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Anne M. Johansen

Carey A. Gazis

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Geographic classification of U.S. Washington State wines using elemental and water isotope composition

Shirley Orellana^a, Anne M. Johansen^{a,*}, Carey Gazis^b

^a Department of Chemistry, Central Washington University, 400 E. University Way, Ellensburg, WA 98926, United States

^b Department of Geological Sciences, Central Washington University, 400 E. University Way, Ellensburg, WA 98926, United States

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ABSTRACT

Wine fraud leaves wineries vulnerable to damage in reputation and potential lost revenue. To reduce this risk for wines from Washington State (WA), USA, advanced analytical instrumentation and statistical methods were employed to geographically classify 133 wines from 4 major wine producing regions, including 70 wines from WA. Analyses of 37 elements and 2 water isotopes were performed with Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry and Cavity Ring-Down Spectroscopy, respectively. Linear discriminant analysis resulted in 96.2% discrimination, achieved with 11 parameters (Mn, Zn, Pb, Ni, As, D/H, La, Ce, Si, Zr and Sr) that were linearly combined into 3 functions. WA wines were uniquely distinguished in large part with low D/H ratios and Mn concentrations derived from the isotopically light precipitation and volcanic loess soils encountered in this region, respectively. This study is the first of its kind to focus on the authentication of WA wines.

1. Introduction

The state of Washington (WA) is now the second largest premium wine producer in the United States (US) (WSWC, 2015). With a \$4.8 billion economic impact in business profits and \$61.9 million in state taxes, wine fraud could have a significant negative effect on the industry. Adulteration, false statement of age and geographic origin, and relabeling of inferior wines are the most common forms of wine fraud (Versari, Laurie, Ricci, Laghi, & Parpinello, 2014). In an effort to reduce this potential risk in WA, here we hypothesize that concentrations of a few key trace elements that are unique to the soils of eastern WA, combined with water stable isotope ratios are effective in classifying WA wine to its geographic origin. Through the use of state-of-the-art analytical instrumentation, chemical signatures of WA wines are compared with those from wines produced in other regions of the world. Currently, 14 different American Viticulture Areas (AVA) are registered in WA, including Yakima Valley, WA's first AVA, established in 1983; and Columbia Valley, the state's largest AVA, containing more than 43,000 acres. All AVAs except for Puget Sound's AVA are considered sub-appellations of the Columbia Valley AVA, as they are either adjacent or surrounded by the Columbia Valley AVA. These AVAs are largely situated on loess derived from windblown silt derived largely from Pleistocene glacial deposits mixed with volcanic ash from current and past Cascade volcanos (McDonald, Sweeney, & Busacca, 2012).

These sediments overly the Columbia River Basalt (CRB) bedrock that dominates central and eastern WA. The region lies in the rain-shadow of the Cascade Mountain Range, with an annual rain fall between 6 and 8 in. and 300 days of sunshine per year (Cascades Volcano Observatory). These geological and climatic conditions are likely to set WA wines apart chemically and isotopically from all other wines.

Elemental concentrations of inorganic chemical components and stable isotope ratios, combined with multivariate statistics have proven to be particularly useful in chemically characterizing wines as shown in the review article by Versari et al. (2014). These tracers remain unchanged in bottled wine and provide a snapshot of the grape's journey from bud to bottle, carrying characteristics of the soil, climate, wine making process and storage (Pepi & Vaccaro, 2018; Versari et al., 2014). The majority of abundant mineral elements found in wine originate from the grape itself through absorption from the soils where the grapes are grown which reflects the elemental profile of the soil (Cheng, Zhai, & Taylor, 2015; Cozzolino, 2015). Factors that affect the elemental uptake by plants include age, root depth, soil pH, rainfall and temperature and will vary widely from region to region (Greenough, Longrich, & Jackson, 1997). Most elements, including Si, K, Ca, Fe, Mn, Rb, Sr, Ti and Ni, bear a mineralogical signature, related to the "terroir" of wine, others, like As, B, Pb, Cd, Cu, Sn and S, may be introduced through fertilizer and pesticide application and during wine making and storage (Di Paola-Naranjo et al., 2011; Hopfer, Nelson,

* Corresponding author.

E-mail address: johansea@cwu.edu (A.M. Johansen).

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Collins, Heymann, & Ebeler, 2015; Pepi & Vaccaro, 2018). Wines from WA State are largely produced in regions that are overlain with loess, alluvial sediments, and deposits from cataclysmic glacial floods, underlain by thousands of feet of CRB lava flows (McDonald et al., 2012). Basalt is generally high in Ca, Sc, Cr, Mn, Fe, Ni and Sr and low in Rb, while the sandy loess may be depleted in some elements, including Sc, Mn, and Fe (Cascades Volcano Observatory; Faure, 1998; McDonald et al., 2012; Pogue & Pitcavage, 2012). The alkaline nature of these soils, averaging a pH of 8, also leads to lower bioavailability of Mn and Fe, as these elements tend to form insoluble oxides at neutral pH (Pogue & Pitcavage, 2012). Throughout soil formation, windborne volcanic ash has been deposited by eruptions of current and past volcanoes of the Cascade Mountains, thereby making for a complex and unique chemical soil signature that may serve as an effective discriminator for the classification of WA wines.

The high sensitivity and specificity toward multielement analysis make the Inductively Coupled Plasma Mass Spectrometry (ICPMS) one of the most frequently used instruments to chemically characterize wine. For instance, in a study carried out on Argentine wines, researchers were able to correctly discriminate 4 geographic regions with only 5 elements: Ba, As, Pb, Mo, and Co (Azcarate, Martinez, Savio, Camina, & Gil, 2015). They were able to reach discrimination rates higher than 96% for prediction and validation data sets, deeming this technique trustworthy in validating the geographical origin and authenticity and wines. In another study carried out on South African wines, successful classification was achieved within the district of Stellenbosch (Coetzee, van Jaarsveld, & Vanhaecke, 2014). Suitable indicators for these regions included B, Ba, Cs, Cu, Mg, Rb, Sr, Tl and Zn. In addition, isotopic ratios of Sr and Pb have been used to differentiate wines from various regions as elements are taken up by roots and transferred to the grape in the same isotopic proportions as found in the soil (Baffi & Trinchnerini, 2016; Bora et al., 2018). In this context Sr and Pb isotopic ratios are of particular interest due to the radiogenic nature of ^{87}Sr which leads to variations of $^{87}\text{Sr}/^{86}\text{Sr}$ ratios with bedrock age and the fact that the various isotopes of Pb are products from the decay of U and Th which allow for differentiation between Pb sources (Bora et al., 2018; Geana, Sandru, Stanciu, & Ionete, 2017).

Stable isotopes of O and H in water are another excellent classifier to further constrain the geographic origin of wine due to their systematic variations with different climatic and geographic conditions (Dansgaard, 1964; Ingraham & Caldwell, 1999; Raco, Dotsika, Poutoukis, Battagliani, & Chantzi, 2015). Stable water isotopes are described with the notations delta deuterium (δD) and delta oxygen-18 ($\delta^{18}\text{O}$), where $\delta = (R_{\text{sample}} - R_{\text{standard}}) * 1000 / R_{\text{standard}}$ and R is the ratio D/H for δD and $^{18}\text{O}/^{16}\text{O}$ for $\delta^{18}\text{O}$. These isotopes are fractionated during evaporation and condensation, causing systematic geographic variation in the respective ratios. As a result, precipitation δD and $\delta^{18}\text{O}$ vary with latitude, elevation, distance from coast, topography, amount of precipitation, and season (Ingraham, 1998), suggesting that wines can be used to reconstruct weather and climate (Ingraham & Caldwell, 1999). After precipitation and water uptake into the plant, enrichment of the heavier isotopes, D and ^{18}O , occurs in leaves and fruit casing as the lighter water preferentially evaporates (Dunbar & Wilson, 1983; Raco et al., 2015). Consequentially, δD and $\delta^{18}\text{O}$ are expected to be higher in grape juice compared to the water taken up by the vine. However, since fractionations between precipitation and grape juice are consistent, δD and $\delta^{18}\text{O}$ can be powerful tools to discriminate wines from different regions and reflect the variations in precipitation. In a study performed in Brazil, $\delta^{18}\text{O}$ in wine water was efficient in differentiating the 3 regions studied (Dutra et al., 2011). Scientists in Romania found that $\delta^{18}\text{O}$ displayed the strongest relationship with climatic conditions and effectively discriminated wines to their geographical region (Dinca et al., 2016). By using C and O stable isotope ratios and concentrations of 10 trace metals, 90.4% classification of the Romanian wines and verification of vintage year were achieved. In a recent study carried out on Chinese wines, $\delta^{18}\text{O}$ also played a

significant role in discriminating between wines from different regions (Fan et al., 2018). The European Union implemented stable isotope applications in official food analyses in 1990, including the isotope ratios of H, C, and O of water and ethanol within wines. With great success they have built a robust database that helps detect adulterated wine (Christoph, Hermann, & Wachter, 2015). For WA State wines, produced at high latitude and in the rain shadow of the Cascade Mountains, significantly lighter δD and $\delta^{18}\text{O}$ are expected compared to those from other regions.

Numerous studies exist that use trace elements and isotopes to geographically classify wines world-wide (e.g., (Cheng et al., 2015; Coetzee et al., 2014; Dinca et al., 2016; Durdic et al., 2017; Dutra et al., 2011; Pepi & Vaccaro, 2018)); however, few focus on the US and none on WA. The closest region from which analyzed wines have been published is from the Okanagan Valley, north of WA in Canada. In this region soils are composed of igneous rocks that carry significantly different signatures compared to the basalt derived loess (Greenough et al., 1997; Taylor, Longerich, & Greenough, 2003). In the present study, elemental and water isotope analyses were performed to characterize WA wines with the goal of differentiating them chemically from those produced in California (CA), Europe (EU), and South America (SA). Using state-of-the-art analytical instrumentation, specific signatures are sought that are based on characteristic soil and climate features of eastern WA. Through this effort, a database and statistical model are built that can help detect wine fraud of WA wines.

2. Materials and methods

2.1. Sample collection and preparation

The 133 wine samples collected for this study stemmed from 4 major wine producing regions: Washington State, USA, (WA, N = 70), California State, USA, (CA, N = 17), Central Europe (EU, N = 33) and South America (SA, N = 13). Of these 133 samples, 116 were red wines of all varieties, and 17 were white wines from WA. EU wines originated from 5 and SA wines from 2 different countries. While collection was performed between December 2016 and May 2018, vintages ranged from 1994 (N = 1) to 2017 (N = 3), with 77% of samples from a 4-year period between 2013 and 2016.

Wine samples were collected in 20-mL amber glass vials with Teflon lined caps that had been acid cleaned in 5% nitric acid (HNO_3) and stored in ultrapure water until use (see SI for more detail). Before analysis, a 2.00 mL aliquot of wine was filtered through a 0.2 μm pore-sized syringe filter, into a new acid cleaned amber glass vial. To achieve a tested adequate dilution of 1:20, 1.00 mL of the filtered wine was diluted to 20.0 mL with nanopure water containing 1.0% ultrapure HNO_3 and 0.5% ultrapure HCl directly into an acid cleaned 15-mL HDPE test tube for analysis on the ICP-MS (Godshaw, Hopfer, Nelson, & Ebeler, 2017). All acid cleaning and sample/standard preparation took place in a class 1000 clean laboratory.

2.2. Multielement analysis

Multielement analysis was performed in-house with the 8900 Triple Quadrupole ICP-MS from Agilent using Collision/Reaction Cell (CRC) to resolve spectral interferences. This technique provides unsurpassed sensitivity and selectivity for (ultra)trace elements (Balcaen, Bolea-Fernandez, Resano, & Vanhaecke, 2015; Hopfer et al., 2015). Collision and reaction gases were He, H_2 and O_2 , and provided effective removal of interferences, including Ar interferences of $^{75}\text{As}^+$, $^{40}\text{Ca}^+$, $^{56}\text{Fe}^+$ and $^{80}\text{Se}^+$ (Godshaw et al., 2017).

An internal standard (ISTD) and three series of mixed calibration standards were prepared in 1.0% HNO_3 and 0.5% HCl. The first set of seven mixed elemental calibration standards were made by serial dilution of a combination of 4 separate mixed standards purchased from Agilent resulting in mixed working standards containing 62 elements.

These elements were Li, Be, B, Na, Mg, Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Hf, Ta, W, Re, Ir, Pt, Au, Hg, Tl, Pb, Th, and U. Standard concentration levels ranged from 0.001 to 100.0 ppb in order to bracket the elemental abundances observed in diluted wine samples. To calibrate the system for the higher concentrations observed of the major cations, including Na, Mg, K, and Ca, a second set of mixed calibration standards was prepared from a mixed major cation standard purchased from Fluka. In addition, a single-element S standard from Agilent was diluted appropriately to quantify S at the high concentrations observed due to sulfite addition to wines.

2.3. Stable isotope analysis

Isotopic ratio determinations for O ($\delta^{18}\text{O}$) and H (δD) in wine water was performed with a Picarro L2130-i equipped with an Induction Module (IM) for sample introduction, which extracts water from samples. Out of 6 replicate runs for each sample, the first 2 data points were discarded due to memory effects and the remaining 4 data points were averaged for use. The resulting average standard deviation per sample was determined to be 0.2 and 0.6 per mil for $\delta^{18}\text{O}$ and δD , respectively.

2.4. Statistical analysis

One-way analysis of variance (ANOVA), principal component analysis (PCA) and linear discriminant analysis (LDA) were performed using SPSS software (ver. 24.0, IBM). ANOVA in combination with Tukey's test established significant concentration/isotope ratio differences between region pairs. PCA was used for data reduction by identifying components that correlate with each other, thereby helping with the interpretation of LDA results. LDA, with the forward step-wise method, was used to discriminate between wines geographically (Azcarate et al., 2015; Cheng et al., 2015).

Elements for which 50% or more of the samples fell below the detection limit (BDL, defined as the average of blank readings plus 3 times the standard deviation of the blanks) were removed from further analyses. This was the case for 25 out of the 62 elements: Li, Be, V, Cr, Ga, Se, Nb, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Te, Hf, Ta, W, Re, Ir, Pt, Au, Hg, Tl and U. ANOVA, PCA and LDA were performed on the remaining 37 elements and two water isotopes, i.e., $\delta^{18}\text{O}$ and δD . Outputs were inspected in combination to identify and interpret components that maximized inter-regional differences.

3. Results and discussion

3.1. Variable means and associations

Variable means, standard deviations, significant regional differences, and component associations with PC loadings $\geq |0.4|$ are presented in Table 1 in order of increasing atomic number. PCA resulted in 10 PCs with eigenvalues > 1 , that together account for 77.7% of the variance in the data (Table S1). PCA shows, for instance, that (i) the first PC, i.e., PC1, accounts for the largest variance (25.0%) and correlates with rare earth elements (REE), and (ii) the last PC, i.e., PC10, accounts for the smallest variance (2.9% variance) and is associated with Cu only. For an overview of the dataset, 14 descriptive variables were selected based on their data reduction capacity and regional discriminating potential as deduced from the PCA and LDA (Table 2): Mg, Si, Mn, Zn, Rb, Sr, Ni, As, Pb, Zr, La, Ce, $\delta^{18}\text{O}$ and δD . In addition, S and Cu are further investigated as they are known additives and/or stem from the wine making equipment. These 16 variables appear in bold in Table 1 and are visualized in Fig. 1A, B, and 2 (and Figs. S1–S3) grouped by abundance in box-and-whisker plots. Outliers were omitted from graphs to not skew the axis range. Horizontal lines above or below the box-and-whiskers indicate significant differences between two

regions as determined with Tukey's test, with one star representing a p -value < 0.05 and two stars a p -value < 0.001 . In addition, the middle row at the bottom of each plot notes whether the element was significant in (i) the PCA (i.e., factor loadings $\geq |0.4|$) and if so, in which PC it appeared, and/or (ii) the LDA. Results of the element analysis in the context of PCA and LDA are presented first, followed by the water isotope ratios.

3.2. Multielement analysis

Selected elements are discussed generally in order of increasing atomic number as they appear in Table 1, which is different from the order in which they appear in the PCA and the LDA. The higher concentration elements Mg, Si, Mn, Rb and Sr are considered to be soil derived, entering the grape through root uptake (Fig. 1A and S1). Mg appears in PC3 (variance 8.3%, Table S1) associated with other major cations, K and Ca. Mg concentrations are highest in CA wines, and significantly so compared to EU and SA ($p < 0.05$), however, neither of these major cations are used in the LDA. Si has highest loading in PC8, correlating with Th, and is the 9th of the 11 significant components that discriminate between regions in the LDA, in particular between EU and SA (Table 2). Similar to Mg, Si concentrations are significantly higher in CA wines compared to all other regions ($p < 0.001$, Fig. S1).

Mn and Rb (Fig. 1A) show the same concentration pattern across regions and have high factor loadings in PC2. PC2 accounts for 14.3% of the variance and also correlates strongly with Co, Ni, and Cs, and moderately with Zn. Tukey's tests reveal that Mn and Rb show significant inter-regional variability as indicated by the 4 and 5 horizontal bars stretching across the various data pairs, respectively (Fig. 1A). Note that a maximum of 6 comparison pairs are possible for the 4 regions. In many studies world-wide, Mn and Rb have proven to be important independent classifiers (Durdic et al., 2017; Fan et al., 2018; Pepi & Vaccaro, 2018; Taylor et al., 2003). In the present study, only Mn is used in LDA, where it appears as the first classifier, separating CA from SA (Table 2). Zn and Ni are the second and fourth elements in the LDA and display similar patterns as Mn and Rb (Fig. 1B and S2, respectively). Zn is evenly split between PC2 and PC4 (6% variance), latter of which is associated the Pb and seems anthropogenic in nature (*vide infra*). Thus, given the soil-derived nature of Mn and Rb, and the strong correlations with Co, Ni, and Cs it can be deduced that these elements may also be soil-derived.

In terms of WA wines, the first transition series of elements consisting of Mn, Fe, Co, Ni, Cu and Zn, generally show significantly lower concentrations compared to those in the other regions (Table 1, Figs. 1 and S2). These elements form insoluble oxides that may be unavailable for plant uptake in the high pH loess soils of the Columbia (Pogue & Pitcavage, 2012). Sr is another important classifier found to discriminate between wines world-wide, here it is the 11th component in the LDA, separating EU and SA (Table 2, Fig. S2) (Coetzee et al., 2014; Fan et al., 2018; Pepi & Vaccaro, 2018; Versari et al., 2014). Sr correlates with As, Na, and B in PC6 (4.7% variance). Sr often correlates with Ca as it substitutes into its place in the mineral structures; however, due to the diversity of wines and their soil origin, a correlation was not seen in these diverse samples (Marchionni et al., 2016). B is known to have high concentrations in wines, dried fruit and nuts. It can stem from the application of fertilizers, however, it also leaches naturally into groundwater from dissolution of minerals in soils (Barth, 2000; Mattos, Hippler, Boaretto, Stuchi, & Quaggio, 2017). Its presence in wine is usually attributed to soil sources and its isotopic ratio has been investigated for the geographic classification of Brazilian wines (Coetzee et al., 2014; Cozzolino, 2015). Thus, PC6 appears to carry some characteristics of soils. In summary, the higher concentration components discussed above seem to group into 4 different, most likely soil-derived sources, represented by PC2, PC3, PC6 and PC8. Additional potential soil sources associated with PC1 and PC7 are discussed below, with the low-concentration elements.

Table 1
Regional average variable concentrations and stable isotope ratios with standard deviations, and assigned PC.

Variable	PC ^a	California (N = 17)	Europe (N = 33)	South America (N = 13)	Washington (N = 70)	p-value ^b
B (ppm)	6	7.17 ± 1.44	6.04 ± 1.26	7.85 ± 1.97	5.97 ± 2.26	*
Na (ppm)	6	19.6 ± 10.5	13.6 ± 5.8	27.7 ± 7.4	16.8 ± 13.3	*
Mg (ppm)	3	209 ± 211	98.4 ± 35.5	93.0 ± 12.2	130 ± 112	*
Al	1, 4	448 ± 153	488 ± 234	385 ± 95	370 ± 193	*
Si (ppm)	8	23.8 ± 5.8	10.8 ± 4.1	13.1 ± 2.5	18.2 ± 5.5	**
P (ppm)	4-	183 ± 39	113 ± 30	135 ± 37	154 ± 44	**
S (ppm)	7-	149 ± 20	141 ± 37	153 ± 27	119 ± 44	**
K (ppm)	3	2,050 ± 2,006	1,112 ± 177	1,263 ± 224	1,488 ± 123	ns
Ca (ppm)	3	105 ± 74	67.7 ± 15.2	70.3 ± 8.7	76.4 ± 67.9	ns
Ti	8	64.0 ± 38.8	43.1 ± 38.3	45.2 ± 34.1	43.5 ± 50.5	ns
Mn (ppm)	2	1.74 ± 0.72	0.947 ± 0.264	1.52 ± 0.83	0.739 ± 0.193	**
Fe (ppm)	4	1.75 ± 0.75	2.03 ± 1.16	2.08 ± 0.66	1.08 ± 0.72	**
Co	2	4.91 ± 1.56	3.13 ± 1.56	3.37 ± 2.06	2.87 ± 1.07	**
Ni	2	34.3 ± 10.9	18.9 ± 6.5	14.4 ± 5.3	14.2 ± 7.5	**
Cu	10	165 ± 194	162 ± 162	116 ± 90	100 ± 101	ns
Zn	4, 2	792 ± 381	580 ± 298	537 ± 189	417 ± 207	**
As	6, 5-	2.41 ± 1.80	1.51 ± 1.16	3.92 ± 1.93	2.49 ± 1.62	**
Rb (ppm)	2	2.40 ± 0.78	1.30 ± 0.49	2.09 ± 0.16	0.751 ± 0.332	**
Sr (ppm)	6	1.22 ± 0.39	0.832 ± 0.534	1.14 ± 0.23	0.694 ± 0.190	**
Y	9, 4	0.442 ± 0.206	0.566 ± 0.467	0.321 ± 0.145	0.515 ± 0.716	ns
Zr	7	20.7 ± 18.4	18.9 ± 18.9	14.9 ± 14.1	15.7 ± 17.1	ns
Cs	2	11.7 ± 9.4	5.00 ± 3.37	4.04 ± 3.92	1.86 ± 1.67	**
Ba	7, 2, 8	462 ± 147	240 ± 97	233 ± 112	291 ± 128	**
La	7	3.85 ± 5.67	5.91 ± 6.08	1.64 ± 4.14	4.89 ± 6.21	ns
Ce	1	0.263 ± 0.173	1.01 ± 1.47	0.283 ± 0.135	0.320 ± 0.486	**
Pr	1	0.037 ± 0.022	0.117 ± 0.162	0.039 ± 0.021	0.047 ± 0.076	*
Nd	1	0.170 ± 0.102	0.465 ± 0.626	0.171 ± 0.089	0.203 ± 0.311	*
Sm	1	0.037 ± 0.024	0.092 ± 0.115	0.040 ± 0.027	0.048 ± 0.075	*
Eu	9, 1	0.028 ± 0.011	0.040 ± 0.035	0.023 ± 0.009	0.030 ± 0.039	ns
Gd	1	0.041 ± 0.019	0.092 ± 0.109	0.034 ± 0.023	0.065 ± 0.095	ns
Dy	1	0.046 ± 0.018	0.083 ± 0.113	0.033 ± 0.020	0.059 ± 0.082	ns
Ho	1	0.013 ± 0.005	0.017 ± 0.015	0.0084 ± 0.0042	0.015 ± 0.017	ns
Er	1	0.047 ± 0.019	0.074 ± 0.096	0.037 ± 0.020	0.053 ± 0.051	ns
Tm	1	0.0090 ± 0.0046	0.0095 ± 0.0054	0.0057 ± 0.0029	0.0093 ± 0.0071	ns
Yb	1	0.066 ± 0.024	0.061 ± 0.043	0.050 ± 0.019	0.067 ± 0.049	ns
Pb	4	4.68 ± 2.01	13.77 ± 8.13	6.39 ± 4.33	4.96 ± 4.96	**
Th	8	0.120 ± 0.067	0.140 ± 0.106	0.135 ± 0.142	0.206 ± 0.241	ns
Delta O18	5, 2	7.88 ± 4.29	5.33 ± 2.95	3.77 ± 1.89	1.13 ± 2.14	**
Delta D	5, 2	8.14 ± 22.89	8.15 ± 10.94	-6.51 ± 14.79	-43.79 ± 13.81	**

Unless otherwise notes, values are in ppb.

^a Number of PC with factor loadings > |0.4| for that variable. For more than one PC, order is in decreasing absolute loading. A negative sign after the number indicates negative loading.

^b P-values: ns = not significant; * < 0.05; ** < 0.001.

Table 2

LDA variables in order of entering in stepwise forward method, with region pairs for discriminating power, standardized canonical discriminant function coefficients, and potential source with PC.

Variables	Between Groups	Function			Potential Source
		1	2	3	
1 Mn	CA and SA	0.081	0.025	-0.749	Soil (PC2)
2 Zn	EU and SA	0.239	0.322	0.413	mixed
3 Pb	CA and SA	0.263	-0.729	0.011	Anthrop. (PC4)
4 Ni	SA and WA	0.06	0.602	0.398	Soil (PC2)
5 As	SA and WA	-0.074	0.328	-0.687	Soil (PC6)
6 Delta D	EU and SA	0.955	0.045	0.112	Precip. (PC5)
7 La	EU and SA	0.314	-0.254	0.46	Soil (PC7)
8 Ce	CA and SA	0.124	-0.389	0.272	Soil (PC1)
9 Si	EU and SA	-0.445	0.325	0.626	Soil (PC8)
10 Zr	EU and SA	-0.515	0.2	0.075	Soil (PC7)
11 Sr	EU and SA	0.347	0.372	-0.147	Soil (PC6)

S and Cu are typically associated with the grape growing and winemaking processes, however, neither prove to be significant in classifying the regions with LDA. S is associated moderately with PC7 (4.3% variance) in a negative fashion. PC7 is also positively correlated with Zr and La, both of which are important in LDA (Table S1, *vide infra*). S is significantly lower in WA compared to all other regions (Fig.

S1). S is added to wine in the form of sulfur dioxide (SO₂) to protect from oxidation and microbial spoilage during storage. Because SO₂ is an irritant and can be harmful to sensitive consumers legal limits exist in wines. In the US, this limit is 350 mg L⁻¹, as SO₂, or 175.2 mg L⁻¹ (ppm) as S, which is exceeded in 7% of WA and 29% of CA wines. In Europe, the limit of 100 ppm S for red wines, is exceeded in 91% of the wines, while in SA, the legal limit of 125.1 ppm S is exceeded in 85% of the wines.

Copper sulfate (CuSO₄) is used as a fungicide and added to wine to remove reduced S species associated with off-odors and flavors. Cu can also be introduced to a wine from copper and brass in wine-making equipment. Cu shows no significant differences between regions (Fig. 1A); however, it is the only element with high loading in PC10 (2.8% variance). Given that Cu is not associated with similar soil-derived elements, it can be deduced that its source in wine relies on other factors, such as from the addition of CuSO₄ and/or the use of Cu containing equipment.

Of the less abundant elements, As and Pb (Fig. 1B) can have mineralogical sources in soils but are also frequently considered to be anthropogenically derived from the application of pesticides, fungicides and fertilizer and from the deposition of ambient aerosol particles (Angelova, Ivanov, & Braikov, 1999; Cozzolino, 2015; Kristensen, Taylor, & Evans, 2016; Vystavna, Zaichenko, Klimenko, & Ratsep, 2017). Arsenic is significant in the LDA and shows up in PC6 (variance

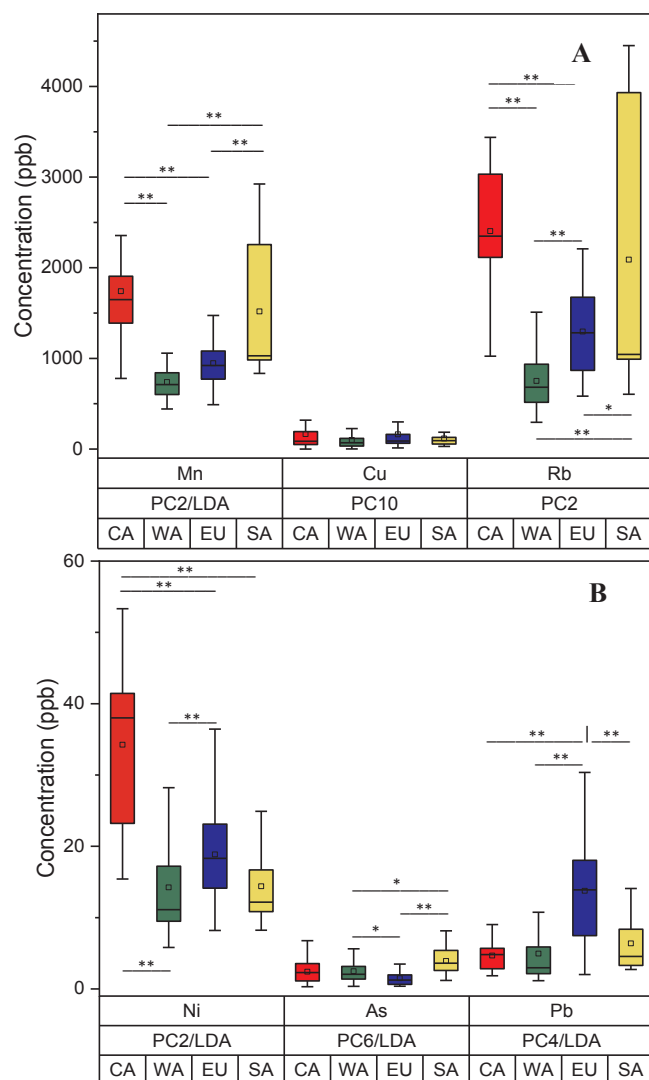


Fig. 1. Box-and-whisker plots of (A) Mn, Cu, and Rb; and (B) Ni, As, and Pb in each region. See text for more detail.

4.7%), where it is associated with B, Na and Sr. Given As' association with soil-derived tracers and the relatively low concentrations, one likely deduction is that As in the tested wines also stems from natural sources rather than from the application of pesticides. Although concentrations for As in SA wines are significantly higher compared to the other areas, concentrations fall below the EPA drinking water standard of 10 ppb and the International Office of Grapes and Wines (OIV) wine standard of 200 ppb (OIV, 2018). Some soils contain significant Pb, e.g. Low-Ca granites (Faure, 1998), which may dominate over the anthropogenic signature; however, in regions of long industrialization, a buildup of Pb from particulate emission of fossil fuel combustion and subsequent deposition onto the grape have led to increased concentrations observed in wines (Angelova et al., 1999; Cozzolino, 2015; Kristensen et al., 2016; Vystavna et al., 2017). Pb can also make it into the wine from the solder used to fuse copper and stainless-steel piping. Pb concentrations in EU wines are significantly higher compared to the other regions, exceeding the EPA drinking water action level of 15 ppb in 42% of the samples, however, all wines fall below the OIV standard for Pb of 150 ppb (Fig. 1B) (OIV, 2018). All WA and CA samples are below the EPA drinking water standard and only one SA sample exceeds this limit. Pb shows up in PC4 (6.0% variance), associated with Fe, which seems to indicate that it may be stemming from the wine making equipment. Pb is important in the LDA.

La and Zr (Fig. S3) are both significant in discriminating between regions in LDA. La is more important; yet, neither of them display significant differences within the various regions. La and Zr correlate with PC7 (4.3% variance), which also shows a weak association with the crustal tracer Ba. Only one reference was found that reported La and Zr as tracers to discriminate wines, in the wine-producing region of Basilicata in Southern Italy (Galgano, Favati, Camso, Scarpa, & Palma, 2008). Here, PC7 could be representative of a basaltic signature from the CRB as these soils are known to be high in Ba and Zr (Ferns, Streck, & McLaughry, 2017).

Ce (Fig. S3) is the lowest concentration element that is present in the LDA and it appears in the component with the highest variance, PC1 (variance 25%), along with all other rare-earth elements (REE). REE are characteristic to the soil and have been found to be present at different concentrations in soils and wine grapes grown in different regions in Italy, suggesting that they could be useful tracers for authentication of wine (D'Antone, Punturo, & Vaccaro, 2017). In the present LDA, La and Ce are in positions 7 and 8, discriminating between EU and SA, and CA and SA, wines, respectively. REE concentrations are within the ranges found in grape juices analyzed in the Italian studies. EU wines tested in the present study had significantly higher REE concentrations than wines from the other three regions.

3.3. Water isotope analysis

Water isotope ratios, $\delta^{18}\text{O}$ and δD , are presented in box-and-whisker plots in Fig. 2. Five comparison pairs between the 4 regions show significant differences in isotope ratios between regions. CA wines have significantly higher and WA wines have significantly lower $\delta^{18}\text{O}$ compared to the other regions. For δD , the pattern is almost identical, except that CA and EU wines are not significantly different from each other (Fig. 2B). The isotopic composition of wine is fundamentally derived from the local meteoric water. The uptake into grapes and the winemaking process cause systematic fractionations that results in the wines being enriched in heavier isotopes (Dunbar & Wilson, 1983). However, previous studies (e.g., (Ingraham & Caldwell, 1999)) indicate that this fractionation is similar from one wine to another. Thus, the smaller δD and $\delta^{18}\text{O}$ of WA wines is explained by a known decrease in

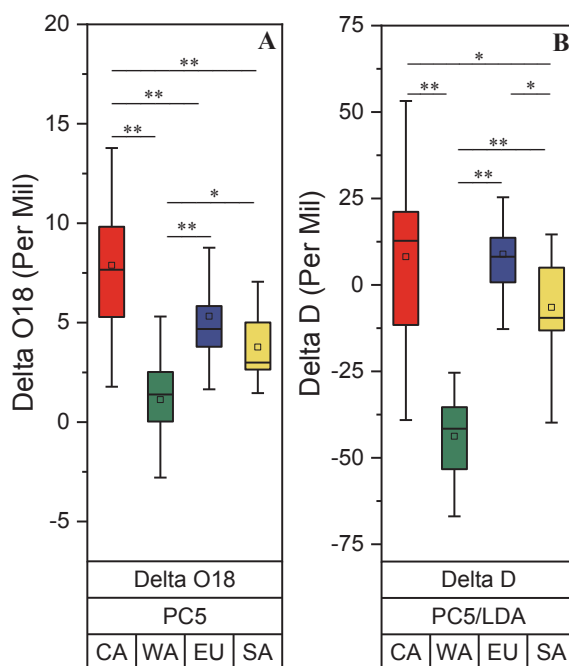


Fig. 2. Box-and-whisker plot of $\delta^{18}\text{O}$ (A) and δD (B) in each region. See text for more detail.

δD and $\delta^{18}O$ of precipitation with increasing latitude due to rain out from moist air masses originating in the tropics combined with a decrease in δD and $\delta^{18}O$ in precipitation moving inland from the Pacific Ocean (Dansgaard, 1964). Both $\delta^{18}O$ and δD appear in PC5 (5.4% variance), correlating only moderately negatively with As, for no obvious reason. In the LDA, δD is identified as the 6th discriminator. Other studies that use $\delta^{18}O$ and elemental analyses found that $\delta^{18}O$ contributes significantly to regional discrimination in wines from Brazil, Romania, China and Italy (Dinca et al., 2016; Dutra et al., 2011; Fan et al., 2018; Raco et al., 2015). It was unclear, however, if δD was measured in these studies.

3.4. Linear discriminant analysis

The forward step-wise method resulted in 11 components being most significant in geographically discriminating wines assigning them to the 4 regions (Table 2). Variables by order of entering in the step-wise LDA are Mn, Zn, Pb, Ni, As, δD , La, Ce, Si, Zr and Sr. Each component and potential provenance was discussed in detail above and a suggested source for each variable is indicated in the last column in Table 2. For optimal separation of regions, LDA combined the 11 components in 3 linear functions with standardized coefficients for each component listed. These are much like factor loadings in PCA and can thus be used to interpret the correlations of variables with the function. For instance, Function 1 (F1) is predominant in δD , Function 2 (F2) in Pb and Ni, and Function 3 (F3) in Mn, As, and Si (i.e., bold entries in Table 2). Positive vs. negative loadings have no physical meaning unless inspected in combination with the coefficients given to each function (*vide infra*).

Classification results of the model show that overall 96.2% of samples were correctly assigned to their respective region (Table 3). Within each region, classification was 88.2%, 100%, 92.3% and 97.1%, for CA, EU, SA, and WA wines, respectively. Five misclassified cases consisted of 2 from CA, one from SA and 2 from WA. In Fig. 3, all 133 wine samples are plotted using the three functions from LDA. Regions are color-coded and the 5 misclassified cases are encased. Examination of the plot from various angles (Figs. S4 and S5) together with the coordinates of the group centroids (Table S3) provides an indication of which functions are best at separating the various regions. For instance, F1, with the high absolute loading of δD , has good discriminating power for WA relative to all regions. Analogously, F2 is best at discriminating between CA and all other regions. F2 is associated with a negative coefficient for Pb (-0.729 , Table 2) and positive coefficient for Ni (0.602, Table 2). The strong discriminating power of F2 for CA is reflected in the large coefficient for F2 at the centroid of the CA group (3.178, Table S3). In combination, these two coefficients have a physical meaning. For instance, the negative Pb coefficient is weighted more strongly with the large positive coefficient of F2, resulting in low Pb in CA compared to the other regions (Fig. 1B).

Two of the five mis-assigned samples have strong justifications for

Table 3
Classification results by region for CA, EU, SA and WA samples.

	Original region	Predicted Group Membership				Total
		CA	EU	SA	WA	
Count	CA	15	0	1	1	17
	EU	0	33	0	0	33
	SA	0	0	12	1	13
	WA	1	1	0	68	70
%	CA	88.2	0	5.9	5.9	100
	EU	0	100	0	0	100
	SA	0	0	92.3	7.7	100
	WA	1.4	1.4	0	97.1	100

96.2% of original grouped cases correctly classified.

their wrongful classification. The CA sample that was classified to be WA with 95.1% confidence, contained grapes from WA but was made and bottled in CA. Thus, it could be argued that it was entered wrong into the model. The SA sample classified to be WA was on the cusp between the two regions with 49.5% vs. 50.5% confidences of being from SA vs. WA, respectively. This sample has water isotope ratios which are typical of those observed in WA wine, which is consistent with the fact that this wine stems from the southern-most wine growing region of Argentina, namely Patagonia, with latitude and climatic conditions similar to WA.

The three remaining mis-assigned samples show robust classification rates and thus have no justification for why they did not fall into their respective groups. For instance, the WA sample classified to be from CA, displayed a solid 91.6% confidence to be from WA, 8.3% from SA, and only 0.02% from its alleged CA origin. This wine's vintage is from 1998, which is the second oldest wine in the study by an average of 16 years. It is feasible that climate, soil and wine growing and making have changed significantly over this long-time frame; however, the oldest wine sample in the study, from 1994 and the same AVA, namely Columbia Valley, classified perfectly into its region. The remaining two misclassified wines are a CA wine classified as from SA, with 97.9% confidence, and a WA wine from Walla Walla thought to stem from EU, with 74.5% confidence.

To test the robustness of the model, 80% of samples were randomly assigned to training and the remainder to prediction sets. Average discrimination rates of 8 such model runs resulted in 96.07% and 91.20%, in the training and prediction sets, respectively (Table S4). Combining WA and CA wines into one North America (NA) region resulted in two functions with somewhat different components, the same classification result of 96.2% and 5 mis-assigned samples (Tables S5 and S6, Fig. S6).

4. Conclusions

Excellent geographical classification was obtained for 133 wines from 4 major wine producing regions using 11 elemental and a water isotopic parameter. The water isotope, δD , deemed particularly important in discriminating WA wines from all others. This is likely driven by isotopically lighter precipitation and soil water at such northern latitudes and the fact that the Columbia Valley lies in the rain shadow of the Cascade mountain range where the heavier water has been preferentially removed by rain-out effects, leaving lighter water in precipitation that progresses eastward. In addition, characteristically low concentrations in the first series of transition elements, in particular Mn and Fe, set apart WA wines from the others as a result of the high soil pH and chemical composition of the loess-derived soils in this wine growing region. The linear combination of Pb and Ni seems to be important in discriminating CA wines, while the linear combination of Mn, As and Si, provided significant discrimination power for SA wines. The remaining elements in the LDA model were Zn, La, Ce, Zr and Sr. To the best of our knowledge, this is the first study of this kind to chemically characterize and geographically assign WA wines compared to those produced in other parts of the world. These results provide a preliminary tool that can be used to effectively authenticate WA wines.

Abbreviations and nomenclature

$$\delta D \text{ or } \delta^{18}O = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} 1000\%$$

where R_{sample} is the ratio of the heavy isotope over the lighter isotope, and R_{standard} is the corresponding ratio observed in a standard, in this case Standard Mean Ocean Water (SMOW). The units in this notation are per mil (‰) difference from the standard.

Conflict of interest

The authors declare that there are no conflicts of interest.

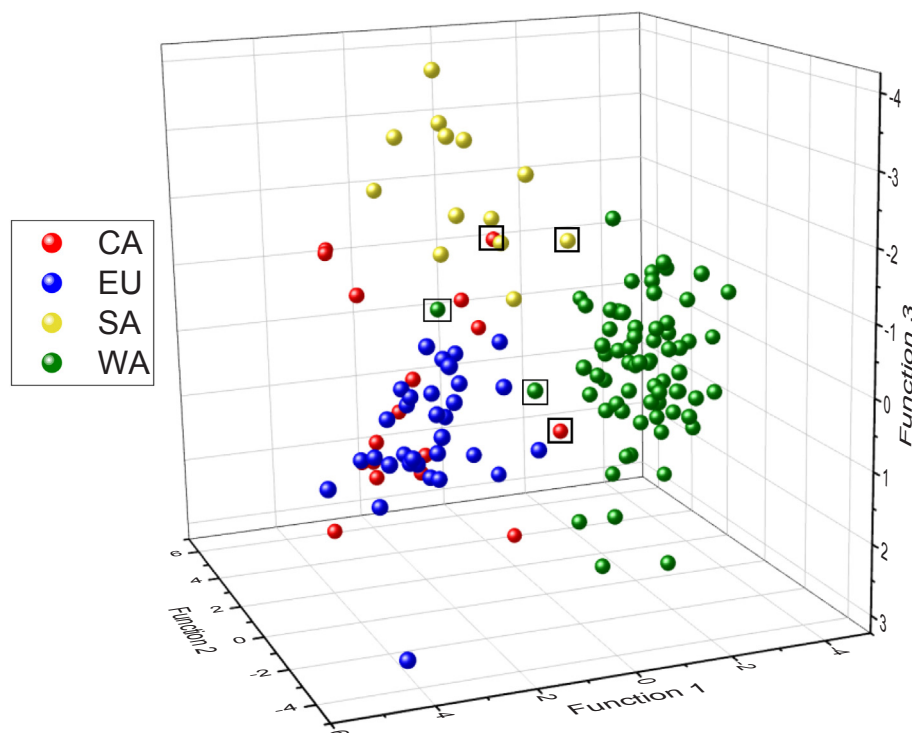


Fig. 3. Classification plot of each wine sample in the 3 dimensions provided by the three LDA functions from Table 2. Encased data symbols are the 5 misclassified samples.

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Appendix A. Supplementary material

A few details regarding the analytical and statistical methods appear in the SI section. In addition, 6 Tables and 3 Figures are shown. The Tables contain ancillary statistical output of the analyses described with the text, including the PCA loading matrix and LDA statistics, while the Figures show plots from different angles for better visualization. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2019.100007>.

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