- PXRF was used to scan different vegetation types at variable moisture
- PXRF accurately quantified Pb, Fe, Cd, and Cu for dry, powder samples
- PXRF reasonably determined Zn and Cu for field moist samples

Elemental Assessment of Vegetation via Portable X-ray Fluorescence (PXRF) Spectrometry

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ABSTRACT

Elemental concentrations in vegetation are of critical importance, whether establishing plant essential element concentrations (toxicity vs. deficiency) or investigating deleterious elements (e.g., heavy metals) differentially extracted from the soil by plants. Traditionally, elemental analysis of vegetation has been facilitated by acid digestion followed by quantification via inductively coupled plasma (ICP) or atomic absorption (AA) spectroscopy. Previous studies have utilized portable X-ray fluorescence (PXRF) spectroscopy to quantify elements in soils, but few have evaluated the vegetation. In this study, a PXRF spectrometer was employed to scan 228 organic material samples (thatch, deciduous leaves, grasses, tree bark, and herbaceous plants) from smelter impacted areas of Romania, as well as NIST certified reference materials, to demonstrate the application of PXRF for elemental determination in vegetation. Samples were scanned in three conditions: as received from the field (moist), oven dry (70°C), and dried and powdered to pass a 2 mm sieve. Performance metrics of PXRF models relative to ICP atomic emission spectroscopy were developed to asses optimal scanning conditions. Thatch and bark samples showed the highest mean PXRF and ICP concentrations (e.g., Zn, Pb, Cd, Fe), with the exceptions of K and Cl. Validation statistics indicate that the stable validation predictive capacity of PXRF increased in the following order: oven dry intact < field moist < oven dried and

powdered. Even under field moist conditions, PXRF could reasonably be used for the determination of Zn (R^2_{val} 0.86; RPD 2.72) and Cu (R^2_{val} 0.77; RPD 2.12), while dried and powdered samples allowed for stable validation prediction of Pb (R^2_{val} 0.90; RPD 3.29), Fe (R^2_{val} 0.80; RPD 2.29), Cd (R^2_{val} 0.75; RPD 2.07) and Cu (R^2_{val} 0.98; RPD of 8.53). Summarily, PXRF was shown to be a useful approach for quickly assessing the elemental concentration of vegetation. Future PXRF/vegetation research should explore additional elements and investigate its usefulness in evaluating phytoremediation effectiveness.

INTRODUCTION

Soils impacted by metal pollution are common worldwide, often occurring in areas of industrial processing (e.g., petrochemical refining, mining, smelting). Industrial metal pollution can stem from an acute source such as a spill, or via long term accumulation by eolian deposition of dusts (Wuana and Okieimen, 2011). Alternately, metals can also occur naturally in soils as a result of parent material weathering (Micó et al., 2006) or irrigation with metal laden groundwater or surface waters (Roberts et al., 2007); in fact, some metals are also plant essential elements (e.g., Cu, Mg, Zn, etc.). In the soil, such metals pose limited risks to humans, mostly through dermal exposure and direct contact. However, unique plant species are known to differentially accumulate different metals, via absorption of metal-laden water, whereby the metals accumulate in plant organs (e.g., roots, stems, leaves, fruits, seeds). For example, *Alyssum* spp., *Brassica pekinensis, Noccaea (Thlaspi) caerulescens, Arabidopsis halerii, Sedum alfredii,* and *Pteris* spp. have been linked to Ni, Pb, and As uptake, respectively (Courbot et al., 2007; Lochlainn et al., 2011; Craciun et al., 2012; Hanikenne et al., 2008, 2013; Halimaa et al., 2014; Li et al., 2003; Srivastava et al., 2006; Xiong, 1997; Zhang et al., 2011). While plant uptake of metals can be of concern if the plants are used as food for human consumption (Millis et al., 2004; Podar et al., 2004; Podar and Ramsey, 2005), certain plant species can also be used as an effective phytoremediation option for metal-laden soils (Brooks et al., 1998; Pollard et al., 2014). Furthermore, a metal hyperaccumulator plant species can be utilized for the purpose of phytomining; where a crop is grown to extract metal from the soil to be harvested and processed for metal recovery (Ali et al., 2013; Barceló and Poschenrieder, 2003; Brooks et al., 1998). Ali et al. (2013) note that the quantity of metal recovered by vegetative hyperaccumulators can vary from a few hundred to several thousand mg kg^{-1}; the best choices for active *phytomining* are plants featuring a rapid growth rate, aboveground biomass production, highly branched root

system to exploit the maximum amount of contaminated soil, ability to translocate metals from roots to shoots, and easy cultivation/harvest.

For decades, the analytical standard for elemental analysis of plant materials has been atomic absorption spectrophotometry (AAS) (Hanlon, 1998) or inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Isaac and Johnson, 1998). In such methods, vegetative samples are subjected to acid digestion while heating, placing all elements into an acidic solution which can then be aspirated across a torch for elemental quantification. While accurate, this approach is time consuming and requires extensive laboratory equipment and consumables. Laboratory-based X-ray fluorescence has also been successfully used to quantify elements in plant material (Watson, 1998), though some limitations such as its inability to detect light elements (e.g., N, B) were noted. By contrast, portable X-ray fluorescence (PXRF) spectrometry is a proximal sensor system which has been shown to provide multi-elemental quantification in soils (Weindorf et al., 2014), water (Pearson et al., 2017), and several other matrices. The key advantages of PXRF relative to traditional laboratory based approaches are that it is fast (60-90 sec), requires no consumables, and is field portable.

A few studies have previously established the applicability of PXRF to vegetation. McLaren et al. (2011) used a Bruker Tracer III-V PXRF (equipped with He purge and Si-Pin detector) to evaluate elemental concentrations in cotton, corn, and wheat. They found significant linear relationships between PXRF and acid digest datasets for Ca, Co, Cr, Fe, K, Mn, Ni, P, S, Si, and Zn, concluding that PXRF "provides an efficient alternative to traditional plant digestion." Paltridge et al. (2012a) used laboratory based energy dispersive XRF to determine Fe and Zn in rice and pearl millet grain. They found strong R^2 values (0.79 to 0.98) between XRF and ICP methods, concluding that XRF is a convenient and economical approach for elemental determination. Similarly, Paltridge et al. (2012b) found strong correlations between the two methodologies for Zn, Fe, and Se in wheat. Moreover, Sacristán et al. (2016) successfully used PXRF to predict Cu in lettuce (*Lactuca sativa* L.).

In Romania, extensive metals pollution has been documented in soils; the result of mining and smelting operations in numerous communities (Krüger and Carius, 2001). Previous studies have applied PXRF to examine the spatial variability of metal pollution in soils (Weindorf et al., 2013; Paulette et al., 2015), but fewer studies have evaluated the vegetation supported therein

and none have applied PXRF for such evaluation. Importantly, areas in the immediate vicinity of the smelters are used today for active agricultural production, causing concerns over potential metal contamination of harvested crops. Such contamination could occur as soil attached to the outer epidermis of root (e.g., carrots, *Daucus carota*) or underground stems (e.g., potatoes, *Solanum tuberosum*), as dust deposited on the leaves of leafy vegetables (e.g., lettuce, *Lactuca sativa*; spinach, *Spinacia oleracea*; cabbage, *Brassica oleracea*), or accumulated in the shoots by transporters for plant nutrient analogs (Krupa et al., 2002) (e.g., Pb^{2+} substituting for Zn^{2+}; Cd^{2+} entering Ca^{2+} transporters).

While previous studies established the potential for PXRF analysis of vegetative materials, they stopped short of evaluating a broad range of organic matrices or considering its use for quantification of toxic metals contained therein. As PXRF quickly gains in popularity consideration of its broader use on organic matrices seems timely. Thus, the objective of this study was to statistically evaluate the relationships between ICP- and PXRF-determined elemental data under different scanning conditions (moisture, homogenization) to establish the validity of PXRF for direct elemental analysis of vegetative matrices. We hypothesize that PXRF will provide qualitative estimations of elemental concentrations for field moist samples, and more definitive (quantitative) elemental determinations for samples which have been dried and/or homogenized.

MATERIALS AND METHODS

General Occurrence and Features

Fieldwork for this study was conducted in two locations, Zlatna and Copşa Mică, Romania (Fig. 1). Both cities, located in central part of the country, are known as polluted areas due to industrial mining, ore processing, and smelting operations.

Zlatna processed non-ferrous ores between 1747 and 2002. The main industrial unit in Zlatna was represented by Ampelum S.A. producing H_2SO_4, Cu, $FeSO_4$, and $MgSO_4$. Mining and processing activities generated silica dust, gasses (e.g., SO_2, CO_2, CO, and various N oxides), and metals released as powders and aerosols (especially Pb). Zlatna is situated in Alba County in the Zlatna-Ighiu Depressional corridor of the Ampoi River Valley, a narrow valley with an east-west orientation (Popa, 2005), where the low wind (0.06 m s^{-1}) creates frequent periods (approx.
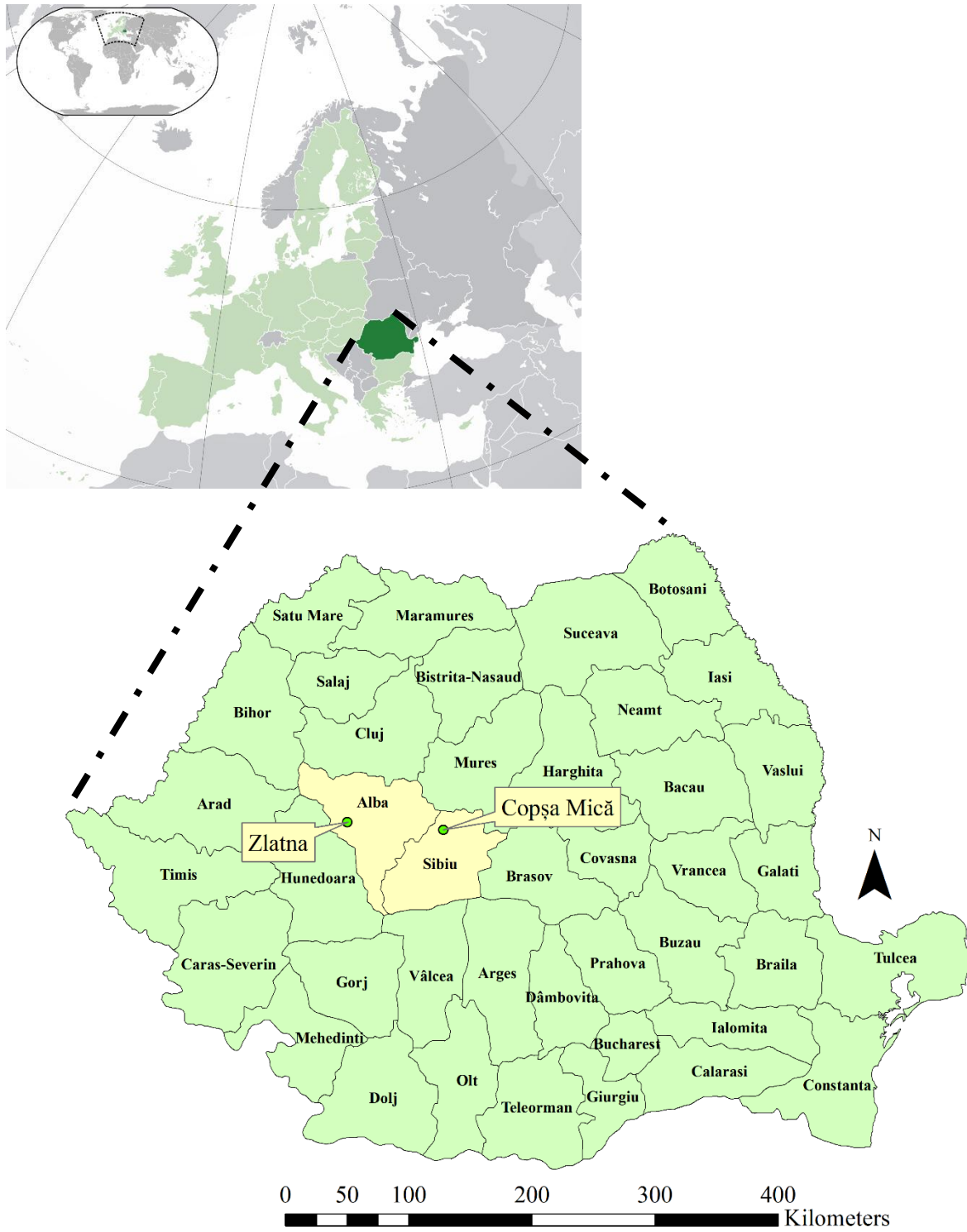
Fig. 1. Location of Zlatna and Copşa Mică, Romania.

half year) of atmospheric calm allowing metal deposition throughout the valley. The climate is generally wet and cool, with average annual temperature and precipitation between 6-8°C and 800-1,000 mm, respectively (Roşu, 1973). The main vegetation is forest, consisting largely of beech (*Fagus sylvatica*) and sessile oak (*Quercus petrea*). Per the *Romanian System of Soil Taxonomy*, Florea and Munteanu (2012) [US Taxonomic equivalent given per Soil Survey Staff, 2014] characterize 63% of soils in the area as Luvisols [Hapludalfs] (mostly on hills) and 27% as Cambisols [Inceptisols] (mostly Dystricambosols [Dystrudepts] developed on acid rocks and Eutricambosols [Eutrudepts] developed on carbonatic diluvium). On steep slopes, Regosols and Lithosols [Udorthents] comprise ~2.5% while floodplain Alluviosols [Udifluvents] cover ~7.5% of the area (Paulette et al., 2007; Florea and Munteanu, 2012). The latter oft present gleyic properties (Lazăr, 2011). Notably, with the exception of Eutricambosols, the soils are naturally acidic, thus enhancing the risk of metal translocation into plants. Kabata-Pendias (2011) note that metals are potentially bioavailable under acidic (pH 5.60 – 6.15) conditions.

The environmental impact of pollution emissions is augmented through aeolian transport throughout the valley and by water erosion of nearby spoil piles which often carries pollution into the Ampoi River. As a consequence of the dispersion of Pb, Cd, Cu, and Zn aerosols and H_2SO_4, the vegetation is severely impacted with slopes frequently devoid of vegetation (Popa, 2005).

By comparison, Copşa Mică is a small town in Sibiu County in the Tărnava Mare Valley at the confluence with the Visa Valley surrounded by hills. Prevailing winds typically follow an east-west transect, with calm conditions 40 to 64% of the time (ARPM, 2011; Iordache, 2009). Climate is temperate, with mild winters and warm summers featuring an average annual temperature of 8.5°C and annual average precipitation of 1,300 mm. Kabata-Pendias (2011) argues that in temperate climates the residence time of trace elements in soils can be considerable: Cd 75 to 380 y; Hg 500 to 1,000 y; and for Ag, Cu, Ni, Pb, Se, and Zn, 1,000 to 3,000 y. Natural vegetation of the area is characterized by temperate grasslands (e.g., *Festuca rubra*, *Poa pratensis*, *Agrostis tenuis*, *Phragmites australis*) and beech forest along with *Robinia pseudoacacia*, the latter of which has mostly been planted.

Pollution of the area came from SOMETRA S.A., which opened in 1939 and specialized in non-ferrous metallurgy and S.C. CARBOSIN S.A, a carbon black-producing company operating

from 1935 to 1993. Copşa Mică pollution is so rife, it was featured on the cover of *National Geographic* magazine in June, 1991 (Thompson, 1991); at that time, it was declared the most polluted town in Europe (Harford, 2013). Citing British scientists, Shukman (2007) reported "the vegetation had a Pb content 22 times above the permitted level." Finally, Copşa Mică was nominated as the world's worst place for non-ferrous metal smelter pollution by Mines and Communities (2006). The key pollutants of concern are Pb, Zn, Cd, SO_2, and C dust.

Geologic floodplain deposits are alluvial material, fine sands, silt, and clay at the surface and coarse sand and gravels with depth. On terraces and hills, Pliocene silt-sand mixtures with intercalated clays are common as yellow-gray powders, generally of horizontal stratification (ASSA GRUP S.R.L., 2009). The main soil types in the area are Haplic Regosols and/or Regosols [Udorthents], Albic Luvisols [Hapludalfs], Haplic Phaeozems [Argiudolls] on terraces and hills and Fluvisols [Udifluvents] on floodplains (RECARE Project, 2013).

The natural regeneration of vegetation in Copşa Mică is very slow, especially forest. In areas of high and very high pollution, vegetative growth is stagnant (Direcţia Silvică Sibiu, 2008). Morărescu (2014) conducted a study in Copşa Mică to investigate the risk of pollutants on human health; connections were found between the elevated Pb and Cd in soil, dust, and vegetables and levels of those metals in children's blood. The Cd content in vegetables produced in the subjects' gardens was between 5 to 20 mg kg^{-1} dry matter, two orders of magnitude greater than that permitted by EU food safety regulations (Morărescu, 2014).

Vegetation was characterized by the following species typical of each matrix evaluated: grasses (*Arrhenatherum elatius, Bromus arvensis, Calamagrostis epigejos, Carex hirta, Carex* sp., *Elymus repens, Festuca* sp., *Phragmites australis, Poa trivialis, Poa* sp.), herbaceous (*Achillea millefolium*, *Adonis vernalis*, *Artemisia vulgaris*, *Chaerophyllum* sp., *Chelidonium majus*, *Dryopteris filix-mas*, *Equisetum telmateia*, *Eupatorium cannabinum*, *Euphorbia* sp., ~~*Frangula alnus*~~, *Galium mollugo*, *Galium* sp., *Gentiana* sp., *Humulus lupulus, Ligustrum* sp., *Medicago sativa, Mentha longifolia, Rorippa austriaca, Rubus idaeus, Rumex sanguineus, Salvia pratensis, Sambucus nigra, Stellaria media, Tusilago* sp.), and deciduous trees (*Acer campestre*, *Acer negundo*, *Acer pseudoplatanus*, *Acer* sp., *Alnus glutinosa, Betula pendula, Betula* sp., *Catalpa* sp., *Fagus sylvatica, Frangula alnus, Fraxinus excelsior, Fraxinus* sp., *Hippophae rhamnoides, Populus alba, Populus nigra, Populus tremula, Populus sp., Prunus avium*, *Prunus*

spinosa, *Robinia pseudoacacia*, *Quercus* sp, *Salix alba, Salix caprea, Salix purpurea*, *Salix triandra*, *Salix* sp.). Leaf litter (thatch) and bark were inherited mostly from deciduous tree species.

Field Methods

Sampling occurred in June, 2017 in and around Copşa Mică and Zlatna, Romania (Fig. 1). Each sampling site was chosen at random, but with deference to variability in vegetative type, land use, and site access. At each site, the location was geolocated via an E-trex global positioning system receiver (Garmin, Olathe, KS) with an accuracy of ~2 m. Five different types of organic materials were sampled: thatch (O horizons), deciduous leaves, grasses, tree bark, and herbaceous plants. With the exception of the former, all other samples were clipped with a small pruning shears and placed in sealed plastic bags for transport to the laboratory. The shears were cleaned between each sampling to prevent any possible cross contamination. Bark was peeled off both live and dead trees; thicknesses ranged from 0.1 mm to 1 cm. Thatch was gently collected by hand, carefully excluding mineral soil; it was also bagged as other samples. Thatch was observed in various states of decay; mostly as Oi and Oe horizons. All samples were placed in a cooler on wet ice, then refrigerated at 4°C until laboratory analysis began within 48 h.

Laboratory Methods

In the laboratory, samples were scanned with PXRF in three conditions: 1) as received from the field (e.g., field moist), 2) oven dry (70°C), and 3) ground to pass a 2 mm sieve. Grinding of samples was facilitated using a small electric grinder. Compressed air was used to clean the grinder and sieve between sample processing to prevent cross contamination. In preparation for PXRF scanning, the vegetation was compressed into a small bundle (~2 cm thick) and secured on both ends with rubber bands (Fig. 2). As many organic samples feature an inherently low matrix density, bundling effectively increased the probability of matter interacting with X-rays. Devoid that, X-rays may be transmitted through organic matter resulting in suboptimal elemental fluorescence. Samples were placed in a hooded test stand with a Prolene thin film protecting the aperture of the PXRF from vegetative debris. For bark, samples were placed with the outer bark surface facing toward the PXRF aperture. In instances where lichens were interlaced with bark, efforts were made to scan the most densely lichen-laden portion of the bark. Loose thatch which
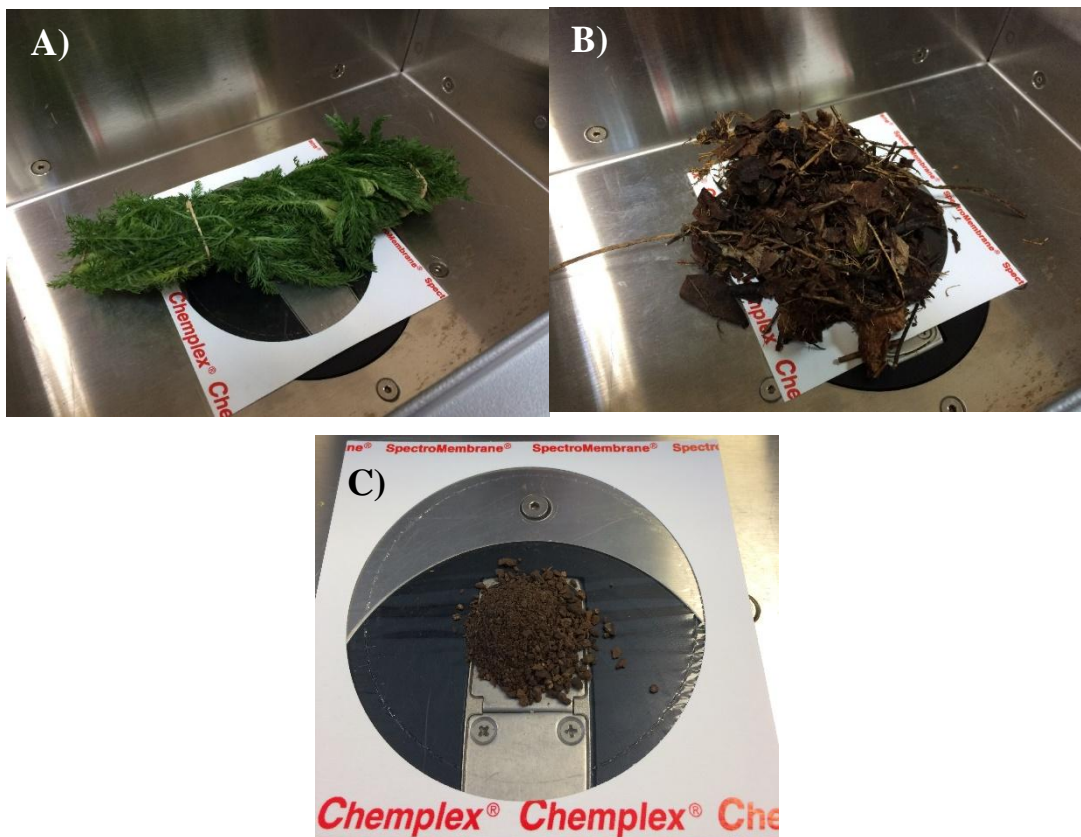
Fig. 2. A) Bundled vegetation placed directly above the PXRF aperture and secured with rubber bands, B) loose thatch massed in a small pile directly above the PXRF aperture, and C) powdered (ground < 2 mm) bark massed in a small pile directly above the PXRF aperture. All scans shown above were conducted inside a hooded test stand.

was impossible to mass into a bundle was placed upon the thin film directly as a small pile (Fig. 2).

Sample scanning was facilitated using an energy dispersive DP-6000 (Olympus, Waltham, MA) PXRF spectrometer operated in *Soil Mode* on line power (115 VAC). Samples were bombarded with X-rays from a Rh tube operated at 10 to 40 keV as a sequence of three beams each set to scan for 30 sec; thus, one complete scan was accomplished in 90 sec. Each field moist and oven dry sample was scanned once, while ground powders were scanned in triplicate and reported as a mean. Multiple scans per sample were not attempted on the former due to inherent sample variability in its native condition. For example, bark samples featured highly variable lichen content across their surface. Prior to scanning, the instrument was allowed to warm up for 30 min, then calibrated using a 316 calibration alloy coin. Instrument performance was verified via scanning of NIST certified reference materials (NIST 1515 and 1547). The following data was obtained (PXRF/**NIST**) [all in mg kg^{-1}]: NIST 1515 Ca (17,667/**15,250**), Cl (474/**582**), Fe (165/**82.7**), Mn (90/**54.1**), K (20,755/**16,080**), Rb (11.1/**10.2**), S (2,076/**1,800**), Sr (20.9/**25.1**), Zn (10.7;**12.45**); NIST 1547 Ca (17,817/**15,590**), Cl (225/**361**), Fe (324/**219.8**), Mn (139/**97.8**), K (33,040/**24,330**), Pb (2.5; **0.869**), Rb (21/**19.65**), S (1,834/**2,000**), Sr (46.1/**53**), Zn (17.8/**17.97**). The aforementioned PXRF data reported was the mean of two individual scans for each NIST sample. It is likely that the variability between NIST certified reference material and PXRF data obtained is due to a matrix density effect. To offset that, correction factors (Table 2) were applied in the same manner as Koch et al. (2017) for Ca, Fe, K, Mn, Pb, Sr, and Zn. Both Cd and Cu had no correction factors applied as they were not present in the NIST materials scanned.

Acid digestion of powdered samples was per Galvak et al. (2005) using HNO_3 and $HClO_4$ in sequence combined with heating at 150°C and 180°C for 1 h each, respectively, in a DigiPREP digestion system (SCP Science, Montreal, QC, Canada). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis was accomplished with an iCAP 7400 model instrument (Thermo Fisher Scientific, Waltham, MA, USA). High purity argon was used as plasma, auxiliary, and nebulizer gas. The data acquisition integration and wash times were 30 s, with three repetitions, and averaged over the three (Bettinelli et al., 2000). The calibration standards were custom ICP standards 1, 2, 3, 4, and 5 (SCP Science, Montreal, QC, Canada),

each diluted to six levels (L1 through L6). Check calibration standards (L2 and L4) were inserted after very 50 samples to check consistency.

Select samples were characterized at 20 keV by an S-4300 (Hitachi, Schaumburg, IL, USA) field emission scanning electron microscope fitted with an energy dispersive X-ray microanalysis (EDAX, Mahwah, NJ, USA) system. The samples were mounted onto Al sample mounts and coated with a thin layer of Au/Pd for conductivity before imaging/EDS analysis. Tomašević et al. (2005) note that since "particles deposited on leaves have complex shapes, quite different from an ideal flat sample, there may be over- or underestimation of the actual atomic concentration, but this does not prevent identification of the most important particle types."

Statistical Methods

Summary statistics were calculated for each organic matrix at both sites. Further, Pearson correlation analysis was conducted to evaluate significant interactions between elements detected. Data quality levels were assessed per the US EPA (2007) whereby definitive level (DL), quantitative screening (Quant), qualitative screening (Qual), and below detection limit (BDL) statistical standards are as follows: $R^2 = 0.85 - 1.00$ and RSD % $\leq 10\%$; $R^2 = 0.70 - 1.00$ and RSD % $< 20\%$; $R^2 = <0.70$ and RSD % is $< 20\%$; elemental concentration is below the detection limit, respectively. To improve the capability to fit models and objectively test their results, the elements with missing PXRF and/or ICP values were removed prior statistical modeling. Simple linear regression (SLR) was used to correlate between the PXRF elemental data for each organic matrix and ICP measured elements powder samples using R 2.11.0 (R Development Core Team, 2014). For individual element, the whole dataset was randomly split into 70% calibration and 30% validation sets. Model assessment for both SLR was done using root mean squared error (RMSE), residual prediction deviation (RPD), the coefficient of determination (R^2) and the ratio of performance to interquartile range (RPIQ) (Chakraborty et al., 2017a,b).

$$R \quad = 1 - \frac{\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i - \bar{Y}_i)^2} \qquad (1)$$

$$RMSE \; = \sqrt{\sum_{i=1}^{n}\left[\frac{(\hat{Y}_i - Y_i)^2}{n}\right]} \qquad (2)$$

$$RPD = \left[\frac{1/(n-1)\sum_{i=1}^{n}(Y_i-\bar{Y}_i)^2}{1/n\sum_{i=1}^{n}(Y_i-\hat{Y}_i)^2}\right]^{0.5}_{Validation} \qquad (3)$$

where, $Y_i, \hat{Y}_i$, $\bar{Y}_i$ and n denote observed target variable, predicted target variable, mean of the target variable and sample number, respectively. Notably, RPIQ is an indicator of the goodness of the calibration models and was calculated as the interquartile range IQ/RMSE of prediction:

$$IQ = Q3-Q1 \qquad (4)$$

where Q3 and Q1 are the third and first quartile, respectively.

RESULTS AND DISCUSSION

One key advantage of PXRF is its ability to simultaneously report multiple elements, inclusive of both metals and plant essential elements. Devoid the latter, plants are unable to complete their life cycle given that their function is irreplaceable by other elements (Marschner, 1995; Barker and Pilbeam, 2007). However, several metals dualistically function as plant essential elements. Based on the amounts needed by the plants, the mineral elements which serve as essential plant nutrients have been grouped into macronutrients and micronutrients. A few mineral elements, other than the macro- and micronutrients, have been shown to positively affect only a limited number of plant species; they are classified as *beneficial* mineral nutrients (Marschner, 1995; Barker and Pilbeam, 2007). The general plant nutrient classes are given in Table 1. And as a baseline for comparison, Epstein and Bloom (2005) offer the following as general ranges of elemental concentrations in crop plants (dry weight basis): P (0.15-0.5%), S (0.1-1.5%), K (0.8-8.0%), Ca (0.1-6.0%), Mg (0.05-1.0%), Fe (20-600 mg kg^{-1}), Mn (10-600 mg kg^{-1}), Zn (10-250 mg kg^{-1}), Cu (2-50 mg kg^{-1}), Ni (0.05-5.0 mg kg^{-1}), B (0.2-800 mg kg^{-1}), and Cl (10-80,000 mg kg^{-1}).

The results of elemental concentrations in the various organic matrices tested as part of this study are given in Tables 3a and 3b. Samples from Copşa Mică and Zlatna were kept separate given the different types of smelting conducted at the two sites. For several elements, PXRF determined concentrations generally increased in the following order: wet < oven dry < powder. The magnitude of increases were both element and matrix dependent. The summary statistics presented in Tables 3a and 3b include all discreet values (with correction factors applied) reported for each moisture condition. Correction factors were derived from mean recoveries of

Table 1. General grouping of plant nutrients.

Group of mineral nutrients	Elements	Amounts needed by plants
Macronutrients	N, P, K, S, Mg, Ca	< 0.1% of dry mass
Micronutrients	Fe, Mn, Zn, Cu, B, Mo, Cl, Ni	< 0.01% of dry mass
Beneficial	Na, Si, Co	

Table 2. Elemental mean recovery by portable X-ray fluorescence spectrometry and correction factor applied based upon NIST certified reference values.

Element	NIST 1515	NIST 1547	Mean Recovery	Correction Factor
Ca	1.16	1.14	1.15	0.87
Fe	2.00	1.47	1.73	0.58
Mn	1.66	1.42	1.54	0.65
K	1.29	1.36	1.32	0.76
Sr	0.83	0.87	0.85	1.17
Zn	0.86	0.99	0.92	1.08
Pb		2.88	2.88	0.35

Table 3a. PXRF and ICP elemental data summary for five vegetative samples types from Copşa Mică, Romania.

Ca (Calcium)	Wet			Dry			Powder			ICP		
	n† Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 6,821 - 40,269	19,755	23	7,079 - 62,430	19,325	23	16,881 - 53,420	37,830	23	8.90 - 44.94	30.07
Deciduous Tree Leaves	25 980 - 13,064	5,162	25	4,291 - 53,714	18,294	25	2,656 - 37,148	16,391	25	6.38 - 71.30	34.75
Grass	24 435 - 5,699	1,736	24	1,344 - 15,279	6,699	24	2,837 - 9,488	5,793	24	5.23 - 24.26	11.96
Herbaceous	23 789 - 8,868	3,421	23	6,958 - 58,682	27,699	23	5,698 - 42,811	21,986	23	16.47 - 205.35	84.00
Thatch	24 4,253 - 145,989	34,066	24	10,284 - 143,541	59,136	24	8,703 - 65,213	37,150	24	10.38 - 64.60	33.86

Mn (Manganese)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 38 - 229	104	23	38 - 196	114	23	53 - 284	105	23	0.31 - 0.79	0.38
Deciduous Tree Leaves	25 9 - 70	32	25	28 - 161	71	25	35 - 160	64	25	0.32 - 0.53	0.39
Grass	24 14 - 151	44	24	50 - 185	112	24	62 - 255	115	24	0.33 - 0.65	0.41
Herbaceous	19 5 - 70	23	23	11 - 255	98	23	21 - 135	72	23	0.35 - 0.93	0.51
Thatch	24 83 - 461	200	24	111 - 586	329	24	101 - 545	207	24	0.39 - 1.54	0.63

Cd (Cadmium‡)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	18 13 - 100	50	17	16 - 110	46	23	14 - 187	60	23	0.31 - 0.97	0.52
Deciduous Tree Leaves	11 9 - 27	13	12	10 - 49	18	21	10 - 51	15	25	0.29 - 0.58	0.32
Grass	9 11 - 26	15	2	19 - 22	21	7	12 - 18	14	24	0.28 - 0.32	0.30
Herbaceous	15 9 - 18	13	6	12 - 31	19	14	11 - 27	15	23	0.29 - 0.40	0.32
Thatch	18 12 - 63	33	20	15 - 760	83	22	15 - 265	57	24	0.32 - 1.31	0.50

Pb (Lead)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 73 - 9,878	2,559	23	62 - 10,774	2,598	23	100 - 10,955	3,291	22	0.31 - 117.35	29.54
Deciduous Tree Leaves	20 1 - 99	14	25	1 - 114	20	25	2 - 160	22	5	0.04 - 1.01	0.34
Grass	20 1 - 214	16	23	2 - 267	20	24	3 - 233	21	2	0.82 - 3.09	1.96
Herbaceous	17 1 - 39	5	23	1 - 123	17	23	2 - 133	19	1	--	1.52
Thatch	24 21 - 3,838	964	24	23 - 35,620	3,031	24	19 - 14,314	2,143	20	2.40 - 101.07	19.11

Cu (Copper‡)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	22 12 - 839	229	23	9 - 941	238	23	27 - 1,365	335	23	0.29 - 1.43	0.58
Deciduous Tree Leaves	1 --	7	15	5 - 20	13	21	5 - 5,405	275	25	0.28 - 1.41	0.37
Grass	2 24 - 34	29	10	11 - 41	19	24	14 - 174	51	24	0.31 - 0.85	0.39
Herbaceous	0 --	--	17	6 - 42	20	22	6 - 62	22	23	0.29 - 0.51	0.38
Thatch	23 7 - 727	172	24	13 - 8,492	639	24	34 - 2,419	341	24	0.28 - 2.59	0.67

Sr (Strontium)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 34 - 173	80	23	26 - 150	78	23	46 - 150	98	23	0.17 - .037	0.26
Deciduous Tree Leaves	25 3 - 72	14	25	3 - 202	43	25	4 - 152	43	25	0.15 - 0.39	0.26
Grass	14 1 - 10	4	24	4 - 35	12	24	5 - 40	13	24	0.15 - 0.22	0.18
Herbaceous	18 2 - 19	8	23	8 - 110	45	23	11 - 113	51	23	0.18 - 0.82	0.38
Thatch	24 14 - 87	50	24	23 - 168	89	24	26 - 158	90	24	0.18 - 0.38	0.26

Fe (Iron)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 70 - 13,300	3,899	23	151 - 12,791	4,827	23	239 - 12,268	3,950	22	0.83 - 1.59	9.50
Deciduous Tree Leaves	25 8 - 367	67	25	39 - 1,391	228	25	57 - 518	150	6	0.04 - 1.59	0.49
Grass	22 13 - 1,696	207	24	135 - 2,052	403	24	59 - 2,032	291	5	0.17 - 8.11	2.73
Herbaceous	23 7 - 136	40	23	55 - 1,679	449	23	56 - 1,793	365	12	0.09 - 4.04	1.44
Thatch	24 405 - 11,698	4,666	24	807 - 58,736	10,693	24	648 - 13,018	5,638	24	0.87 - 51.74	17.84

Zn (Zinc)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 233 - 13,751	4,425	23	395 - 14,887	4,949	23	590 - 29,669	7,120	23	0.81 - 36.29	11.97
Deciduous Tree Leaves	25 9 - 917	117	25	44 - 2,283	324	25	65 - 2,431	377	7	0.93 - 8.56	2.71
Grass	24 7 - 630	86	24	50 - 1,381	280	24	76 - 1,237	304	5	0.06 - 3.88	1.56
Herbaceous	22 7 - 81	34	23	36 - 774	278	23	66 - 733	279	13	0.12 - 3.49	0.90
Thatch	24 377 - 14,240	4,160	24	658 - 152,193	13,960	24	779 - 37,354	7,334	24	0.78 - 48.27	12.86

K (Potassium)	Wet			Dry			Powder			ICP		
	n Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg	n	Range (mg kg⁻¹)	Avg
Bark	23 2,047 - 17,668	6,133	23	1,969 - 16,170	5,919	23	1,090 - 12,908	5,407	23	4.45 - 32.70	11.79
Deciduous Tree Leaves	25 2,696 - 13,633	5,975	25	3,848 - 110,276	28,029	25	5,873 - 50,130	22,478	25	21.89 - 128.42	67.01
Grass	24 1,512 - 13,151	6,810	24	2,075 - 103,217	39,787	24	6,233 - 79,113	38,609	24	21.08 - 113.45	77.79
Herbaceous	23 2,472 - 7,524	5,081	23	14,264 - 218,309	61,754	23	16,158 - 101,269	47,408	23	26.93 - 239.41	136.76
Thatch	24 913 - 9,638	3,563	24	2,978 - 25,107	6,960	24	1,632 - 9,964	4,085	24	6.07 - 20.36	11.74

†Only discreet values reported; 'ND' non-detected elements omitted
‡No correction factor applied as either none present in NIST certified reference materials or levels were below PXRF detection limit

Table 3b. PXRF and ICP elemental data summary for five vegetative samples types from Zlatna, Romania.

Calcium (Ca) — mg kg⁻¹

Ca	Wet n†	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	3,300 - 46,675	20,814	22	4,166 - 54,002	26,843	22	9,158 - 59,283	35,230	22	7 - 77	35
Deciduous Tree Leaves	20	936 - 12,342	5,126	20	5,557 - 47,876	21,636	20	7,264 - 34,640	16,533	20	15 - 84	43
Grass	22	591 - 4,802	1,837	21	2,747 - 64,314	11,721	22	3,010 - 16,655	7,200	22	5 - 24	13
Herbaceous	22	726 - 11,853	3,302	22	6,742 - 87,318	25,793	22	4,864 - 36,256	16,198	22	8 - 95	43
Thatch	21	1,871 - 125,638	66,718	21	19,775 - 236,130	91,687	21	20,435 - 73,549	44,444	21	14 - 51	27

Manganese (Mn) — mg kg⁻¹

Mn	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	32 - 661	167	22	59 - 746	246	22	58 - 561	179	22	0.31 - 1.47	0.54
Deciduous Tree Leaves	20	8 - 619	91	20	44 - 1,402	253	20	39 - 1,042	163	20	0.34 - 4.21	0.76
Grass	22	15 - 232	46	21	51 - 627	190	22	67 - 567	172	22	0.35 - 1.36	0.55
Herbaceous	22	6 - 112	32	22	27 - 1,063	194	22	33 - 754	110	22	0.34 - 2.67	0.64
Thatch	21	33 - 5,400	1,181	21	172 - 6,536	1,548	21	122 - 3,167	811	21	0.38 - 5.72	1.34

Cadmium‡ (Cd) — mg kg⁻¹

Cd	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	13	14 - 20	16	10	11 - 23	16	15	10 - 19	14	22	0.28 - 0.33	0.31
Deciduous Tree Leaves	10	10 - 18	13	3	15 - 19	17	17	10 - 17	14	20	0.29 - 0.34	0.31
Grass	11	9 - 19	14	4	17 - 24	21	7	12 - 18	15	22	0.28 - 0.32	0.30
Herbaceous	11	10 - 15	13	4	15 - 20	18	14	11 - 29	14	22	0.29 - 0.45	0.31
Thatch	5	12 - 25	20	1	--	35	9	12 - 19	14	21	0.29 - 0.34	0.31

Lead (Pb) — mg kg⁻¹

Pb	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	1 - 6,565	496	22	3 - 3,486	249	22	2 - 3,219	385	7	0.09 - 34.04	11.21
Deciduous Tree Leaves	10	1 - 2	1	18	1 - 6	3	19	1 - 3	2	0	--	--
Grass	10	1 - 3	1	19	2 - 16	5	22	2 - 16	4	0	--	--
Herbaceous	5	--	1	21	1 - 14	4	21	1 - 13	2	1	--	0.02
Thatch	21	7 - 94	41	21	15 - 287	92	21	16 - 398	82	7	0.01 - 4.90	1.03

Copper‡ (Cu) — mg kg⁻¹

Cu	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	20	11 - 3,360	559	22	9 - 5,831	521	22	18 - 3,292	541	22	0.31 - 3.91	0.95
Deciduous Tree Leaves	3	7 - 15	10	20	8 - 45	23	19	6 - 51	23	20	0.29 - 0.41	0.35
Grass	1	--	7	17	13 - 71	33	22	28 - 99	56	22	0.32 - 0.41	0.37
Herbaceous	0	--	--	21	7 - 82	32	22	6 - 47	25	22	0.30 - 0.42	0.36
Thatch	21	18 - 1,214	346	21	74 - 2,368	586	21	59 - 1,378	428	21	0.33 - 1.52	0.68

Strontium (Sr) — mg kg⁻¹

Sr	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	4 - 170	50	21	4 - 191	60	22	5 - 216	67	22	0.15 - 0.52	0.24
Deciduous Tree Leaves	17	1 - 19	8	20	2 - 47	25	20	5 - 55	28	20	0.15 - 0.32	0.23
Grass	9	1 - 5	3	20	3 - 32	13	22	3 - 30	12	22	0.15 - 0.22	0.17
Herbaceous	18	1 - 22	6	22	8 - 173	37	22	10 - 112	35	22	0.17 - 0.59	0.25
Thatch	21	6 - 156	63	21	34 - 149	81	21	38 - 151	80	21	0.17 - 0.28	0.21

Iron (Fe) — mg kg⁻¹

Fe	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	53 - 12,621	2,861	22	240 - 9,098	2,058	22	87 - 8,488	2,271	18	0.29 - 30.46	8.35
Deciduous Tree Leaves	20	6 - 146	53	20	99 - 755	265	20	60 - 305	125	3	0.01 - 0.27	0.12
Grass	22	11 - 292	64	21	85 - 2,025	437	22	67 - 462	172	3	0.08 - 0.63	0.33
Herbaceous	22	6 - 133	42	22	98 - 1,775	456	22	45 - 648	170	9	0.11 - 2.95	0.71
Thatch	21	309 - 11,725	4,445	21	777 - 32,883	7,497	21	795 - 18,990	4,584	21	2.17 - 62.53	20.14

Zinc (Zn) — mg kg⁻¹

Zn	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	39 - 1,757	505	22	40 - 4,964	656	22	63 - 2,095	597	14	0.05 - 5.58	1.38
Deciduous Tree Leaves	19	8 - 475	106	20	51 - 1,207	349	20	40 - 1,193	346	7	0.25 - 2.95	1.86
Grass	22	4 - 110	29	21	52 - 678	193	22	50 - 585	199	1	--	0.88
Herbaceous	22	4 - 424	58	22	75 - 1,656	322	22	54 - 1,353	262	4	0.48 - 6.11	3.21
Thatch	21	91 - 9,788	1,253	21	318 - 11,037	1,654	21	195 - 7,800	1,233	14	0.28 - 14.59	3.78

Potassium (K) — mg kg⁻¹

K	Wet n	Wet Range	Wet Avg	Dry n	Dry Range	Dry Avg	Powder n	Powder Range	Powder Avg	ICP n	ICP Range	ICP Avg
Bark	22	1,531 - 14,663	5,873	22	1,910 - 19,551	8,143	22	1,594 - 31,749	7,470	22	4.67 - 87.63	15.48
Deciduous Tree Leaves	20	2,877 - 8,520	5,073	20	10,053 - 57,919	31,426	20	6,620 - 51,210	20,911	20	14.72 - 112.4	70.45
Grass	22	2,938 - 13,341	6,854	21	19,208 - 103,399	54,468	22	24,226 - 73,222	47,939	22	11.13 - 110.89	79.93
Herbaceous	22	2,350 - 10,063	6,317	22	16,992 - 170,219	69,799	22	13,430 - 126,653	46,629	22	18.92 - 176.7	104.22
Thatch	21	682 - 15,435	6,918	21	3,319 - 30,980	10,834	21	2,126 - 18,487	5,151	21	5.99 - 20.81	10.06

†Only discreet values reported; 'ND' non-detected elements omitted
‡No correction factor applied as either none present in NIST certified reference materials or levels were below PXRF detection limit

NIST vegetative samples relative to reported PXRF values (Koch et al., 2017). However, only samples with discreet values for wet, dry, and powdered conditions were included in SLR modeling; this allows for correlation comparisons between moisture conditions based upon a fixed number of samples. Notably, the number of samples reported for each of the four assessments (PXRF field moist, dry, powder, and ICP) were variable based upon instrumental detection limits. For example, Cu was only detected in one deciduous tree leaf sample from Copşa Mică under wet conditions, but upon drying and then powdering, Cu was detected in 15 and 21 samples, respectively.

In many instances, the highest elemental concentrations were found in bark and thatch. In Copşa Mică, Paulette et al. (2015) previously identified high concentrations of Pb and Cu, with substantive concentrations of V, Mn, and Zn as well. PXRF determined Pb concentrations in powders were similarly high; herbaceous, grass, and deciduous samples averaged 19, 21, and 22 mg kg^{-1} while thatch and bark were 2,143 and 3,291 mg kg^{-1}, respectively. Similarly, mean PXRF determined Zn concentrations in powders were 279, 304, and 377 mg kg^{-1} for herbaceous, grass and deciduous samples; thatch (7,334 mg kg^{-1}) and bark (7,120 mg kg^{-1}) were orders of magnitude higher. This suggests concentration of metals in recalcitrant organics (thatch, bark) which are not actively growing, or in fact may be degrading. Concentrations in thatch support similar findings of Paulette et al. (2015) in mineral soils, where means of 6,455 mg kg^{-1} (Zn) and 4,957 mg kg^{-1} (Pb) were reported. Contrary to the pattern of highest elemental concentrations occurring in thatch and bark, K and Cl showed the highest mean PXRF powder concentrations in grass (38,609; 8,142 mg kg^{-1}) and herbaceous (47,408; 6,457 mg kg^{-1}) samples, respectively. Data for Cl was not shown in Table 2 as it was not part of the suite of elements determined via ICP. The high concentration of K and Cl may be a reflection of solubility of these ions in solution. Both K and Cl are quite mobile in the phloem of plants (also S, P, Mg); Fe, Mn, Zn, Cu, and Mo feature intermediate mobility whereas Ca, Sr, Ba, Li, Si, and B are immobile (Epstein and Bloom, 2005). Even so, Ca levels were high, ranging from powder means of grass at 5,793 mg kg^{-1} to bark at 37,830 mg kg^{-1}. However, the observed levels of Ca, K, and Cl fit well within common plant concentrations established by Epstein and Bloom (2005). Epstein and Bloom (2005) also note that heavy metal mobility in the phloem of plants is oft associated with their precipitation as various oxides, hydroxides, or carbonates at slightly alkaline pH; complexation with organic acids such as malate and other metal-chelating compounds may increase mobility

within the phloem, but are species specific. Elemental concentrations of P, K, Mg, Zn, Cu, and B tend to be higher in phloem than xylem, while the reverse is true for Mn and Ca (Miranda et al., 2002; Epstein and Bloom, 2005).

In Zlatna, Weindorf et al. (2013) found that Pb, As, Co, Cu, and Cd exceeded governmental action limits in soils for more than 50% of the area. In support of these findings, our study revealed elevated levels of Pb and Cu, especially in bark and thatch samples. Powder samples of the former yielded means of 385 and 82 mg kg^{-1} (Pb) and 541 and 428 mg kg^{-1} (Cu), respectively. While some Pb was smelted in Zlatna, the main focus there was the exploitation of Cu whereas the RomPlumb smelter operated in Copşa Mică depositing much higher Pb levels to the surrounding environment. In fact, deciduous, grass, and herbaceous powder samples in Zlatna featured mean Pb concentrations of 2, 4, and 2 mg kg^{-1}, respectively; far lower than comparable vegetation in Copşa Mică. Not surprisingly, high concentrations of Cl, K, and Ca were found in Zlatna samples. Among the types of samples evaluated, grass featured the highest mean PXRF powder concentrations of Cl (7,930 mg kg^{-1}) and K (47,939 mg kg^{-1}). By contrast, it also showed the lowest Ca content (mean 7,200 mg kg^{-1}), while the means of bark and thatch both exceeded 35,000 mg kg^{-1}. Across all sample types, our study produced a mean powder Cd concentration (13.9 mg kg^{-1}) roughly double that of mineral soil (7.6 mg kg^{-1})(Weindorf et al., 2013).

For many elements (e.g., Zn, Pb, Cd, Fe) thatch and bark showed the highest mean ICP concentrations. Yet K was consistently highest in herbaceous and grass samples. The two elements showing the most stability in ICP results across samples types were Sr (0.17 – 0.25 mg kg^{-1} Zlatna; 0.18 – 0.38 mg kg^{-1} Copşa Mică) and Cd (0.30 – 0.31 mg kg^{-1} Zlatna; 0.30 – 0.52 mg kg^{-1} Copşa Mică).

Performance metrics of PXRF models relative to ICP are given in Table 4 and Figs. 3, 4, 5, and 6. SLR models were executed for predicting the ICP-based elemental results for various matrices using PXRF elemental data as inputs. In the moist conditions model, results were less robust except for Pb (R^2_{cal} 0.74; $RMSE_{cal}$ 11.74), Zn (R^2_{cal} 0.76; $RMSE_{cal}$ 3.91), Cu (R^2_{cal} 0.77; $RMSE_{cal}$ 0.22) and Cd (R^2_{cal} 0.56; $RMSE_{cal}$ 0.16). Validation statistics indicate that even under field moist conditions, PXRF can reasonably be used for the determination of Zn (R^2_{val} 0.86; $RMSE_{val}$ 3.50) and Cu (R^2_{val} 0.77; $RMSE_{val}$ 0.37). Surprisingly, the predictive capability of

Table 4. Performance metrics of calibration and validation models for elemental determination via portable X-ray fluorescence spectrometry vegetation in three moisture conditions in Romania. All values are in mg kg^{-1}.

Element	R^2_{cal}	$RMSE_{cal}$[a]	RPD_{cal}[b]	$Bias_{cal}$[c]	$RPIQ_{cal}$	R^2_{val}	$RMSE_{val}$[d]	RPD_{val}[e]	$Bias_{val}$[f]	$RPIQ_{val}$
					Moist					
Pb	0.74	11.74	1.98	0.00	1.65	0.40	19.68	1.32	-2.74	1.03
Fe	0.49	7.96	1.41	-9.03×10^{-17}	1.77	0.19	14.16	1.13	-3.50	0.88
Zn	0.76	3.91	2.07	-3.37×10^{-17}	1.83	0.86	3.50	2.72	-0.80	2.29
K	0.01	50.19	1.01	2.71×10^{-16}	1.53	0.00	51.23	1.01	1.29	1.45
Ca	0.001	28.48	1.00	4.29×10^{-16}	1.00	0.00	24.78	1.00	0.46	1.08
Mn	0.54	0.40	1.48	-3.60×10^{-19}	0.44	0.30	0.48	1.21	-0.01	0.39
Cu	0.77	0.22	2.14	-1.53×10^{-17}	1.75	0.77	0.37	2.12	-0.07	0.92
Sr	0.006	0.09	1.00	0.00	0.83	0.05	0.08	1.03	-0.005	1.53
Cd	0.56	0.16	1.53	4.75×10^{-18}	1.48	0.59	0.09	1.61	0.022	2.54
					Dry					
Pb	0.33	18.74	1.24	4.03×10^{-17}	1.04	0.39	19.90	1.31	-2.81	1.02
Fe	0.57	7.30	1.54	-6.19×10^{-17}	1.93	0.09	16.84	0.95	0.38	0.74
Zn	0.86	3.02	2.68	-3.65×10^{-17}	2.37	0.08	28.77	0.33	3.87	0.27
K	0.62	31.38	1.62	1.81×10^{-16}	2.45	0.26	44.23	1.17	6.26	1.68
Ca	0.05	27.69	1.03	-2.037×10^{-16}	1.02	0.04	25.27	0.98	1.94	1.06
Mn	0.72	0.31	1.90	-2.16×10^{-18}	1.46	0.57	0.45	1.29	0.03	0.42
Cu	0.68	0.27	1.78	-1.70×10^{-18}	1.46	0.04	1.20	0.65	0.20	0.28
Sr	0.20	0.08	1.12	4.17×10^{-19}	0.92	0.31	0.07	1.22	0.00	1.80
Cd	0.54	0.16	1.50	-7.93×10^{-18}	1.46	0.22	0.12	1.17	0.01	1.85
					Powder					
Pb	0.71	12.27	1.89	4.03×10^{-17}	1.58	0.90	7.93	3.29	-1.14	2.57
Fe	0.74	5.73	1.96	-6.19×10^{-17}	2.45	0.80	7.00	2.29	-0.88	1.78
Zn	0.93	2.10	3.86	-3.65×10^{-17}	3.41	0.61	5.84	1.63	0.80	1.37
K	0.65	30.03	1.69	1.81×10^{-16}	2.56	0.56	34.07	1.52	3.08	2.18
Ca	0.13	26.47	1.08	-2.03×10^{-16}	1.07	0.11	23.37	1.06	0.66	1.15
Mn	0.82	0.25	2.37	-2.16×10^{-18}	1.94	0.63	0.35	1.66	0.01	0.54
Cu	0.82	0.20	2.38	-1.70×10^{-18}	1.94	0.98	0.09	8.53	-0.001	3.72
Sr	0.20	0.08	1.12	4.17×10^{-19}	0.92	0.46	0.06	1.37	0.001	2.03
Cd	0.97	0.04	5.97	-7.93×10^{-18}	5.79	0.75	0.07	2.07	0.001	3.27

[a]RMSE$_{cal}$, root mean squared error of calibration set

[b]RPD$_{cal}$, residual prediction deviation of calibration set

[c]Bias$_{cal}$, bias of calibration set

[d]RMSE$_{cal}$, root mean squared error of validation set

[e]RPD$_{cal}$, residual prediction deviation of validation set

[f]Bias$_{cal}$, bias of validation set
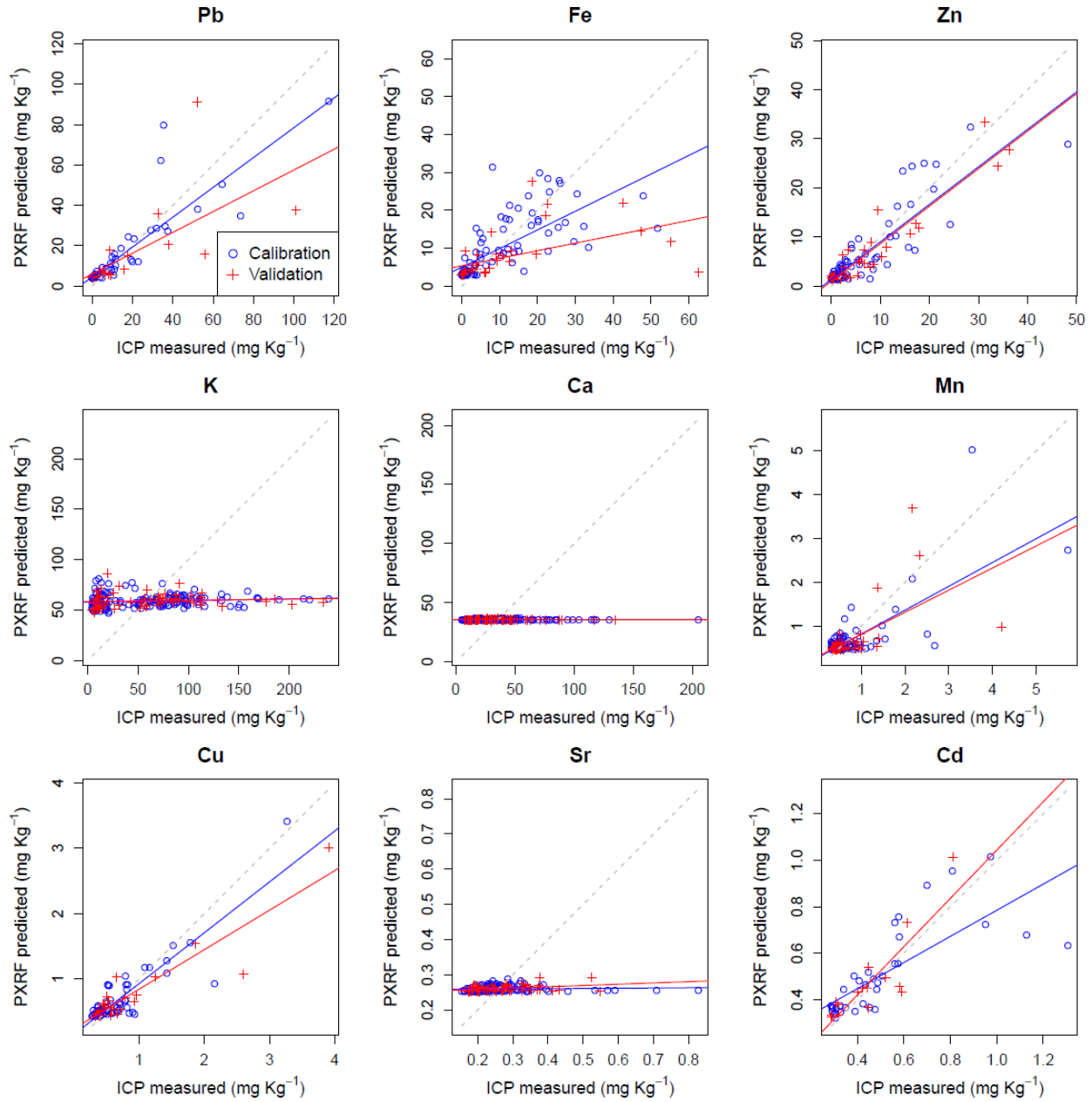
Fig. 3. PXRF predicted vs. ICP determined elemental concentrations across five vegetative sample types in Romania; determinations made on field moist samples.
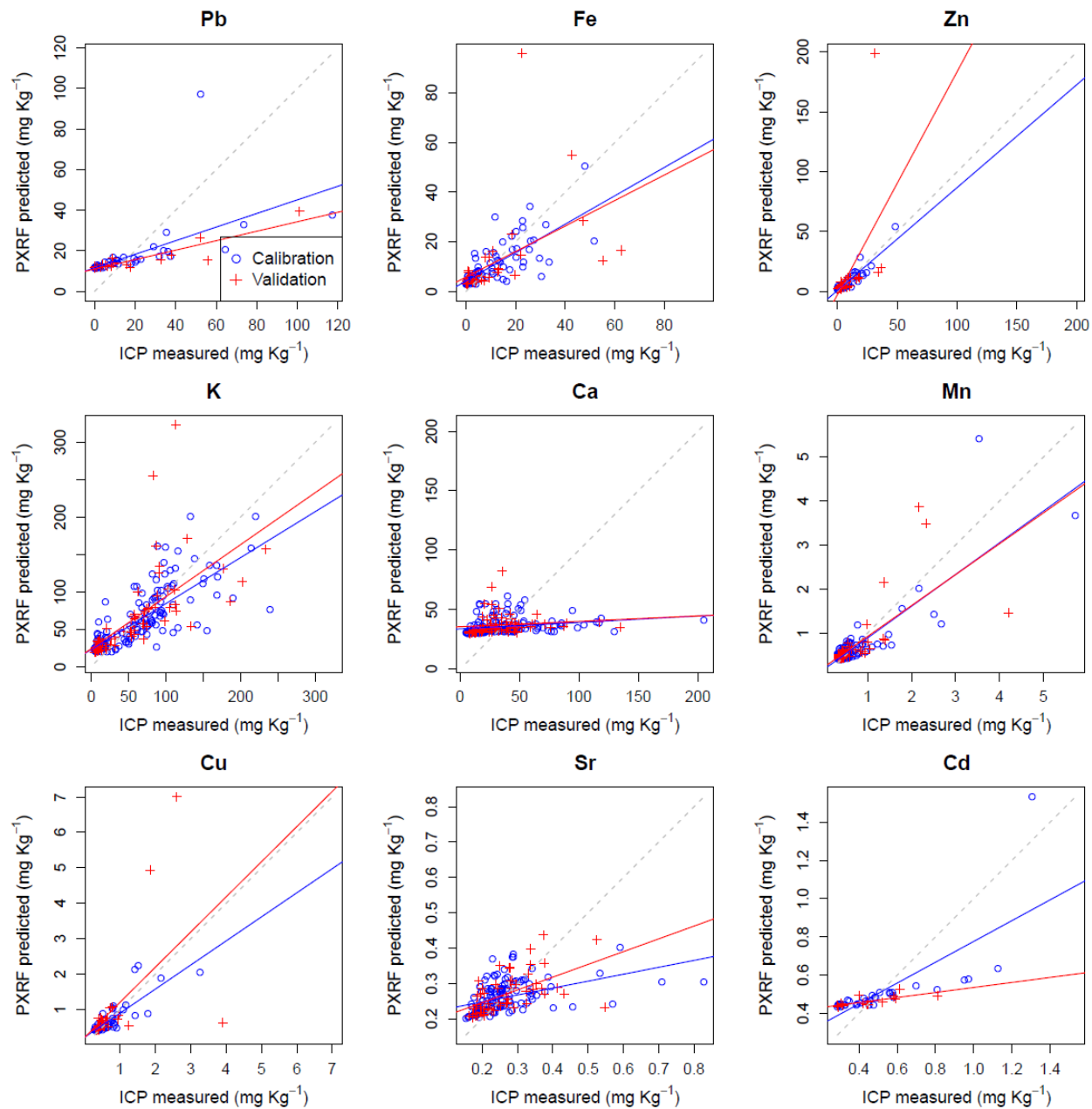
Fig. 4. PXRF predicted vs. ICP determined elemental concentrations across five vegetative sample types in Romania; determinations made on oven dried, intact samples.
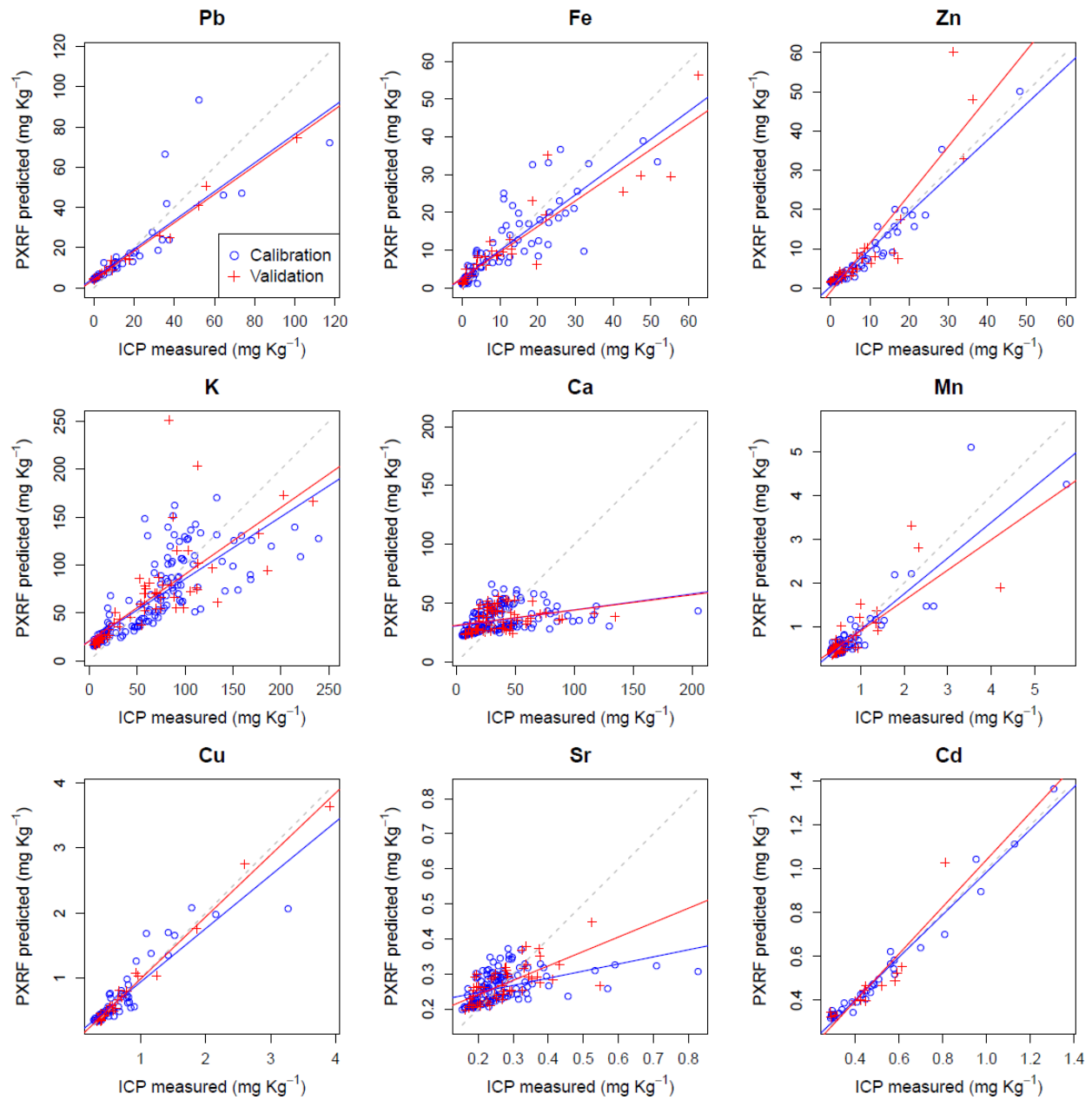
Fig. 5. PXRF predicted vs. ICP determined elemental concentrations across five vegetative sample types in Romania; determinations made on oven dried, powder (< 2 mm) samples.

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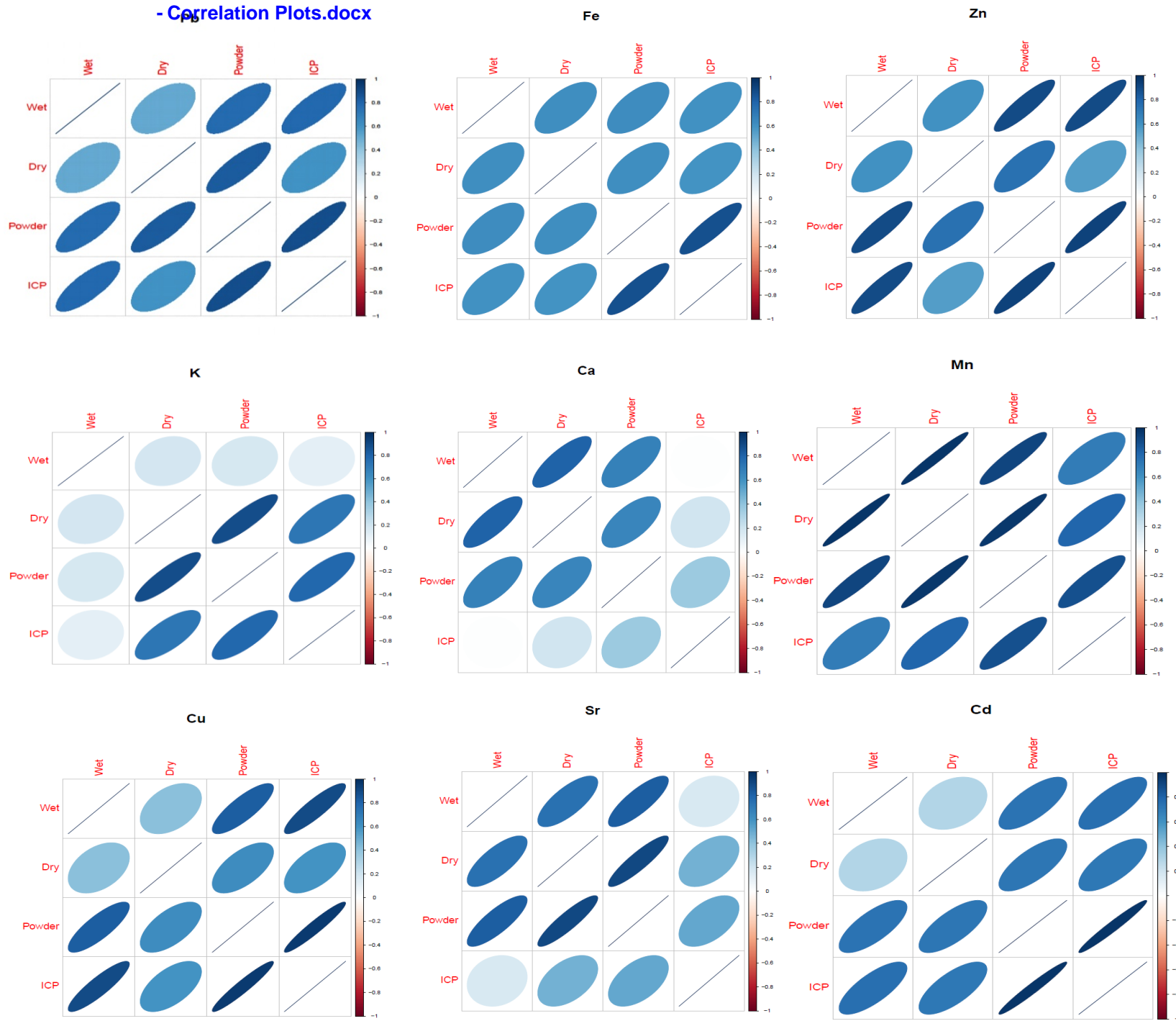
Fig. 6. Correlation plots showing different degrees of linear associations between ICP and PXRF measured values for three different vegetative matrix moisture conditions from Romania. The more linear the plot, the stronger the correlation.

PXRF for dry intact samples worsened slightly relative to scanning under field moist conditions while considering the validation statistics. Notably, the highest validation R^2 was produced by both Pb and Mn (0.39). Although, Zn (R^2_{cal} 0.86; $RMSE_{cal}$ 3.02), Cu (R^2_{cal} 0.68; $RMSE_{cal}$ 0.27) and Cd (R^2_{cal} 0.54; $RMSE_{cal}$ 0.16) in dry intact samples produced reasonable calibration performance, these results were not maintained in the validation sets indicating overfitted calibration algorithms. However, drying and powdering samples allowed for stable validation prediction of Pb (R^2_{val} 0.90; $RMSE_{val}$ 7.93), Fe (R^2_{val} 0.80; $RMSE_{val}$ 7.00), and Cd (R^2_{val} 0.75; $RMSE_{val}$ 0.07). Under the same conditions, Cu produced R^2_{val} of 0.98 and $RMSE_{val}$ of 0.09. Validation RPD values indicated strong, stable models at 3.29, 2.29, 2.07, and 8.53, respectively (Chang et al., 2001). Furthermore, the best RPIQ values for both calibration set (5.97) and validation set (3.72) for powder data were produced by Cd and Cu, respectively. Most of the elements under moist condition showed different degrees of underestimation at higher analyte values, possibly due to lack of samples with higher ends of the property scale (Fig. 3). Notably, Sr was unable to yield reasonable correlation with PXRF data in any condition, since a large number of Sr values were near the detection limit of PXRF (<5 mg kg^{-1}), ultimately increasing the standard error of the measurements. The high agreement between ICP Cu and PXRF Cu supported the findings of Sacristán et al. (2016) who used PXRF spectra derived parameters [maximum height (Hmax) and Full Width at Half Height (FWHH)] to satisfactorily predict Cu concentration in lettuce samples. Still, based on our earlier research (Pearson et al., 2017), the applicability of PXRF spectra derived parameters for predicting elements cannot be generalized. Figure 6 represents correlation plots showing different degrees of linear associations between ICP and PXRF measured values for three different vegetative matrix conditions. As expected, except for Ca and Sr, most of the elements exhibited strong positive correlation between their powder PXRF data and ICP data, further supporting the SLR-based results.

General Field Observations

In Copşa Mică and Zlatna, the PXRF was used on-site to make some rapid assessments of various organic materials. The outer bark (rhytidome) of a deciduous tree near the Copşa Mică dispersion stack was first scanned, followed by a scan of the live periderm beneath. The selected tree (*Robinia pseudoacacia*) was ~25 y old, and subsequently planted after cessation of activities at the Copşa Mică smelter. Exploratory results revealed excessive Pb concentrations in the

rhytidome (2,061 mg kg^{-1}), while the underlying periderm contained just 9 mg kg^{-1}. This suggests substantive Pb accumulation in non-living tissue, while the live inner tissues (or bark) remains relatively low. Further, Pulford and Watson (2003) note that "partitioning of the metals within the stem can vary within a tree. Wood and bark are important sinks for biologically available metals, with additional sink tissue being formed each growing season." As evidence, Pulford et al. (2002) found consistently higher concentrations of Cu, Ni, Zn, and Cd in the rhytidome of willow clones relative to concentrations in periderm of the same clone. In some instances, the differences were modest, yet in others, rhytidome samples were orders of magnitude higher than the periderm. Scanning electron microscopy analysis (Fig. 7) reveals the pitted nature of the bark matrix, which effectively traps particulate pollution dust in the rhytidome. Microprobe (EDAX) analysis of individual grains (Fig. 7) shows the Pb content orders of magnitude higher than the vegetative matrix immediately adjacent. By comparison, Fig. 8 shows two types of matrices in a thatch sample: organic and mineral particulate. While EDAX analysis of the latter shows a strong likelihood of the particulate being ZnS, even the organic matrix features appreciable Pb and Zn across an organic surface which appears to be relatively devoid of mineral pollutants. This could either indicate a particle of mineral pollutant behind the organic structure being detected by energy penetrating beneath the organic structure, or some level of pollutant may have been integrated into the organic matrix. Leaves of the same tree were also field tested and shown to contain (moist) Pb concentrations of 20 mg kg^{-1}, indicating possible translocation from the contaminated mineral soil (>20,000 mg kg^{-1}; Paulette et al., 2015) into the leaves and bark. This was further investigated by recording PXRF values of various bark samples from both the out-facing and in-facing sides. Results show marginally lower values from the in-facing sides of thicker bark samples, while the thin samples showed no change in readings, no matter the orientation. This suggests that the depth of X-ray transmisivity is unable to detect trace elemental differences at the millimeter scale. Rather, the X-rays simply pass through each bark sample. In all likelihood, Pb is most abundant closest to the surface and diminishes deeper into the tree tissue.

Barks that possess lichens showed considerably higher Pb concentrations than their lichen-free counterparts due to some lichen's propensity to hyperaccumulate metals. For example, Sarrett et al. (1998) found lichen *Diploschistes muscorum* to be an effective hyperaccumulator of Pb, whilst *Xanthoria parietina* effectively tolerated high Pb levels. Since many of the trees were
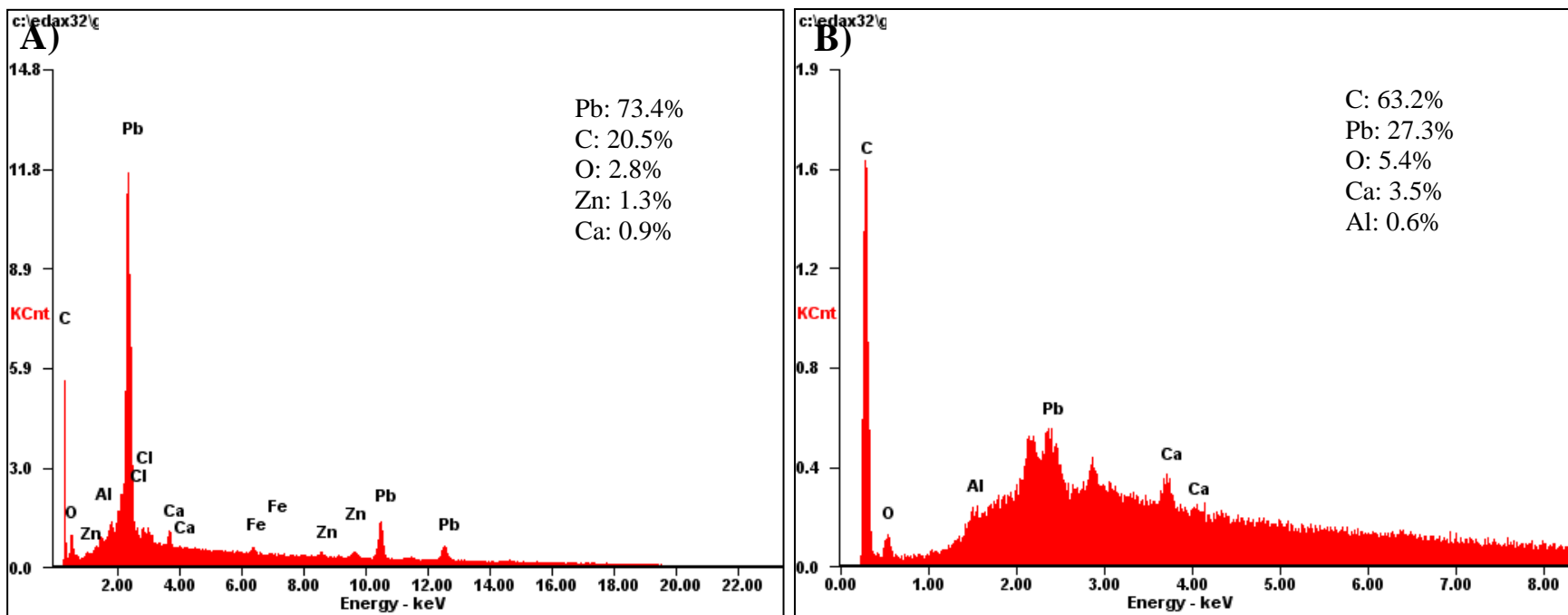
Fig. 7. A) EDAX microprobe analysis of particulate matter (suspected aeolian smelter pollution; red +), B) EDAX microprobe analysis of organic, vegetative bark matrix (green +) featuring much lower levels of particulate smelter pollution, and C) scanning electron micrograph showing the vesicular nature of the bark, its propensity to trap particulate smelter pollution, and microprobe scan locations. The featured bark sample was obtained from a tree located 500 m north of the dispersion smokestack; 75 m north of the Târnava Mare River in Copșa Mică, Romania.

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A)

C: 47.9%
Pb: 21.8%
Zn: 9.5%
O: 9.4%
Fe: 3.9%
Si: 2.8%
Cu: 1.4%
Ca: 1.2%

B)

Zn: 49.6%
S: 23.3%
C: 18.5%
Cd: 5.3%
O: 1.8%
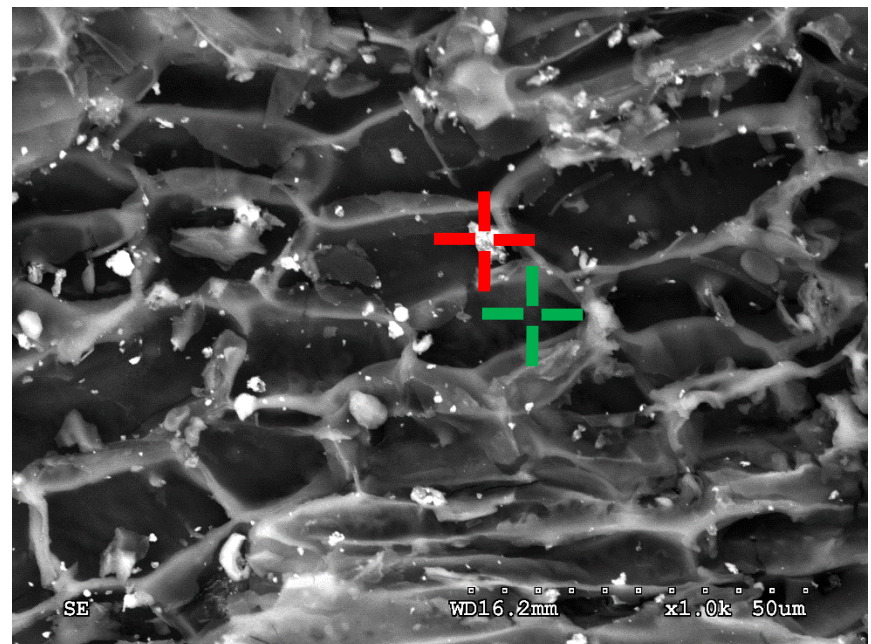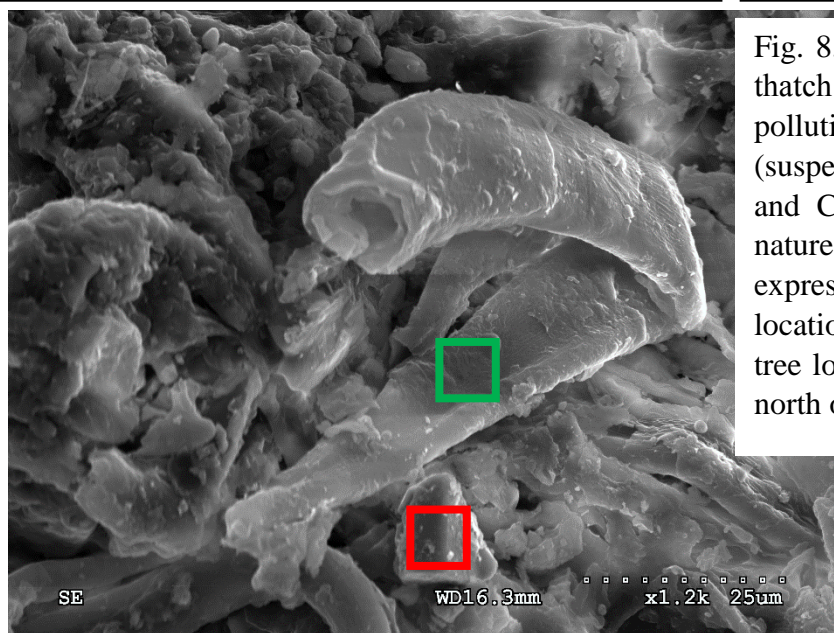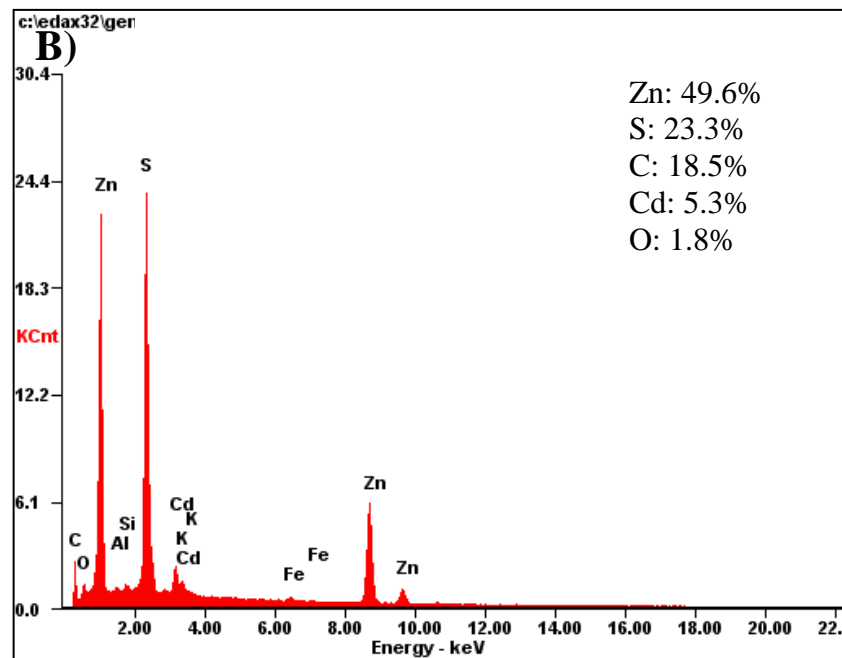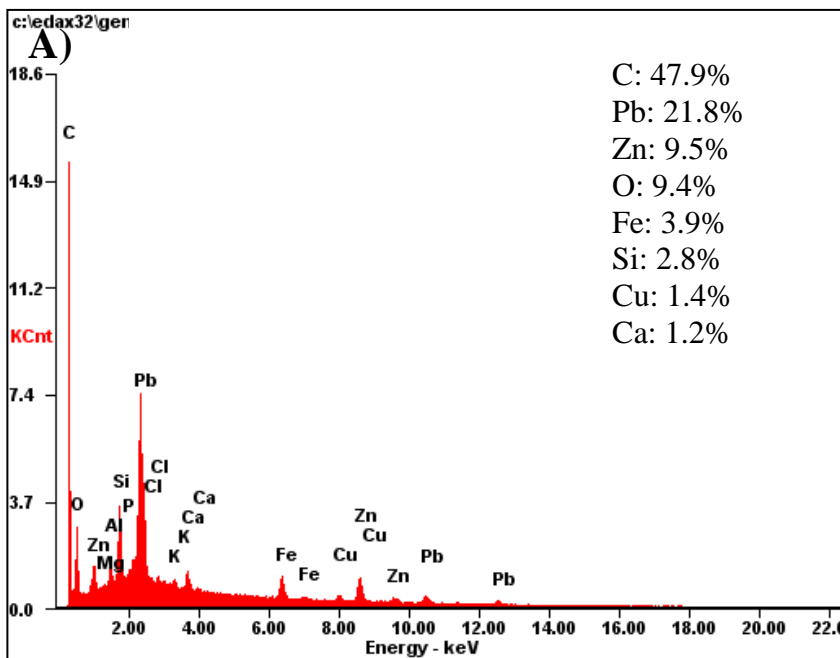
Fig. 8. A) EDAX microprobe analysis of organic, vegetative thatch matrix (green □) featuring moderate levels of smelter pollution, B) EDAX microprobe analysis of particulate matter (suspected aeolian smelter pollution, specifically ZnS; red □), and C) scanning electron micrograph showing the organic nature of the thatch, laminar coatings and particulate expressions of smelter pollution, and microprobe scan locations. The featured thatch sample was obtained from a tree located 500 m north of the dispersion smokestack; 75 m north of the Târnava Mare River in Copşa Mică, Romania.

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established after the cessation of works at the smelting plant, the question remains as to how the epiphytic lichens were exposed to such high elemental concentrations. Evaluating epilithic lichen colonization in smelter impacted waste, Rola et al. (2016) suggests that the main source of elemental input to lichen thalli is atmospheric deposition as lichens have no roots; biomineralization and surface complexation are other sources of potential elemental input. In our study, it was noteworthy that lichens were more prolific on dead wood bark relative to live tree bark, though the two types of samples were not independently collected.

Grasses typically showed undetectable or low trace element concentrations compared to all other vegetation samples. As such, the grasses currently growing in Zlatna and Copşa Mică will be ineffective phytoremediators. This is unfortunate, since the mowing and removal of hyperaccumulator grasses has proven both a cost effective and practical remediation strategy. However, other studies have shown the potential of certain grass species for remediation. Golda and Korzeniowska (2016) reported variable success in root/shoot accumulation of Cd amended soil growing *Poa pratensis*, *Lolium perenne*, and *Festuca rubra*. Chen et al. (2004) investigated metal laden soils and found *Vetiveria zizanioides* could re-adsorb 98%, 54%, 41%, and 88% of the initially applied Pb, Cu, Zn, and Cd, respectively. However in our study, removal of leaf-litter from wooded areas could provide a strong alternative, particularly in Copşa Mică.

A sample of cow dung in a field near the Copşa Mică smelter contained 4 mg kg^{-1} Pb in the excrement, which was typical of grasses in Copşa Mică. Another confounding discovery was that of a bracket fungi found on *Robinia pseudoacacia* in a highly contaminated zone of the Copşa Mică forest; the fungi was completely devoid of Pb. The surrounding thatch contained ~665 mg kg^{-1} Pb, yet the bracket fungi effectively acted as a biological filter to prevent the uptake of Pb into its tissues.

CONCLUSIONS

This research evaluated the efficacy of PXRF spectrometer for determining elemental concentration in vegetation relative to more expensive and time consuming approaches (e.g., acid digestion followed by quantification via ICP). Organic material samples from smelter impacted areas of Romania were evaluated, inclusive of thatch, deciduous leaves, grasses, tree bark, and herbaceous plants. Samples were scanned in three conditions: as received from the field (moist), oven dry (70°C), and dried and powdered to pass a 2 mm sieve. Performance

metrics of PXRF models relative to ICP atomic emission spectroscopy were developed to asses optimal scanning conditions. In most instances, the highest elemental concentrations (Zn, Pb, Cd, Fe) were found in bark and thatch samples, which suggests concentration of metals in recalcitrant organics which are not actively growing, or in fact may be degrading. In contrast to the pattern of highest elemental concentrations occurring in thatch and bark, K and Cl showed the highest mean PXRF powder concentrations in grass and herbaceous samples, which was expected since they are mobile elements. Validation statistics show that PXRF could be used for the determination of Zn (R^2 0.86; RPD 2.72) and Cu (R^2 0.77; RPD 2.12) even under field moist conditions, while dried and powdered samples allowed for stronger validation prediction of Pb (R^2 0.90; RPD 3.29), Fe (R^2 0.80; RPD 2.29), Cd (R^2 0.75; RPD 2.07) and Cu (R^2 0.98; RPD of 8.53). In sum, PXRF was shown to be a useful approach for quickly assessing multiple elements in vegetation. Scanning electron microscopy analysis further explained the pitted nature of the bark matrix, which effectively trapped particulate pollution dust in the rhytidome. Future PXRF/vegetation research work should focus upon quantification of additional elements and investigating its usefulness in evaluating phytoremediation effectiveness and assessment of plant essential elements.

ACKNOLEDGEMENTS

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