Limitation of multi-elemental fingerprinting of wheat grains: effect of cultivar, sowing date, and nutrient management

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Abbreviations: PCA=principal component analysis; AM=animal manure; NPK=mineral fertilisers; LTE=long term experiment;

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ABSTRACT

Multi-element fingerprinting demonstrates some potential for tracing the origin of agricultural products but not for discriminating among crop cultivars and nutrient management (source, rate). With principal component analysis (PCA) and univariate statistics, we examined 19 elements in grains from two winter wheat cultivars (Hereford, Mariboss) grown with different rates of animal manure (AM) or mineral fertilisers (NPK) in a long-term field experiment and two sowing dates (early, timely).

Nitrogen, Cd and Mn related to NPK, and Mo and Na to AM. Barium, Fe, and P reflected nutrient rate; these elements increased with nutrient rate regardless of source. Unmanured grains were enriched in Cu. Mariboss was characterized by higher concentrations of Sr, Ba and Sc compared to Hereford with Sr in grain as the main separator. Univariate statistics showed higher concentrations of N, P, Mg, Ba, Cu, Mo and Zn in early sown than in timely sown wheat. Compared with Hereford grains Mariboss was higher in P, Mg, Ba, Cu and Sr but lower in Mn, Mo and Zn. Thus, confounding effects of cultivar, sowing date, nutrient source and rate limits the potential of multi-element analysis in discriminating among agricultural products from different sites and cropping systems.

1. Introduction

Wheat accounts for 60 % of the production of small grain cereals in the European Union. In Denmark, winter wheat (*Triticum aestivum* L.) occupies 25% of the arable land, corresponding to 40 % of the area cropped with cereals. The majority of cereals grown in Denmark are used for animal feeding. On farms specialized in livestock production high yielding cultivars of autumn sown wheat are preferred and usually grown with application of animal manure. Historically, breeding programmes for wheat have targeted disease resistance, grain yield potentials and crop response to mineral fertilisers (Graham et al., 1999; Rengel et al., 1999) while the elemental composition of grains has been of secondary importance when developing new cultivars.

The element contents in grain have been shown to differ among wheat cultivars (Fan et al., 2008; Svecnjak et al., 2013; Zhao et al., 2009), but cultivar properties may be of minor importance relative to the impact of soil parent material and crop management. Increasing rates of mineral N fertiliser may increase trace element concentrations in wheat grains (Gooding et al., 2012; Kutman et al., 2011; Shi et al., 2010; Svecnjak et al., 2013). Moreover, a range of elements has been found to differ in concentration between crops dressed with mineral fertiliser or animal manure (Christensen and Elsgaard, 2014; Fan et al., 2008; Hamnér and Kirchmann, 2015; Kirchmann et al., 2009). However, effects of soil type, climate, cultivar, and management factors are often confounded, and we found little conclusive evidence in the literature on the specific impact of animal manure versus mineral fertilisers on the elemental composition of grains from different wheat cultivars.

Studies based on multi-element analysis and multivariate statistics have been applied to fingerprint grains of different geographical origin (Høgh-Jensen et al., 2006; Husted et al., 2004;

Zhao et al., 2011, 2013) and to discriminate among different management systems such as organic versus conventional cropping (Gundersen et al., 2000; Laursen et al., 2011, 2013). Although multi-element fingerprinting demonstrates some potential for authentication of agricultural products, this approach has not yet been successful in discriminating among crop cultivars, nutrient source, and nutrient addition rate as the effect of these factors were confounded.

To examine if these confounding factors violate the concept of multi-element fingerprinting, we analysed for 38 elements (19 elements above detection limits) in grains from two winter wheat cultivars grown on soils with long-term application of different rates of nutrients in either animal manure or mineral fertilisers. The wheat cultivars were planted at two dates spaced one month apart (early and timely sowing). Using multivariate statistics and univariate analyses, our aim was to isolate the effect of sowing date, nutrient source, and nutrient addition rate on the elemental composition of grains from two winter wheat cultivars.

2. Materials and methods

2.1 The Askov Long-Term Experiments (Askov-LTE)

The Askov-LTE was established in 1894 on the Lermarken site at Askov Experimental Station, Denmark (55º 28' N, 09º 07' E).The soil is a light sandy loam derived from glacial deposits and classified as Ultic Hapludalf (USDA Soil Survey Staff). The topsoil (0-20 cm) has 10% clay (< 2 µm), 12% silt (2-20 µm), 43% fine sand (20-200 µm) and 35% coarse sand (200-2000 µm).Soil pH is maintained in the range 5.5-6.5 by addition of magnesium-enriched lime every four years. Sulphur is applied annually at a rate of 12.5 kg S ha^{-1}. Annual average precipitation and temperature is 862 mm and 7.7 ºC, respectively (1961-1990 averages).

The Askov-LTE encompasses four separate fields (termed B2, B3, B4 and B5) and a four-course crop rotation of winter wheat (*Triticum aestivum* L.), silage maize (*Zea maize* L.), and spring barley (*Hordeum vulgare* L.) undersown with a grass-clover mixture that is cut twice in the subsequent production year. The crop rotation is fixed and each crop is present every year in a separate field. The fields are subject to conventional management and crop protection measures (e.g. pesticides) applied when needed.

The core experimental treatments (established in 1894/1923) of the Askov-LTE are unmanured plots and plots receiving different rates (½, 1, and 1½ times the standard rate for a given crop) of nitrogen (total-N), phosphorus (P) and potassium (K) in animal manure (AM; cattle slurry since 1973) and mineral fertilisers (NPK). Mineral fertiliser N, P and K are added in calcium-ammonium-nitrate, triple-super-phosphate and potassium chloride. Within each field, the nutrient treatments are replicated and randomized. Averaged across the rotation (no additions to the grass-clover crop), 1 AM and 1 NPK represents an annual input of 100 kg total-N ha^{-1}, 20 kg P ha^{-1} and 80 kg K ha^{-1}. Christensen et al. (2006) provide further details on the experimental layout of the Askov-LTE.

2.2 Study of elemental composition of wheat grains

For the present study, we relied winter wheat grains harvested in 2015 in 3 replicates of the unmanured, 1 AM, 1½ AM, 1 NPK, and 1½ NPK plots in the B3-field. For winter wheat, 1 AM and 1 NPK correspond to 150 kg total-N ha^{-1}, 30 kg P ha^{-1} and 120 kg K ha^{-1}. In the growing season 2014-2015, each nutrient plot in the B3-field was divided into four subplots to accommodate two winter wheat cultivars (Hereford™, Sygenta, CH and Mariboss™, Nordic Seeds, DK) and two sowing dates (early sowing, 20 August 2014; timely sowing, 18 September

2014). This provided a split-split plot experimental design with three replicates of five nutrient treatments, two sowing dates and two cultivars. Hence, 60 samples of grains were retrieved for chemical analyses. The wheat was established after the grass-clover crop which was terminated by herbicide (glyphosate applied 8 August 2014) and ploughing (20 August 2014). In March 2015, AM and NPK was surface-applied onto the emerging wheat crop. The wheat was harvested at physiological maturity on 20 August 2015 and grain samples were subsequently oven-dried at 80 ºC before analyses.

2.3 Chemical analyses

Grain samples were ground to <0.5 mm (CT 193 Cyclotec™ Sample Mill, Denmark) and sub-samples analysed for 37 elements (Ag, Al, As, Au, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Te, Th, Ti, Tl, U, V, W and Zn) at Bureau Veritas Mineral Laboratories (Vancouver, Canada). A 1 g split of the ground sample was treated with cold nitric acid and then digested in a hot water bath. Aqua Regia (equal parts of nitric and hydrochloric acid) and distilled water was added and the sample further digested in a heating block. Multi elemental analysis was performed with ICP-MS (Inductively Couple Plasma-Mass Spectrometry) using a NexION 300 (PerkinElmer, USA).

The concentration of N was analysed by dry combustion on a separate subsample of ground wheat grain using a Flash 2000 Organic Elemental Analyser (Thermo Fisher Scientific, USA).

The following 19 elements were found in concentrations above the analytical detection limits (ADL) and used in the subsequent data analyses: Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, P, Pb, S, Sc, Sr, Ti and Zn. Supplementary Table S1 presents analytical detection limits for the 37 elements and overall mean values for elements with concentrations above detection limits.

2.4. Data analysis

2.4.1 Multivariate analysis

Multivariate analysis was performed using R (version 3.2.4) with the mdatools package (DOI: 10.5281/zenodo.59547). Principal component analysis (PCA) was used for unsupervised pattern recognition to determine associations between management factors (nutrient source (AM, NPK), nutrient rate (unmanured, ½, 1, 1½), wheat cultivar, wheat sowing date) and grain elemental composition. The elements related to a given management factor were further subject to response analysis using univariate statistics.

The PCA reduces the number of original variables (X) into fewer latent variables (principal components). The goal of the PCA is to explain as much of the variability as possible with as few principal components. The first principal component (PC1) follows the direction of maximum variance in the data, then the second principal component (PC2) is orthogonal to PC1 alongside the second maximum variance, and so on (Esbensen et al., 2002). There are three main outcomes in PCA: the scores, the loadings and the residuals. The scores show the position of the samples being projected into the PC space and can be used to visualise any form of relationship among the samples (trends, clusters, outliers). The loading are unit vectors in the X space that defines the direction of the principal components, therefore they show which variables are responsible for a particular trend (Bro and Smilde, 2014). Residuals are mainly used to identify samples, which are not well explained by the principal components (e.g. outliers).

2.4.2 Response analysis

The element responses isolated by the PCA and ascribed to different management factors were analysed by univariate statistics using the model:

$$y_{ijkl} = \mu + B_i + Tj + BT_{ij} + P_k + BP_{ik} + TPjk + BTP_{ijk} + V_l + BV_{il} + PV_{kl} + TPV_{jkl} + TV_{jl} + BTV_{ijl} + BPV_{ikl} + BTPV_{ijkl} + \varepsilon_{(ijklm)}$$

with three replicates (i), five nutrient treatments (j), two sowing dates (k), and two wheat cultivars (l). In the model:

y_{ijk} =observation of the l^{th} wheat cultivar subject to different sowing date (k^{th}) and to different nutrient treatments (j^{th})

μ =grand mean

B_i =random effect of the i^{th} block NID (0, σ_b^2)

T_j =fixed effect of the j^{th} nutrient treatment

P_k =fixed effect of the k^{th} sowing date

TPjk=interaction between the j^{th} treatment and the k^{th} sowing date

V_l =fixed effect of the l^{th} cultivar

PV_{kl}=interaction between the k^{th} sowing date and the l^{th} cultivar

TPV_{jkl}=interaction between the j^{th} nutrient treatment, the k^{th} sowing date and the l^{th} cultivar

TV_{jl}=interaction between the j^{th} sowing date and the l^{th} cultivar

$\varepsilon_{(ijklm)}$ =random experimental error (0, σ_e^2)

The data was analysed using SAS 9.3 and a mixed model procedure (SAS Institute, USA). Interactions and main effects were declared significant at $p<0.05$. Variables were checked for assumptions of normality and homogeneity of variances by plotting residuals versus predicted values. Log transformations were performed for Mn, Mo, Sr and Cu. Nitrogen and Zn were transformed using an inverse function. The transformations were based on the Box-Cox power transformation series. Least square means were separated using the Tukey mean separation procedure in SAS proc mixed. Differences are reported at $p < 0.05$ significance level and means separated into groups by letters.

3. Results

3.1 Elemental fingerprints of wheat grains

The concentrations were below analytical detection limits for 19 out of the 38 elements (Supplementary Table S1) and these elements are not considered further. A nonparametric Spearman test showed that management factors (nutrient source, nutrient rate, sowing date and cultivar) were not correlated.

The data set was mean centred and standardized prior to PCA modelling. Standardisation was performed since the elements were reported in different units (% for macro-nutrients and mg kg^{-1} for other elements). The final model included seven principal components, but 76 % of the structural information was explained by only five components (PC1, 28 %; PC2, 24 %; PC3, 9 %; PC4, 8 %; PC5, 7 %). The two remaining components were mainly responsible for random variation. Figures 1 and 2 show scores and loadings plot for the components. The points on scores plots are coloured with a colour gradient reflecting the five nutrient treatments and concentrations of different elements.

The first principal component clearly separated effects of nutrient rate and the second component was responsible for effects of nutrient source (AM and NPK) (Fig. 1, panel A and B). For example, grains from NPK plots were characterized by higher concentrations of Cd (>0.05 mg kg^{-1}), Mn (> 22.6 mg kg^{-1}) and N (>1.51 %) while grains from AM plots were characterized by higher concentrations of Mo (>0.445 mg kg^{-1}) and Na (>0.004 mg kg^{-1}) (Fig. 1, panels C to F).

Thus, Cd, Mn and N were related to use of mineral fertilisers, and Mo and Na to use of animal manure.

In the first component, the nutrient rate was reflected in grain contents of Ba, Fe, and P (Fig. 1, panels G to I). Grain P concentration increased stepwise from unmanured (0.23 to 0.27 % P) to 1 NPK and 1 AM (0.27 to 0.31 % P): grains from 1½ NPK and 1½ AM plots showing the highest P concentrations (>0.31 % P). A similar pattern was seen for concentrations of Fe (Fig. 1, panel H): unmanured plots (0.003 mg Fe kg^{-1}), 1 NPK and 1 AM plots (0.004-0.006 mg kg^{-1}), and 1½ NPK and 1½ AM plots (>0.006 mg kg^{-1}). The clustering of Ba was less clear (Fig. 1, panel I), although the analysis suggests a higher Ba content in grains from NPK and unmanured plots (>0.004 mg kg^{-1}) than in grains from AM plots. Concentrations of Cu in grains were higher for unmanured (> 4.26 mg kg^{-1}) compared with the other treatments (Fig. 1, panel J). Thus, concentrations of Ba, Fe and P in grain increased with nutrient rate regardless of nutrient source, and grains from unmanured plots were enriched in Cu.

Component 3 (not shown) clustered data according to nutrient source but showed a less clear separation than found in Fig. 1. Component 4 made a clear distinction between cultivars (Fig. 2) with Mariboss characterized by higher concentrations of Sr, Ba and Sc compared to Hereford. Sr in grain was the main separator (Hereford, 1.4-2.1 mg Sr kg^{-1}, Mariboss, 2.1-4.0 mg Sr kg^{-1}). Ba showed a less clear separation than Sr (Fig. 2, panel A to D). Component 5 (not shown) reflected mainly the nutrient source with a clear distinction between grains from unmanured and from nutrient treated plots. This was related to a higher content of Ca and Cu in grains from unmanured plots.

3.2 Univariate statistical analysis

A univariate analysis was performed for the following elements: N, P, S, Mg, Ba, Cu, Mn, Mo, Sr, and Zn. The elements Fe, Cd, and Na were not analysed by ANOVA as the chemical analyses showed a low and discrete concentration pattern for these elements. One general trend (not considering S, Mn and Sr) was a higher concentration of elements in grains from early sown wheat than in grains from timely sown wheat (Table 1).

The highest concentration of N was found in wheat grown with 1½ NPK and the lowest in grains from 1 AM, 1½ AM, and unmanured plots. No differences were seen between wheat cultivars. The interaction between nutrient treatment and sowing time showed that early sown wheat from 1½ NPK had a significantly higher N content than any other treatments (Fig. 3).

According to the PCA, nutrient addition rate was reflected in grain concentrations of P, Fe and Ba. The P content was significantly higher for grains from 1 AM, 1½ AM and 1½ NPK plots compared with 1 NPK and unmanured plots. The P concentration in early sown wheat was higher than in grains from timely sown wheat, and grains from Mariboss were higher in P than grains from Hereford (Table 1). There was a significant interaction between nutrient treatment and sowing date with higher P in early sown wheat dressed with 1½ AM (Fig. 3).

The PCA revealed that Cd and Mn concentrations were related to NPK application. Differences in Mn content were found for nutrient treatment and cultivar, but not for sowing date. The highest Mn concentration was registered for grains from 1 NPK and 1½ NPK plots (Table 1), and grains from Hereford were higher in Mn than grains from Mariboss. There was a significant interaction between nutrient treatment and cultivar with higher Mn concentrations for the 1 AM treated Hereford grains. The lowest grain Mn concentration was found for Mariboss grown on unmanured plots (Fig. 4).

The PCA showed that concentrations of Mo and Na reflected AM treatments. Concentrations of Mo differed between nutrient source, wheat cultivar and sowing date. Mo concentrations were higher for AM than for NPK and unmanured treatments, Hereford held more Mo than Mariboss, and early sown wheat was higher in Mo than timely sown wheat (Table 1).

Grains from Mariboss held more Sr than grains from Hereford (Table 1), and the Sr content was higher in grains from NPK plots than in grains from AM and unmanured plots. There was a significant interaction between sowing date and nutrient treatment for Sr. When dressed with 1½ AM, early sown wheat was higher in Sr than timely sown wheat (Fig. 3). Wheat cultivar and sowing date interacted significantly with higher Sr concentration in Mariboss regardless of sowing time. Grains from 1½ NPK and 1½ AM plots had the highest Ba concentrations while grains from unmanured plots had the lowest concentrations (Table 1). Mariboss was higher in Ba than Hereford, and early sown wheat was higher in Ba than timely sown wheat.

The PCA indicated that Cu concentrations peaked in grains from unmanured plots. This was confirmed by univariate statistics (Table 1). Mariboss grains were higher in Cu than Hereford and early sowing gave higher Cu concentrations than timely sowing. There was a significant interaction between nutrient treatment and wheat cultivar with higher Cu concentrations in Mariboss grown under unmanured conditions (Fig. 4). The nutrient treatments 1 NPK, 1 AM and 1½ AM showed similar Cu contents regardless of cultivar. The nutrient treatment interacted with sowing date. Grains from early sown wheat grown with AM were higher in Cu than grains from 1½ NPK (Fig. 3).

The concentrations of S, Mg and Zn were not identified in the PCA as elements of discriminating power. Although S tended to increase with increasing nutrient rate, differences between nutrient source, nutrient rate, cultivar and sowing date were not significant (Table 1). Small, but significant, differences were seen for Mg. The concentration of Zn was higher in grains from early sown wheat, and higher in Hereford than in Mariboss. Increasing the nutrient rate from 1 to 1½ AM or NPK increased grain Zn contents.

4. Discussion

Previous studies based on multi-element element analyses and principal component analyses indicate some potential for tracing the geographical origin of wheat grains (Zhao et al., 2011, 2013), for discrimination between products from conventional and organic cropping systems (Gundersen et al., 2000; Laursen et al., 2011, 2013), and for separating crops grown on different soils (Høgh-Jensen et al., 2006; Husted et al., 2004). However, identification of specific management practices has been challenging due to confounding factors in the analyses. Thus Zhao et al. (2011) and Høgh-Jensen et al. (2006) were unable to isolate effects of crop cultivar and fertilisation rate because these were confounded with effects associated with site and crop management. In a study of three barley cultivars grown on three Danish arable soils, Husted et al. (2004) found that the principal component analysis separated multi-element data according to soil type whereas the influence of soil chemistry and possibly also climate and management was too large to allow a unique elemental fingerprint for cultivars. Using multi-isotope analyses to discriminate between plant products grown in organic and conventional systems, Laursen et al. (2013) were able to detect the use of animal manure but was unable to discriminate between plants receiving N from mineral fertiliser or from leguminous green manure.

Thus, a wide range of factors affects the predictive power of multi-element fingerprinting, including parent material, atmospheric depositions, weather conditions during the growing season (e.g. affecting translocation of elements from shoots to grain), fertilisation, crop protection and rotation. In most studies, the effect of these factors is confounded and results are often inconclusive with respect to reasons behind higher or lower concentrations of elements in grains. To examine the predictive power of element fingerprinting, the impact of individual management parameters has to be known.

We examined the elemental composition of grains from two contemporary wheat cultivars as affected by nutrient source (animal manure or mineral fertiliser), nutrient application rate and sowing date, and to what extent element fingerprinting based on multivariate and univariate analyses separated these factors. In this context, the Askov-LTE provides a unique research platform with plots treated with different rates of mineral fertiliser or animal manure for more than a century (Christensen et al., 2006). By subdividing the nutrient treated plots into four subplots, we were able to accommodate two wheat cultivars and two sowing dates.

The main elements separating nutrient sources were N, Cd and Mn for mineral fertiliser, and Mo and Na for animal manure. This aligns with Laursen et al. (2011) who found Mn and Cd associated with the application of mineral fertilisers. The presence of Cd in mineral fertiliser relates to the P component originating from sedimentary rock (Stacey et al., 2010). Previous field studies have shown that the addition of P fertiliser increases the concentration of Cd in crops whereas P added with animal manure does not (Christensen and Elsgaard, 2014).

We found that animal manure could be recognized by Mo and Na. This is in accordance with Laursen et al. (2011), who related Mo to soil incorporation of animal manure or leguminous crop residues. A higher concentration of Mo in animal manure ascribes to cattle fed with legumes that are known to be higher in Mo than non-legumes (Grattan et al, 2004). However, Kirchmann et al. (2009) found no differences in the Mo content of wheat grown with long continued application of animal manure and mineral fertilisers. The Na signature for animal manure can be ascribed to supplements of minerals and feed concentrates (e.g. oil-seed rape cake).

The concentration of Ba has been identified as a fingerprint of the geographical origin of grains (Laursen et al., 2011; Zhao et al., 2013). Hence, our study based on one specific location suggests that for Ba (and Fe), the effect of nutrient application rate can be confounded with that of location when attempting to verify the geological origin of wheat. One effect of rate could be a greater root development with increasing nutrient application rate (Rasmussen et al., 2015). Higher Fe, Zn and Cu concentrations in grain have been related to increasing N rates (Gooding et al., 2012; Kutman et al., 2011; Shi et al., 2010). We surmise that the somewhat higher Cu content in grains from unmanured soil is explained by a dilution effect as a larger crop biomass is obtained by application of fertiliser (Hamnér and Kirchmann, 2015). In a previous study, a dilution effect for Cu, Zn and Mg was found across different harvesting periods dating back to 1843 (Fan et al., 2008). This was explained by change of cultivars from long stem to semi-dwarf and dwarf cultivars. Li et al. (2007) found higher contents of Cu, Zn and Fe in wheat grains from unfertilised treatments compared to wheat treated with organic manure and mineral fertiliser. For six differently sited fertility experiments in Sweden, Hamnér and Kirchmann (2015) found similar contents of Cu in wheat grains from unfertilised soils and soils receiving animal manure and mineral fertilisers; for three other sites addition of mineral fertiliser (but not animal manure) halved grain Cu contents. We found that the Cu content in grains decreased with increased addition of mineral fertiliser whereas concentrations of P, Ba and N increased.

Higher concentrations of N in grain due to early sowing has been observed previously (Batten and Khan, 1987; Milford et al., 1993) and may be ascribed to the development of larger root system and biomass associated with an earlier sowing date (Barraclough and Leigh, 1984). However, early sowing does not always influenced grain N concentrations (Rasmussen and Thorup-Kristensen, 2016). We found that early sowing of winter wheat accomplishes higher concentrations in grains of N, P, Mo, Ba, Cu, Mn, and Sr, indicating that early sowing can be an effective management option to improve grain quality. Furthermore, our study shows that in fingerprinting studies the effects of different sowing dates across regions represents a confounding factor affecting the interpretation of grain concentration results.

The two wheat cultivars were also separated by elemental composition; the main separator being higher concentrations of Sr in Mariboss than in Hereford grains, suggesting differences in cultivars may inflate the use of Sr in determining the geographical origin of winter wheat (Laursen et al., 2011; Zhao et al., 2013). The different concentration of Sr is ascribed to different discrimination for uptake of Ca and Sr that show high chemical similarity (Mengel and Kirkby, 1987). The genotypic distinction between Ca and Sr uptake highlight the potential of elemental fingerprints of wheat cultivars. However, the content of Sr in grain relates not just to wheat cultivar but also to nutrient treatment. Separation of cultivars was found only for grains grown with AM and NPK and not for grains retrieved from unmanured plots, again emphasizing the abundance of confounding factors in previous fingerprinting studies.

5. Conclusions

In the present study, we were able to separate fertiliser type and rate by multi-element fingerprinting. In addition, elemental fingerprinting separated the two wheat varieties, and the two sowing times were separated with univariate statistics. The PCA analysis showed that N, Cd and Mn relate to application of mineral fertilisers and the presence of Mo and Na relate to application of manure. The nutrient rate was reflected in grain contents of N, Ba, Fe, and P. Grains from unmanured plots were enriched in Cu. Mariboss was characterized by higher contents of Sr, Ba and Sc compared to Hereford with Sr in grain as the main separator. The univariate statistical analysis showed higher contents of N, P, Mg, Ba, Cu, Mo and Zn in grains from early sown wheat than in grains from timely sown wheat. Compared with the Hereford cultivar, grains from Mariboss were higher in P, Mg, Ba, Cu and Sr but lower in Mn, Mo and Zn. Hence, our study shows that cultivar, sowing date, nutrient source and nutrient application rate all affects the elemental composition of grains. We conclude that confounding effects derived from these factors may seriously limit multi-element analyses in discriminating between agricultural products from different sites and cropping systems. The multitude of factors that affect the elemental composition of wheat grains questions the rationale of multi-element fingerprinting as a tool in product authentication.

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FIGURE LEGENDS

Fig. 1. Principal component analysis (PCA) with panel B showing the loadings and panels A to J the scores. PC1 and PC2 are plotted in panel A and B showing the nutrient treatments and the corresponding elements. Panels C to J provide a visualization of the score plots coloured with respect to the concentration of the individual elements (Cd, Mn, N, Mo, P, Fe, Ba and Cu). PC1 describes nutrient rate and PC2 for nutrient source (AM or NPK).

Fig. 2. Loadings (Panel B) and score plot (Panel A) for PC1 and PC4. Note the separation given toward the vertical axis due to accumulation of Sr. Panel C and D show the score plot coloured with respect to the concentrations of Sr and Ba, respectively, to show the difference between cultivars.

Fig. 3. Effect of sowing date and nutrient treatment on concentrations of N, P, Sr and Cu in wheat grains. Different letters indicate statistical difference ($p < 0.05$).

Fig. 4. Effect of cultivar and nutrient treatment on concentrations of P, Mn, Sr and Cu in wheat grain. Different letters indicate statistical difference ($p < 0.05$).