Differential Absorption of Metals from Soil to Diverse Vine Varieties from the Valley of Tulum (Argentina): Consequences To Evaluate Wine Provenance

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We report the effect of vine variety on the absorption of metals from soil and follow the variety from wine through juice, verifying which metals could be used to assess wine provenance. Eleven metals were determined by atomic absorption spectroscopy in 32 soils, 16 grapes juices, and 18 wines sampled from a single vineyard having four red grape varieties (Cabernet Sauvignon, Bonarda, Malbec, and Syrah). The K nearest neighbor method allows us to distinguish among different soils, juices, and wines. Linear discriminant analysis affords descriptors to point out differences, mainly Mg, Mn, Ca, K, and Na. Data analysis evidenced that some elements have equivalent concentrations in soil, juice, and wine, while others did not. Canonical analysis shows good correlation between grape juice and wine with their provenance soil. We suggest using Mg as a marker of wine provenance, while Mn could be used to evaluate differences between wine varieties associated with plant physiology.

INTRODUCTION

The word "terroir" designates the association between geography, geology, climate, and winemaking practices that give wine of a particular provenance a singular character, which is frequently appreciated by consumers (*1*). The soil, for their physical structure and their chemical composition, interferes directly in the development of roots and, as a consequence, in the transport of water and mineral substances to the grapes. Vineyard soils should contain all of the indispensable elements for the nutrition of the grape and should fix them without being retained in exaggerated form. The water transports minerals and other elements necessary for their growth from the soil toward the plant. The ionic concentration of this solution is a function of the nature of the vineyard soil and fertilization (*2*).

Minerals are located mainly in the solid parts of the grapes, namely, skin (1−2%), the cellular walls of the pulp (0.8−2.8%), seeds (2−4%), and woody parts (2−3%) (*3*). Cations and anions are present in the grape juice in the form of both inorganic and organic salts (sulphates, phosphates, tartrates, malates, etc.). Salts subsist in the wine but in little proportions in some cases, producing settling during fermentation and the aging of wine (*4*). Additionally, the concentration of metals is sometimes modified during winemaking by the addition of bentonite and similar compounds used to clarify the wine (*5*).

The berry of the grape has a high complexity, which is the reason for its quality, but is also a factor of variability and originality between different regions and viticulture practices (*6*). Therefore, the chemical composition of wine varies from one region to another, and it is influenced by climate and regional geology as well as viticulture practice, transport and storage of the grapes, must, and winemaking techniques (*5*, *7−15*). The use of fertilizers, inorganic pesticides, and additives may change the natural metal contents in wines (*5*, *13*, *16−18*). As a function of their concentrations and different properties, metals in wine could be classified in three groups: major, minor, and trace elements.

In wine, most elements are present naturally, and their concentration patterns have already been used for years to evaluate wine provenance (*5*). Different physicochemical parameters have been used to differentiate wines according to their origin. However, metals are the most used to prove the relationship between the wine, the grape juice, and the soil composition (*9*). Almeida and Vasconselos (*14*) found a significant correlation between the multielemental composition of wine and the respective grape juice as well as between the wine and the provenance soil. Castiñeira et al. (*5*) studied the changes of minerals during the German white winemaking process.

The province of San Juan is situated in the center-west of Argentina. It is the second wine producer in Argentina, with an

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increasing part of its production dedicated to exportation, facing the need of traceability to improve the access to exigent markets.

The traceability could be defined as the ability to track the origin of a given food from its origin to the consumer through several steps, including transportation from farm to food manufacturers, processing, distribution to retailers, etc. (from farm to fork). Different approaches are possible to trace the origin of foodstuffs; however, the commonly accepted concept in food trade is based on the ability to follow the route of a given product by means of papers (certificates of origin, informatics systems giving information on the seller and the retailer, etc.). Geomatic traceability is also possible with some products but not with any. In wine, the concept of traceability is important because the provenance of a given wine usually defines its price as well as consumer confidence. In this context, winemakers and regulatory agencies introduced the certification of wine origin as a way to verify the provenance of wines. Chemical characterization is included among requisites necessary to obtain the certification of origin in wines, thus giving rise to the concept of chemical traceability.

In the present work, we evaluate the concentration of 11 metals (Mg, Ca, Fe, Mn, Zn, Cu, Cr, Co, Ni, K, and Na) in grape juices, wines, and soils from a vineyard located in the Valley of Tulum (Province of San Juan, Argentina). Our main goal was to investigate the effect of vine variety on the absorption of metals from soil and follow them from wine through grape juice, looking to verify which metals could be used to effectively assess wine provenance.

MATERIALS AND METHODS

Reagents and Materials. All reagents were of analytical grade. Ultrapure water (Millipore, Milli-Q system) was used to prepare standard solutions, dilutions, and blanks. AccuStandard atomic absorption spectrometry standard solutions (Fluka), 1000 mg L^{-1} in 1% HNO$_3$ (63%) (Merck), were used as stock solutions for calibration and in spike recovery studies. A 0.1% lanthanum oxide (Baker) solution in HCl (37%) (Baker), 0.1% CaCl$_2$ (Baker) solution in ultrapure water, and 30% H$_2$O$_2$ (Merck, Suprapure) were used as matrix modifiers (molecular suppressor). All glassware used was left in sulfuric−nitric acid solution 1:2 v/v HNO$_3$ (63%)−H$_2$SO$_4$ (98%) (Merck) overnight and then washed with ultrapure water.

Sampling. The sampling area (Valley of Tulum) is located at both riverbanks of the river San Juan between 31° and 32° south latitude. The altitude varies from 650 to 750 m above sea level. The main varieties of grapes in this area are Malbec, Cabernet Sauvignon, and Syrah. The ground used for the vineyards is stony and aluvional, with alternations of gravel, sand, slime, and clay. It is virginal or little cultivated, which is ideal for the cultivation of vines. The median annual temperature is 17 °C. The rainfalls are scarce (90 mm per year), with dry summers, which are reflected in the excellent levels of health of the vines, but force additional irrigation with either surface (ice-melting rivers) or groundwater. The winds are generally soft, granting excellent ventilation to the vineyard.

Soil. The vineyard studied, "Doña Filomena", is situated in the "Valley of Tulum" and is one of the oldest vineyards of this area. Additionally, this vineyard provides grapes to the "Augusto Pulenta" cellar, which provided the wines used for this study. The geographic coordinates are 31° 38′ (south) and 68° 21′ (west). It has 90 ha cultivated in trellis with four grapevine varieties: Cabernet Sauvignon, Bonarda, Malbec, and Syrah. Grapevines are drip (trickle) irrigated using water withdrawn from the San Juan River, which flows down from the Andes Mountains. Thirty-two soil samples were collected from the corresponding vineyard plots: nine from Cabernet Sauvignon plots, three samples from Bonarda parcels, 12 samples from Malbec plots, and eight samples from Syrah parcels. The number of soil samples for different varieties is proportional to the surface of the corresponding plots. Samples were collected using stainless steel shovels and were stored in individual plastic bags at darkness. Soils were sampled in depths from 10 to 20 cm, to avoid surface contamination by pollution of the surrounding environment and

to reduce the effects of fertilizers and variable organic matter content (*14*), and 50 cm from the side of the plot to reduce the effects of fertilizers and variable organic matter content (*19*).

Grape Juice. Samples of grapes were also obtained from the vineyard "Doña Filomena", collected one or two days before harvest, using plastic grooves and stored in plastics bags at 4−8 °C until preparation of the juice within 48 h of collection. Grape samples were collected from vineyard plots corresponding to the four grapevine varieties studied (Cabernet Sauvignon, Bonarda, Malbec, and Syrah). A total of 180 bunches were collected and classified according to their variety. Then, groups of 10 bunches were pooled and crashed to obtain the juice. Thus, we analyzed 18 samples of juice: six Cabernet Sauvignon, two Bonarda, six Malbec, and four Syrah. The number of juice samples is also proportional to the surface of the plots where each variety was grown to keep similar proportions during data treatment.

Wine. Eighteen samples of wine were obtained from "Augusto Pulenta" cellar. Wines were produced in the cellar using grapes from the same parcels that we used for soil and grape sampling. Winemaking used the same yeast and almost the same procedures, times, and temperatures for all varieties studied. Briefly, grapes were harvested and transported in plastic boxes to the cellar, where woody parts were separated before the grapes were pressed, where juice was stored in refrigerated stainless steel tanks where the pH was adjusted. Thereinafter, dry yeast was added to produce alcoholic fermentation, followed by separation of seeds and skin with further malolactic fermentation. Wine samples were collected after winemaking in refrigerated stainless steel tanks, followed by filtration without using bentonite or other additives, bottling, and stabilization at 20−22 °C during 1 year. Samples were transported to the laboratory in commercial 750 mL glass bottles and stored in the dark at 4−8 °C until analysis within 2 months. All wines (*n* = 18) were produced from a single grape variety (monovarietal) and corresponded to the same vintage: six samples from Cabernet Sauvignon, two samples from Bonarda, six samples from Malbec, and four samples from Syrah. The number of samples was proportional to the soil surface dedicated to each cultivar by the cellar to keep similar proportions during data treatment.

Elemental Analyses. Among analytical techniques used to evaluate metals, we can mention those used most: flame atomic absorption spectroscopy and flame atomic emission spectroscopy (FAAS-FAES) (*9*, *11*, *15*, *20*−*22*) as well as inductively coupled plasma mass spectrometry (ICP-MS) (*5*, *7*, *10*, *12*−*14*, *16*−*18*, *23*). In our case, elemental analyses were carried out on a Perkin-Elmer 3110 flame atomic absorption spectrometer (FAAS). Air-acetylene flame and monoelement hallow cathode lamps were used for measuring 11 minerals by atomic absorption spectrometry: Mg, Ca, Fe, Mn, Zn, Cu, Cr, Co, Ni, K, and Na.

Metals in Soil. Soils were dried at 30−40 °C, homogenized, and sieved using acrylic sieves (four different sizes) to avoid interferences observed when using metallic sieves (*14*). Each sample, consisting of the particle fraction < 63 μm, was divided into two subsamples, and each subsample was analyzed in triplicate.

Bioavailable Fraction. The method used for the analysis of bioavailable metals in soil was an extraction with dietilentetraminopentacetic acid (DTPA) as a chelating agent (*24*) and triethanolamine (TEA). This solution simulates the ability of plant roots to uptake nutrients from the soil. Brief procedure: 10 g of dried soil was weighed into an erlenmeyer flask, and 20 mL of extraction solution (0.01 M CaCl$_2$ + 0.005 M DTPA + 0.1 M TEA) was added (*24*, *25*). The resulting suspension was shaken for 2 h on a mechanical shaker and filtered through Whatman-42 paper. Blank solutions, containing only reagents without soil, were used to subtract metal contents arising from reagents and laboratory tools.

Pseudo-Total Fraction. The method used for the analysis of pseudo-total metals fraction in soil was using aqua regia (HNO$_3$−HCl, 1:3 v/v) (*24*, *25*). Brief procedure: 0.5 g of dried soil was weighed into an erlenmeyer flask and 7.5 mL of HCl (36.5%) and 2.5 mL of HNO$_3$ (63%) were added, followed by gentle shaking overnight at room temperature (23 ± 2 °C). Afterward, the flask was placed on a hot plate in a ventilated hood and heated until the formation of moist salts, which were allowed to cool and diluted to 100 mL in a volumetric flask. Blanks were prepared using the same protocol without soil. Recovery studies were also carried out by adding variable amounts of individual atomic absorption spectrometry standard solutions (AccuStandard, 1000 mg L^{-1} in 1% nitric acid)

Table 1. Analytical Parameters for Wine, Juice, and Soil (Pseudo-Total) Samples

element	wavelength (nm)	Wine–Grape Juice			soil (*pseudo*-total fraction)		
		% recovery[a]	LOD (mg L^{-1})	LOQ (mg L^{-1})	% recovery[a]	LOD (mg g^{-1})	LOQ (mg g^{-1})
Ca	422.7	86	0.16	0.51	95	0.08	0.25
Co	240.7	90	0.10	0.30	105	0.03	0.10
Cr	357.9	96	0.03	0.10	85	0.015	0.05
Cu	324.8	85	0.005	0.015	82	0.002	0.007
K	766.5	99	0.30	0.93	89	0.01	0.03
Fe	248.3	108	0.15	0.48	118	0.05	0.15
Mg	285.2	102	0.006	0.018	93	0.003	0.009
Mn	279.5	85	0.025	0.08	85	0.012	0.04
Na	589.0	108	0.012	0.036	98	0.003	0.01
Ni	232.0	104	0.41	1.29	114	0.20	0.60
Zn	213.0	96	0.013	0.042	89	0.007	0.02

[a] Percent of recovery from spiked samples.

Table 2. Concentration of Bioavailable Metals Measured in Soil by FAAS (mg g^{-1})[a]

element	Cabernet Sauvignon (*n* = 9)		Bonarda (*n* = 3)		Malbec (*n* = 12)		Syrah (*n* = 9)	
	median	range	median	range	median	range	median	range
K	0.25	0.21–0.41	0.17	0.14–0.21	0.05	0.02–0.20	0.09	0.05–0.40
Na	0.09	0.03–0.17	0.07	0.04–0.12	0.07	0.03–0.09	0.06	0.02–0.11
Mg	0.08	0.06–0.15	0.06	0.05–0.09	0.09	0.04–0.20	0.09	0.04–0.15
Ca	1.14	0.86–2.49	0.79	0.62–1.08	1.02	0.30–1.88	1.48	0.73–1.86
Fe	0.002	0.001–0.004	0.003	0.002–0.005	0.005	0.002–0.007	0.005	0.003–0.01
Zn	0.001	<LOD–0.002	0.0008	0.0006–0.0009	0.0007	0.0004–0.003	0.0007	0.0004–0.003
Mn	0.008	<LOD–0.013	0.007	0.006–0.009	0.005	<LOD–0.009	0.005	0.002–0.01
Cu	0.003	0.002–0.004	0.002	0.002–0.003	0.002	0.001–0.009	0.003	0.001–0.006
Ni	LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cr	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD–<LOQ
Co	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

[a] LOD (mg g^{-1}): Co (0.002), Zn (0.00003), Mn (0.0005), Ni (0.0008), and Cr (0.0007). LOQ (mg g^{-1}): Co (0.006), Zn (0.00008), Mn (0.0016), Ni (0.0026), and Cr (0.002).

to yield double of the starting concentration for each element before shaking started. Spiked samples were further analyzed to evaluate the recovery percentage.

Metals in Juice and Wine. Grape juice was prepared in the laboratory. Grapes collected at each plot were crushed, and the juice was separated from woody parts, skins, and seeds by filtration and stored in glass bottles. For wine preparation, the corks were carefully removed from the bottle, and an aliquot was decanted into a 50 mL beaker (*5*). Considering our previous experience (*26*) and reports on matrix effects (*14, 18*), we decided to test two different methods: (A) Direct 1:1 dilutions using 1% HNO$_3$ (63%) or (B) microwave-assisted digestion. We obtained better recoveries using the method B (recoveries were between 86 and 114%, **Table 1**). Following is a brief description of method B: Samples were mineralized using a microwave oven (Anton Paar 3000); thus, 5 mL of raw sample was introduced in quartz vessels, followed by the addition of 6 mL of concentrated nitric acid. Vessels were cap closed and heated in the microwave oven using the following power sequence: starting a 15 min ramp until reaching 350 W, holding for 45 min (maximal *T* = 169 °C; max pressure = 75 bar) and a final 15 min step disabling power to reach pressure equilibration. Mineralized samples were quantitatively transferred to 25 mL volumetric flasks, and the volume was completed using ultrapure water followed by filtration using 0.45 μm filters (Millipore, HAWG04756). Samples were prepared in duplicate. A triplicate spiked sample was also prepared to verify recovery percentages of different elements. So, 5 mL of sample was introduced into the quartz vessel, followed by the addition of variable amounts of individual atomic absorption spectrometry standard solutions (AccuStandard, Fluka, 1000 mg L^{-1} in 1% nitric acid) to yield double of the starting concentration for each element. The rest of the procedure was the same used for nonspiked samples. Appropriate dilutions and the addition of matrix modifiers (CsCl, La$_2$O$_3$, CaCl$_2$, and H$_2$O$_2$) were performed before measuring individual elements. Repeatability of atomic absorption measurements was usually ≥97% comparing values obtained from triplicates (three measurements on each duplicate sample or spiked sample). The stability of the equipment was verified by measuring the calibration curve before and after sample measurements. Blank solutions, containing only reagents without wine/juice, were used to deduct the content of metals arising from reagents and laboratory tools. The limit of detection (LOD) was established considering three standard deviations (SD) of absorption of the blank, while the limit of quantification (LOQ) was set considering 10 SD. Analytical parameters, instrumentation conditions, and recovery percentages are summarized in **Table 1**.

Statistical. Multivariate statistical methods allowed verification of the contribution of each variable to the model and its capacity to discriminate one category from another.

Data Set. The contents of metals in grape juice, wine, and soil samples were taken as chemical descriptors. Each data matrix was composed of 11 columns (the analyzed elements), using one additional column containing the grape variety as the dependent categorical variable. First, we tested the normal distribution of our data set. None of the studied metals were normally distributed, so the statistical analysis was based on nonparametric techniques. Thus, we report the median and the interquartile range as a measure of data dispersion (*8*).

Stepwise Linear Discriminant Analysis (SLDA). SLDA was applied to the data set to identify those variables that could help to distinguish among categories (varieties of grape juice, wine, or provenance soil) according to *F* value. However, because of the non-normal distribution observed, we did not use SLDA but K nearest neighbor (KNN) to calculate the rate of classification.

KNN Method. KNN is a nonparametric method of classification (it does not formulate a hypothesis on the distribution of the variables used). This procedure classifies an object in the category that contributes the greatest number of the K nearest known objects (*11*). The objective of applying this method was to classify an unknown sample in terms of its mineral characteristics into one of the groups corresponding to the varieties of grapevine in study. Considering the size of the data set, we decided to use the cross-validation method to validate results.

Canonical Correlation (CC). CC was used to find multivariate relationships between levels of elements in soils, grape juice, and

Table 3. Concentration of Pseudo-Total Metals and pH Measured in Soil by FAAS (mg g^{-1})^a

element	Cabernet Sauvignon (n = 9)		Bonarda (n = 3)		Malbec (n = 12)		Syrah (n = 9)	
	median	range	median	range	median	range	median	range
K	3.51	2.61−5.14	2.48	2.37−2.53	2.78	1.35−4.27	2.86	2.42−2.11
Na	2.35	0.94−3.26	2.30	2.15−2.49	2.31	1.52−2.89	2.27	1.77−3.40
Mg	6.91	3.20−10.94	5.41	4.84−6.84	7.87	4.60−14.26	8.82	6.26−10.80
Ca	10.65	4.90−17.61	17.63	15.63−24.16	21.61	15.81−31.92	23.49	16.05−31.23
Fe	19.87	11.55−26.51	17.66	16.68−21.67	20.36	15.76−25.95	22.38	16.29−28.62
Zn	0.08	0.05−0.11	0.06	0.05−0.07	0.06	0.05−0.10	0.07	0.05−0.08
Mn	0.50	0.38−0.71	0.47	0.43−0.60	0.49	0.19−0.76	0.51	0.19−0.77
Cu	0.03	0.02−0.04	0.03	0.02−0.04	0.02	0.01−0.05	0.03	0.01−0.05
Ni	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cr	0.04	<LOD−0.13	0.09	0.08−0.13	0.11	<LOD−0.14	0.12	0.09−0.16
Co	<LOQ	<LOD−<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
pH	7.47	7.30−7.62	7.72	7.57−7.73	7.59	7.40−7.78	7.57	7.47−7.85

^{a} LOD (mg g^{-1}): Co (0.03), Zn (0.007), Mn (0.01), Ni (0.2), and Cr (0.015). LOQ (mg g^{-1}): Co (0.1), Zn (0.02), Mn (0.04), Ni (0.6), and Cr (0.05).

Table 4. Concentration of Metals, pH, and °Brix Measured in Grape Juice by FAAS (mg L^{-1})^a

element	Cabernet Sauvignon (n = 6)		Bonarda (n = 2)		Malbec (n = 6)		Syrah (n = 4)	
	median	range	median	range	median	range	median	range
K	846	474−974	632	608−652	769	700−1096	1009	826−1089
Na	29	20−35	48	46−49	40	31−62	67	60−81
Mg	112	101−127	80	77−82	94	83−119	132	106−158
Ca	136	96−187	114	108−120	114	108−169	145	114−181
Fe	1.3	0.8−2.1	0.7	0.7−0.8	1.1	0.3−1.4	2.0	1.4−4.3
Zn	0.4	0.2−1.1	0.4	0.4−0.5	0.3	0.2−0.4	0.5	0.3−0.5
Mn	0.3	0.2−0.6	0.2	0.1−0.4	0.2	0.1−0.4	0.5	0.3−0.6
Cu	2.1	1.0−3.2	1.3	0.9−1.8	2.5	2.0−4.2	3.8	2.8−4.0
Ni	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cr	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Co	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
pH	3.90	3.73−3.96	4.16	4.13−4.18	4.18	4.13−4.24	4.10	4.08−4.14
°**Brix**	23.5	23.2−25.2	22.2	22.1−22.2	25.6	25.0−26.2	24.0	24.0−25.5

^{a} LOD (mg L^{-1}): Ni (0.4), Cr (0.03), and Co (0.1).

wines. Specifically, this analysis allows us to investigate paired relationships between studies matrices: soil−juice, juice−wine, and soil−wine. The statistical package, STATISTICA 7 from Statsoft (*27*), was used for discriminant analysis and CC, while the SAS package (*28*) was used for KNN method with cross-validation.

RESULTS AND DISCUSSION

Soil. *Content of Bioavailable Metals in Soil.* The median contents of the exchangeable cations and the range are presented in **Table 2**. Ca and K presented the major concentrations in analyzed plots, while Na and Mg showed similar contents. Concentrations of bioavailable Cr, Co, and Ni were very low, usually below the LOD in studied soil.

From **Table 2**, we can observe that concentrations of potassium were higher in plots corresponding to Cabernet Sauvignon and Bonarda in comparison to plots of Malbec and Syrah. The median content of bioavailable Ca was higher in plots of Syrah, intermediate in plots of Cabernet Sauvignon and Malbec, and lower in parcels of Bonarda. The median concentration of bioavailable Mn was higher than the limit, indicating its deficit in soils (< 0.001 mg g^{-1}) (*29*). Available Zn in soils varied among studied plots, with some of them having contents below the concentration essential for vineyards (> 0.0008 mg g^{-1}) (*25*).

Chemometrics of Bioavailable Metals in Soil. KNN allows 85% discrimination among different plots studied (data not shown). The application of forward stepwise LDA affords seven descriptors: Mn, Fe, K, Cr, Na, Ca, and Zn.

Pseudo-Total Metal Content in Soil. Results for pseudo-total metals in studied vineyard parcels are presented in **Table 3**. These results show that Ca and Fe are in major proportion than the rest of studied metals, showing a big difference in the iron concentration with respect to the analysis of the bioavailable fraction (**Table 2**). The median amount of Mg is higher than that corresponding to K and Na, while minor metals decreased in the order Mn > Zn > Cr > Cu. The levels of Ni and Co were below the LOQ (mostly < LOD).

Fe is one of the most important constituents of the ground (*30*). The parcels analyzed show similar contents of iron. Concentrations of Ca and Mg in soils are strongly influenced by the composition of parent rocks (*13*). The Ca content was similar in Malbec and Syrah plots, intermediate in Bonarda parcels, and lower in Cabernet Sauvignon plots. We observed that plots corresponding to Bonarda had the lowest content of Mg. On the other hand, parcels corresponding to Cabernet Sauvignon showed the highest levels of potassium.

The median level of Mn in the vine-growing soils studied was 490 mg kg^{1-}, which is similar to that found in vineyard soils of the Penedés area (NE Spain) (*24*). Additionally, studied soils have median levels of Zn (67.5 mg kg^{1-}) similar to that reported by Ramos (*25*).

Chemometrics of Pseudo-Total Metal in Soil. KNN allows us to distinguish with 93% accuracy between parcels corresponding to different vine varieties. Although it is supposed that the bioavailable fraction should give a more close picture on metals effectively absorbed by plants from soils, we obtained better results using the pseudo-total content during analysis of

Table 5. Concentration of Metals and pH Measured in Wines by FAAS (mg L^{-1})^a

element	Cabernet Sauvignon ($n = 6$)		Bonarda ($n = 2$)		Malbec ($n = 6$)		Syrah ($n = 4$)	
	median	range	median	range	median	range	median	range
K	1714	1618−2589	1673	1639−1697	1631	1274−1716	1639	1451−1962
Na	55	18−64	50	48−53	41	21−64	81	68−115
Mg	121	109−154	94.5	93.5−94.5	95	90−119	132	106−138
Ca	262	72−301	226	169−277	283	211−331	262	205−337
Fe	2.6	2.1−3.7	4.2	4.2−4.3	2.53	2.05−4.26	2.85	2.77−2.90
Zn	1.0	0.7−1.9	0.95	0.95−0.99	1.10	0.86−1.58	0.84	0.66−0.99
Mn	1.0	0.9−1.1	0.79	0.78−0.79	1.15	1.07−1.29	1.36	1.00−1.58
Cu	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ−0.46	<LOQ	<LOD−0.15
Ni	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cr	0.4	0.3−0.5	0.60	0.43−0.99	0.31	<LOQ−0.76	0.48	0.20−0.76
Co	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
pH	3.81	3.80−3.83	3.77	3.76−3.78	3.87	3.85−3.88	3.77	3.75−3.78

^{a} LOD (mg L^{-1}): Ni (0.4), Cr (0.03), and Co (0.1).
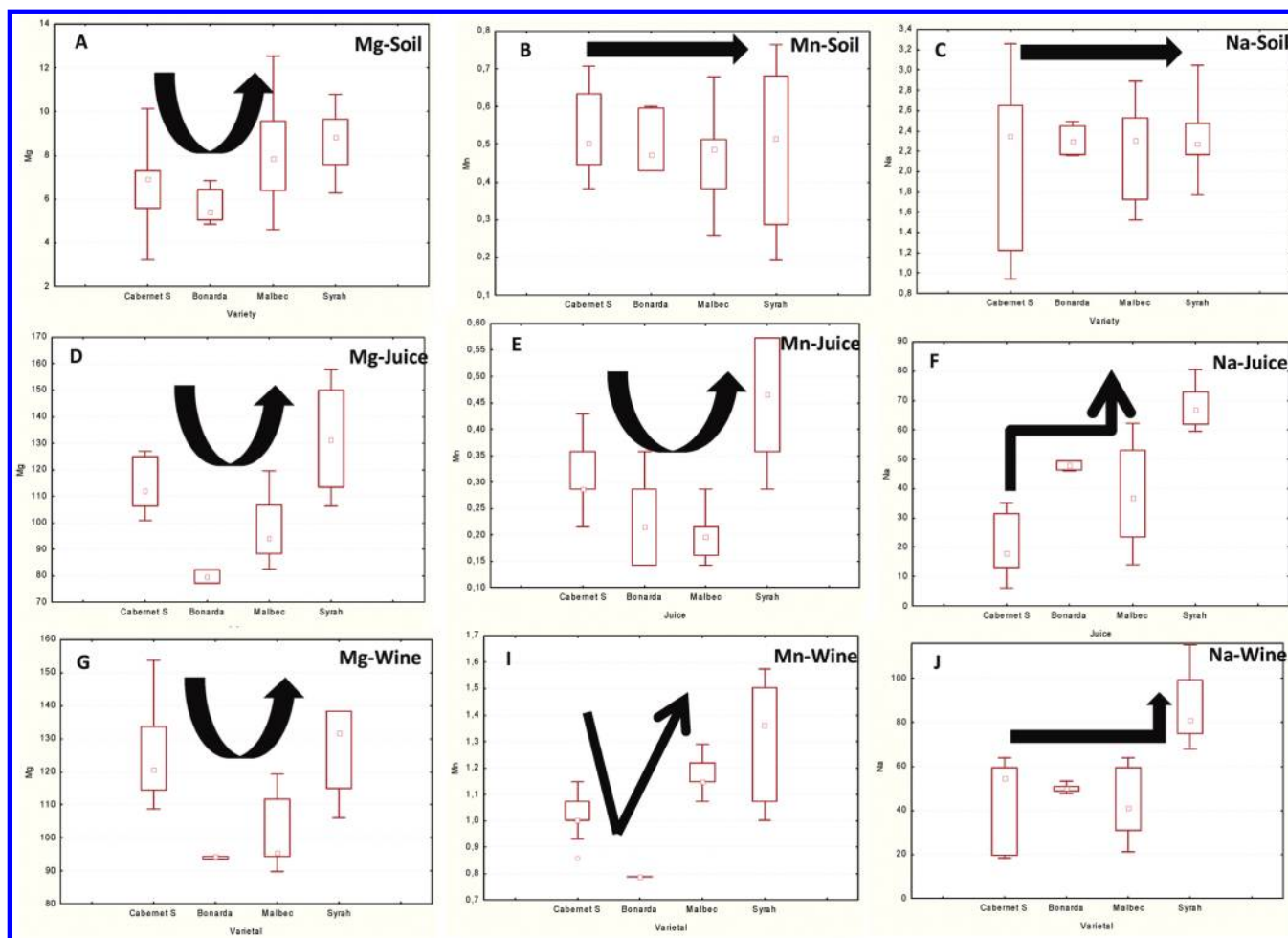

Figure 1. Multiple box-and-whisker plots showing the median and range of Mg, Mn, and Na in wine, grape juice, and soil samples, according to grapevine variety.

correspondence between soils, grape juice, and wines. SLDA affords six descriptors, Cr, Ca, K, Mg, Zn, and Cu, to differentiate between plots with different varieties.

Wine and Grape Juice. *Content of Metals in Wine and Grape Juice.* The contents of the metals analyzed in grape juice and wines, corresponding to four varieties of grapevine, are presented in **Tables 4** and **5**. The median contents of major elements decrease in the order K > Ca > Mg > Na for grape juice and wines. The median contents of minor and trace elements decrease in the order Cu > Fe > Zn > Mn for grape juice and Fe > Mn

> Zn > Cr > Cu for wines. Co and Ni were below the LOD in juice and wine, while Cr was under the LOD for grape juice.

The pulp of grape is rich in cations, with potassium and calcium as the major elements. The concentrations of K and Ca vary with the nature of the soil, the viticulture practices, the grape variety, and the climatic conditions (*4*). The contents of K and Ca were lower in juice than in wine (**Tables 4** and **5**), which could be explained as a consequence of differences in the pH of juice (3.8−3.9) and wines (3.3−3.4), leading to increased dissolution of both elements because of the pK_a of tartaric acid (3.036), which is
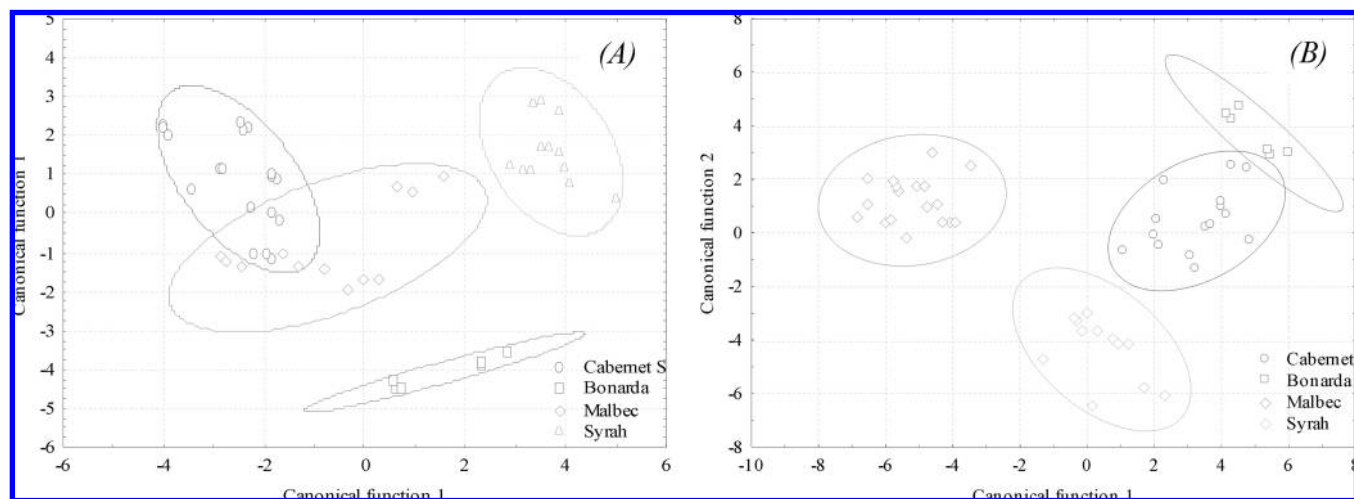
Figure 2. Distribution of grape juice (**A**) and wines (**B**): Cabernet Sauvignon, Bonarda, Malbec, and Syrah in the plane defined by the first two canonical functions of SLDA.

added during winemaking to reduce the pH of wine (*31*). Additionally, the low amount of Ca and K in studied juice can be partly explained because we discarded seeds and skin immediately after juice extraction in the laboratory, while red winemaking conserves seeds to give the color to the wine. The median concentration of potassium was quite different within the four juice varieties but similar among studied wines. Similar values between diverse wine varieties could be explained by settling of potassium bitartrate during the stabilization and aging at cool temperatures (*10, 22, 32*). The content of K found in wine during this work is similar to that cited by Cabanis et al. (*3*). However, median concentrations of Ca found during this work are higher than those reported in the literature (*3, 4*).

The presence of Mg in wines is dependent on the natural content in grape (*8*). The median content of Mg was similar in both grape juice and wine varieties analyzed during this work (**Tables 4** and **5**) and similar to values reported in the literature for wines (100 mg L^{-1}) (*3, 4*). A previous report on selected white wines showed a relatively wide variation in the content of Mg according to the wine variety (*22*). Our present results confirm such variation, now observed in four red wine varieties. However, the observed variation is equivalent between soil, juice, and wines (**Figure 1A,D,G**).

The concentration of Mn in must depends on the uptake capacity of grapevine to this element, the contribution of the ground, and agricultural practices (*12, 13*). Conversely, plants absorb Zn from the ground in small quantities (*8, 12, 13, 32*). Levels of Mn and Zn are increased during winemaking, which has been attributed to contamination by contact with equipment of the cellar (*8*).

We observed that median values of Zn and Mn were higher in wines than in grape juices (**Tables 4** and **5**). However, differences in the content of Zn between studied varieties were not evident in juices or in wines (**Tables 4** and **5**). On the contrary, the concentration of Mn showed a trend in juices that is similar to the corresponding wine variety but not related to the content of Mn in soil (**Figure 1B,E,I**).

The content of sodium was quite different for four juice varieties studied but quite similar in wines, except for Syrah, which presented the highest sodium concentration in studied wines. Previous reports on the sodium concentration in wines show that this element is in variable concentrations (10–250 mg L^{-1}) (*33*) according to the geographical origin of the vineyard: cultivated to the border of the sea, on dry lagoons, irrigated with saline waters

(Argentina, Australia) (*4, 12*). Our present results show that levels of sodium in wines from Tulum Valley (San Juan, Argentina) are in good agreement with limits establishment by OIV (*33*).

The presence of Fe and Cu in must and wines comes from the vineyard soil, the equipment used in the wine cellar, and the use of fertilizers or pesticides containing these elements in their composition (*9, 12, 13, 32*). The quantification of Fe and Cu is important in must and wines because these elements are responsible for ferric and cupric cases, respectively, during wine aging (*35*).

We observed that the content of Fe was increased in wines in relation to grape juices, which is in agreement with a previous report by Castiñeira et al. (*5*). The increment observed in the concentration of Fe from juices to wines was strongly dependent on the studied varietal, with Bonarda showing the highest increase (ca. 6-fold) and Syrah with the lowest one (similar median values) (**Tables 4** and **5**). An explanation could be the contamination from tubes, tanks, and pumps (*12, 13, 32*). All wine samples studied had contents of Fe below 7 mg L^{-1} (range 2.05–4.30 mg L^{-1}), which is considered the minimum concentration required to form ferric cases (*13*).

The concentration of Cu is affected by the winemaking process and can decrease by a factor of 5–20 after fermentation, depending on the wine variety and winemaking process. It is easily converted to CuS and Cu_2S (*1, 5*). The limit for cupric cases in wines established by OIV is 1 mg L^{-1}. In our case, the content of Cu in juice ranged from 0.9 to 4.20 mg L^{-1}, while the corresponding value in wines was mostly below the limit of quantification (0.15 mg L^{-1}). These results are consistent with those reported by Castiñeira et al. (*5*).

Co and Ni were below the detection limit in grape juices and wines. In contrast, Cr was not detected in juices, but its content in wines ranged from 0.2 to 0.99 mg L^{-1}. The presence and increment of Cr in wines could be the result of the contact with pumps, pipelines, and metallic containers (*12–14*).

As can we see, the concentration of many metals changes from must to wine depending on the grapevine variety and the winemaking process. Castiñeira et al. (*5*) reported that the magnitude of these changes is different, depending more on the element and its concentration than on the winemaking process or wine origin.

Chemometrics of Metals in Grape Juice and Wine. The KNN method was applied to the juice data set, looking for classification of samples according to their variety using cross-validation, Mahalanobis's distance, and $k = 5$. Applying KNN, 100% of juice samples were correctly assigned to their

corresponding varieties (data not shown). SLDA was also used to reduce or to eliminate irrelevant information, showing the most relevant data (*11*). SLDA afforded seven descriptors: Na, Mn, Mg, Cu, Ca, K, and Fe that allowed us to distinguish between juice varieties. As we can see from **Figure 1D–F**, Mg, Mn, and Na allow us to distinguish among four studied varieties of grape juice. The highest levels of Mn and Na allow differentiation of Syrah juice (**Figure 1E,F**), while Bonarda presents the lowest levels of Mg (**Figure 1D**) and Cabernet Sauvignon presents the lowest values of Na (**Figure 1F**).

The application of KNN also allows 100% discrimination among four wine varieties (data not shown). LDA pointed to Mn, Mg, Na, Fe, Zn, K, Ca, and Cr for distinguishing between different wines. **Figure 1G,I,J** shows that Mg, Mn, and Na medians allow differentiation between wine varieties. Conversely, K and Ca median concentrations were quite similar in studied wines, not allowing discrimination between varieties (**Table 5**). Our present results do not agree with previous reports using K as one of the parameters allowing discrimination between two Andalusian DO fine wines (*35*) or classifying wines from Canary Islands, respectively (*21*).

From **Figure 1**, we can observe that Mg and, to a lesser extent Mn, exhibit a visual correlation between its content in both grape juice and wine, for Cabernet Sauvignon, Bonarda, Malbec, and Syrah varieties. A graphical representation of studied juices and wines, in the plane defined by the first two canonical functions, is shown in **Figure 2**, where it is statistically demonstrated that juices and wines from the same vineyard can be clearly differentiated in function of their variety.

Correspondence between Provenance Soil, Grape Juice, and Wine. The use of chemical (metals) patterns to evaluate the geographical provenance of wines may suggest that the elements are mainly regulated by their movement from rock to soil and from soil to grape (*14*). The overall soil–plant interaction is highly complex, and soil–wine element relationships are further complicated by wine-processing effects (*10*). Greenough et al. (*36*) suggested that the elemental composition of wines could be largely related to solubility factors of particular elements, normally present in soils.

As we can see from **Figure 1C,F,J**, sodium is present in similar quantities in the analyzed parcels but has different profiles in both juices and wines. This could indicate a selective absorption of this mineral by vine, confirming the theory that the vine is a sodium-phobic plant, absorbing little content of Na from soil, allowing growth in highly saline soils (*6*).

Also from **Tables 3–5**, we can observe that K and Ca have a different behavior in comparison to Na. This is mainly because there are some degrees of correspondence between levels of K and Ca in both soils and grape juices, which is lost in the corresponding wines.

The vine plant takes up Mg (lithophile element) easily from the soil, mainly because it tends to be water-soluble as a result of extremely low ionic potentials. Kment et al. (*13*) and Frías et al. (*34*) found that the Mg content in wine depends on its concentration in the vineyard soil, being one of the elements useful to evaluate wine provenance. Our present study fully corroborates those results. Furthermore, we demonstrate that the relative levels of Mg are conserved from provenance soil, through grape juice (must) until wine (**Figure 1A,D,G**).

Also, **Figure 1** shows that the mineral profile of Mn (lithophile element) is similar in wines and grape juices but different from the original soil where the grapevines grew (**Figure 1B,E,I**). In this respect, our present results do not agree with the previous one from Kment et al. (*13*), finding positive correlation between levels of Mn in soil and wines. Furthermore, differences observed for

relative levels of Mn among provenance soils and grape juices point out the need for considering plant physiology, in addition to geochemistry, to assess reliable markers for wine traceability.

Although bivariate correlation between individual elements at each matrix (soil, juice and wine) is possible, we were interested in evaluating the correspondence of used matrices by a multivariate method. Among available methods, CC allows us to fully verify correlation between different data sets at the multivariate level. However, only two data matrices can be analyzed by CC. So, we used CC to verify the correlation between (a) provenance soils and grape juices varieties, (b) grape juices and wine varieties, and (c) soils and wines (Cabernet Sauvignon, Bonarda, Malbec, and Syrah) considering those variables pointed out by LDA as the most significant to evidence differences among studied varieties.

So, we defined three data sets: Set 1 includes values for pseudo-total elements in soil (K, Mg, Ca, and Fe), set 2 includes the variables for juices (K, Mg, Ca, Fe, Na, and Mn), while set 3 includes the variables for studied wines (K, Mg, Ca, Fe, Na, and Mn), plus a new variable accounting for grapevine variety at each matrix. The analysis showed a significant correlation of 91% ($p = 0.002$) between the multielemental composition of soil and grape juice, with Mg being one of the most important variables to correlate both data sets. Similarly, grape juices and wines present a correspondence of 94%, with Mg and K being the best elements to explain the relationship between wine variety and its respective grape juice. Finally, we observed a significant correspondence (r, 0.79; $p = 0.0001$) between soil and wine sets. This last result indicates that the metal content in vineyard soil is reflected in wine with an overall correlation of 79%, pointing out Mg and K as most important variables to associate wines with their provenance soil at a multivariate level.

Potassium was used by Latorre et al. (*20*) to characterize wines according to geographical origin; Kment et al. (*13*) as well as our current results show the importance of Mg in predicting wine provenance. The minor correspondence between soil and wine data set (79%), in comparison with either soil–juice or juice–wine (91 and 94%, respectively) could be due to the influence of both winemaking technology and plant physiology. The remaining 21% could be attributed to supplementary factors, like climate and other environmental issues, agricultural practices, grape maturity at harvesting, etc.

So far, we conclude that the metal profile can be used for distinguishing among wines of different origins, with special emphasis on the content of Mg and consideration of CCs to fully demonstrate correspondence at the multielemental level. However, it should be considered that the metal profile does not depend exclusively on the geochemistry of provenance soil but is affected by the winemaking process and the grape variety. Mn seems to be a good marker associating grape variety with the corresponding wine. Additional studies are necessary to confirm if these results are also valid to both different grapevines growing in Argentina and wines, grape juices, and soils from other regions.

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