society of Chemistry, UK.

Reid, R. L., A. J. Post, and G. A. Jung. 1970. Mineral composition of forages. West Virginia University, Agricultural Experiment Station, Morgantown. Bulletin 589T.

Reidinger, S., M.H. Ramsey, and S.E. Hartley. 2012. Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer. New Phytol. 195(3): 699–706.

Rosen, C.J., and P.M. Bierman. 2005. Using manure and compost as nutrient sources for vegetable crops. University of Minnesota, Extension Service. http://www.extension.umn.edu/distribution/horticulture/M1192.htmL.

Sahraoui, H., and M. Hachicha. 2017. Effect of soil moisture on trace elements concentrations. J. Fundam. Appl. Sci. 9(1): 468–484.

Salesh, N. 1988. Enhancement effect in XRF analysis. J. Radioanal. Nucl. Chem. 120(1): 161–165

Shepherd, M., J. Webb, and L. Philipps. 2002. Tools for managing manure nutrients. In UK organic research: Proceedings of COR conference. Aberystwyth. 165-168.

Sitko, R. 2009. Quantitative X-ray fluorescence analysis of samples of less than "infinite thickness": Difficulties and possibilities. Spectrochim. Acta - Part B At. Spectrosc. 64(11–12): 1161–1172.

Smagunova, A. N., B. Oyuntsetseg, S.D Pan'kov. 2012. Determination of optimum conditions for X-ray fluorescence analysis using coupling equations. J. Anal. Sci. Methods Instrum. 2(2): 81–86.

Stockmann, U., H.J. Jang, B. Minasny, and A. B. McBratney. 2006. The effect of soil moisture and texture on Fe concentration using portable X-ray fluorescence spectrometers. In: A.E. Hartemink and B. Minasny (eds.), Digital Soil Morphometric, Progress in soil science, Springer International Publishing Switzerland.

Towett, E.K., K.D. Shepherd, and B. L. Drake. 2016. Plant elemental composition and portable X-ray fluorescence (pXRF) spectroscopy: Quantification under different analytical parameters. X-Ray Spectrom. 45(2): 117–124.

USDA-NRCS. 2003. National planning procedures handbook. United States Department of Agriculture, Natural Resource Conservation Service.

USDA-NRCS. 2010. Statement of work: comprehensive nutrient management plan (code 102), Pensylvania

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Comstock Publishing Associates, Ithaca, NY.

Weindorf, D.C., and S. Chakraborty. 2016. Portable X-ray fluorescence spectrometry analysis of soils. Methods Soil Anal. 1(1): 1-8.

Weindorf, D.C., R. Sarkar, M. Dia, H. Wang, Q. Chang, B. Haggard, A. McWhirt, and A. Wooten. 2008. Correlation of X-ray fluorescence (XRF) spectrometry and inductively coupled plasma atomic emission spectroscopy (ICP-AES) for elemental determination in composted products. Compost Sci. Util. 16 (2): 79–82.

Whitehead, D. C. 1966. Data on mineral composition of grassland herbage from the Grassland Research Institute, Hurley, and the Welsh Plant Breeding Station, Aberystwyth. Grassland Research Institute, Hurley, Maidenhead Berks England. Technical report no. 4.

Willis, J. P., and A. R. Duncan. 2008. Understanding XRF spectrometry, Vol I. PANanalytial B.V., Almelo, The Netherlands.

Yamada, Y. 2014. Sample preparation for X-ray fluorescence analysis I . Outline of sample preparation. Rigaku J. 30(1): 26–29.

Chapter 2: Moisture Effect on Elemental Concentration Determination in Manure using Portable X-ray Fluorescence Spectroscopy

Introduction

A Comprehensive Nutrient Management Plan (CNMP) is a conservation plan for animal feeding operations (AFO). It is used to help AFO owners manage on-farm nutrients and meet local, state, and federal water quality goals and regulations (USDA-NRCS, 2003). Manure and wastewater handling and storage, land treatment practices, nutrient management, record keeping and feed management are the major components of a CNMP (USDA-NRCS, 2010). The major objective of conservation planning standard (Ac. 590) is to budget and supply plant nutrients, properly utilize manure and organic by-products in plant nutrition, maintain or improve soil health and minimize surface and groundwater pollution due to agricultural nonpoint sources (NRCS-WV, 2010; Evanylo and Beegle, 2006).

Phosphorus (P) is one of the important macronutrients essential for growth and development of a plant. It is a building block of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in plant cells and responsible for energy storage and transfer. Plants acquire all of their P from the soil. Weathering process, external applications of manure, fertilizers, and crop residues add P to soil. In animal farms, manure is a major source of soil P. Since the nitrogen (N)-to-P ratio in manure is less than what is required by crop plants, N-based manure applications will lead to excess soil P and thus potential losses to water bodies (Maguire, 2014). High soil test P, soil erosion, and runoff are major contributors to P loss from farms. Since there is no gaseous form of P, amounts applied in excess of plant removal could be a potential source to nearby water bodies. Therefore, P mass balance analysis is essential for the sustainability of animal farms (Maguire, 2014).

Mineral composition data, especially P, of soil and manure are important components of P mass balance evaluations and the CNMP development process. However, getting mineral composition data is time-consuming and costly. Conventionally, minerals are quantified with expensive and time-consuming wet-chemical methods. This involves acid digestion of samples followed by spectroscopic techniques (Karla, 1998). Although they provide accurate quantification of minerals, they are not free from serious shortcomings. They are costly, time-consuming, and generate hazardous waste.

Mineral concentrations in manure can also be predicted from ‘book values’ (Isleib, 2016), however, the actual concentration varies from farm to farm depending on the feed, type, and amount of bedding, amount of water used, manure handling and storage. Thus, they are used for prediction of manure composition in the absence of manure tests and do not give exact concentrations (Isleib, 2016) and are not accurate for P (Brackenrich et al., 2016). Thus, only appropriate sampling and testing can provide accurate mineral concentrations and in turn, realistic manure management plans (Martin, 2009; Peters and Combs, 2003). Simplification of the mineral analysis process could expedite the planning process at a reduced cost. X-ray fluorescence (XRF) could overcome many of these disadvantages of wet chemical analysis and allow quick determination of P concentrations.

X-ray photons are emitted from an X-ray tube by the interaction of electrons with a metal anode. When the energy of the incident X-ray is greater than the binding energy of electrons in the shell, inner electrons are ejected leaving a vacant space. In order to fill this vacant space the electron from a higher shell moves to the inner shell emitting secondary X-ray radiation of energy/wavelength characteristic to each element. The emitted radiation is then detected. X-ray energy is inversely proportional to wavelength and is expressed as keV (kilo-electron volt) (Kalnicky and Singhvi, 2001). Portable XRF (PXRF) units are Energy Dispersive (ED) systems with low power X-ray tubes (10-40W) in comparison to benchtop units (50-300W). They are light and convenient to use either in benchtop or field-analysis modes.

When a sample is scanned, the resulting spectrum identifies the element (peak position or energy); area under the peak (intensity) is proportional to concentration (Willis and Duncan, 2008; Weindorf et. al., 2014). Although PXRF can measure most elements, air attenuation of low energy X-rays restricts the measurement of light elements in the periodic table, especially below silicon (Potts and West, 2008) except when equipped with a unique chamber capable of working under vacuum, or a helium atmosphere (Bueno Guerra et al., 2014; Brouwer, 2013). The critical penetration and escape depths are the depth of the sample beyond which almost all of emitted x-rays are absorbed, which is very low (in µm range) for light elements (Potts and West, 2008). PXRF may be qualitative, semi-quantitative, or quantitative as an analytical technique. The quantitative data are obtained by calibrating the XRF with reference wet chemical methods (Maarschalkerweerd and Husted, 2015) or standard addition methods (Reidinger et al., 2012).

Some portable units are comparable to benchtop XRF in elemental quantification and limits of detection (Bueno Guerra et al., 2014) but superior in terms of portability, cost-effectiveness, simplicity of operation, potential for in-situ measurement, and analysis of large samples (Bueno Guerra et al., 2014; AMC, 2008). In addition, it can be a superior alternative to wet chemistry in terms the cost- and time-effectiveness, and non-destructive analysis of samples (Reidinger et al., 2012).

Water molecules scatter primary X-rays and absorb characteristic X-rays of the analyte, thus reducing the photon counts (Ge et al., 2005; Stockmann et al., 2006). Increased moisture content reduces the apparent concentration and fosters a lower precision, poorer detection limit and reduced accuracy (Stockmann et al., 2006). Kalnicky and Singhvi, (2001) mention that presence of moisture, typically above 20%, will attenuate x-rays and dilute the concentration of elements affecting quantification of elements. This effect is larger for light elements (below 5 Kev) and may be negligible for the heavy elements like Pb.

Weindorf et al. (2008) concluded that PXRF (Alpha series, Innov-X, USA) could be a potential tool for quantification of Ni, Cu, Zn, Se, Mo, and Pb determination in single compost feedstock of composted dairy manure. McWhirt et al. (2012) in an experiment with compost samples using PXRF (Delta Premium DP-4000, Olympus Innov-X, USA) indicated that the results for Ca, Cr, Cu, Fe, K, Mn, P, and Zn were in close agreement with ICP values. However, there is a lack of information for analysis of heterogeneous manure samples, including optimal sample preparation (moisture content) using the PXRF equipped with vacuum, which improves light element measurement.

We hypothesize that PXRF will be useful in the determination of forage elemental composition because our unit has vacuum capability, and PXRF has been used successfully to detect and quantify light elemental composition in different plant, soil, geological and archeological samples.

The overall goal of this study is to evaluate the suitability of PXRF to facilitate the development of CNMPs, specifically, the determination of P in manure. Because other elements are obtained simultaneously, results for K, Ca, Mg, Fe, Cu, and Zn are also reported. To initiate this process we assumed that water content would be the limiting factor for manure analyses. Thus, the specific objective was to quantify the effect of manure moisture content on elemental concentrations.

Material and Methods

Sample collection and preparation

Thirty five manure samples were collected from cattle farms of West Virginia representing various beef and dairy cattle manure. These include decomposed feces, decomposed mixtures of bedding, feces, and urine, and decomposed feces from pasture. In addition, five poultry manure samples were also taken representing broiler, layer, and turkey. Thus, total number of samples collected were 40.

Samples were refrigerated as received and spread on a filter paper over aluminum foil and oven dried at 50^0C for 72 hours. Samples were made stone free using mortar and pestle followed by milling in cyclone mill (FOSS Tecator 1093, FOSS North America, Eden Prairie, MN). The whole sample was allowed to pass through a 0.5 mm screen. Ground samples were subsampled using alternate scooping method into three parts: for PXRF scanning, the wet chemical analysis in WVU lab and wet chemical analysis in West Virginia Department of Agriculture (WVDA), Moorefield, WV.

Wet Chemical Analysis

Wet chemical elemental concentration were determined by microwave (MARS Xpress, CEM Inc., Matthews, NC)-digesting samples in triplicate followed by Elemental quantification through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima DV 2100, Perkin Elmer, Norwalk, CT). A Certified Reference Material (CRM), 1573a-tomato leaf, was digested with samples to check the accuracy of digestion. Exactly 10 mL 70% nitric acid was added to 0.5 g sample in digestion vessels and placed in a fume hood for 1 hour to eliminate the gases produced.

Then the tubes were placed in a microwave, heated for 15 minutes at 200 ^{0}C followed by holding at 200 ^{0}C for 15 minutes and allowed to cool overnight. The digested liquid was transferred into test tubes, diluted and final volume was made 50 mL using volumetric flasks. The diluted liquid was filtered before analysis by ICP-OES. Additionally, a part of samples was sent to WVDA for elemental analysis.

PXRF Scanning

Samples were scanned using portable XRF (Tracer III-SD; Bruker Elemental, Kennewick, WA) equipped with a rhodium tube from which X-rays are emitted, and a Peltier-cooled, silicon PIN diode detector, with a voltage of 15 KeV and anode current of 26 uA without a filter. It was operated on PC trigger mode and vacuum (<10 torr) was used for all scans. Samples were placed in double open-ended sample cups (series: 1500, Chemplex Industries Inc., USA) over a thin X-ray film (4 um prolene). The cups containing ground samples were placed in the nose of the PXRF and covered with the X-ray shielding lid.

Simulating higher moisture levels

Manure samples were adjusted to 4 different moisture level using a gravimetric technique. Ground samples were weighted in sample cups and known mass of water was added. They were allowed to equilibrate for 15 hours and moisture content was determined to be 60-70 %. Each sample was scanned twice with PXRF for 180s. They were air-dried and to reduce the moisture content to 40-50%, 20-30%, and 10-20% with subsequent PXRF readings taken at each moisture level. Moisture content was determined gravimetrically.

Data Collection and Statistical Analysis

Spectrum for each analysis was collected using S1PXRF software (Bruker Elemental, WA, USA). The intensity of P, K, Ca, Mg, Fe, Cu and Zn in count per second (cps) and elemental concentration of P, K, Ca and Mg in weight percent were obtained from the same software. A standard-based calibration (obtained from personal communication with Dr. Lee Drake, Senior Application Scientist, Bruker AXS, Albuquerque, NM) for plant samples was used to obtain elemental concentration.

Data were organized in Microsoft Excel and analyzed by JMP (JMP®, Version Pro 12.2, SAS Institute Inc., Cary, NC, Copyright ©2015), SAS (SAS®, Version 9.4, SAS Institute Inc., Cary, NC, Copyright ©2002-2012), and R (R Foundation for Statistical Computing, Vienna, Austria). Regression analysis, two sample t-test, and repeated measures ANOVA was used for data analysis. Samples at different moisture level were considered as repeated. The intensity and elemental concentrations obtained from PXRF scanning were compared with the ICP-OES measured values to determine the effect of moisture content on elemental quantification. Likewise, wet chemical analysis results from WVDA and WVU lab were also compared. Significance criterion (alpha) for all tests was 0.05.

Result and Discussion

Reference standard

All of the elements measured by ICP-OES had average Relative Standard Deviation (RSD) below 10%. The RSD for CRM was below 10% for all elements determined, except for Cu and Zn (Table 2.1). The RSD below 10% is acceptable for plant elemental analysis (Bueno Guerra et al., 2014).

Sample description

The summary statistics of the elemental concentration of P, K, Ca, Mg, Fe, Cu, and Zn determined by ICP-OES are presented in Table 2.2 and summary statistics of West Virginia Department of Agriculture (WVDA)-determined concentration of P, K, Ca, Mg and Cu are presented in Table 2.3. ICP-determined concentration had RSD ranging from 25% for Mg to 120% for Cu. All the elements under consideration were in RSD of nearly 50% except Mg and Cu. WVDA determined elemental concentration had RSD from 26% for Mg to 130% for Cu. Both ICP and DOA determined concentration had comparable RSDs. Moreover, a dataset of K, Mg, and Fe was symmetrical while moderately to highly right-skewed for P, Ca, Cu, and Zn (Tables 2.2, 2.3).

Comparability between ICP-OES and WVDA measured concentrations

There was no difference (Welch two-sample t-test) between ICP and WVDA measured elemental concentration (Table 2.4).

Regression between ICP and WVDA measured concentration were significant for all of the elements compared (P, K, Ca, Mg and Cu) with r^2 values from 0.92 to 0.99 and $p<0.001$. The slopes of the regression lines were between 0.91 and 1.01 (Fig: 2.1).

The CNMP uses manure test results from WVDA laboratory. There was close agreement between ICP and WVDA measured elemental concentration of P, K, Ca, Mg, Fe, and Cu. Thus, ICP values can be used as reference standard for evaluating PXRF to support CNMP.

PXRF analysis of manure samples

PXRF intensity

Manure samples had average intensity ranging from 4800 cps for Mg to 432,900 cps for Ca. The intensities had RSD ranging from 9% for Cu to 62% for Cu. All of the datasets were right-skewed. K and Mg were symmetrical while P, Ca, Mg, Fe, Cu, and Zn were moderate to highly skewed (Table 2.5).

Regression models between ICP-determined concentration and PXRF counts were significant ($p<0.001$) for elements under consideration- P, K Ca, Mg, Fe, Cu and Zn (Fig 2.2). The relationships were stronger ($r^2>0.90$) for all elements except Mg ($r^2=0.59$) and Zn ($r^2=0.56$).

PXRF determined concentrations

Manure samples (measured with PXRF) had average elemental concentrations ranging from 1,900 mg/kg for P to 20,900 mg/kg for Ca (Table 2.6). The RSDs were from 38% for K to 71% for Mg. Compared to the ICP determined concentration RSDs were lower for P and K, higher for Ca, Mg and comparable for Fe (Table 2.2 and 2.5). All of the datasets were moderate to highly right-skewed except K (almost symmetrical).

Regression models between ICP and PXRF measured concentrations were significant ($p<0.001$) for elements - P, K, Ca, Mg, and Fe (Fig 2.3). The relationships were stronger ($r^2>0.66$) for all elements except Mg ($r^2=0.25$). Compared to the regression with intensities, PXRF determined concentration produced lower r^2 values for P (r^2 decreased from 0.93 to 0.66) and Mg (r^2 decreased from 0.59 to 0.25). Moreover, r^2 values for K and Fe were slightly lower while comparable for Ca (Fig 2.2 and 2.3).

The concentration of P, K, Ca, Fe, and Cu can reliably be measured in dry and ground manure samples with PXRF. Likewise, compromising some accuracy, Mg and Zn can also be measured with PXRF. McWhirt et al. (2012) also found that P, K, Ca, Fe, Cu, and Zn could be measured in compost samples using portable XRF. The reduced accuracy in Mg measurement might be due to the absorbance of the characteristics X-ray before reaching the detector. Likewise, reduced accuracy in Zn measurement may be due to the use of same factory setting (current, voltage, and filter) used for light elements. Bruker Elemental has recommended using different factory settings for elements above Fe in periodic table. Reduced strength of the relationship with PXRF concentration suggests that a calibration for plant samples is not appropriate for manure samples or the need for a calibration update.

Effect of moisture in manure samples

PXRF intensities

Average intensity (cps) of P, K, Ca, Mg, and Fe decreased with increasing moisture content of sample but a slight increasing trend was observed for Cu and Zn beyond moisture range of 10-20% (Table 2.8 and Fig 2.4). Relative standard deviations were comparable for the intensities measured with oven dried (<10%) and 10-20% moisture ranges except Ca and Cu (slightly increased with moisture). Beyond 10-20% moisture range RSDs for P, Cu, and Zn were decreasing, Ca and Fe was almost constant, Mg was almost constant except elevated at 30-40% moisture range, and K was almost constant except being elevated at 60-70% moisture range (Table 2.8 and Fig 2.5).

Regression models between ICP determined concentration and PXRF counts were significant ($p<0.001$) for elements under consideration- P, K Ca, Mg, Fe, Cu and Zn (Table 2.9). Moisture content did not affect the strength of relationship for Cu ($r^2=0.91$) however strength slightly decreased at 60-70% moisture range for Ca and Fe. The strength of relationship decreased with increasing moisture content for P, K, and Mg except being increased at 40-50% moisture range. Likewise, the strength decreased for Zn up to 20-30% moisture range and increased thereafter (Table 2.9 and Fig 2.6).

PXRF measured concentrations

The average concentration of P, K, Ca, Mg, and Fe decreased with increasing moisture content of the sample (Table 2.10, Fig 2.7). Relative standard deviations were comparable for P, Ca and Fe over the moisture range (Fig: 2.8) however increasing after 10-20% moisture range for K. Mg RSD increased at the beginning and decreased beyond 20-30% moisture range (Table 2.10 and Fig 2.8). Datasets were right skewed except potassium.

Repeated measures ANOVA results demonstrated that there was evidence of significant relationship and moisture effects on the P, K, Ca and Fe concentration in manure (Table 2.11).

Mean P and K concentration measured at 0-10% and 10-20% was not significant. Likewise, mean P concentration at 20-30% and 40-50% moisture level and K concentration at 40-50% and 60-70% moisture levels were not significant (Table 2.12). However, mean Ca and Fe concentrations at each preceding moisture level was not significant (Table 2.12).

Regression models between ICP and PXRF measured concentration in manure sample with 10-20% moisture were significant ($p<0.001$) for all elements. Beyond 20% models were significant for all elements except Mg (Table 2.12). In the case of P, the strength of the relationship continued decreasing ($r^2=0.94$ to 0.53) except at the moisture range of 40-50% ($r^2=0.80$) with increasing moisture content. Similar trends were observed for K, Ca and Fe, however, the strength of relationship remained almost constant for Mg beyond 20-30% moisture range. (Table 2.13, Fig 2.9)

Moisture significantly affected the elemental determination in manure samples (Fig 2.10 and 2.11). The effect was more pronounced for P, K, Mg and less for Ca, Fe, Cu, and Zn because light elements have lower energy level. The characteristics X-rays produced will be be absorbed by the moisture, thus reducing the PXRF intensity (Ge et al., 2005; Stockmann et al., 2006). However, elemental concentration in manure samples can be measured, without losing accuracy, up to 20% moisture. Beyond which moisture correction is advisable especially for elements like P and K.

Though this method has potential to determine elemental concentration directly in field, moisture is a major challenge. If drying and grinding is not possible, samples can be mixed thoroughly in field to meet the condition of homogeneity and air dried to reduce moisture content. Alternatively, a moisture correction model could be developed.

Conclusion

Since the PXRF results were in close agreement with ICP-OES and WVDA results, it can be used to determine P, K, Ca, Mg, Fe, Cu and Zn concentrations in manure samples. It removes several disadvantages of traditional wet chemical analysis techniques. The result can be obtained quickly at a reduced cost. Though moisture content of the sample affected the result, it can be used for the samples up to 20% moisture level without losing accuracy. Above 20%, moisture correction is advisable especially for the elements like P and K.

This method could be a very useful technique for the purpose of CNMP development. The results obtained in benchtop mode were comparable to ICP-OES values. If this method is used directly in the field for the unprocessed sample the results may be better than ‘Book Values’. However, at this stage drying and grinding of the sample is recommended. Additional work is recommended to extend this to infield analysis.

Literature Cited

Analytical Methods Committee (AMC), Royal Society of Chemistry. 2008. Evaluation of analytical instrumentation. Part xxiii. Instrumentation for portable x-ray fluorescence spectrometry. Accred. Qual. Assur. 13:453–464

Brackenrich, J., L.M. McDonald, T. Basden and K. Shaffer. 2016. Optimizing feeding strategies to minimize phosphorus losses from roofed winter feeding areas. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America Annual Meeting, Phoenix, AZ. Nov. 6-9, 2016.

Brouwer, P. 2013. Theory of XRF. PANanalytial B.V., Almelo, The Netherlands.

Bueno Guerra, M.B., E. de Almeida, G.G.A. Carvalho, P.F. Souza, L.C. Nunes, D.S. Júnior, and F.J. Krug. 2014. Comparison of analytical performance of benchtop and handheld energy dispersive X-ray fluorescence systems for the direct analysis of plant materials. J. Anal. At. Spectrom. 29(9): 1667.

Evanylo, G.K. and D. Beegle.2006. Introduction to nutrient management. In K. C. Haering and G. K. Evanylo. Mid-Atlantic nutrient management handbook. Mid-Atlantic Regional Water Program, USA.

Ge, L., W. Lai, and Y. Lin. 2005. Influence of and correction for moisture in rocks, soils and sediments on in situ XRF analysis. X-Ray Spectrom 34(28): 28–34.

Isleib, J. 2016. Manure provides plant nutrients, but not always at book value. Michigan State University Extension.

Kalnicky, D.J., and R. Singhvi. 2001. Field portable XRF analysis of environmental samples. J. Hazard. Mater. 83(1–2): 93–122.

Karla, Y. P. 1998. Handbook of reference methods for plant analysis. CRC Press Taylor & Francis Group.

Maarschalkerweerd, M., and S. Husted. 2015. Recent developments in fast spectroscopy for plant mineral analysis. Front. Plant Sci. 6(March): 1–14.

Maguire, R.O. 2014. Importance of farm phosphorus mass balance and management options. Virginia Cooperative Extension. Publication No. CSES-98P.

Martin, J. 2009. Manure sampling for nutrient management planning. Agronomy Facts 69. Penn State Extension.

McWhirt, A., D.C. Weindorf, and Y. Zhu. 2012. Rapid analysis of elemental concentrations in compost via portable X-ray fluorescence spectrometry. Compost Sci. Util. 20(3): 185–193.

NRCS-WV. 2010. Conservation practice standard, Nutrient Management (Ac.) 590. Natural Resources Conservation Service. West Virginia.

Peters, J. and S. Combss. 2003. Sampling livestock waste for analysis. In: J. Peters, S.M. Combss, B. Hoskins, J. Jarman, J.L. Kovar, M. E. Watson, A.M. Wolf and N. Wolf. Recommended methods of Manure Analysis, Cooperative Extension Publishing, University of Wisconsin-Extension.

Potts, P. J., and M. West. 2008. Portable X-ray fluorescence spectrometry: capabilities for in situ analysis. The Royal Society of Chemistry, UK.

Reidinger, S., M.H. Ramsey, and S.E. Hartley. 2012. Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer. New Phytol. 195(3): 699–706.

Stockmann, U., H.J. Jang, B. Minasny, and A. B. McBratney. 2006. The effect of soil moisture and texture on Fe concentration using portable X-ray fluorescence spectrometers. In: A.E. Hartemink and B. Minasny (eds.), Digital Soil Morphometric, Progress in soil science, Springer International Publishing Switzerland.

USDA-NRCS. 2003. National planning procedures handbook. United States Department of Agriculture, Natural Resource Conservation Service.

USDA-NRCS. 2010. Statement of work: comprehensive nutrient management plan (code 102), Pensylvania

Weindorf, D.C., N. Bakr, and Y. Zhu. 2014. Advances in portable X-ray fluorescence (PXRF) for environmental, pedological, and agronomic applications. Adv. Agron. 128:1–45.

Weindorf, D.C., R. Sarkar, M. Dia, H. Wang, Q. Chang, B. Haggard, A. McWhirt, and A. Wooten. 2008. Correlation of X-ray fluorescence (XRF) spectrometry and inductively coupled plasma atomic emission spectroscopy (ICP-AES) for elemental determination in composted products. Compost Sci. Util. 16 (2): 79–82.

Willis, J. P., and A. R. Duncan. 2008. Understanding XRF spectrometry, Vol I. PANanalytial B.V., Almelo, The Netherlands.

Tables

Table 2.1: Average Relative Standard Deviation (RSD) for three replications of manure sample digestion and RSD for SRM digested with manure samples (n=40).

Elements	Average RSD for manure samples (%)	RSD for SRM (%)	Difference between standard and ICP concentration for SRM (%)
P	8.7	5.53	4.09
K	8.35	3.93	4.80
Ca	7.78	4.25	2.42
Mg	8.13	5.10	14.13
Fe	7.77	8.25	13.64
Cu	9.54	23.21	42.34
Zn	8.45	14.82	97.89

Table 2.2: Summary Statistics for ICP determined elemental concentrations (mg/kg) in manure samples (n=40).

Statistic	Elemental Concentrations (mg/kg)						
	P	K	Ca	Mg	Fe	Cu	Zn
Mean	6800	20200	26700	5500	4300	500	200
Minimum	2200	2700	10500	2100	600	0	100
Maximum	21900	38000	84100	8100	10000	2600	600
SD	3600	9500	14200	1400	2800	600	100
RSD (%)	52.94	47.03	53.18	25.45	65.12	120	50
Skewness	2.11	0.22	1.92	0.17	0.60	1.69	1.03
Kurtosis	6.72	-0.85	5.66	-0.16	-0.63	2.37	1.26

Table 2.3: Summary Statistics for WVDA determined elemental concentrations (mg/kg) in manure samples (n=40).

Statistic	Elemental Concentrations (mg/kg)				
	P	K	Ca	Mg	Cu
Mean	6600	19700	24600	5300	600
Minimum	2100	2500	10300	2000	10
Maximum	21600	35300	84800	8700	2400
Standard Dev	3500	9000	14200	1400	650
RSD (%)	53.03	45.69	57.72	26.42	130
Skewness	2.19	0.11	2.26	0.31	1.60
Kurtosis	7.40	-0.94	7.31	0.45	1.67

Table 2.4: Mean comparison between ICP and WVDA measured concentrations (mg/kg) in manure samples (n=40).

Element	t-value	Degree of freedom	p-value
P	0.275	78	0.784
K	0.238	78	0.813
Ca	0.676	78	0.501
Mg	0.568	78	0.572
Cu	0.067	78	0.946

Table 2.5: Summary statistics for PXRF intensities (cps) of oven dried manure samples scanned at 180 seconds (n=40).

Statistic	PXRF intensities (cps)						
	P	K	Ca	Mg	Fe	Cu	Zn
Mean	33000	256900	432900	4800	408600	85600	59800
Minimum	16300	61600	183900	3800	110700	43300	44200
Maximum	78700	453500	1236200	5800	906600	288100	84000
Standard Dev	12600	93800	195800	440	213300	53400	11300
RSD (%)	38.18	36.51	45.23	9.17	52.20	62.38	18.90
Skewness	1.63	0.17	1.66	0.28	0.55	1.99	0.56
Kurtosis	3.35	-0.52	5.88	-0.27	-0.37	4.75	-0.43

Table 2.6: Summary statistics of PXRF determined concentrations (mg/kg) in oven-dried manure samples (n=40).

Statistic	PXRF determined elemental concentration (mg/kg)				
	P	K	Ca	Mg	Fe
Mean	5600	14500	20900	3400	1900
Minimum	2700	2000	5400	1000	100
Maximum	10600	26200	74400	12100	4800
Standard Dev	2100	5000	12800	2400	1300
RSD (%)	37.50	34.48	61.24	70.59	68.42
Skewness	0.69	-0.12	1.91	2.32	0.63
Kurtosis	-0.47	0.40	6.64	6.25	-0.27

Table 2.7: Welch two-sample t-test results between ICP and PXRF measured concentration (mg/kg) (n=40).

Element	Degrees of freedom (df)	t value	p-value
P	78	-1.83	0.072
K	78	3.34	0.002
Ca	78	1.94	0.055
Mg	78	4.59	<0.001
Fe	78	4.95	<0.001

Table 2.8: Summary statistics for PXRF intensities (cps) of manure samples adjusted to four moisture ranges (10-20%, 20-30%, 40-50%, and 60-70%) (n=40).

Moisture range	Statistic	PXRF intensities (cps) P	K	Ca	Mg	Fe	Cu	Zn
10-20%	Mean	28700	225300	388700	4400	389500	83000	57800
	Minimum	16500	56300	152800	3700	114500	38300	39700
	Maximum	67800	382400	1229800	5400	841600	283500	80600
	Standard Dev	11000	78500	196800	400	195900	54800	11400
	RSD	38.29	34.83	50.63	9.50	50.28	66.09	19.76
	Skewness	1.74	-0.07	2.01	0.70	0.69	2.03	0.30
	Kurtosis	3.36	-0.61	7.35	-0.24	-0.07	4.43	-0.62
20-30%	Mean	22300	171800	315000	3900	347800	83600	62000
	Minimum	7900	50600	131400	1000	88800	45400	46600
	Maximum	38800	337100	967100	5400	762600	254000	83900
	Standard Dev	8000	62400	162000	700	180000	47100	9600
	RSD	35.99	36.31	51.43	18.55	51.77	56.29	15.42
	Skewness	0.40	0.33	1.93	-1.68	0.67	2.04	0.26
	Kurtosis	-0.41	0.22	5.86	5.77	-0.09	4.34	-0.68
40-50%	Mean	20200	138300	275400	3800	317700	85200	65200
	Minimum	7000	41700	123400	2600	97900	45100	46600
	Maximum	42100	221200	901400	5000	705000	241200	85400
	Standard Dev	6700	47500	147200	400	164200	43100	9900
	RSD	32.99	34.35	53.43	10.26	51.70	50.63	15.13
	Skewness	1.12	-0.19	2.24	0.02	0.73	1.98	0.06
	Kurtosis	1.78	-0.75	7.63	3.15	-0.04	4.23	-0.67
60-70%	Mean	17000	126500	218500	3700	271600	85400	68600
	Minimum	10200	33200	91200	3200	94300	49000	53200
	Maximum	31700	301300	661000	5200	693500	209600	86500
	Standard Dev	4800	55800	113300	300	137200	35700	9200
	RSD	28.03	44.07	51.86	9.35	50.51	41.88	13.34
	Skewness	1.23	0.80	2.50	2.39	1.10	1.77	0.16
	Kurtosis	1.36	1.13	7.74	8.71	1.34	3.23	-0.70

Table 2.9: Regression between PXRF intensities (cps) of manure samples adjusted to four moisture ranges and ICP determined concentrations (mg/kg) (n=40).

Experiment	Element	r^2	RMSE	Intercept	Slope	p-value
ICP Vs 10-20% moisture range	P	0.84	1472	-1801	0.30	<0.001
	K	0.85	3689	-5036	0.11	<0.001
	Ca	0.93	3725	-391	0.07	<0.001
	Mg	0.45	1049	-4484	2.24	<0.001
	Fe	0.94	719	-1073	0.01	<0.001
	Cu	0.91	192	-417	0.01	<0.001
	Zn	0.52	76	-153	0.01	<0.001
ICP Vs 20-30% moisture range	P	0.59	2340	-876	0.34	<0.001
	K	0.80	4317	-3227	0.14	<0.001
	Ca	0.92	4002	183	8.42	<0.001
	Mg	0.20	1273	2111	0.87	<0.001
	Fe	0.94	687	-935	0.02	<0.001
	Cu	0.91	197	-575	0.01	<0.001
	Zn	0.27	94	-124	0.01	<0.001
ICP Vs 40-50% moisture range	P	0.80	1635	-2957	0.48	<0.001
	K	0.83	3969	-5037	0.18	<0.001
	Ca	0.94	3291	832	0.09	<0.001
	Mg	0.40	1101	-3167	2.27	<0.001
	Fe	0.94	651	-958	0.02	<0.001
	Cu	0.91	187	-701	0.01	<0.001
	Zn	0.36	88	-189	6.59	<0.001
ICP Vs 60-70% moisture range	P	0.53	2499	-2567	0.55	<0.001
	K	0.70	5263	2117	0.14	<0.001
	Ca	0.83	5909	1736	0.11	<0.001
	Mg	0.30	1190	2945	2.23	<0.001
	Fe	0.89	924	-927	0.02	<0.001
	Cu	0.91	184	-955	0.02	<0.001
	Zn	0.41	84	-282	0.01	<0.001

Table 2.10: Summary statistics for PXRF determined concentrations (mg/kg) of manure samples adjusted to four moisture ranges (10-20%, 20-30%, 40-50%, and 60-70%) (n=40).

Moisture range	Statistic	PXRF determined concentrations (mg/kg)				
		P	K	Ca	Mg	Fe
10-20%	Mean	5000	13000	19500	2900	1800
	Minimum	2400	1600	5200	1000	100
	Maximum	9200	22000	74800	12000	4900
	Standard Dev	1900	4500	12800	2400	1300
	RSD	37.32	34.31	65.80	80.71	70.58
	Skewness	0.71	-0.47	2.18	2.63	0.80
	Kurtosis	-0.39	0.29	7.85	7.48	0.05
20-30%	Mean	4100	10200	17300	2800	1700
	Minimum	2200	800	5300	900	100
	Maximum	8300	19600	56500	12000	4600
	Standard Dev	1500	4400	10300	2200	1200
	RSD	35.70	42.76	59.79	80.18	68.67
	Skewness	0.97	-0.02	1.73	2.74	0.64
	Kurtosis	0.56	-0.22	4.37	8.11	-0.20
40-50%	Mean	3700	7200	15200	2200	1500
	Minimum	2100	0	5400	1100	200
	Maximum	6900	12400	51800	8700	4300
	Standard Dev	1300	3300	8700	1400	1000
	RSD	35.04	45.11	57.62	62.27	67.49
	Skewness	1.00	-0.29	2.23	3.52	0.98
	Kurtosis	0.15	-0.71	7.42	14.42	0.80
60-70%	Mean	3000	5900	11900	1900	1200
	Minimum	1400	-800	4500	1100	200
	Maximum	6900	15500	36500	8400	4100
	Standard Dev	1000	3700	6600	1100	800
	RSD	34.01	63.88	55.49	55.79	70.54
	Skewness	1.68	0.38	2.41	5.72	1.50
	Kurtosis	4.73	-0.18	7.24	34.86	3.09

Table 2.11: Repeated measures ANOVA results for the effect of moisture on elemental concentration (mg/kg) measured by PXRF (n=40).

Element	Effect	Degrees of freedom (df)	F value	p-value
P	Moisture	4	59.71	<0.001
K	Moisture	4	61.18	<0.001
Ca	Moisture	4	10.80	<0.001
Fe	Moisture	4	31.26	<0.001

Table 2.12: Welch two-sample t-test results for the effect of moisture on elemental concentration (mg/kg) measured by PXRF (n=40).

Element	PXRF measured concentration	Two sample t-test		
		t value	df	p-value
P	<10% and 10-20%	1.44	78	0.15
	10-20% and 20-30%	2.35	78	0.022
	20-30% and 40-50%	1.36	78	0.18
	40-50% and 60-70%	2.79	78	0.006
K	<10% and 10-20%	1.43	78	0.156
	10-20% and 20-30%	2.84	78	0.005
	20-30% and 40-50%	3.46	78	<0.001
	40-50% and 60-70%	1.72	78	0.08
Ca	<10% and 10-20%	0.49	78	0.62
	10-20% and 20-30%	0.82	78	0.41
	20-30% and 40-50%	0.99	78	0.32
	40-50% and 60-70%	0.99	78	0.32
Fe	<10% and 10-20%	0.37	78	0.71
	10-20% and 20-30%	0.19	78	0.85
	20-30% and 40-50%	0.98	78	0.32
	40-50% and 60-70%	1.61	78	0.11

Table 2.13: Regression between PXRF concentration of manure samples adjusted to four moisture ranges and ICP determined concentrations (mg/kg).

Experiment	Element	r^2	RMSE	Intercept	Slope	p-value
ICP Vs 10-20% moisture range	P	0.49	2607	48	1.36	<0.001
	K	0.78	4459	-4385	1.89	<0.001
	Ca	0.90	4613	6263	1.05	<0.001
	Mg	0.23	1246	4632	0.28	0.002
	Fe	0.85	1070	634	2.05	<0.001
ICP Vs 20-30% moisture range	P	0.42	2775	249	1.60	<0.001
	K	0.65	5693	2247	1.76	<0.001
	Ca	0.84	5754	4935	1.26	<0.001
	Mg	0.08	1364	4978	0.18	0.082
	Fe	0.77	1356	728	2.05	<0.001
ICP Vs 40-50% moisture range	P	0.61	2285	-1212	2.18	<0.001
	K	0.82	4044	1058	2.65	<0.001
	Ca	0.92	4062	3064	1.56	<0.001
	Mg	0.08	1362	4826	0.29	0.076
	Fe	0.90	864	356	2.63	<0.001
ICP Vs 60-70% moisture range	P	0.36	2924	462	2.15	<0.001
	K	0.71	5147	7613	2.15	<0.001
	Ca	0.81	6194	3604	1.94	<0.001
	Mg	0.10	1346	4662	0.41	0.078
	Fe	0.82	1212	719	3.07	<0.001

Figures

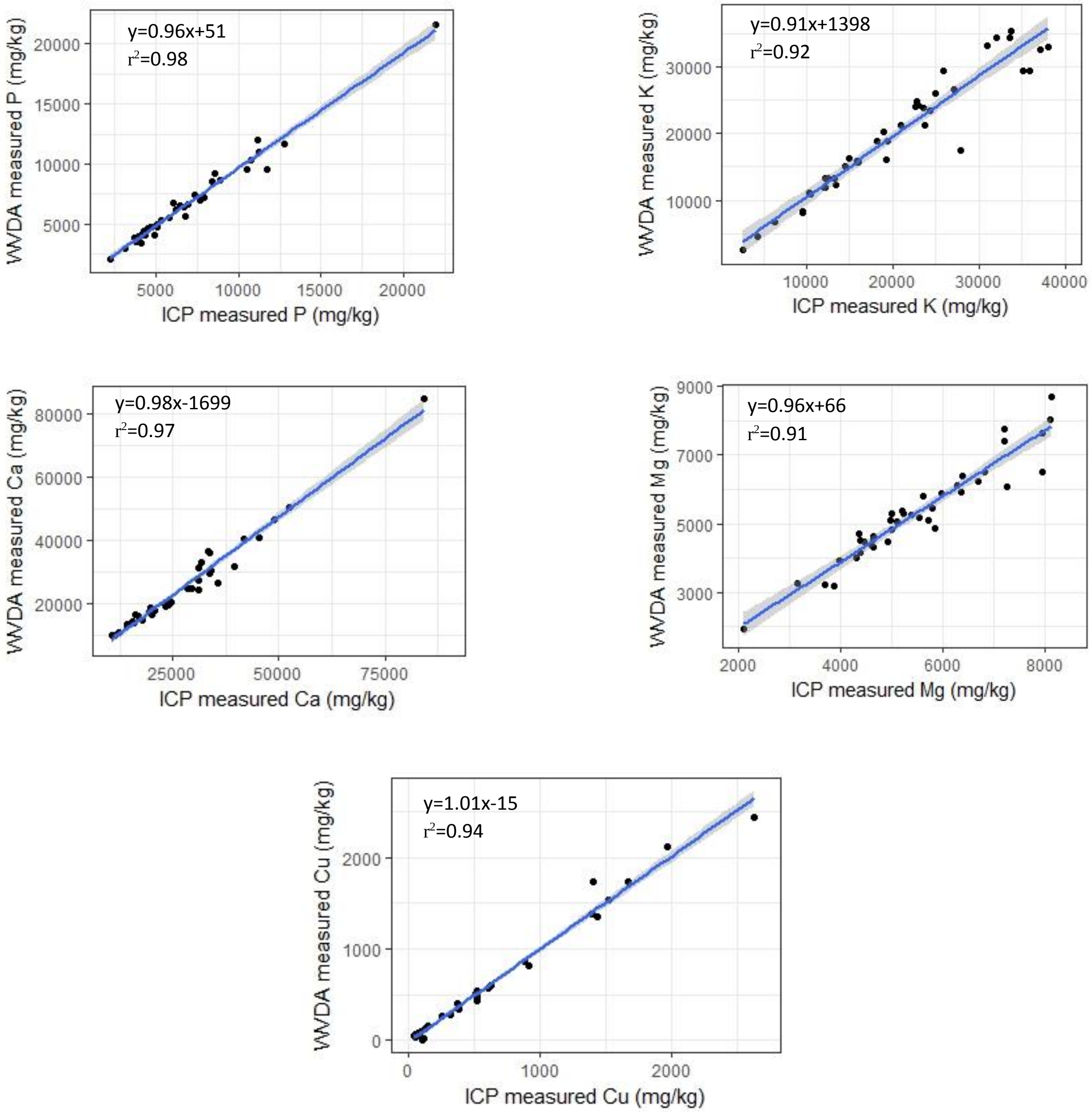


Fig 2.1: Regression plots between ICP and WVDA measured P, K, Ca, Mg, and Cu concentration. The shaded portion shows the standard error.

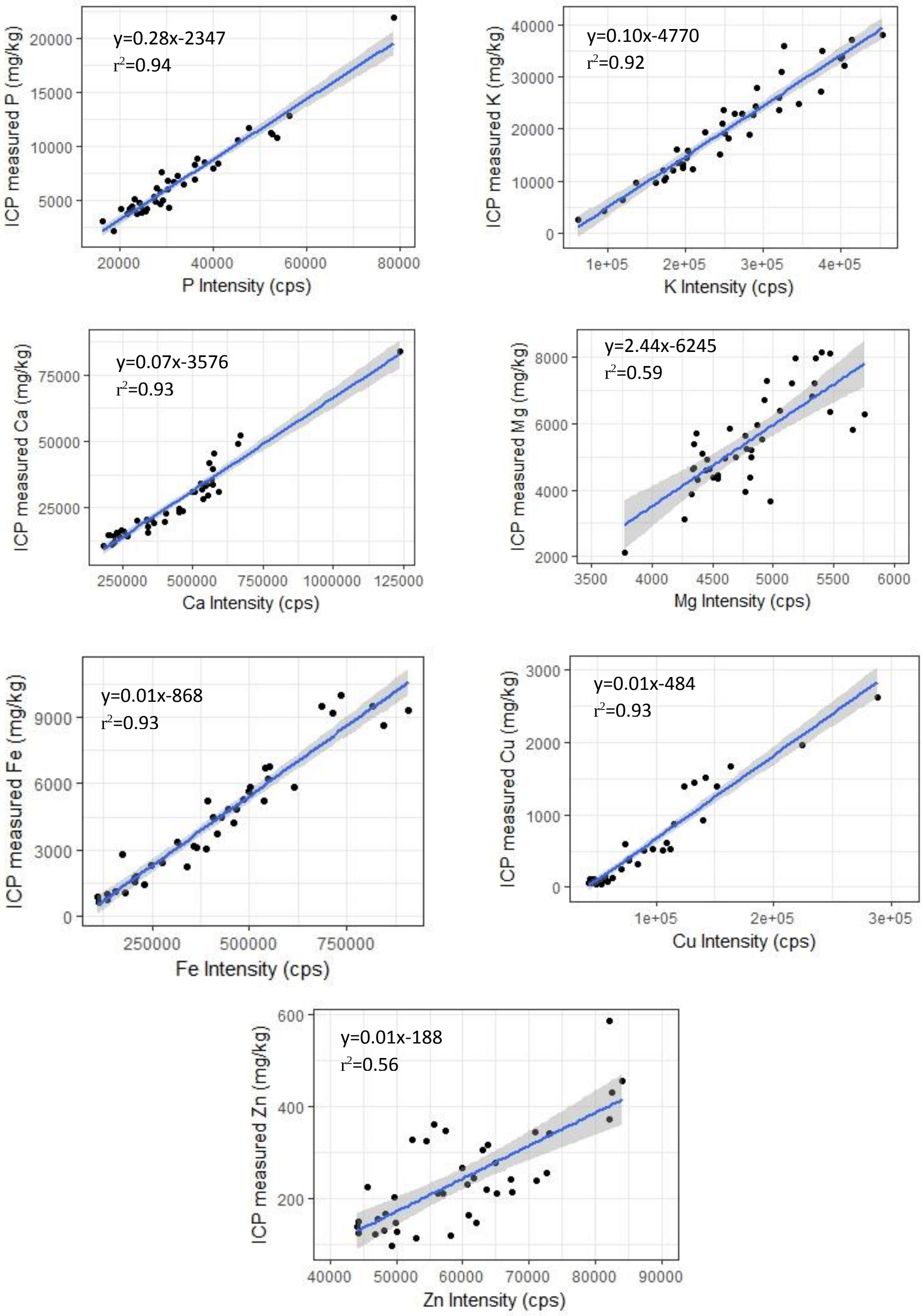


Fig 2.2: Regression plots between ICP measured concentration and PXRF intensities for P, K, Ca, Mg, Fe, Cu and Zn in oven dried manure samples. The shaded portion shows the standard error.

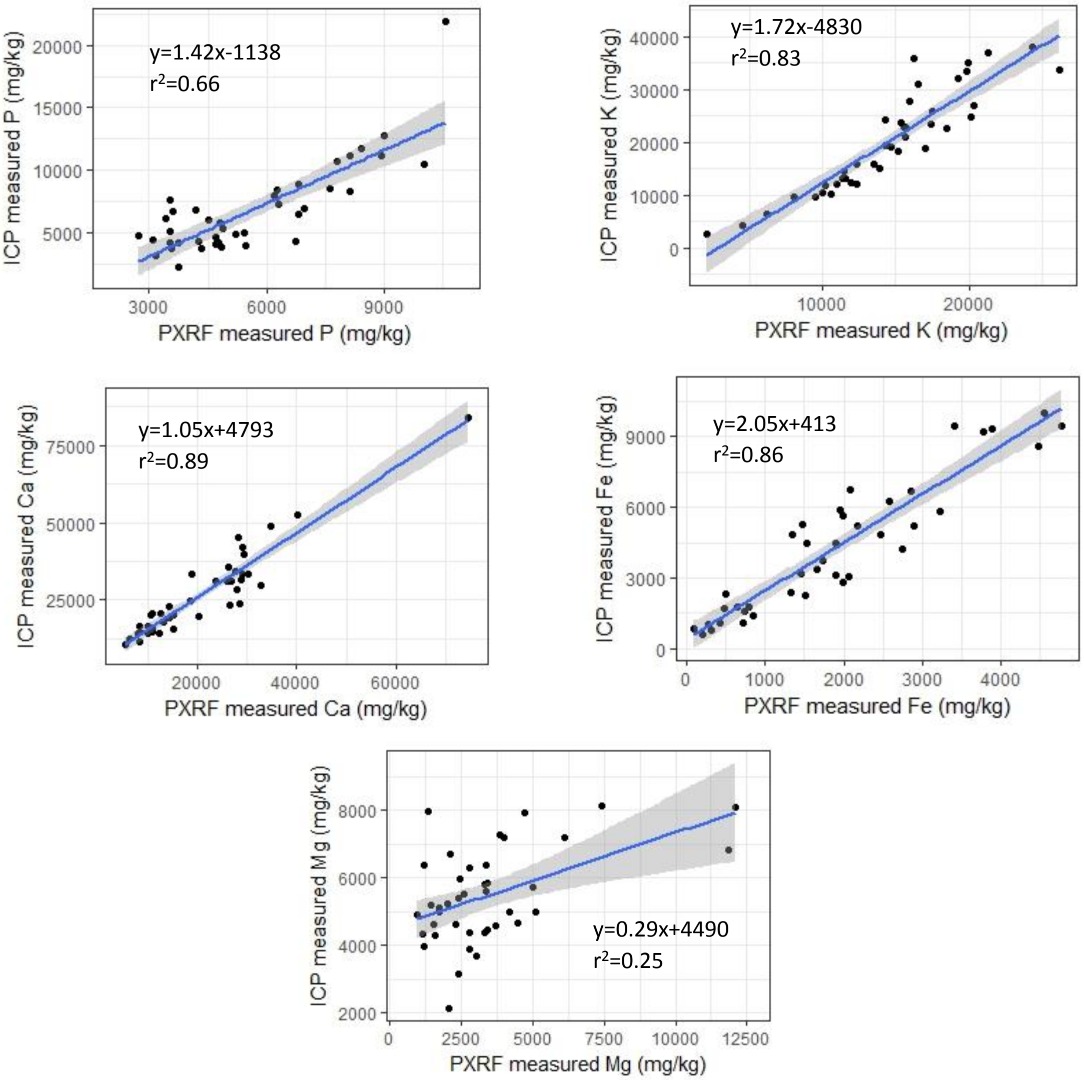


Fig 2.3: Regression plots between ICP and PXRF measured concentration for P, K, Ca, Mg and Fe in oven dried manure samples. The shaded portion shows the standard error.

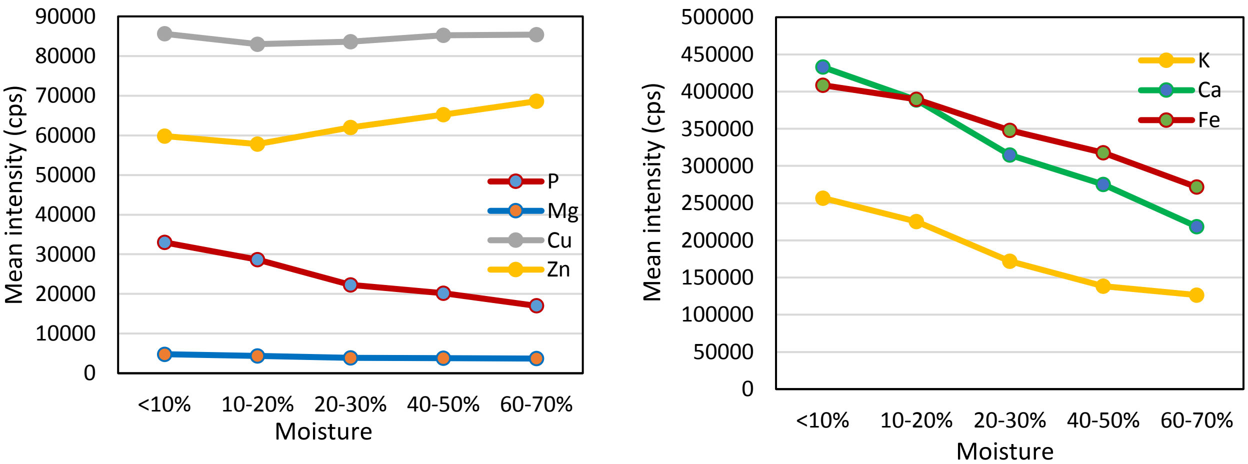


Fig 2.4: Mean comparison of P, K, Ca, Mg, Fe, Cu and Zn intensities measured at five moisture ranges

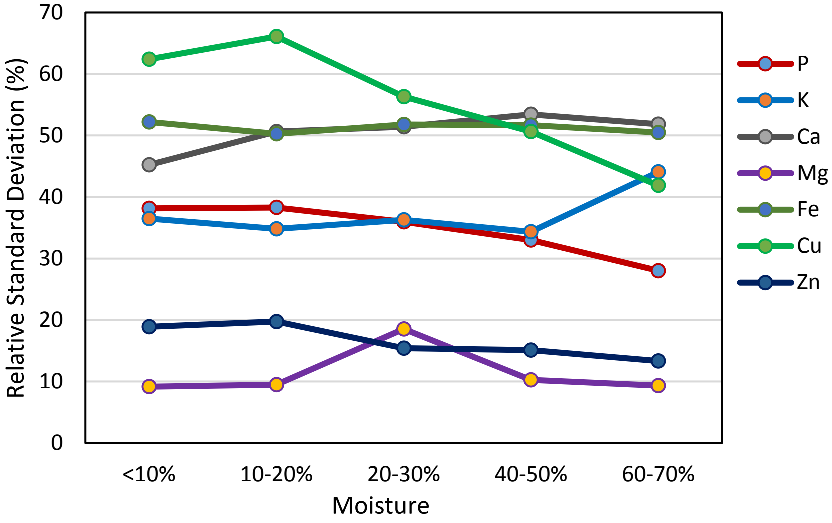


Fig 2.5: Relative Standard Deviation of PXRF intensities measured at five moisture ranges

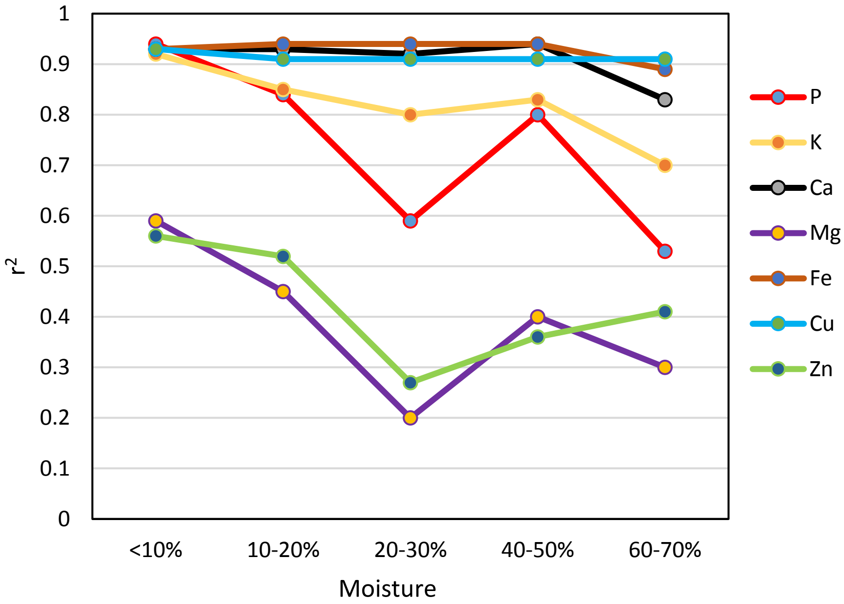


Fig 2.6: r^2 values (regression between ICP measured concentration PXRF intensities) produced over different

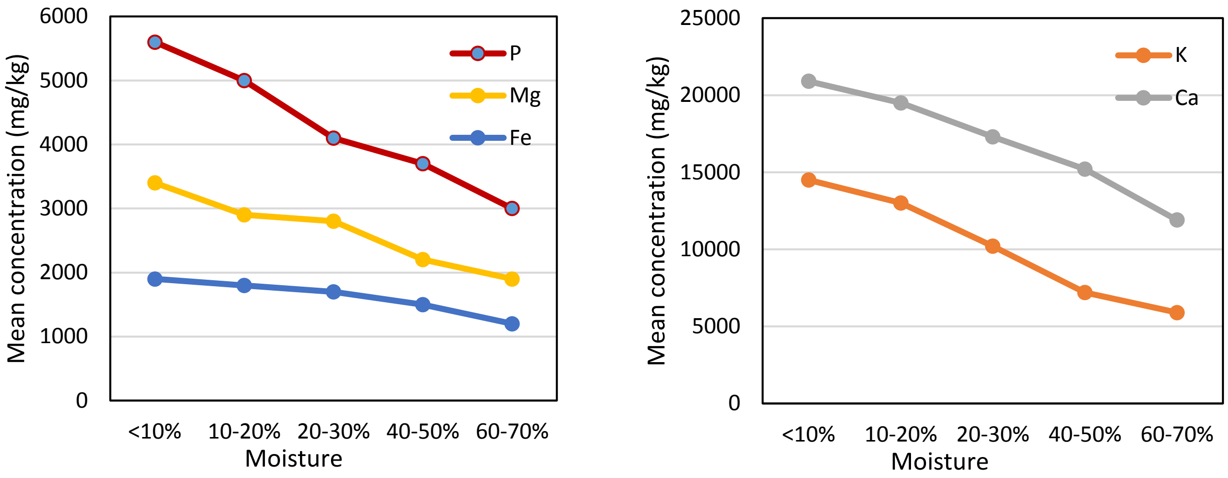


Fig 2.7: Mean comparison of P, K, Ca, Mg and Fe concentrations measured at five moisture ranges

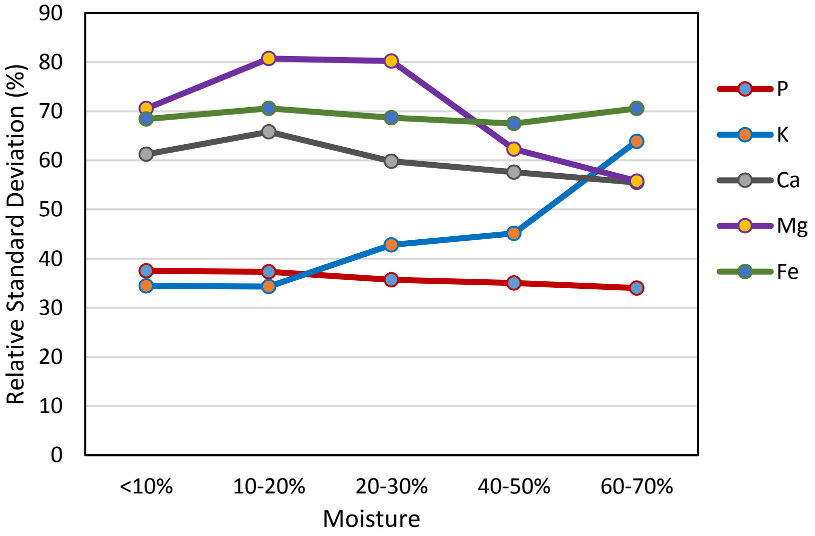


Fig 2.8: Relative Standard Deviation of PXRF concentration measured at five moisture ranges

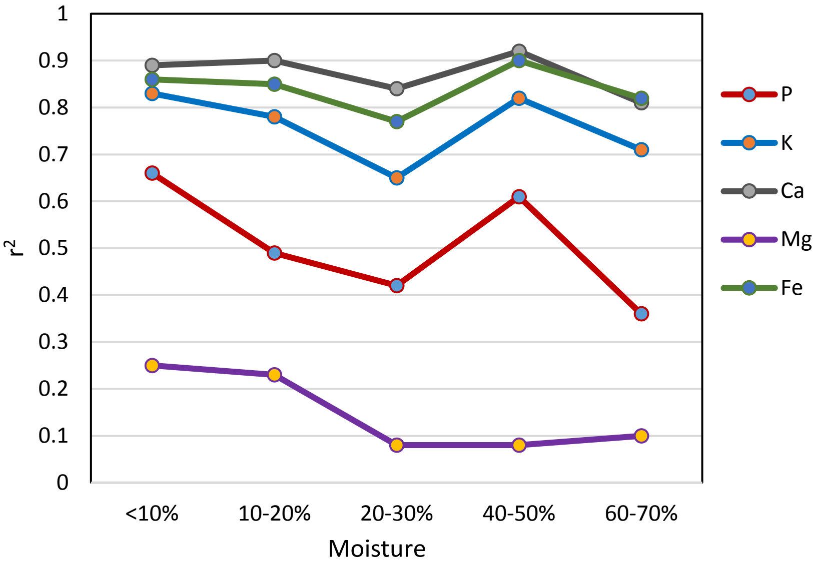


Fig 2.9: r^2 values (regression between ICP and PXRF measured concentration) produced over different moisture range

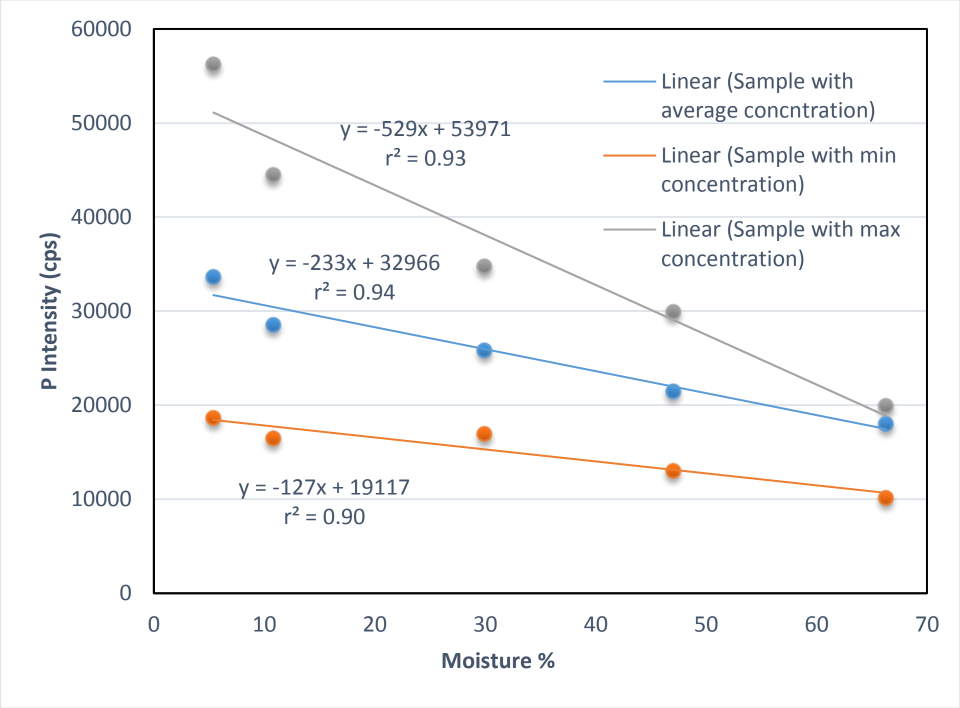


Fig 2.10: Regression between moisture content and P Intensity by sample for 3 samples varying in P concentration.

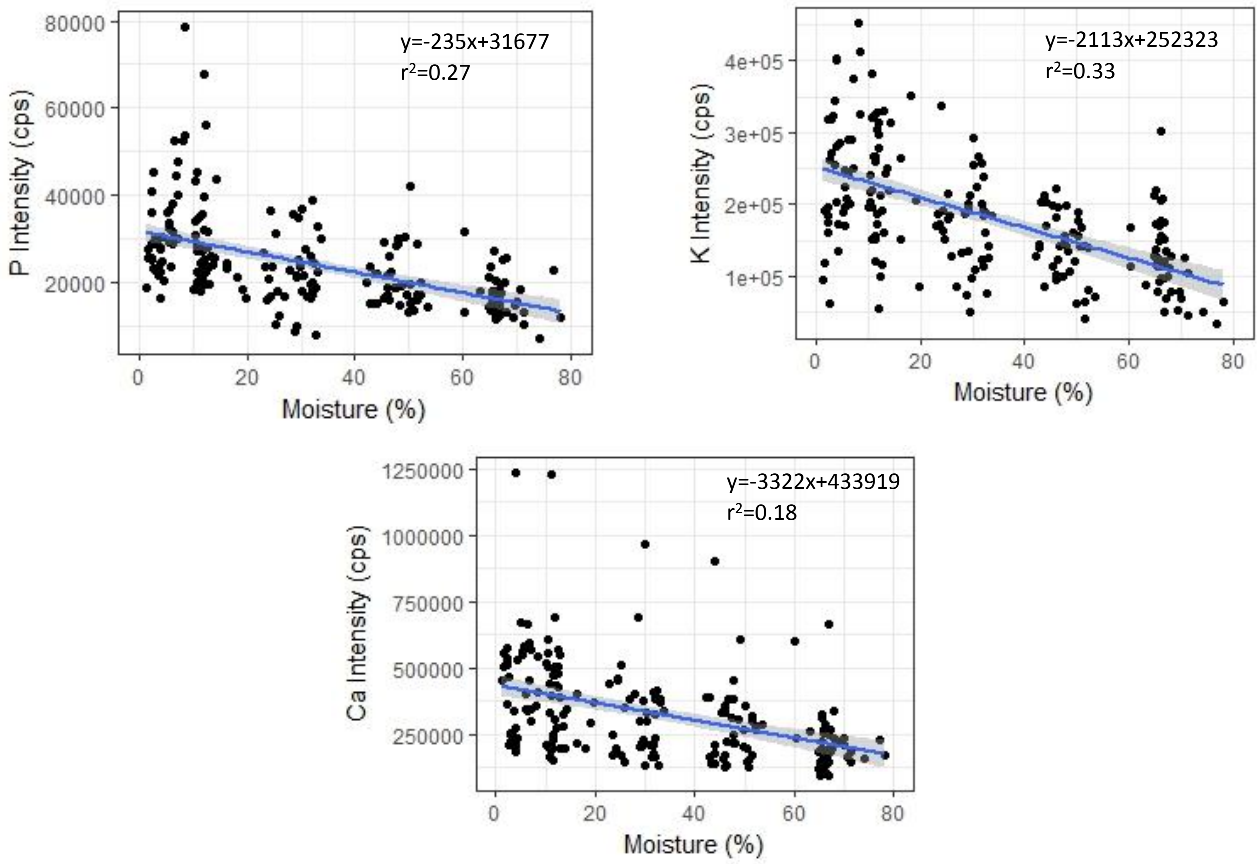


Fig 2.11: Regression between moisture content and P, K, and Ca Intensity. The shaded portion shows standard error.

Chapter 3: Particle Size Effect on Elemental Concentration Determination in Forage using Portable X-ray Fluorescence Spectroscopy

Introduction

Forage is the major feed and source of minerals for ruminant farm animals. The performance and health of the animal are directly influenced by the mineral content of the forages (Minson, 1990; Van Soest, 1994). The deficiencies or excesses of specific mineral elements in forage decrease animal performance and economic return (Reid et al., 1970). The quantity of forage essential to each animal type is determined by the expected level of animal performance, quality of the forage, and its mineral content. Thus, knowing elemental composition (Mg, Ca, P, K, Fe, Cu, and Zn) in forage sources is crucial for farmers and/or nutritionists in formulating feeding plans for animals. In addition, large herds require enormous amounts of feed and forage for daily consumption indicating the need to know the nutritional status of the feed and forages frequently for appropriate feeding planning. Since the nutritional status of the forage changes over time, regular sampling and analysis are crucial for ration balancing. However, this increases the monetary burden to farmers (Berzaghi et al., 2005). However, getting mineral composition data is time-consuming and costly. Conventionally, minerals are quantified with expensive and time-consuming wet-chemical methods. This involves acid digestion of samples followed by spectroscopic techniques (Karla, 1998). Although they provide accurate quantification of minerals, they are not free from serious shortcomings. They require destructive sampling, are costly, time-consuming, and generate hazardous waste. X-ray fluorescence (XRF) could overcome many of these disadvantages of wet chemical analysis and allow quick determination of P concentrations.

X-ray photons are emitted from an X-ray tube by the interaction of electrons with a metal anode. When the energy of the incident X-ray is greater than the binding energy of electrons in the shell, inner electrons are ejected leaving a vacant space. In order to fill this vacant space the electron from a higher shell moves to the inner shell emitting secondary X-ray radiation of energy/wavelength characteristic to each element. The emitted radiation is then detected. X-ray energy is inversely proportional to wavelength and is expressed as keV (kilo-electron volt)

(Kalnicky and Singhvi, 2001). Portable XRF (PXRF) units are Energy Dispersive (ED) systems with low power X-ray tubes (10-40W) in comparison to benchtop units (50-300W). They are light and convenient to use either in benchtop or field-analysis modes.

When a sample is scanned, the resulting spectrum identifies the element (peak position or energy); area under the peak (intensity) is proportional to concentration (Willis and Duncan, 2008; Weindorf et. al., 2014).). Although PXRF can measure most elements, air attenuation of low energy X-rays restricts the measurement of light elements in the periodic table, especially below silicon (Potts and West, 2008) except when equipped with a unique chamber capable of working under vacuum, or a helium atmosphere (Bueno Guerra et al., 2014; Brouwer, 2013). The critical penetration and escape depths are the depth of the sample beyond which almost all of emitted X-rays are absorbed, which is very low (in µm range) for light elements (Potts and West, 2008). PXRF may be qualitative, semi-quantitative, or quantitative as an analytical technique. The quantitative data are obtained by calibrating the XRF with reference wet chemical methods (Maarschalkerweerd and Husted, 2015) or standard addition methods (Reidinger et al., 2012).

Some portable units are comparable to benchtop XRF in elemental quantification and limits of detection (Bueno Guerra et al., 2014) but superior in terms of portability, cost-effectiveness, simplicity of operation, potential for in-situ measurement, and analysis of large samples (Bueno Guerra et al., 2014; AMC, 2008). In addition, it can be a superior alternative to wet chemistry in terms of cost- and time-effectiveness, and non-destructive analysis of samples (Reidinger et al., 2012).

The texture of sample surface is extremely important in XRF measurement. The surface of the sample should have mirror-finish, which can only be realized with fine powders and metals (Willis and Duncan, 2008). PXRF intensity increases with decrease in particle size of sample and is more sensitive for the light elements (Maruyama et al., 2008). Particle size effect is more pronounced in light elements than heavy elements because of lower penetration depth and measurement surface located on or in the proximity of the surface. In addition, there is shadowing effect if the sample is of heterogeneous particle size. Incoming X-rays will not irradiate all particles and thus no fluorescence will be received from shadowed particles (Yamada, 2014). The best approach to deal with a sample particle size is to grind to size less than the measurement depth of the elements to be determined (Willis and Duncan, 2008). However, grinding samples to fine power is not always beneficial; there is possibility of contamination by blades in grinding mill.

Raw peak count and spectral resolution increase with increasing scan time (McLaren et al., 2012, Kalnicky and Singhvi, 2001). Along with element of interest and sample matrix, detection limit depends on scan time. Scanning samples for longer time improves detection limit, however, the number of samples analyzed will be reduced (Kalnicky and Singhvi, 2001) and radiation damage is possible.

A few studies (Towett et al., 2016, Kalcsits ,2016, Bueno Guerra et al., 2014, McLaren et al., 2012, Reidinger et al., 2012, Paltridge et al. 2012a, and 2012b), have indicated the possible use of PXRF for the analysis of plant tissue. However, there is a lack of information for analysis of heterogeneous forage samples, including optimal sample preparation (particle size) and instrumental parameters (scan time).

We hypothesize that PXRF will be useful in the determination of forage elemental composition because our unit has vacuum capability, and PXRF has been used successfully to detect and quantify light elemental composition in a different plant, soil, geological and archeological samples.

The overall goal of this study was to evaluate the suitability of PXRF for P determination in forage samples. Because other elements are obtained simultaneously, results for K, Ca, Mg, Fe, Cu, and Zn are also reported. To initiate this process we assumed that particle size of the sample would be the limiting factor for forage analyses. Thus, the specific objective was to quantify the effect of forage particle size and scan time on elemental concentrations.

Material and Methods

Sample collection and preparation

Hay samples (n=42) were collected from hay bales of different West Virginia farms using a plunger corer in summer 2016. Samples were cored from hay bales representing legumes, grass-legume mixture, mixed grasses, first cut, and regrowth cut. 30 samples were subsampled in the field and sent to Cumberland Valley Analytical Services (CVAS), Waynesboro, PA for wet chemical analysis.

Collected samples were placed in paper bags and dried in an oven at 60^{0}C for 72 hours. Milling of the dried samples was done in cyclone mill (FOSS Tecator 1093, FOSS North America, Eden Prairie, MN). The whole sample was allowed to pass through a 2 mm screen and then subsampled into three parts using the alternate scooping method. Two subsamples were re-ground separately and allowed to pass through the screens of 1 mm and 0.5 mm respectively. Thus, each hay samples had three subsamples with particles passing screens with 2 mm, 1mm and 0.5 mm hole size.

In order to further investigate the effect of particle size of the sample, two particle sizes were selected. 1-2 mm (samples that pass through 2 mm sieve but retained on a 1mm sieve) were obtained from existing 2 mm samples and 0.25 to 0.5 mm (samples that pass through 0.5 mm sieve but retained on a 0.25 mm sieve) were obtained from existing 0.5 mm samples.

Wet Chemical Analysis

Each hay sample were digested thrice in the microwave (MARS Xpress, CEM Inc., Matthews, NC) and the elemental concentration was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima DV 2100, Perkin Elmer, Norwalk, CT). A Certified Reference Material (CRM), 1573a-tomato leaf, was digested with samples to check the accuracy of digestion. Exactly 10 mL 70% nitric acid was added to 0.5 g sample in digestion vessels and placed in a fume hood for 1 hour to eliminate gases produced. Then tubes were placed in a microwave, heat for 15 minutes at 200 ^{0}C followed by holding at 200 ^{0}C for 15 minutes and allowed to cool overnight. The digested liquid was transferred into test tubes, diluted and final volume was made 50 mL using volumetric flasks. The diluted liquid was filtered before analysis by ICP-OES. The WVU ICP-OES determined concentrations are abbreviated as ICP values.

PXRF Scanning

Samples were scanned using portable XRF (Tracer III-SD; Bruker Elemental, Kennewick, WA) in benchtop mode equipped with a rhodium tube from which X-rays are emitted, and a Peltier-cooled, silicon PIN diode detector, with a voltage of 15 KeV and anode current of 26 uA without a filter. It was operated on PC trigger mode and vacuum (<10 torrs) was used for all scans. Samples were placed in double open-ended sample cups (series: 1500, Chemplex Industries Inc., USA) over a thin X-ray film (4 um prolene). The cups containing ground samples were placed in the nose of the PXRF and covered with a X-ray shielding lid. All samples were scanned for 180s. Some forage samples (n=29) were also scanned for the 60s and 120s for scan time analysis.

Data Collection and Statistical Analysis

Spectrum for each analysis was collected using S1PXRF software (Bruker Elemental, WA, USA). The intensity of P, K, Ca, Mg, Fe, Cu and Zn in count per second (cps) and elemental concentration of P, K, Ca and Mg in weight percent were obtained from the same software. A standard-based calibration (obtained from personal communication with Dr. Lee Drake, Senior Application Scientist, Bruker AXS, Albuquerque, NM) for plant samples was used to get elemental concentration.

Data were organized in Microsoft Excel and analyzed by JMP (JMP®, Version Pro 12.2, SAS Institute Inc., Cary, NC, Copyright ©2015), SAS (SAS®, Version 9.4, SAS Institute Inc., Cary, NC, Copyright ©2002-2012), and R (R Foundation for Statistical Computing, Vienna, Austria). Regression analysis, matched paired t-tests, and repeated measures ANOVA followed by Tukey Kramer adjustment was used for data analysis. Scan time and particle size were considered as repeated. The intensity and elemental concentrations obtained from PXRF scanning were compared with the ICP-OES measured values to determine the effect of sample particle size and scan time on elemental quantification. Likewise, wet chemical analysis results from CVAS and WVU lab were also compared. Significance criterion (alpha) for all tests was 0.05.

Result and Discussion

Wet chemical elemental concentrations

The average relative standard deviation (RSD) for three sets of digestion, RSD for SRM digestion and difference between standard value and ICP determined value of CRM have been presented in Table 3.1. The RSD below 10% is acceptable for plant elemental analysis (Bueno Guerra et al., 2014).

Sample description

Forage samples had variable RSD for different element ranging from 19 % for Mg to 64% for Zn. All the elements under consideration were in RSD of nearly 19% to 32% except Zn (Table 3.2). Moreover, 30 subsets of the sample analyzed by CVAS had RSD ranging from 17% for K to 45% for Fe (Table: 3.3).

Comparability between ICP-OES and CVAS concentration

The comparability evaluation between ICP-OES and CVAS determined elemental concentration was done by using linear regression analysis (Fig 3.1). Regression models were significant for P, K, Ca, and Mg with r^2 values of 0.88, 0.85, 0.74, and 0.81 respectively. The slopes of the regression lines were between 0.66 and 0.75. The models for Fe, Cu, and Zn were not significant.

PXRF analysis of forage samples

PXRF intensity

The summary statistics PXRF intensities for three particle sizes of the sample scanned at 180 seconds is presented in Table 3.4. The intensities had RSD ranging from 2.29% for Cu (1mm samples) to 35.45% for Fe (1mm samples). RSD was comparable for each particle size of the samples. RSD of the PXRF intensities were lower than the RSD of the elemental concentration (reference standard) determined using ICP. Symmetry in the distribution of dataset was evaluated using the measure of skewness. All of the datasets were right skewed except Cu measured with 0.5 mm samples. The P, Mg, and Cu were symmetrical (skewness between -0.5 to 0.5) while the K, Ca, Mg and Zn were moderate to highly right-skewed (skewness greater than 0.5).

Regression models between PXRF counts and ICP determined concentration were significant for P, K, Ca, Fe and Zn while not significant for Cu in the samples of all particle sizes (Fig 3.2). Mg was not significant for 0.5 mm sample while significant for 1mm and 2 mm samples. P, K, Ca and Fe had R^2 values ranging from 0.7 to 0.93 while 0.23 to 0.47 for Zn. The particle size of the sample did not produce a significant impact on the strength of relationship for all elements (Fig 3.2).

PXRF determined concentrations

The elemental concentrations had RSD ranging from 10.53% for Mg (2 mm samples) to 84.21% for Fe. RSD was comparable for each particle size of the samples (Table 3.5). All of the datasets were moderate to highly right-skewed.

Furthermore, these datasets were checked for normal distribution using Shapiro-Wilk W (goodness-of-fit) test. All elements had lack of normality of distribution, which was then corrected by the Ln-transformation. Then the data were analyzed using Proc Mixed of SAS as repeated measures ANOVA, followed by multiple comparisons on Least Square (LS) means with Tukey-Kramer adjustment for multiple comparisons.

Repeated measures ANOVA results demonstrate that there is an evidence of significant relationship and particle size of the sample affects P concentration (F= 4.22, 2 df, p=0.018). The LS means were significant (P<0.001) at each particle size of the samples. Differences in the least square means were significant (p=0.018) only for the particle size of 2 mm and 1mm samples.

However, the similar analysis with K (F=2.33, 2df, p=0.114) and Ca (F=2.47, 2df, p=0.091) demonstrate that there is no evidence of the relationship and particle size of the samples were not significant. Differences in LS mean were not significant to any pair of particle sizes.

Likewise, there is an evidence of significant relationship and the particle size of the sample affects Mg concentration (F=10.75, 2df, P<0.001). The least square means were significant (P<0.001). Differences in the least square means were also significant for 1mm and 2 mm samples (P<0.001) and 0.5 mm and 2 mm samples (P<0.001).

Regression models between PXRF determined and ICP determined concentration were significant for P, K, Ca and Fe while not significant for Mg in the samples of all particle sizes (Fig 3.3). The particle size of the sample did not produce a significant impact on the strength of relationship for all elements. The strength of the relationship was lower for the PXRF determined concentration in comparison to the intensities indicating the need for some revision in the calibration.

The concentration of P, K, Ca, and Fe can reliably be measured in dry and ground forage samples with PXRF. Some elements indicated a significant effect of particle while others not. Since, the particle size of the sample was determined by passing the sample through the screen (2 mm, 1mm, and 0.5 mm), each sample was a mixture of the particles below the determined size. The elements under consideration had very low penetration depth, generally determined at the surface. The smaller particles settle at the bottom of the sample cups during measurement. This might be the cause of getting similar results from the samples of all particle sizes.

PXRF analysis of new particle sizes

In order to further, investigate the effect of particle size of the sample, two particle size of the samples were selected from the existing samples. 1-2 mm (samples that pass through 2 mm sieve but retained on a 1mm sieve) were obtained from existing 2 mm samples and 0.25-0.5 mm (samples that pass through 0.5 mm sieve but retained on a 0.25mm sieve) were obtained from existing 0.5 mm samples.

PXRF Counts: New particle size

The summary statistics PXRF intensities for two new particle sizes of the sample scanned at 180 seconds are presented in Table 3.6. The intensities of 0.25-0.5 mm samples had RSD ranging from 3.97% for Cu to 25.69% for Ca and intensities of 1-2 mm samples had RSD ranging from 7.83% for Cu to 29.60 for Ca. RSD was approximately 4% higher in larger particle size of the samples than the smaller one except for Fe (approximately 4% lower). All of the datasets had a different degree of right skewness. The right skewness of the dataset increased with the particle size except for the Zn. The differences in the intensity between two particle sizes of the samples for each element were significant (Matched Paired t-test). There is decreasing trend in the photon counts with an increase in particle size of the samples.

Regression models between PXRF counts and ICP determined concentration were significant for P, K, Ca and Fe while not significant for Mg in the samples of all particle sizes. (Fig 3.4) The strength of relationship decreased with increase in the particle size of the sample.

PXRF measured concentration: New particle size

The summary statistics of PXRF determined concentration of P, K, Ca, and Mg has been presented in Table 3.11. The elemental concentrations had RSD ranging from 11.54% for Mg to 27.27% for P. RSD was comparable for both particle size of the samples. All of the datasets were moderate to highly right-skewed.

The matched paired t-test result is presented in table 3.8. Forage samples with larger particle size had a greater bias (mean difference) with ICP determined concentration except for Ca. The bias was not significant for the P measured by PXRF in 0.25-0.5 mm samples. Likewise, in PXRF analysis particle size of the sample produced a significant bias for all analyzed elements.

Moreover, regression models between PXRF and ICP measured concentration were significant for P, K and Ca ($P<0.001$) while not significant for Mg in the samples of both particle sizes (Fig 3.5). The strength of relationship decreased with increase in the particle size of the sample. Likewise, the r^2 values were lower in comparison to the regression between PXRF intensity and ICP measured concentration, adding insights for the need of the calibration equation revision.

Effect of scan time

Doubly repeated measures ANOVA followed by Tukey-Kramer adjustment was used to analyze the effect of particle size, scan time and their interaction in PXRF measured concentration of P, K and Ca. The particle size of the sample was significant while scan time and interaction between scan time and particle size of the sample was not significant (Table 3.9).

The regression analysis between ICP determined concentration and PXRF determined concentration/intensity at different scan time produced similar r^2 (data not shown) values further confirming the nominal effect of the scan time on elemental quantification.

Elemental concentration (P, K, Ca, Fe) in dry and ground forage samples can reliably be measured by PXRF. The particle size of the sample significantly affects the result; however, compromising some accuracy PXRF can be reliably used to measure P concentrations. Scanning time was not significant indicating the potentiality of getting a result even faster, however, longer scanning time increases detection limit. Reduced strength of the relationship with PXRF concentration indicates the need for calibration update.

Mg, Cu and Zn were significantly different from the ICP values. The weak relationship of Mg might be due to the absorption of the characteristic X-ray before reaching the detector. However, in case of Cu and Zn presence of very low concentration affected the result. The weaker strength in case of Cu and Zn might be due to the use of same factory setting used for other light elements.

Conclusion

Since the PXRF results were in close agreement with ICP and CVAS results, it can be used as a prominent technique in elemental (P, K, Ca, and Fe) concentration determination in forage samples. It removes several disadvantages of traditional wet chemical analysis techniques. The result can be obtained quickly at a reduced cost. The scan time did not affect the result indicating the potentiality of getting a result even faster. The particle size of the sample affects the result, however, compromising some accuracy, it can be used for larger particle size of the samples. This method could be helpful in preparing feeding plans for animal farms and even promote hay marketing based on the mineral composition. To obtain better result drying and grinding of the sample is recommended, which would still be cheaper and quicker than wet chemical analysis. Additional work is recommended to extend this to in-field analysis.

Literature Cited

Analytical Methods Committee (AMC), Royal Society of Chemistry. 2008. Evaluation of analytical instrumentation. Part xxiii. Instrumentation for portable x-ray fluorescence spectrometry. Accred. Qual. Assur. 13:453–464

Berzaghi, P., L. Serva, M. Piombino, M. Mirisola, and F. Benozzo. 2005. Prediction performances of portable near infrared instruments for at farm forage analysis. Ital. J. Anim. Sci. 4(SUPPL. 3): 145–147.

Brouwer, P. 2013. Theory of XRF. PANanalytial B.V., Almelo, The Netherlands.

Bueno Guerra, M.B., E. de Almeida, G.G.A. Carvalho, P.F. Souza, L.C. Nunes, D.S. Júnior, and F.J. Krug. 2014. Comparison of analytical performance of benchtop and handheld energy dispersive X-ray fluorescence systems for the direct analysis of plant materials. J. Anal. At. Spectrom. 29(9): 1667.

Kalcsits, L.A. 2016. Non-destructive measurement of calcium and potassium in apple and pear using handheld X-ray fluorescence. Front. Plant Sci. 7:442.

Kalnicky, D.J., and R. Singhvi. 2001. Field portable XRF analysis of environmental samples. J. Hazard. Mater. 83(1–2): 93–122.

Karla, Y. P. 1998. Handbook of reference methods for plant analysis. CRC Press Taylor & Francis Group.

Maarschalkerweerd, M., and S. Husted. 2015. Recent developments in fast spectroscopy for plant mineral analysis. Front. Plant Sci. 6(March): 1–14.

Maruyama, Y., K. Ogawa, T. Okada, and M. Kato. 2008. Laboratory experiments of particle size effect in X-ray fluorescence and implications to remote X-ray spectrometry of lunar regolith surface. Earth Planets Sp. 60: 293–297.

McLaren, T.I., C.N. Guppy, and M.K. Tighe. 2012. A rapid and nondestructive plant nutrient analysis using portable X-ray fluorescence. Soil Sci. Soc. Am. J. 76(4): 1446–1453.

Minson, D.J. 1990. Forage in ruminant nutrition. Academic Press, San Diego, USA.

Paltridge, N.G., L.J. Palmer, P.J. Milham, G.E. Guild, and J.C.R. Stangoulis. 2012a. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. Plant Soil 361(1–2): 251–260.

Paltridge, N.G., P.J. Milham, J.I. Ortiz-Monasterio, G. Velu, Z. Yasmin, L.J. Palmer, G.E. Guild, and J.C.R. Stangoulis. 2012b. Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat. Plant Soil 361(1–2): 261–269.

Potts, P. J., and M. West. 2008. Portable X-ray fluorescence spectrometry: capabilities for in situ analysis. The Royal Society of Chemistry, UK.

Reid, R. L., A. J. Post, and G. A. Jung. 1970. Mineral composition of forages. West Virginia University, Agricultural Experiment Station, Morgantown. Bulletin 589T.

Reidinger, S., M.H. Ramsey, and S.E. Hartley. 2012. Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer. New Phytol. 195(3): 699–706.

Towett, E.K., K.D. Shepherd, and B. L. Drake. 2016. Plant elemental composition and portable X-ray fluorescence (pXRF) spectroscopy: Quantification under different analytical parameters. X-Ray Spectrom. 45(2): 117–124.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Comstock Publishing Associates, Ithaca, NY.

Weindorf, D.C., N. Bakr, and Y. Zhu. 2014. Advances in portable X-ray fluorescence (PXRF) for environmental, pedological, and agronomic applications. Adv. Agron. 128:1–45.

Willis, J. P., and A. R. Duncan. 2008. Understanding XRF spectrometry, Vol I. PANanalytial B.V., Almelo, The Netherlands.

Yamada, Y. 2014. Sample preparation for X-ray fluorescence analysis I . Outline of sample preparation. Rigaku J. 30(1): 26–29.

Tables

Table 3.1: Average Relative Standard Deviation (RSD) for 3 replications of forage sample digestion and RSD for SRM digested with forage samples (n=42).

Elements	Average RSD for forage samples (%)	RSD for SRM (%)	Difference between standard and ICP concentration for SRM (%)
P	6.3	5.53	4.09
K	7.19	3.93	4.80
Ca	4.24	4.25	2.42
Mg	5.82	5.10	14.13
Fe	10.01	8.25	13.64
Cu	12	23.21	42.34
Zn	20.75	14.82	97.89

Table 3.2: Summary Statistics for ICP determined elemental concentrations (mg/kg) forage samples (n=42).

Statistic	Elemental Concentration (mg/kg)						
	P	K	Ca	Mg	Fe	Cu	Zn
Mean	2100	17100	4700	1800	200	11	41
Minimum	1000	11500	2700	1100	44	7	23
Maximum	3500	27400	9000	2600	448	17	200
Standard Dev	600	4000	1500	350	105	3	26
RSD (%)	28.57	23.39	31.91	19.44	52.50	27.27	63.41
Skewness	0.40	1.13	1.46	0.42	0.75	0.37	5.65
Kurtosis	-0.29	0.33	1.42	-0.70	-0.51	-0.81	29.07

Table 3.3: Summary Statistics for CVAS determined elemental concentrations (mg/kg) in 30 subsets of forage samples.

Statistic	Elemental Concentration (mg/kg)						
	P	K	Ca	Mg	Fe	Cu	Zn
Mean	2500	22900	4900	2000	500	7	22
Minimum	1600	16300	3200	1500	80	5	16
Maximum	3900	33600	7400	3000	6100	12	30
Standard Dev	600	3900	1000	350	225	2	4
RSD (%)	24	17	20.41	17.50	45	28.57	18.18
Skewness	0.36	1.12	0.58	0.28	1.85	1.10	0.65
Kurtosis	-0.79	0.73	-0.19	-0.08	1.82	0.15	-0.93

Table 3.4: Summary statistics for PXRF intensities (cps) for 3 different particle size forage samples (n=42).

Method	Statistic	PXRF intensities (cps)						
		P	K	Ca	Mg	Fe	Cu	Zn
PXRF (0.5 mm)	Mean	15700	284900	146800	3600	98800	55200	55500
	Minimum	10300	188700	100500	3300	63600	50200	49900
	Maximum	21900	399300	294700	4000	205300	58800	67100
	SD	2900	54200	41900	200	31500	1800	2600
	RSD (%)	18.47	19.02	28.54	5.56	31.88	3.26	4.68
	Skewness	0.34	0.56	1.86	0.30	1.38	-0.25	1.99
	Kurtosis	-0.62	-0.46	2.71	-0.90	1.61	0.23	6.73
PXRF (1 mm)	Mean	15300	276800	141800	3600	99300	54600	55000
	Minimum	10400	188500	95100	3300	61700	50800	50200
	Maximum	21600	402200	290700	3900	210900	58600	60600
	SD	2700	53800	39100	200	35200	1600	2000
	RSD (%)	17.65	19.44	27.57	5.56	35.45	2.93	3.64
	Skewness	0.34	0.73	1.89	0.38	1.45	0.45	0.74
	Kurtosis	-0.61	-0.41	3.55	-0.82	1.58	0.63	0.85
PXRF (2 mm)	Mean	15400	271700	149000	3500	96500	52100	52400
	Minimum	10300	181500	100100	3200	58700	47200	47100
	Maximum	22000	383800	302200	3900	211100	57200	59100
	SD	2800	52400	42400	200	33300	1900	2300
	RSD (%)	18.18	19.29	28.46	5.71	34.51	3.65	4.39
	Skewness	0.32	0.56	1.75	0.25	1.42	0.18	0.75
	Kurtosis	-0.44	-0.53	2.60	-0.65	1.88	0.19	0.94

Table 3.5: Summary statistics for PXRF determined concentrations (mg/kg) for 3 different particle size forage samples (n=42).

Method	Statistic	PXRF determined elemental concentration (mg/kg)				
		P	K	Ca	Mg	Fe
PXRF (0.5 mm)	Mean	2500	16300	6100	2000	180
	Minimum	1700	11200	2800	1700	0.2
	Maximum	4300	25700	15600	2700	620
	Standard Dev	600	3300	2400	300	150
	RSD	24.00	20.25	39.34	15.00	83.33
	Skewness	1.31	1.03	1.76	1.01	1.01
	Kurtosis	0.63	0.73	3.93	-0.36	0.45
PXRF (1 mm)	Mean	2400	16100	6000	2000	190
	Minimum	1700	11300	2500	1600	1
	Maximum	4300	26100	15600	2800	530
	Standard Dev	600	3400	2300	300	160
	RSD	25.00	21.12	38.33	15.00	84.21
	Skewness	1.35	1.13	1.87	0.98	0.66
	Kurtosis	0.73	0.87	4.86	0.18	-0.81
PXRF (2 mm)	Mean	2500	16000	6300	1900	190
	Minimum	1700	10700	3000	1600	1
	Maximum	4500	25200	10500	2400	670
	Standard Dev	600	3400	1900	200	160
	RSD	24.00	21.25	30.16	10.53	84.21
	Skewness	1.43	1.02	0.66	0.97	1.10
	Kurtosis	1.05	0.69	-0.22	0.03	0.78

Table 3.6: Summary statistics for PXRF intensities (cps) for 2 different particle size (new particle size) forage samples (n=42).

		PXRF intensities (cps)						
Method	Statistic	P	K	Ca	Mg	Fe	Cu	Zn
PXRF (0.25-0.5 mm)	Mean	13400	264200	130800	3300	77100	52900	53400
	Minimum	8900	178200	87400	2900	54900	48800	48600
	Maximum	19100	393500	226700	3700	111400	57800	67700
	SD	2400	51700	33600	200	16800	2100	3200
	RSD	17.91	19.57	25.69	6.06	21.79	3.97	5.99
	Skewness	0.59	0.81	1.36	0.33	0.43	0.40	2.20
	Kurtosis	0.17	0.14	1.47	0.07	-1.03	0.03	8.57
PXRF (1-2 mm)	Mean	10800	208800	86500	2900	58000	43400	42200
	Minimum	7000	135800	56200	2600	43900	37300	36100
	Maximum	16700	345600	185300	3300	95000	51000	50400
	SD	2200	44800	25600	200	9600	3400	3700
	RSD	20.37	21.46	29.60	6.90	16.55	7.83	8.77
	Skewness	0.95	1.07	2.03	0.64	1.33	0.41	0.48
	Kurtosis	1.21	1.35	5.11	-0.13	3.75	-0.28	-0.29

Table 3.7: Summary statistics for PXRF determined concentrations (mg/kg) for two new particle size of forage samples (n=42).

		PXRF determined elemental concentration (mg/kg)			
Method	Statistic	P	K	Ca	Mg
PXRF (0.25-0.5 mm)	Mean	2200	15800	5800	2500
	Minimum	1500	10800	2600	2200
	Maximum	3800	25900	12700	3300
	Standard Dev	600	3400	2000	300
	RSD	27.27	21.52	34.48	12.00
	Skewness	1.39	1.17	1.23	1.18
	Kurtosis	1.36	1.54	2.45	0.68
PXRF (1-2 mm)	Mean	1900	13100	4300	2600
	Minimum	1200	8200	2300	2000
	Maximum	3500	24100	10700	3300
	Standard Dev	500	3100	1400	300
	RSD	26.32	23.66	32.56	11.54
	Skewness	1.73	1.51	2.24	0.66
	Kurtosis	2.76	3.49	9.18	-0.32

Table 3.8: Matched paired t-test between ICP and PXRF measured concentration (mg/kg) for for 2-particle size (new particle size) of forage samples (n=42).

Experiment	Element	Bias (mg/kg)	t-ratio	P value
ICP vs PXRF 0.25-0.5 mm	P	52	0.97	0.334
	K	1409	-5.42	<0.001
	Ca	-1044	6.91	<0.001
	Mg	-750	10.48	<0.001
ICP vs PXRF 1-2 mm	P	275	-5.08	<0.001
	K	4049	-13.34	<0.001
	Ca	452	-2.67	0.01
	Mg	-829	10.56	<0.001
PXRF 0.25-0.5 mm vs PXRF 1-2 mm	P	327	-9.41	<0.001
	K	2640	-13.94	<0.001
	Ca	1497	-9.41	<0.001
	Mg	79	3.16	0.003

Table 3.9: Repeated measures ANOVA results for the effect of scan time and particle size on PXRF measured elemental concentration (n=29).

Element	Effect	Degree of freedom (df)	F value	p-value
P	Particle size	1	5.09	0.0373
	Time	2	0.02	0.9804
	Particle size*Time	2	0.31	0.7368
K	Particle size	1	6.75	0.0185
	Time	2	0.92	0.4059
	Particle size*Time	2	0.3	0.7403
Ca	Particle size	1	3.78	0.0101
	Time	2	3.12	0.0502
	Particle size*Time	2	4.86	0.0694

Figures

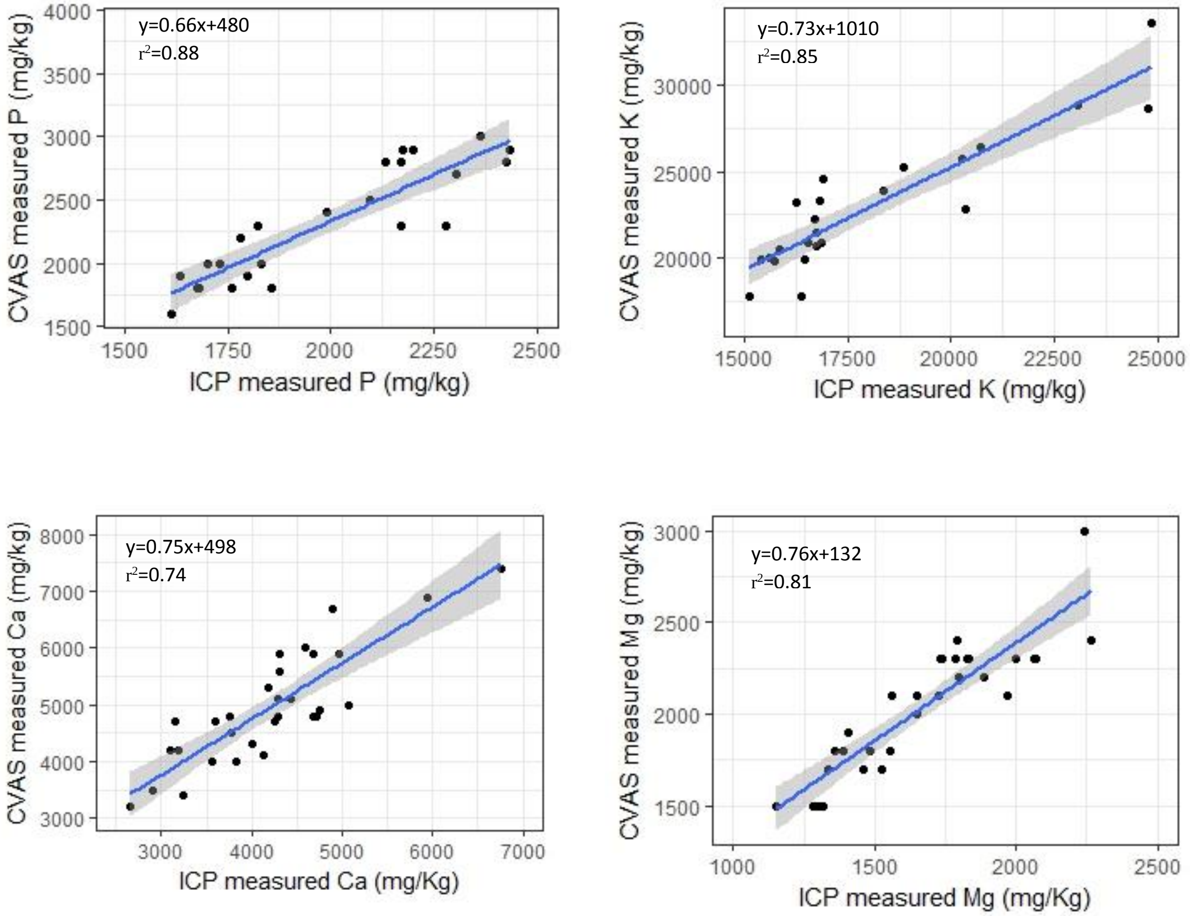


Fig 3.1: Regression plots between ICP and CVAS measured P, K, Ca and Mg. The shaded region shows standard error.

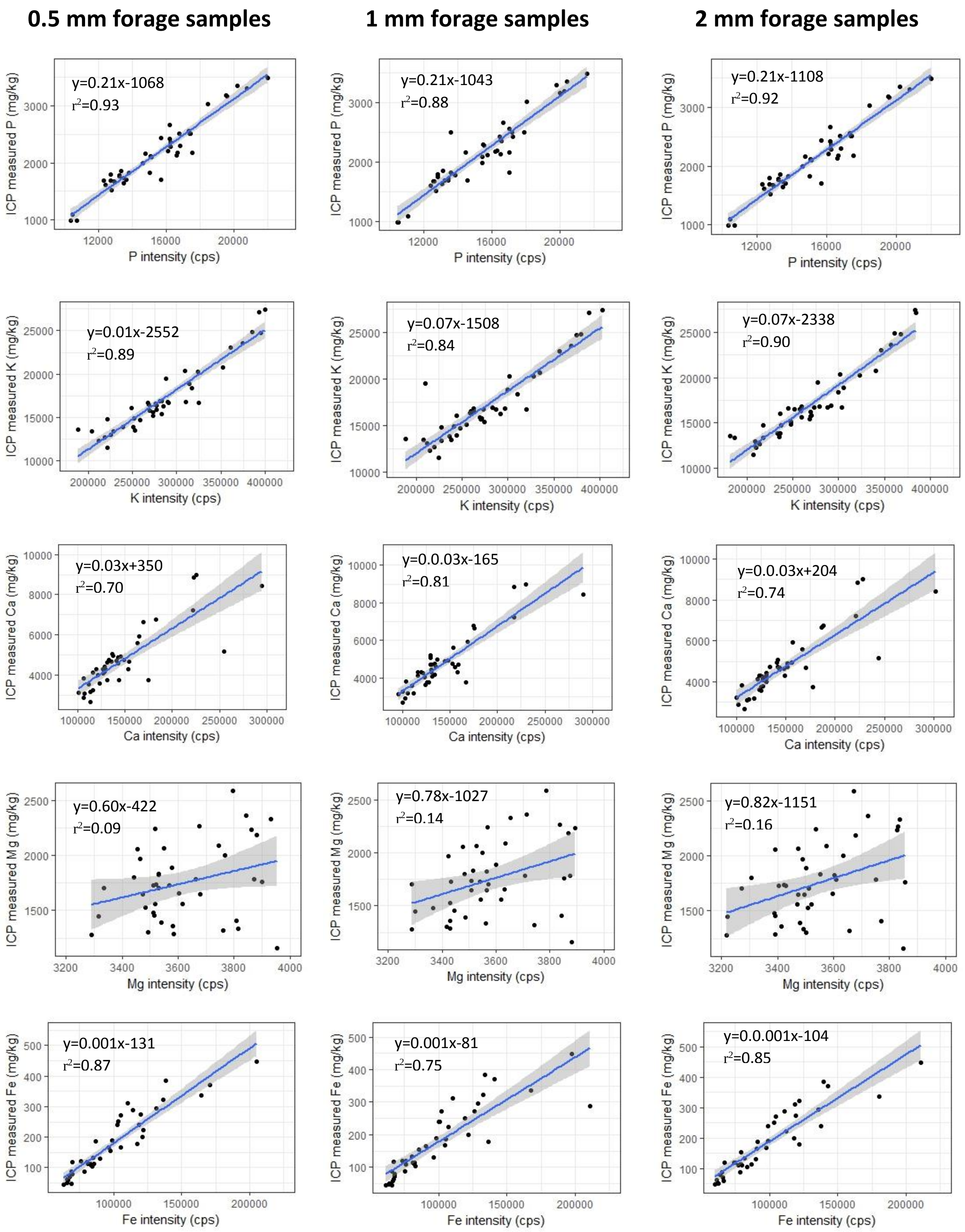


Fig 3.2: Regression plots between ICP measured concentration and PXRF intensity for P, K, Ca, Mg, and Fe. The shaded portion shows the standard error.

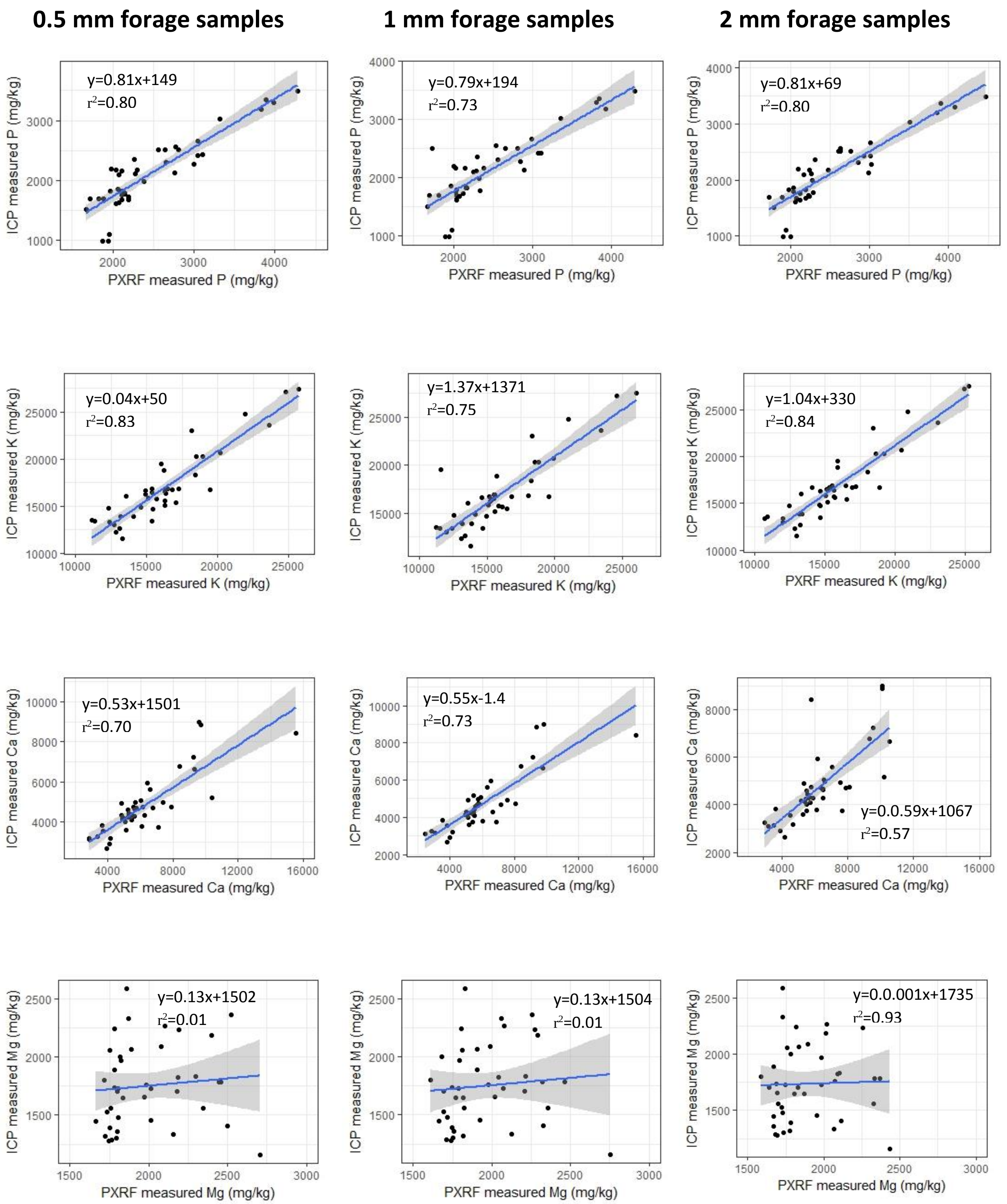


Fig 3.3: Regression plots between ICP measured concentration and PXRF measured concentration for P, K, Ca and Mg. The shaded portion shows the standard error.

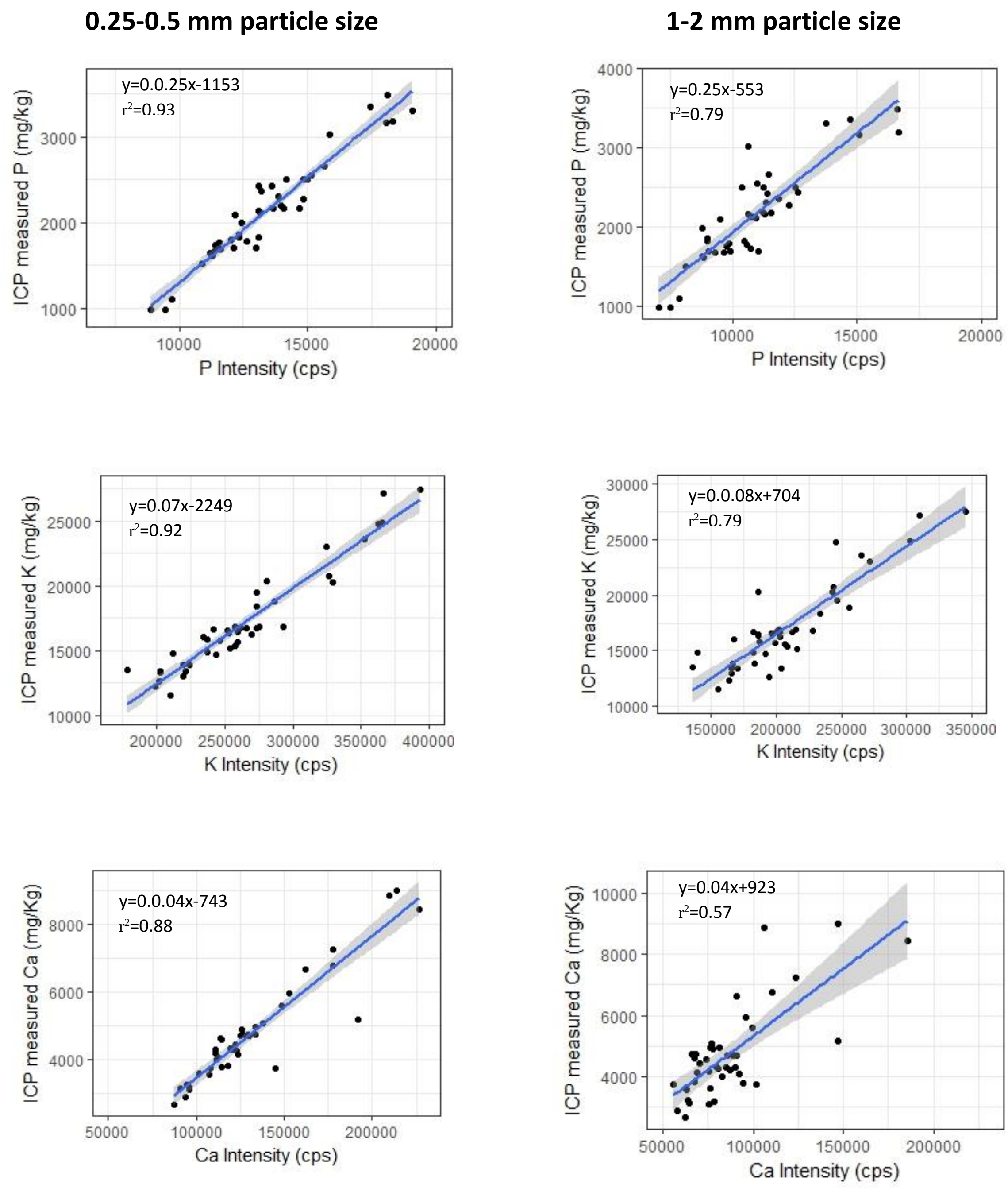
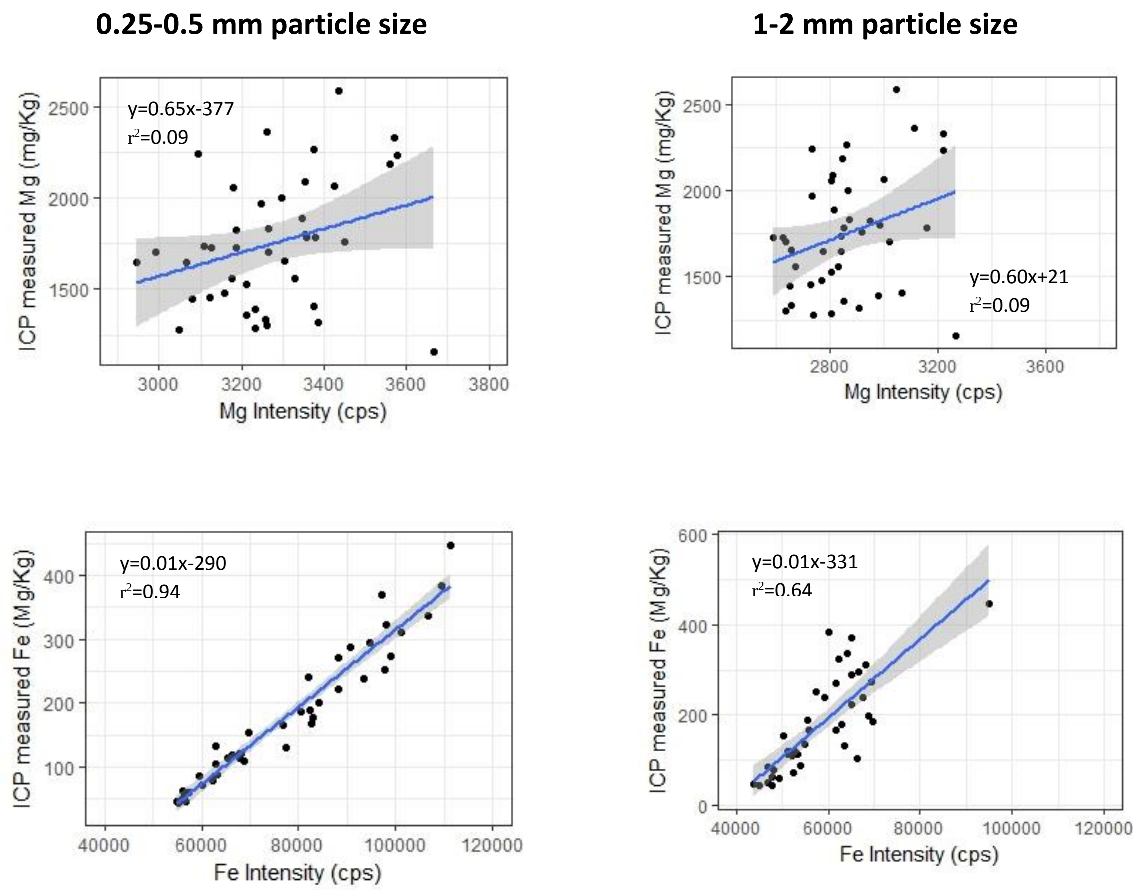


Fig 3.4 (a): Regression plots between ICP measured concentration and PXRF intensity for P, K, and Ca for two new particle size of forage samples. The shaded portion shows the standard error.



0.25-0.5 mm particle size

1-2 mm particle size

Fig 3.4 (b): Regression plots between ICP measured concentration and PXRF intensity for Mg, Fe and Zn for two new particle size of forage samples. The shaded portion shows the standard error.

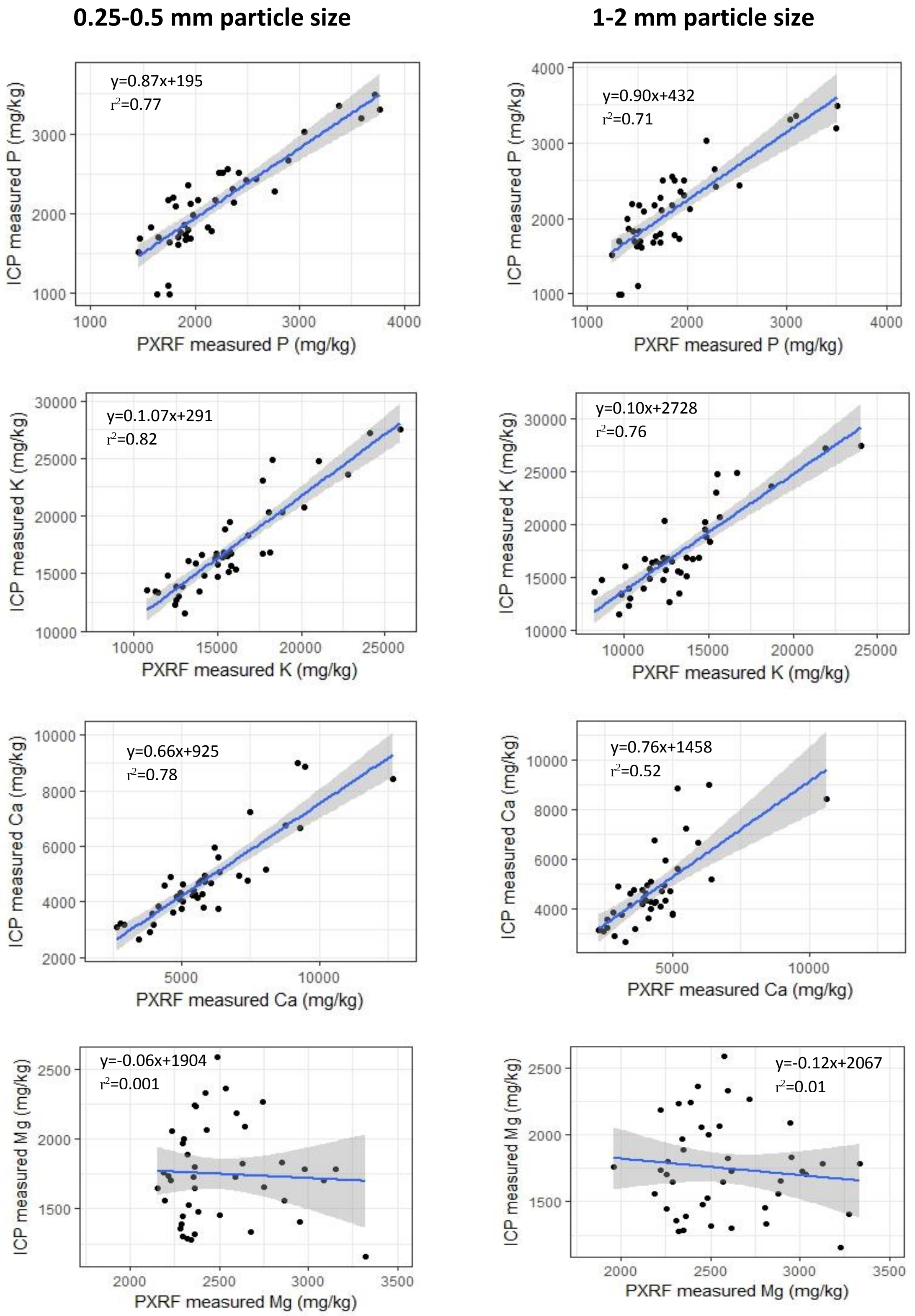


Fig 3.5: Regression plots between ICP and PXRF measured P, K, Ca, Mg and Fe concentrations. The shaded region shows confidence interval.

Chapter 4: Conclusion

A phosphorus mass balance across animal-based farms could be part of the solution to water quality problems. Comprehensive Nutrient Management Planning (CNMP) does this. The major basis of the CNMP is the P data from soil and manure. Any cost-effective and rapid P measurement technique would expedite CNMP planning process at a reduced cost. Accurate data on manure can be obtained using PXRF. The units are equipped with low power X-ray tubes, light and convenient to use either in benchtop or field-analysis modes. They are comparable to wet chemical methods in terms of elemental concentration determination but superior in terms of portability, cost-effectiveness, the simplicity of operation, the potential for in-situ measurement, and analysis of large samples. Moreover, the presence of vacuum facility further improves the measurement of light elements.

Moisture significantly affected the elemental determination in manure samples. The effect is more pronounced in elements like P, K, Mg and less pronounced for Ca, Fe, Cu, and Zn. This is because the light elements have lower energy levels. However, elemental concentration in manure samples can be measured, without losing accuracy, up to 20% moisture. Beyond this moisture correction is advisable especially for elements like P and K. Drying and grinding of the manure samples improved results, however, losing some accuracy PXRF can even be used in wet and unground samples.

Forage is the major feed and source of minerals for animals. The performance and health of the animal are directly influenced by the mineral content of the forages. Thus, mineral composition date in forage is the most important part of the animal feeding plan. The mineral composition data can be quickly obtained with PXRF at a reduced cost. The scan time did not affect the result indicating the potentiality of getting a result even faster. The particle size of the sample affects the result, however, compromising some accuracy, it can be used for larger particle size of the samples. To obtain better result drying and grounding of the sample is recommended, which would still be cheaper and quicker than wet chemical analysis.

This simplification of mineral determination techniques can contribute to better whole-farm P management and CNMP development. This will reduce the costs of forage and manure mineral analysis. Knowing forage mineral composition will ultimately increases the efficiency of animal feeding. In addition, this tool will be helpful in price determination of dried hay bales in forage markets. Likewise, the manure composition data at low cost will help farmers plan manure applications to their fields in ways to obtain maximum use efficiency and minimize runoff to water bodies, thus reducing environmental hazards. Nevertheless, this will be a great tool for researchers working in animal feeding management, manure management, and forage breeding activities. This work demonstrated that with the right sample preparation PXRF can obtain results comparable to digestion and ICP. Further study is warranted to extend this analysis to in-the-field measurements.

Appendix:

Appendix 1: Regression between ICP and WVDA determined elemental concentrations (mg/kg) in manure samples (n=40).

Element	r^2	RMSE	Intercept	Slope	p-value
P	0.98	528	50.76	0.96	<0.001
K	0.92	2570	1398	0.91	<0.001
Ca	0.97	2481	-1699	0.98	<0.001
Mg	0.91	418	65.68	0.96	<0.001
Cu	0.99	76	-15.44	1.01	<0.001

Appendix 2: Regression between ICP determined concentration (mg/kg) and PXRF intensities (cps) for oven dried manure samples (n=40).

Element	r^2	RMSE	Intercept	Slope	p-value
P	0.94	916	-2347	0.28	<0.001
K	0.92	2740	-4770	0.10	<0.001
Ca	0.93	3817	-3576	0.07	<0.001
Mg	0.59	909	-6245	2.44	<0.001
Fe	0.93	735	-868	0.01	<0.001
Cu	0.93	166	-484	0.01	<0.001
Zn	0.56	72	-188	0.01	<0.001

Appendix 3: Regression between ICP and PXRF measured concentration (mg/kg) for oven dried manure samples (n=40).

Element	r^2	RMSE	Intercept	Slope	p-value
P	0.66	2117	-1138	1.42	<0.001
K	0.83	3956	-4830	1.72	<0.001
Ca	0.89	4755	4793	1.05	<0.001
Mg	0.25	1232	4490	0.29	<0.001
Fe	0.86	1040	413	2.05	<0.001

Appendix 4: Regression between ICP and CVAS determined elemental concentrations (mg/kg) in forage samples (n=42).

Elements	r^2	RMSE	Intercept	Slope	P-value
P	0.88	142.30	479.85	0.66	<0.001
K	0.85	1225.00	1010.00	0.73	<0.001
Ca	0.74	456.20	498.23	0.75	<0.001
Mg	0.81	131.90	131.52	0.76	<0.001
Fe	0.09	75.63	108.05	0.11	0.10
Cu	0.04	2.44	8.72	0.25	0.32
Zn	0.001	31.46	34.26	0.26	0.85

Appendix 5: Regression between ICP-OES determined concentration (mg/kg) and PXRF intensities (cps) for 3 different particle size of forage samples (n=42).

Experiment	Element	r^2	RMSE	Intercept	Slope	P-value
ICP-OES and PXRF intensities of 0.5 mm forage samples	P	0.93	168	-1068	0.21	<0.001
	K	0.89	1319	-2552	0.00	<0.001
	Ca	0.70	829	350	0.03	<0.001
	Mg	0.09	337	-422	0.60	0.06
	Fe	0.87	38	-131	0.00	<0.001
	Cu	0.05	3	29	0.00	0.15
	Zn	0.47	19	-338	0.01	<0.001
ICP-OES and PXRF intensities of 1mm forage samples	P	0.88	217	-1043	0.21	<0.001
	K	0.84	1622	-1508	0.07	<0.001
	Ca	0.81	653	-165	0.03	<0.001
	Mg	0.14	327	-1027	0.78	0.01
	Fe	0.75	53	-81	0.00	<0.001
	Cu	0.01	3	20	0.00	0.53
	Zn	0.25	23	-319	0.01	<0.001
ICP-OES and PXRF intensities of 2 mm forage samples	P	0.92	176	-1108	0.21	<0.001
	K	0.90	1277	-2338	0.07	<0.001
	Ca	0.74	769	204	0.03	<0.001
	Mg	0.16	325	-1151	0.82	0.009
	Fe	0.85	42	-104	0.00	<0.001
	Cu	0.02	2.55	20	0.001	0.42
	Zn	0.23	23	-244	0.01	0.009

Appendix 6: Regression between ICP and PXRF concentration (mg/kg) for 3 different particle size of forage samples (n=42).

Experiment	Elements	r^2	RMSE	Intercept	Slope	P value
ICP-OES and PXRF concentrations of 0.5 mm forage samples	P	0.80	321	149	0.81	<0.001
	K	0.83	1584	50	1.04	<0.001
	Ca	0.70	836	1501	0.53	<0.001
	Mg	0.01	352	1502	0.13	0.54
ICP-OES and PXRF concentrations of 1mm forage samples	P	0.73	308	194	0.79	<0.001
	K	0.75	1905	1371	1.37	<0.001
	Ca	0.73	789	1.4	0.55	<0.001
	Mg	0.01	352	1504	0.13	0.55
ICP-OES and PXRF concentrations of 2 mm forage samples	P	0.80	266	69	0.81	<0.001
	K	0.84	1505	330	1.04	<0.001
	Ca	0.57	997	1067	0.59	<0.001
	Mg	0.08	400	1735	0.00	0.08

Appendix 7: Regression between ICP determined concentration (mg/kg) and PXRF intensities (cps) for two new particle size of forage samples (n=42).

Experiment	Element	r^2	RMSE	Intercept	Slope	P value
ICP Vs 0.25-0.5 mm	P	0.93	163.70	-1153.00	0.25	<0.001
	K	0.92	1159.00	-2249.00	0.07	<0.001
	Ca	0.88	519.60	-743.00	0.04	<0.001
	Mg	0.09	337.30	-376.91	0.65	0.055
	Fe	0.94	26.75	-290.40	0.01	<0.001
	Cu	0.03	2.53	-0.05	0.00	0.273
	Zn	0.58	17.22	-289.80	0.01	<0.00
ICP Vs 1-2 mm	P	0.79	281.00	-552.88	0.25	<0.001
	K	0.79	1828.00	703.70	0.08	<0.001
	Ca	0.57	994.10	923.40	0.04	<0.001
	Mg	0.09	337.00	20.63	0.60	<0.001
	Fe	0.64	63.70	-330.60	0.01	<0.001
	Cu	0.01	2.57	12.07	0.00	0.852
	Zn	0.01	26.50	19.91	0.00	0.642

Appendix 8: Regression between ICP and PXRF concentration (mg/kg) for 2-particle size (new particle size) of forage samples (n=42).

Method	Element	r^2	RMSE	Intercept	Slope	p-value
PXRF (0.25-0.5 mm)	P	0.77	289.00	195.18	0.87	<0.001
	K	0.82	1688.00	290.90	1.07	<0.001
	Ca	0.78	710.50	924.80	0.66	<0.001
	Mg	0.001	353.00	1904.07	-0.06	0.7497
PXRF (1-2 mm)	P	0.71	322.90	431.75	0.90	<0.001
	K	0.76	1965.00	2728.00	1.10	<0.001
	Ca	0.52	1458.10	1458.10	0.76	<0.001
	Mg	0.01	351.00	2068.61	-0.12	0.4607

Appendix 9: WVDA, ICP, and PXRF measured P concentration (mg/kg) and PXRF intensity of P (cps) in manure samples at different moisture level.

S.No	P conc M WVDA	P conc M ICP	P conc M <10	P conc M 10-20	P conc M 20-30	P conc M 40-50	P conc M 60-70	P Int M <10	P Int M 10-20	P Int M 20-30	P Int M 40-50	P Int M 60-70
1	9592	11711	8422	7655	6273	5455	4475	47662	43248	36371	30090	25492
2	8712	8866	6806	5792	4820	4352	3351	36579	28392	24525	22417	18125
3	6556	6477	6820	5890	5023	4269	3431	33645	28558	25866	21464	18036
4	9240	8576	7601	7067	5984	5490	3098	38039	34048	31132	28493	17558
5	3872	3738	4328	4686	3103	2115	2120	23671	23488	17876	7009	11992
6	7436	7322	6317	6062	5312	4605	2140	32318	30700	27011	23685	13014
7	8536	8364	8135	8250	8250	6938	6896	35939	35844	35844	30514	31728
8	12012	11156	8941	9212	5413	4947	4205	52706	56136	33031	29368	25210
9	3560	3764	3575	3080	2641	2617	2074	21653	18248	15796	14837	12355
10	6160	6178	3408	2792	2588	2529	1886	27919	20791	17864	16698	13521
11	5368	5352	4892	4049	3377	2937	2593	27451	24361	21039	17043	15524
12	4180	4265	3521	2805	2153	2497	3097	22027	19407	7941	15273	16608
13	6952	7635	3544	3262	2296	2764	1387	29008	23065	10515	17077	11502
14	4796	4787	2723	2446	2499	2214	2629	24259	21042	20870	15211	20239
15	4752	5113	3515	3577	3031	2685	1871	23242	22352	18115	15774	13536
16	6732	6089	4527	4121	3835	3129	2955	30245	25715	23429	15879	15782
17	4664	4478	3103	2522	2422	2470	2265	22738	19968	17145	16305	14575
18	4400	4273	4259	3586	2970	2752	2254	22499	18646	16826	15291	12959
19	5632	6813	4175	3450	3063	2452	2596	30200	23237	19609	15265	13453
20	2957	3137	3182	3816	3367	2951	2447	16334	18596	16712	15302	14255
21	4162	4204	3760	3364	2995	2488	2800	20366	18022	16216	13770	14796
22	4972	5055	5432	5348	4422	3786	2570	29152	28152	23806	20146	14706
23	4708	4657	4702	3838	2733	2829	2135	28729	26099	10147	17082	13361
24	3969	3905	4831	4140	3455	3230	2529	24745	21657	18574	17251	13214
25	6380	6693	3618	2839	2752	2736	2659	31617	23839	20192	18818	16305
26	4132	4320	6756	6352	4892	4416	3298	30466	28524	23516	21884	17641
27	4013	3985	5445	4915	3767	3112	2539	25649	22593	18853	16039	13581
28	4057	4939	5221	4613	4021	3489	3294	27582	24321	12518	19058	17948
29	3428	4140	4713	3884	2991	2667	2134	25050	19698	8965	14286	12062
30	4202	4260	4787	5819	4193	3549	2706	25790	31488	22622	18740	16014
31	9504	10529	10014	8959	7566	6092	4601	45373	39746	34901	28753	22731
32	8536	8399	6262	4734	3924	3679	3592	41058	32214	25045	21931	19702
33	6644	6972	6954	5250	3991	3141	2657	36093	27143	21527	17288	15657
34	2064	2246	3734	3315	3362	2465	1798	18640	16472	16936	13043	10190
35	5500	5766	4808	4908	4157	3751	2536	28791	27170	22223	18988	14927
36	7172	7931	6207	4185	3721	3551	3180	39948	25044	21481	19621	18288
37	10956	11218	8123	7126	4987	4886	3663	52509	45419	27480	26045	21353
38	10296	10750	7811	6361	5621	5030	4063	53661	43703	37014	28666	23640
39	21560	21921	10557	8436	6323	6849	4423	78724	67751	38782	42148	27385
40	11660	12800	9015	6735	5811	5426	3550	44501	34768	29953	19944	19944

Appendix 10: WVDA, ICP, and PXRF measured K concentration (mg/kg) and PXRF intensity of K (cps) in manure samples at different moisture level.

S.No	K conc M WVDA	K conc M ICP	K conc M <10	K conc M 10-20	K conc M 20-30	K conc M 40-50	K conc M 60-70	K Int M <10	K Int M 10-20	K Int M 20-30	K Int M 40-50	K Int M 60-70
1	16019	19189	14633	14219	10446	7032	5942	250024	241034	192119	143466	128342
2	21331	23659	15387	12783	9524	7870	5962	248616	195504	161104	142451	120796
3	11869	11958	10204	9287	6130	4629	4013	171445	155767	123236	102055	97735
4	21331	20952	15671	14209	10286	7886	5936	246860	217442	178935	149771	125817
5	13363	12177	12297	9680	3115	2931	1424	208867	161503	88610	50366	65691
6	18841	19424	14293	13033	10245	7721	5005	224523	204349	169931	140805	114893
7	15687	15988	12351	12319	12319	10118	10195	188307	187070	187070	162204	167299
8	15106	14478	11389	12049	7464	5340	3772	201941	211776	141418	113472	99494
9	18758	18209	15151	12298	9943	6980	5632	254828	206105	172977	135187	121025
10	34279	33542	19888	18120	14496	12365	8660	400016	328632	254230	221198	175853
11	20169	18863	16997	15894	10288	7025	7198	281484	266246	193747	142136	150785
12	25979	24865	20180	18595	18871	10114	11920	345647	331059	186982	189496	208278
13	33117	30969	16566	15009	19552	10847	7582	323588	264594	215520	183347	155875
14	34362	32053	19302	17293	16904	10974	15486	404281	350832	337069	204315	301269
15	24153	22936	15583	13526	11342	8004	6442	263230	219565	184625	142445	137282
16	24900	22834	15600	13539	10987	7573	6393	272357	220930	185985	123847	115068
17	29465	25920	17494	17235	13265	10805	8905	319915	320943	238771	198794	169758
18	35275	33776	26185	21969	16172	12421	11520	401811	326043	267934	211933	205896
19	29465	35044	19976	17545	14918	12264	10507	375398	304011	257059	204617	177058
20	15853	15872	13485	13153	10130	8292	6018	202764	195623	164057	145271	127245
21	23987	22665	18470	17086	12129	9677	9802	286386	260694	201959	168398	173656
22	13363	12445	11940	11712	8323	6568	3929	197050	192743	151103	127846	98722
23	16351	14994	13839	13627	12889	5736	4221	243591	243490	134747	120067	104818
24	11122	10251	10550	9330	5690	4235	2669	173420	152098	113350	96118	79389
25	23406	24358	14313	13223	10103	8563	5541	290466	250431	185271	156740	114405
26	8300	9564	7971	6962	4299	3266	1558	135874	121212	96631	85207	68733
27	6781	6363	6191	5110	2791	1658	294	118222	100580	77305	65524	49676
28	12367	13330	11550	8832	13390	4855	3715	190862	152568	127895	106191	93039
29	8126	9587	9451	6709	6613	2404	729	162251	116781	73214	70744	52373
30	11869	12112	10923	11296	6828	4549	2172	183811	190820	132237	102194	77922
31	2515	2687	2047	1550	826	30	-817	61610	56307	50553	41720	33169
32	23738	23496	17374	16376	11675	9333	6410	319847	296569	211863	171532	135091
33	10873	10510	9977	9166	5215	2944	1850	174772	151649	108203	80381	68721
34	4507	4313	4467	3661	3707	1777	73	94278	84712	86744	63406	44825
35	13280	13266	11331	10286	6719	5173	2703	196424	172056	125570	101093	78904
36	26560	27082	20370	16618	11443	8492	6132	374348	279535	200152	155514	126592
37	32951	37966	24383	21052	13371	11552	11389	453518	382423	223866	195806	213967
38	32619	37100	21344	16492	15464	10678	11429	414052	314976	292877	197671	219008
39	29382	35846	16233	15208	10723	9159	7414	326272	313809	191210	177288	149614
40	17430	27907	15906	14346	9603	7030	5009	291462	267954	184050	139612	112537

Appendix 11: WVDA, ICP, and PXRF measured Ca concentration (mg/kg) and PXRF intensity of Ca (cps) in manure samples at different moisture level.

S.No	Ca conc M WVDA	Ca conc M ICP	Ca conc M <10	Ca conc M 10-20	Ca conc M 20-30	Ca conc M 40-50	Ca conc M 60-70	Ca Int M <10	Ca Int M 10-20	Ca Int M 20-30	Ca Int M 40-50	Ca Int M 60-70
1	26700	35533	26246	25885	21196	19567	17726	566931	554084	450496	383585	334620
2	40900	45321	28387	27719	22656	21050	17827	577868	503312	413757	381357	323402
3	29800	33604	30137	28193	22583	20810	16218	550101	498624	409953	359291	283279
4	46400	48899	34717	33440	26805	24397	16911	662449	608140	511674	455720	310478
5	19000	19861	20258	21706	14098	15886	10391	401771	390160	259085	160802	171285
6	40600	41889	29120	27506	23898	21233	11847	558965	518020	441285	385634	232037
7	50600	52490	40167	41859	41859	36929	35051	670154	687406	687406	603546	598236
8	31600	31140	26786	21001	17847	16322	13572	591147	480185	382060	331359	267612
9	17400	20251	15247	14257	12538	11883	9157	336094	294463	249858	217768	166030
10	10300	10514	5433	5249	5315	5440	4480	183934	152770	131381	123425	93934
11	13500	14427	12367	10966	9311	8970	7505	270615	248711	200028	168722	144375
12	10300	11256	8339	7239	9626	7126	8156	213173	194540	132302	145460	162176
13	16300	16701	8293	8461	10143	8116	4827	249714	215154	169708	167513	105764
14	11200	12159	6442	6264	6373	6262	6744	220943	197663	194566	139822	188939
15	13500	14550	8461	7420	7191	6888	4724	205902	165646	146065	127409	91175
16	16700	16297	10215	8656	8045	7949	7943	255306	194942	170038	130498	130594
17	13600	14114	8090	6757	6850	7272	6794	222570	195123	165491	158773	136164
18	13100	14124	10015	8424	7507	6913	6684	237831	187423	164573	138075	132184
19	16800	20064	10567	8956	9116	8252	7993	301452	228886	212005	173747	158741
20	13500	14652	10936	11847	10477	9514	6841	202189	211365	181609	164511	120033
21	14500	15398	10966	10314	9099	8364	7885	232821	211190	175268	153856	145013
22	36100	33605	29047	28337	24004	21290	13678	571055	545819	456605	389426	250149
23	14300	15737	15144	13265	15968	10546	8019	341360	322803	204960	201309	144711
24	33000	31784	28731	25746	21074	19630	13222	531378	474809	382464	346873	232607
25	17800	20611	10937	9832	9752	9910	9090	336267	277747	232618	217911	172041
26	84800	84129	74403	74789	56482	51753	36529	1236183	1229765	967094	901403	661014
27	25000	29554	32808	31200	22792	19252	15111	553987	506592	383333	320673	248070
28	24400	30933	25968	21503	31429	16826	15146	500816	402237	352860	305765	266674
29	30700	34113	27782	24413	29671	16999	13547	528524	427059	300609	286594	221405
30	31800	39587	29501	28499	21379	18705	12440	573680	565784	400813	330800	223500
31	19700	23784	28595	27167	23361	18604	14564	467125	430575	376928	296182	229401
32	17400	19451	14283	11879	10185	10105	9832	364947	305789	230192	206493	181459
33	25100	28466	27924	23791	18026	14988	13625	535586	441726	329572	263879	233240
34	19300	23427	26584	22381	22638	16341	11399	452490	370163	380578	267020	182875
35	27300	31144	23606	23633	20038	18311	11640	508832	469565	370118	312480	207000
36	15100	17728	13109	11967	10609	10400	9153	343373	272561	217809	195761	164277
37	17400	20496	12616	12494	10001	11274	8795	353949	330260	207419	220394	184641
38	20000	23011	14356	12909	11801	11883	8828	405433	343494	298420	246144	190934
39	36600	33267	18881	16433	14453	16062	11979	545395	500745	321874	357800	251831
40	20700	24580	18690	15733	15531	14447	10740	453347	392653	335523	279876	198951

Appendix 12: WVDA, ICP, and PXRF measured Mg concentration (mg/kg) and PXRF intensity of Mg (cps) in manure samples at different moisture level.

S.No	Mg conc M WVDA	Mg conc M ICP	Mg conc M <10	Mg conc M 10-20	Mg conc M 20-30	Mg conc M 40-50	Mg conc M 60-70	Mg Int M <10	Mg Int M 10-20	Mg Int M 20-30	Mg Int M 40-50	Mg Int M 60-70
1	6060	7264	3851	3305	2416	2410	1910	4944	4788	4486	4220	3939
2	7640	7946	4747	4205	2560	2011	1525	5352	4718	4297	4050	3875
3	4830	4998	5089	4225	2767	2041	1940	4692	4437	4241	3916	3778
4	8700	8125	7444	7135	3703	3311	1648	5398	5099	4714	4461	3838
5	3280	3140	2432	2638	1539	5567	1780	4268	4141	3794	2568	3241
6	7400	7199	6111	4978	2898	2277	1457	5344	5019	4529	4236	3679
7	8030	8093	12094	12018	12018	8694	8383	5468	5408	5408	4975	5174
8	7760	7201	3991	3132	2049	1764	1871	5159	5019	4288	4010	3926
9	4000	4299	1568	1258	1396	1357	1479	4373	4016	3797	3723	3505
10	3930	3955	1186	1230	1084	1250	1551	4766	4127	3846	3765	3732
11	5240	5374	2422	1582	1701	1751	1809	4343	4231	4244	3730	3600
12	5040	5098	1733	1560	4147	1118	1805	4412	4352	2706	3774	3917
13	6400	6370	1191	1279	2848	1374	2382	5052	4357	2939	3691	3625
14	5280	5237	2010	2045	1953	1535	1596	4780	4412	4323	3809	4450
15	4480	4911	984	1074	1184	1425	2104	4452	4089	3726	3394	3471
16	5360	5191	1449	1213	1371	1296	1408	4819	4032	3783	3207	3347
17	4720	4344	1175	1339	1329	1531	1524	4543	4259	4040	3929	3653
18	5800	5614	3358	1982	1757	1549	1591	4766	4137	4075	3650	3645
19	6490	7954	1349	1033	1418	1315	1359	5181	4307	4124	3533	3450
20	4540	4627	1563	2244	1175	1448	1344	4339	4368	3944	3940	3629
21	5100	4964	1730	1040	929	1231	1117	4602	4101	3845	3725	3734
22	5280	4991	4179	3933	2801	2452	1851	4823	4713	4388	4087	3601
23	4630	4626	2309	1740	4252	1831	1903	4472	4288	2765	3669	3568
24	4530	4386	3350	2307	1915	2174	1732	4500	4203	3958	3941	3466
25	6220	6687	2138	2209	2118	1999	1953	4930	4254	3898	3813	3559
26	6510	6817	11876	10971	5656	4547	2425	5327	5197	4625	4476	3905
27	4310	4647	4504	4106	2126	1979	1850	4342	4154	3857	3642	3445
28	4860	5850	3438	2199	8392	1644	1618	4638	4123	3031	3806	3686
29	3200	3864	2809	2080	8274	1527	1813	4329	3852	2783	3344	3348
30	4490	4454	3437	2896	2171	1973	1873	4545	4551	4014	3688	3512
31	5100	5701	5018	5155	3417	2606	2032	4361	4291	3999	3740	3491
32	5870	5969	2459	1818	1949	1912	1877	4872	4341	3890	3624	3447
33	4410	4583	3722	2237	1688	1902	1859	4442	3877	3580	3511	3363
34	1950	2106	2086	1986	1879	1442	1536	3770	3689	3659	3373	3192
35	4180	4373	2819	2766	2486	2344	1740	4812	4514	1012	3818	3464
36	5160	5529	2625	1843	2011	2054	1970	4913	4045	3872	3664	3530
37	5910	6356	3390	2451	1672	1841	1789	5471	4907	3685	3719	3734
38	5450	5799	3312	2356	2136	2190	2170	5663	4992	4638	4096	4079
39	6120	6282	2803	2120	1964	2203	1901	5758	5324	4109	4267	3943
40	3220	3673	3018	1990	1950	2051	1929	4975	4578	4051	3735	3529

Appendix 13: WVDA, ICP, and PXRF measured Fe concentration (mg/kg) and PXRF intensity of Fe (cps) in manure samples at different moisture level.

S.No	Fe conc M ICP	Fe conc M <10	Fe conc M 10-20	Fe conc M 20-30	Fe conc M 40-50	Fe conc M 60-70	Fe Int M <10	Fe Int M 10-20	Fe Int M 20-30	Fe Int M 40-50	Fe Int M 60-70
1	1575	729	716	608	594	570	204629	199720	182119	167548	158792
2	9474	3409	3378	2877	2722	2246	684205	622250	550963	525653	454190
3	3117	1895	1846	1496	1469	1125	363938	354745	312576	296401	247272
4	6683	2863	2887	2286	2139	1584	540762	535010	466099	435253	340544
5	2249	1502	1568	980	1308	620	340599	315907	242167	155525	166645
6	9209	3782	3818	3384	3106	1877	712773	718220	639304	591786	436801
7	10014	4545	4623	4623	4336	4062	736079	745370	745370	704970	693480
8	1746	799	634	591	567	494	207327	207628	191022	178812	162760
9	4471	1900	1826	1621	1567	1059	407057	404490	362386	331916	249993
10	4834	1345	1323	1263	1353	996	443992	405255	355271	355034	289573
11	2413	1334	1147	921	892	727	275830	289407	249781	220408	197364
12	3156	1455	1192	2229	1176	1379	357714	333506	273326	282494	296083
13	5282	1477	1551	2512	1529	787	483243	441419	400197	369543	280207
14	1719	469	473	488	539	567	207650	219622	216896	180938	219821
15	5669	1981	1677	1740	1668	1016	497394	424488	407390	378730	296780
16	5866	1953	1556	1456	1547	1433	500456	402174	368808	311076	297624
17	4459	1531	1232	1287	1368	1233	429081	400728	364008	354828	307076
18	5853	3223	2628	2303	2134	1946	615148	508512	481040	436805	401263
19	6738	2078	1662	1739	1555	1498	549465	452439	436972	375441	353004
20	9466	4765	4890	4378	3874	2653	815766	841613	762583	700513	539896
21	8616	4485	4136	3626	3214	3004	843992	787400	694184	618821	576157
22	6238	2573	2597	2245	2082	1343	546190	524217	464204	422301	307562
23	1423	847	684	1316	584	438	228942	234364	185606	176996	147352
24	5229	2894	2617	2227	2113	1409	538524	516644	451033	424109	314709
25	2300	497	432	475	525	504	248748	218536	196220	191666	164339
26	5203	2179	2206	1774	1698	1245	392206	393020	342139	337399	277424
27	4208	2750	2691	2063	1763	1340	458786	461847	389384	343229	279499
28	4834	2460	2041	3683	1665	1427	468194	417614	402915	353962	306639
29	3707	1736	1796	2886	1306	1025	416295	353740	296836	274135	228091
30	3337	1648	1578	1288	1170	713	316531	348662	286171	255034	191680
31	2806	1985	1911	1672	1340	1045	175793	339444	312878	263639	222097
32	1121	417	326	286	317	344	156215	157541	134716	128290	121735
33	1070	720	599	481	410	368	182646	156623	138833	126126	117301
34	3068	2057	1814	1894	1379	913	387626	343046	361701	280149	214217
35	9315	3894	3884	3380	3070	2083	906601	793296	663310	568512	449098
36	776	316	392	361	360	290	134135	135720	122053	115265	102751
37	1800	651	631	411	561	482	208609	205943	136489	153533	148934
38	1012	282	276	257	327	259	136897	139678	128556	119676	110849
39	877	96	61	82	170	162	110710	114511	88804	102480	98766
40	616	202	176	199	210	187	114772	116677	106066	97889	94335

Appendix 14: WVDA and ICP measured Cu concentration (mg/kg) and PXRF intensity of Cu (cps) in manure samples at different moisture level.

S.No	Cu conc M WVDA	Cu conc M ICP	Cu Int M <10	Cu Int M 10-20	Cu Int M 20-30	Cu Int M 40-50	Cu Int M 60-70
1	9	107	53744	53251	58947	63608	65158
2	1350	1439	132730	117481	118756	118132	110464
3	2440	2630	288056	283471	254000	241158	209562
4	1740	1674	163137	147275	140209	136656	136994
5	85	76	58548	55000	65155	64486	65286
6	1530	1524	141672	135928	130638	126710	118259
7	1380	1389	123556	127621	127621	121866	123310
8	269	255	70598	66323	72776	74088	75988
9	37	51	46828	48727	53073	56743	62082
10	71	66	46337	42354	49460	53877	62029
11	42	51	53016	50430	60090	63979	64969
12	43	44	49633	47554	56164	60959	60873
13	69	72	43489	42386	45406	49599	58060
14	79	87	51130	53388	53964	61973	58021
15	11	106	44315	39241	45447	51679	60633
16	14	113	47053	39874	46307	45873	52684
17	76	74	46204	43955	52787	56312	59883
18	39	53	44197	38298	50178	53193	55783
19	57	64	43269	38378	45860	45102	48972
20	84	87	43431	43834	47529	50654	56472
21	95	97	45450	43424	50751	54561	56211
22	2110	1972	224137	224744	208172	199104	168375
23	544	521	111842	106919	103074	103690	95663
24	403	367	76713	71942	78416	79021	78462
25	155	148	56791	51195	58955	63256	67934
26	577	602	74239	72203	75556	75071	75718
27	822	917	140169	134108	127898	123289	113323
28	436	515	89434	85459	85273	88489	87705
29	1730	1401	151301	204721	187903	177340	168097
30	344	381	77406	78711	80161	79236	80379
31	59	68	54413	54613	58708	62627	66382
32	60	54	51984	50040	56144	61030	65350
33	123	125	62954	56139	65549	69441	71488
34	38	47	53371	56715	56645	62418	67391
35	63	75	47789	47326	54214	54542	62221
36	505	513	106165	95674	95059	95327	96711
37	486	524	97654	88433	69891	80455	95451
38	598	620	109336	100526	98366	98552	103562
39	861	879	115533	103984	81987	105209	105228
40	277	312	84779	76750	78524	77890	82943

Appendix 15: ICP measured concentration (mg/kg) and PXRF intensity of Zn (cps) in manure samples at different moisture level.

S.No	Zn conc M ICP	Zn Int M <10	Zn Int M 10-20	Zn Int M 20-30	Zn Int M 40-50	Zn Int M 60-70
1	278	64970	63957	68638	72456	73419
2	359	55734	50861	56793	58780	61320
3	342	70966	69813	75425	75559	76395
4	347	57354	56357	60146	62666	65556
5	146	62014	59490	69258	67960	67665
6	324	54450	53915	56530	59383	65255
7	327	52412	53236	53236	53439	54656
8	340	72984	69982	75680	77420	80088
9	127	50070	52568	56322	60165	65169
10	146	49795	46024	52337	56868	64797
11	164	60815	57776	66964	70057	70805
12	113	52903	50414	58401	62980	62717
13	154	47214	45954	49012	52299	60863
14	95	49258	51206	52170	61223	56547
15	137	44198	39717	46597	52428	61386
16	166	48303	41079	47909	46634	53812
17	129	48111	45638	54615	57597	61449
18	122	46903	39820	52339	54777	58819
19	201	49700	43119	51238	48605	53192
20	148	44251	44592	48679	51829	57886
21	123	44266	42788	50215	54098	55637
22	266	59820	59912	63915	65646	69948
23	238	71153	69415	72972	77040	78027
24	210	56311	55014	63181	65011	67719
25	315	63715	58293	63919	68395	72721
26	224	45597	45501	51482	52984	58074
27	219	63555	62486	68657	69882	71966
28	210	57079	58774	58828	66408	68409
29	244	61555	58977	65458	69829	73539
30	230	60599	62712	66880	68061	73485
31	241	67231	67887	70678	71993	74963
32	211	65172	61704	65627	69661	73664
33	213	67494	60546	69394	73368	74952
34	119	58168	60018	60597	66211	70589
35	305	63025	61687	66918	66364	70601
36	370	82011	78559	83881	85365	84256
37	454	84028	77872	63561	74400	86496
38	430	82428	80610	80654	81517	86143
39	586	81972	79746	66622	84489	84337
40	255	72622	73218	75050	74379	76372

Appendix 16: CVAS, ICP and PXRF measured concentration (mg/kg) and PXRF intensity of P (cps) in forage samples at two different particle size.

S.No	P conc F CVAS	P conc F ICP	P conc FNP <106mm	P conc FNP 0.25-0.5mm	P conc FNP 1-2mm	P Int FNP <106mm	P Int FNP 0.25-0.5mm	P Int FNP 1-2mm
1	2800	2425	3642	2494	2289	21635	13115	211936
2	2800	2131	3560	2366	2024	20499	13088	208467
3	2800	2169	2952	2021	1852	20586	14051	201958
4	2200	1782	2833	2158	1870	17046	12635	227925
5	2300	2168	2796	2198	1669	18997	14700	242854
6	2700	2304	3333	2360	1962	20391	13871	244286
7	1800	1682	2599	1955	1734	16325	11617	206628
8	1900	1635	2504	1752	1490	16204	11167	200477
9	3200	2510	3298	2259	1756	22840	15015	182146
10	3900	3166	2931	2069	1964	22840	18051	302758
11	2900	2173	2864	1738	1516	22094	13671	256105
12	2300	1822	2657	1580	1460	20845	12331	202641
13	2000	1701	2790	1834	1466	19893	12996	183452
14	2500	2095	2562	1813	1564	18652	12153	187388
15	2900	2508	3148	2412	1962	20453	14154	199028
16	2900	2433	3407	2588	2519	19411	13584	204275
17	3300	2659	3546	2895	2273	20806	15678	191363
18	3000	2359	2781	1937	1935	20208	13192	165944
19	2400	1989	2781	1974	1395	18819	12439	166238
20	2000	1732	2560	1903	1921	16307	11379	233889
21	2900	2199	2562	1794	1449	19426	13972	271868
22	3400	3023	3967	3043	2191	21877	15876	185687
23	2900	2556	3256	2311	1852	21673	15172	245968
24	1600	1614	2495	1835	1541	15838	11282	186014
25	1800	1759	2709	1860	1689	16774	11561	182637
26	2300	2278	3918	2761	1727	21435	14830	216393
27	1800	1675	2594	1905	1656	15831	11421	196378
28	1800	1855	2448	1892	1416	16171	12311	165173
29	1900	1799	2486	1931	1727	15888	12023	215313
30	2000	1830	2596	2126	1512	16328	13078	186344
31		3302	4350	3774	3037	23356	19092	265067
32		3189	4392	3594	3492	23267	18353	345583
33		3353	4386	3383	3093	23420	17469	310497
34		3485	5074	3725	3506	26279	18106	194411
35		1700	2273	1650	1321	16614	12110	167543
36		1694	2381	1467	1531	17104	11577	170205
37		2507	3209	2223	1872	22065	14842	246486
38		2116	2742	1959	1746	18814	13129	202907
39		985	2387	1758	1313	13349	9422	135802
40		1512	2036	1453	1242	14682	10876	139153
41		1099	2280	1747	1511	12994	9689	155700
42		984	2246	1632	1345	12520	8866	164184

Appendix 17: CVAS, ICP, and PXRF measured concentration (mg/kg) and PXRF intensity of K (cps) in forage samples at two different particle size.

S.No	K conc F CVAS	K conc F ICP	K conc FNP 0.25-0.5mm	K conc FNP 1-2mm	K Int FNP 0.25-0.5mm	K Int FNP 1-2mm
1	21500	16723	17706	14018	273387	211936
2	19900	15400	16112	13330	257162	208467
3	24600	16897	15758	12317	275331	201958
4	23300	16820	18125	14405	292989	227925
5	25700	20283	18866	14772	329703	242854
6	26400	20727	20157	15623	326494	244286
7	20000	15623	15781	13156	259022	206628
8	22200	16720	15834	12607	266398	200477
9	20700	16730	14959	11242	261263	182146
10	33600	24840	18260	16698	366089	302758
11	25200	18850	15468	14867	286262	256105
12	23200	16263	14839	12157	270022	202641
13	17800	13880	12483	11143	223672	183452
14	20500	15850	13702	11533	237474	187388
15	19800	15733	15024	12431	246004	199028
16	19600	13440	13910	13233	221635	204275
17	21100	14707	14981	12327	243659	191363
18	17600	13367	11453	9840	202151	165944
19	18000	13897	12877	10271	219733	166238
20	23900	18370	16795	15035	272946	233889
21	28800	23050	17686	15410	324568	271868
22	22800	20337	18085	12363	281140	185687
23	28600	24777	21030	15513	363098	245968
24	17800	16370	15268	11614	253141	186014
25	18300	14853	14200	11533	236787	182637
26	17800	15133	15674	13716	254183	216393
27	20900	16553	15547	12837	251644	196378
28	16300	13020	12694	10307	219291	165173
29	20900	16860	15391	13716	257590	215313
30	19900	16473	15484	11893	259674	186344
31		23577	22825	18689	352799	265067
32		27467	25938	24051	393486	345583
33		27173	24107	21961	366715	310497
34		12663	12529	12643	201605	194411
35		16058	13237	10060	233928	167543
36		13420	11261	9853	202338	170205
37		19490	15731	14784	273200	246486
38		16650	14061	12374	241552	202907
39		13540	10772	8219	178248	135802
40		14790	11992	8651	211552	139153
41		11540	13019	9684	209807	155700
42		12283	12414	10246	199282	164184

Appendix 18: CVAS, ICP, and PXRF measured concentration (mg/kg) and PXRF intensity of Ca (cps) in forage samples at two different particle size.

S.No	Ca conc F CVAS	Ca conc F ICP	Ca conc FNP 0.25-0.5mm	Ca conc FNP 1-2mm	Ca Int FNP 0.25-0.5mm	Ca Int FNP 1-2mm
1	5600	4302	4960	4711	110870	90026
2	5900	4687	6057	4564	129797	88931
3	6900	5938	6182	4730	153130	96113
4	5900	4315	4949	4016	119481	85156
5	4000	3824	4136	2790	117682	67629
6	4000	3559	3920	2573	107680	62949
7	4100	4128	5560	3419	123636	69001
8	3500	2905	3821	2846	93672	58009
9	3200	2660	3439	3257	87405	62226
10	4200	3107	2616	2444	95828	75733
11	6700	4902	4600	2974	125599	77352
12	6000	4588	4361	3432	114566	74203
13	5900	4959	5819	4068	133791	81387
14	5300	4181	4813	3888	110593	75221
15	4300	4006	5030	4165	111956	82440
16	4500	3776	5784	5007	114372	94306
17	5100	4287	5753	4378	121740	80513
18	4700	3601	4666	4079	101387	76169
19	4800	3749	5005	3102	107995	56175
20	4200	3189	3942	3589	95739	78585
21	4700	3155	2885	2286	91319	64765
22	5100	4430	5445	3925	122271	70285
23	3400	3242	2749	2584	94300	63705
24	4800	4685	5627	4601	125201	85420
25	4800	4718	5817	4893	126354	91153
26	7400	6760	8763	4307	178056	110835
27	4800	4291	5454	4191	120290	80193
28	5000	5065	6358	4187	137589	76905
29	4700	4246	5407	4307	123329	87509
30	4900	4745	5684	3568	129690	68134
31		8853	9484	5150	209629	106102
32		8997	9220	6318	214304	146706
33		7235	7478	5478	177670	123930
34		8431	12715	10655	226705	185321
35		5596	6319	5162	148697	99785
36		4082	4906	4546	112611	92434
37		5180	8070	6405	191957	146893
38		3745	6327	4964	145051	101847
39		6643	9328	5935	162068	91067
40		4627	5044	3993	114083	67412
41		4944	7072	4695	133598	76052
42		4753	7375	3890	133452	66265

Appendix 19: CVAS, ICP, and PXRF measured concentration (mg/kg) and PXRF intensity of Mg (cps) in forage samples at two different particle size.

S.No	Mg conc F CVAS	Mg conc F ICP	Mg conc FNP 0.25-0.5mm	Mg conc FNP 1-2mm	Mg Int FNP 0.25-0.5mm	Mg Int FNP 1-2mm
1	2100	1651	2151	2288	2945	2777
2	3000	2244	2361	2392	3094	2732
3	2400	2268	2744	2717	3374	2863
4	2200	1800	2359	2258	3352	2988
5	2300	2067	2432	2551	3423	3001
6	2300	2000	2299	2489	3299	2868
7	2300	2063	2234	2449	3179	2806
8	2100	1727	2354	2620	3188	2624
9	1700	1333	2679	2813	3259	2657
10	1500	1154	3322	3225	3667	3270
11	2300	1787	3156	3128	3377	3163
12	2400	1789	2978	3336	3357	2854
13	2300	1834	2850	2953	3265	2873
14	2300	1826	2632	2594	3186	2948
15	2000	1650	2364	2571	3067	2844
16	2300	1741	2214	2221	3110	2842
17	2100	1969	2294	2339	3249	2734
18	2100	1563	2866	2879	3175	2832
19	2300	1732	2590	3015	3125	2592
20	1800	1390	2285	2361	3234	2979
21	1900	1407	2950	3278	3377	3069
22	1800	1557	2196	2190	3328	2675
23	1500	1316	2362	2503	3385	2911
24	1800	1483	2383	2458	3160	2768
25	1700	1524	2325	2482	3212	2805
26	2200	1888	2318	2345	3348	2815
27	1800	1359	2281	2306	3212	2851
28	1700	1458	2504	2808	3124	2731
29	1500	1283	2319	2345	3232	2809
30	1500	1302	2295	2619	3263	2636
31		2186	2597	2219	3561	2848
32		2233	2366	2320	3578	3221
33		1765	2184	1961	3451	2921
34		2364	2539	2432	3261	3112
35		2096	2644	2949	3355	2814
36		1703	3084	3030	3264	3024
37		2332	2420	2599	3572	3224
38		2594	2490	2574	3436	3046
39		1704	2225	2253	2992	2639
40		1656	2749	2894	3304	2658
41		1446	2296	2253	3079	2652
42		1275	2343	2319	3047	2740

Appendix 20: CVAS and ICP measured concentration (mg/kg) and PXRF intensity of Fe (cps) in forage samples at two different particle size.

S.No	Fe conc F CVAS	Fe conc F ICP	Fe Int FNP 0.25-0.5mm	Fe Int FNP 1-2mm
1	283	113	65320	51152
2	576	104	63036	66171
3	236	112	65819	53312
4	217	186	80607	69811
5	239	118	66204	51894
6	149	86	59606	46895
7	276	120	68028	52737
8	6104	113	67860	52558
9	102	51	56003	46780
10	788	240	82095	67623
11	240	59	57442	49411
12	126	62	56026	47853
13	165	87	63249	54049
14	87	45	54911	47926
15	90	71	60195	52515
16	203	178	82919	62819
17	100	79	62328	48003
18	90	44	55189	45000
19	79	46	56756	43884
20	268	134	62852	54851
21	875	114	65803	52212
22	445	190	82411	55520
23	908	154	69700	50332
24	238	110	68635	52086
25	254	323	97994	62237
26	282	311	101008	68266
27	188	166	76706	55725
28	293	239	93453	59077
29	318	223	88160	65121
30	178	251	97606	57390
31		120	67981	51235
32		271	88333	61741
33		370	97038	65144
34		200	84193	68863
35		384	109316	60137
36		448	111440	94997
37		289	90499	65129
38		295	94567	66608
39		273	99100	69364
40		336	106777	64295
41		167	82599	61545
42		131	77365	63675

Appendix 21: CVAS and ICP measured concentration (mg/kg) and PXRF intensity of Cu (cps) in forage samples at two different particle size.

S.No	Cu conc F CVAS (mg/kg)	Cu conc F ICP (mg/kg)	Cu Int FNP 0.25-0.5mm	Cu Int FNP 1-2mm
1	7	8	51279	40117
2	9	10	51527	40689
3	9	10	51219	42817
4	8	12	52075	47429
5	6	11	52086	44598
6	7	9	50713	39209
7	7	9	52743	44221
8	8	9	51995	41443
9	6	9	51409	40842
10	12	11	48755	40882
11	10	9	49693	49081
12	8	10	50530	39883
13	7	11	51494	41205
14	7	8	51302	45558
15	5	9	53571	43581
16	6	8	54052	46978
17	6	9	54727	42807
18	5	7	51546	40157
19	6	7	52646	38447
20	7	9	52539	44036
21	8	11	50002	42187
22	11	14	51173	39795
23	11	13	49588	38017
24	6	12	54293	43042
25	6	13	54771	42966
26	7	14	55372	45208
27	6	11	52946	45145
28	5	13	55425	45298
29	7	17	54063	44918
30	6	15	54551	42847
31		15	54018	42712
32		17	52672	42223
33		12	52020	41717
34		12	55500	46936
35		15	53016	38720
36		14	53490	46448
37		12	53029	46025
38		13	54134	44427
39		9	56674	50315
40		11	53471	37291
41		12	57833	50362
42		8	57689	50967

Appendix 22: CVAS and ICP measured concentration (mg/kg) and PXRF intensity of Zn (cps) in forage samples at two different particle size.

S.No	Zn conc F CVAS	Zn conc F ICP	Zn Int FNP 0.25-0.5mm	Zn Int FNP 1-2mm
1	19	27	50354	38418
2	26	33	51178	38087
3	23	34	51301	41737
4	20	28	51542	45596
5	18	31	52376	42878
6	18	29	50734	36913
7	17	25	53377	42561
8	16	23	52616	39533
9	21	29	51444	39195
10	27	38	48640	39725
11	24	28	49393	49585
12	20	34	49675	38611
13	20	40	51710	39907
14	18	34	50990	44457
15	17	35	53883	41866
16	17	34	54034	45887
17	20	201	67682	42877
18	22	28	51662	38420
19	16	27	52098	36818
20	30	37	52685	42914
21	28	42	49950	40597
22	29	46	51531	38811
23	29	44	49991	36148
24	22	35	53854	42097
25	19	38	54982	41628
26	22	42	55682	43827
27	22	39	52865	44234
28	18	41	55477	43318
29	27	35	53886	43406
30	21	40	54736	41480
31		46	54651	41201
32		49	53230	41782
33		43	52011	41300
34		41	56372	47501
35		48	53585	37443
36		49	53783	45895
37		51	53901	45365
38		51	55297	43577
39		40	57504	49634
40		48	54848	36678
41		47	58073	49454
42		41	57868	50442

Appendix 23: R codes for Moisture analysis in manure samples for Phosphorus

```
#Moisture 1: <10%
P.Manure.I<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P Int M <10`)
summary(P.Manure.I)
qplot(P_moisture_manure$`P Int M <10`, P_moisture_manure$`P conc M ICP`, xlab = "P Intensity (cps)",
ylab = "ICP measured P (mg/kg)", xlim=
        c(15000, 80000)) + theme_bw() + geom_smooth(method="lm")
P.Manure.C<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P conc M <10`)
summary(P.Manure.C)
qplot(P_moisture_manure$`P conc M <10`, P_moisture_manure$`P conc M ICP`, xlab = "PXRF measured
P (mg/kg)", ylab = "ICP measured P (mg/kg)", xlim=
        c(2000, 11000)) + theme_bw() + geom_smooth(method="lm")
t.test(P_moisture_manure$`P conc M <10`, P_moisture_manure$`P conc M ICP`)

#Moisture 2: 10-20%

P.Manure.I2<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P Int M 10-20`)
summary(P.Manure.I2)
qplot(P_moisture_manure$`P Int M 10-20`, P_moisture_manure$`P conc M ICP`, xlab = "P Intensity
(cps)", ylab = "ICP measured P (mg/kg)", xlim=
        c(15000, 70000)) + theme_bw() + geom_smooth(method="lm")
P.Manure.C2<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P conc M 10-20`)
summary(P.Manure.C2)
qplot(P_moisture_manure$`P conc M 10-20`, P_moisture_manure$`P conc M ICP`, xlab = "PXRF
measured P (mg/kg)", ylab = "ICP measured P (mg/kg)", xlim=
        c(2000, 10000)) + theme_bw() + geom_smooth(method="lm")
t.test(P_moisture_manure$`P conc M 10-20`, P_moisture_manure$`P conc M ICP`)

#Moisture: 3:20-30%
P.Manure.I3<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P Int M 20-30`)
summary(P.Manure.I3)
qplot(P_moisture_manure$`P Int M 20-30`, P_moisture_manure$`P conc M ICP`, xlab = "P Intensity
(cps)", ylab = "ICP measured P (mg/kg)", xlim=
        c(5000, 40000)) + theme_bw() + geom_smooth(method="lm")
P.Manure.C3<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P conc M 20-30`)
summary(P.Manure.C3)
qplot(P_moisture_manure$`P conc M 20-30`, P_moisture_manure$`P conc M ICP`, xlab = "PXRF
measured P (mg/kg)", ylab = "ICP measured P (mg/kg)", xlim=
        c(2000, 9000)) + theme_bw() + geom_smooth(method="lm")
t.test(P_moisture_manure$`P conc M 20-30`, P_moisture_manure$`P conc M ICP`)

# Moisture 4: 40-50%
P.Manure.I4<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P Int M 40-50`)
summary(P.Manure.I4)
```

```
qplot(P_moisture_manure$`P Int M 40-50`, P_moisture_manure$`P conc M ICP`, xlab = "P Intensity
(cps)", ylab = "ICP measured P (mg/kg)", xlim=
      c(5000, 45000)) + theme_bw() + geom_smooth(method="lm")
P.Manure.C4<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P conc M 40-50`)
summary(P.Manure.C4)
qplot(P_moisture_manure$`P conc M 40-50`, P_moisture_manure$`P conc M ICP`, xlab = "PXRF
measured P (mg/kg)", ylab = "ICP measured P (mg/kg)", xlim=
      c(2000, 8000)) + theme_bw() + geom_smooth(method="lm")
t.test(P_moisture_manure$`P conc M 40-50`, P_moisture_manure$`P conc M ICP`)

#Moisture 5: 60-70%
P.Manure.I5<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P Int M 60-70`)
summary(P.Manure.I5)
qplot(P_moisture_manure$`P Int M 60-70`, P_moisture_manure$`P conc M ICP`, xlab = "P Intensity
(cps)", ylab = "ICP measured P (mg/kg)") + theme_bw() + geom_smooth(method="lm")
P.Manure.C5<- lm(P_moisture_manure$`P conc M ICP`~ P_moisture_manure$`P conc M 60-70`)
summary(P.Manure.C5)
qplot(P_moisture_manure$`P conc M 60-70`, P_moisture_manure$`P conc M ICP`, xlab = "PXRF
measured P (mg/kg)", ylab = "ICP measured P (mg/kg)") + theme_bw() + geom_smooth(method="lm")
t.test(P_moisture_manure$`P conc M 60-70`, P_moisture_manure$`P conc M ICP`)

##t-test
t.test(P_moisture_manure$`P conc M <10`, P_moisture_manure$`P conc M 10-20`)
t.test(P_moisture_manure$`P conc M 10-20`, P_moisture_manure$`P conc M 20-30`)
t.test(P_moisture_manure$`P conc M 20-30`, P_moisture_manure$`P conc M 40-50`)
t.test(P_moisture_manure$`P conc M 40-50`, P_moisture_manure$`P conc M 60-70`)

## Regression between P and moisture percentage
P.R.I<- lm(Moistue_Regression$P ~ Moistue_Regression$Moisture)
summary(P.R.I)
qplot(Moistue_Regression$Moisture, Moistue_Regression$P, xlab = "Moisture (%)", ylab = "P Intensity
(cps)", xlim=
      c(0, 80)) + theme_bw() + geom_smooth(method="lm")
```

Appendix 24: R codes for particle size analysis in forage samples for Phosphorus

#Intensity

```
P.F.Np.I2<- lm(P_forage_new_size$`P conc F ICP`~ P_forage_new_size$`P Int FNP 0.25-0.5mm`)
summary(P.F.Np.I2)
P.F.Np.I3<- lm(P_forage_new_size$`P conc F ICP`~ P_forage_new_size$`P Int FNP 1-2mm`)
summary(P.F.Np.I3)
qplot(P_forage_new_size$`P Int FNP 0.25-0.5mm`, P_forage_new_size$`P conc F ICP`, xlab = "P Intensity
(cps)", ylab = "ICP measured P (mg/kg)", xlim=
          c(7000, 20000)) + theme_bw() + geom_smooth(method="lm")
qplot(P_forage_new_size$`P Int FNP 1-2mm`, P_forage_new_size$`P conc F ICP`, xlab = "P Intensity
(cps)", ylab = "ICP measured P (mg/kg)", xlim=
       c(7000, 20000)) + theme_bw() + geom_smooth(method="lm")
```

#Concentration

```
P.F.Np.C2<- lm(P_forage_new_size$`P conc F ICP` ~ P_forage_new_size$`P conc FNP 0.25-0.5mm`)
summary(P.F.Np.C2)
P.F.Np.C3<- lm(P_forage_new_size$`P conc F ICP` ~ P_forage_new_size$`P conc FNP 1-2mm`)
summary(P.F.Np.C3)
qplot(P_forage_new_size$`P conc FNP 0.25-0.5mm`, P_forage_new_size$`P conc F ICP`, xlab = "PXRF
measured P (mg/kg)", ylab = "ICP measured P (mg/kg)", xlim=
       c(1000, 4000)) + theme_bw() + geom_smooth(method="lm")
qplot(P_forage_new_size$`P conc FNP 1-2mm`, P_forage_new_size$`P conc F ICP`, xlab = " PXRF
measured P (mg/kg)", ylab = "ICP measured P (mg/kg)", xlim=
       c(1000, 4000)) + theme_bw() + geom_smooth(method="lm")
t.test(P_forage_new_size$`P conc F ICP`, P_forage_new_size$`P conc FNP 0.25-0.5mm`, paired=TRUE)
```