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# Instantaneous accounting for leaf water in X-ray fluorescence spectra of corn grown in manure- and fertilizer-amended soils

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## ABSTRACT

Manure-amended soils have shown a high degree of temporal and geographic variability in labile phosphorus (P). Real time information and practical ways for determining foliar composition are needed to obtain direct evidence of plant uptake, and the supplying capacity of the soil. Field and laboratory experiments were conducted to determine leaf water content effects on spectral characteristics of corn (*Zea mays*) uppermost leaves when grown in mineral P fertilizers- (0–250 mg P $kg^{-1}$) or manure-amended soils (0, 15, and 30 kg P $ha^{-1}$). *In situ* X-ray fluorescence scans of fresh green leaves yielded foliar P concentrations that paralleled those obtained using oven-dried harvested leaf samples. Leaf structure and size of seedlings can cause significant variations in leaf-to-spectrometer contact, inter-element interference, and leaf water content and its attenuating effects on the yield of weak fluorescence radiation of low Z-elements (<19). Intensities of scattered radiation associated with the X-ray tube anode were significantly correlated with leaf water content ($\theta_w$), which was used to (i) assess crop water status and (ii) normalize fluorescence intensities of P to a common basis. The $\theta_w - P$ reduced concentration relationship was best-described by a sigmoidal function

$$y = 0.12 + 0.94/(1 + \exp(-(\theta_w - 0.37)/-0.17))$$

with $r^2 = 0.938$ and RMSE = 0.02. Therefore, we proposed its use to obtain P concentration on a dry weight basis and unbiased estimates of crop P status. The *in situ* fluorescence sensing system presents a new paradigm in nutrient management to insure the sustainability of agroecosystems and development of field-specific nutrient management practices.

## 1. Introduction

Soils repeatedly treated with P-enriched manure were observed to have high P levels in regions of intensive animal agriculture of the US and Western Europe (Koopmans et al., 2003; European Parliament European Council, 2008; Pew Commission on Industrial Farm Animal Production, 2008; Dao and Schwartz, 2011). Current field-scale nutrient management practices also have been observed to introduce significant variability in soil P distribution in the near surface zone of amended soils (Dao et al., 2011; Dao, 2014). The heterogeneity persisted over seasons in conservation production systems, in addition to being temporally dynamic within and across growing seasons (Magid and Nielsen, 1992; Schwartz et al., 2011). Distinct clusters of phosphate- and enzyme-labile organic P were observed within manure treatments of seemingly uniform fields. The geographic variability highlighted the critical need for large scale time-sensitive sensing methods for assessing actual nutrient status, in particular, non-mobile ones in agricultural fields. Increased measurement density across a production field would improve knowledge of site-specific nutritional conditions (Dao et al., 2011).

Typical methods used for assessing P availability in soils and manure, and other organic by-products include plant uptake in controlled environments and the field, incubation and analysis using various extractants, and sequential extractions that partition P into various fractions of varying solubility (Hedley et al., 1982; Brookes et al., 1983; Schwartz and Dao, 2005). In addition, nutrient composition of aboveground biomass or plant parts, frequently leaves, have been used to deduce nutrient availability and status in a field soil, and that changes in the nutrient supply in the soil are reflected in leaves of a plant growing in it (Bould, 1968; Sumner, 1977; Robinson, 1980; Lucena, 1997). Compositional results served as guides for determining amounts of supplemental nutrients in the form of commercial fertilizers and/or organic byproducts. Foliar

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*Abbreviations:* XRFS, X-ray fluorescence spectrometry; Leaf N, uppermost true leaf; Leaf (N+1), next upper leaf to the uppermost true leaf; Z, atomic number.

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composition was often observed to be temporally dynamic and varied widely between plant parts, and specific plant developmental stages or a period of plant growth (Jones, 1970; Sumner, 1977; Barry and Miller, 1989). A critical need exists for a rapid spectroscopic method or a suitable analytical sensor that preferably yields element-specific results in real time. In particular, there is a critical need to re-assess the effects of elevated P levels on plant nutrient use efficiency in many agricultural fields amended with animal manure or other organic nutrient sources. In the short-term, remedial best-nutrient management practices have not brought about the expected improvements in the water quality of impounded water bodies, and streams in watersheds with a high concentration of confined animal feeding operations (Meals et al., 2010).

Trace elements in plant materials, metal-contaminated soils, geological materials, or mineral nutrients in manure solids and soil samples from nutrient-enriched fields have been measured using X-ray fluorescence spectrometry (XRFS) (Queralt et al., 2005; Morgenstern et al., 2010; Chuparinaa and Martynov, 2011; Dao et al., 2011; Paltridge et al., 2012). The multi-element XRFS analytical technique is based on spectral measurements of X-rays of characteristic wavelengths emitted from a sample following irradiation with monochromatic incident X-rays, hence, fluorescence spectrometry (Dziunikowski, 1989). A frequency distribution of energy-proportional signals is generated in the energy-dispersive mode. Elements of high atomic number ($Z$) excited by the incident X-rays, emit fluorescence radiation of short wavelength and high-energy while light elements of low Z (i.e., Na, Mg, Al, P, S, K, etc.) emit fluorescence radiation of long wavelength and low-energy. Recent advances in detector design, sample-detector geometry, filters, and the polarization of incident radiation have greatly improved the detection of light elements ($Z < 19$) that include many plant macronutrients by energy-dispersive XRFS. Although a large number of published studies have addressed micronutrient composition of medicinal plants or crop plants grown on metal-contaminated soils, few studies were focused on macronutrients in crop plants or plant-based materials (Dao and Zhang, 2007; Morgenstern et al., 2010; Chuparinaa and Martynov, 2011). In addition, compositional studies involving hand-held XRF spectrometers were scarce. This trend was related to the fact that many hand-held spectrometers, while having superior detection capability for high-energy fluorescence radiation, had a rather poor analytical sensitivity for the weak-energy fluorescence radiation emitted by low-Z elements. As such, XRFS has not been included and used as a sensing technology for precision crop nutrient management in recent reviews of proximal sensing technologies (Adamchuk et al., 2004; Lee et al., 2010).

To optimize the accuracy of the spectral data of foliar scans for P and other elements of low Z, estimates of mass attenuation can be used to correct for variations in sample matrices, composition, and water content of leaf samples. Empirically, the relationship between the intensities of Compton and Rayleigh scattered radiation and mass absorption have been used to correct for matrix effects in a wide range of geological and environmental samples (Harvey and Atkin, 1982; Liihrmann et al., 1986; Rousseau, 2006). Matrix corrections are necessary when samples are of sufficient thickness to absorb significant fractions of the fluorescent radiation, and when the sample matrix is appreciably different from those of standards used for calibration (Nielson, 1977). To optimize the accuracy of measurements of elements of low Z, such as P, this study was conducted to improve the accuracy of light element determination and derive improved computational and spectral correction procedures for the analysis of fresh moist samples and/or inferring crop water status. In addition, we evaluate the robustness of hand-held XRF spectrometry for detecting foliar compositional changes of corn to added P. Our objective was to derive a system of foliar *in situ* analyses to determine soil P availability and plant uptake because of the minimal sample processing and a high analytical throughput of XRFS. The calibration of foliar nutrient profile during early vegetative growth stages was hypothesized to be invaluable in the diagnosis of nutritional deficiency and forestalling crop suboptimal development, productivity, and potential yield loss. These calibrations would be just as useful in detecting elevated P concentrations in field zones that present risks of offsite losses and water quality impairments.

## 2. Materials and methods

### 2.1. Field studies

Two field studies of the effects of application rates of mineral fertilizer and dairy manure on the uptake of $PO_4$-P during early growth stages were implemented at the Beltsville Agricultural Research Center (39.03°N, 76.85°W). In an on-going study of the long-term effects of conservation production systems and rates of application of dairy manure, the liquid slurry was applied at 0, 15, and 30 kg P $ha^{-1}$ with a 20,000-L liquid tanker-spreader in mid-April. A corn (*Zea mays* L.) crop was planted annually about mid-May since 2005, with an intervening cover crop of winter wheat (*Triticum aestivum* L.) planted in late October. The wheat crop was terminated in mid-April with contact herbicides. Foliar P concentrations of the growing seedlings were determined by scanning the three uppermost leaves using a hand-held energy-dispersive XRF spectrometer (Model XLt3, Thermo Scientific, Niton, Billerica, MA, USA) (see Section 6). A total of 75 geo-referenced scans were made in each P rate treatment to obtain spectral data based on a grid pattern along transects that cut across land contours and manure rate treatments.

In a second field trial, twenty mini-lysimeters made from open-ended HDPE cylinders (50 cm O.D.) were installed in a field following the planting of a no-till corn crop at a stand density of 74,000 plants $ha^{-1}$ in late April of 2011. Each mini-lysimeter was amended with one of five P application rates. Four P rates (0, 56, 224, and 560 kg $ha^{-1}$, or between 0 and 250 mg $kg^{-1}$) were added to the soil surface of the mini-lysimeters as $KH_2PO_4$. A single rate of $Fe^{3+}$ amendment (84 kg $ha^{-1}$ or 150 mg $Fe^{3+}$ $kg^{-1}$) was applied as the 5th P treatment to reduce initial soil available P. Whole plants and the upper three leaves were scanned *in situ* in the field, beginning with V1–V2 stages of corn growth.

### 2.2. Effects of water content in drying leaves

A batch harvest of 12 flag leaves were harvested and on-going drying at 45 °C was interrupted at regular intervals (every 15 min, up to 2 h (h), then every h thereafter, up to 8 h and again during the next day) for X-ray fluorescence scanning. A last complete drying was done at 65 °C for 24 h and fluorescence scans were again obtained to determine intensities of P and Ag characteristic lines.

### 2.3. Spectroscopic foliar analyses

The XLt3 energy-dispersive XRF spectrometer was used to obtain plant compositional data in the field and in the laboratory. The spectrometer was equipped with a 50 kV end window miniaturized X-ray tube that was optimized for analyzing elements of low Z with helium (He) gas purging the X-ray analysis path. A silicon drifted detector, equipped with an Ag anode, was thermoelectrically cooled and attained a spectral resolution of <190 eV for the Mn-Kα line at full width half-maximum. Primary beam filters modified the source radiation for detection of specific Z- ranges of elements, improved fluorescence yield, and, along with the He purge improved the detection limits for elements of low Z (or “light” elements), in particular P.