

Multielement Composition of Wines and Their Precursors Including Provenance Soil and Their Potentialities As Fingerprints of Wine Origin

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The influence of the provenance soil and vinification process on the wine multielemental composition was investigated. For this purpose, two different vineyards from the Douro wine district, Portugal, were selected. Monovarietal grapes from a 10 year old vineyard were used to produce a red table wine, in a very modern winery. Polyvarietal grapes from a 60–70 year old vineyard were used to produce a red fortified wine, similar to Port, through a traditional vinification process. The multielement compositions (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Hf, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Sc, Sr, Ti, Th, Tl, U, V, W, Y, Zn, Zr, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) of soil, grape juices (prepared in the laboratory), and samples collected in the different steps of each winemaking process were measured. Inductively coupled plasma mass spectrometry was used, after suitable pretreatment of the samples (by UV irradiation for liquid samples and high-pressure microwave digestion for soil). Both vinification processes influenced the multielement composition of the wines. Most of the elements presented similar or even lower concentrations in the wine as compared to that observed in the respective grape juice, probably as a result of precipitation or coprecipitation with suspended particles during fermentation and/or wine aging. Evidence of effective contamination during grape pressing, fermentation, and/or fining of wines (depending on the element) was observed for Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn in the fortified wine and Al, Cr, Fe, Ni, Pb, and V in the table wine. Nevertheless, significant correlations were obtained between the multielement composition of the wine and the respective grape juice ($R = 0.997$ and 0.979 for the fortified and table wines, respectively, $n = 31$, $P < 0.01$), as well as between that in the wine (median of the two studied wines) and the provenance soil ($R = 0.994$, $n = 19$, $P < 0.01$), for the set of elements determined in common in the different types of samples. These results are promising concerning the usefulness of the elemental patterns of both soil and wine as fingerprints of the origin of the studied wines. Nevertheless, more wines from the same and other wine districts must be studied in order to consolidate this conclusion. The multielement compositions of the studied wines were compared with those of wines of different characteristics and origins, as well as with the respective legal threshold limit values, when available. Relatively low metal levels, below their threshold limit values, were found in all cases.

KEYWORDS: Wine; multielement composition; vineyard soil; vinification; ICP-MS

INTRODUCTION

Various studies indicate that multielement composition of a wine can be used to obtain information about its origin, making up a way for laying down a wine fingerprint that is able to secure the authenticity of the wine (1–4). The ability for discriminating wines by regions through their trace element patterns suggests that the elements are mainly regulated by their movement from rock to soil and from soil to grape. Greenough et al. (1) have shown that wine multielemental composition was strongly influenced by the solubility of inorganic compounds of the soil.

In principle, the pattern of a wine will reflect the geochemistry of the provenance soil. However, several factors, such as environmental contamination, agricultural practices, climatic changes, and vinification processes, may change markedly the multielement composition of the wine and may endanger the relationship between wine and soil compositions. Another factor that may condition that relationship is related with plant uptake, which can vary with the vine variety. All of these factors may imperil the usefulness of wine multielement composition as a fingerprint of the wine region of origin.

Concerning the influence of wine processing on the elemental pattern of a wine, there is already some information in the literature, which, in some aspects, is even contradictory.

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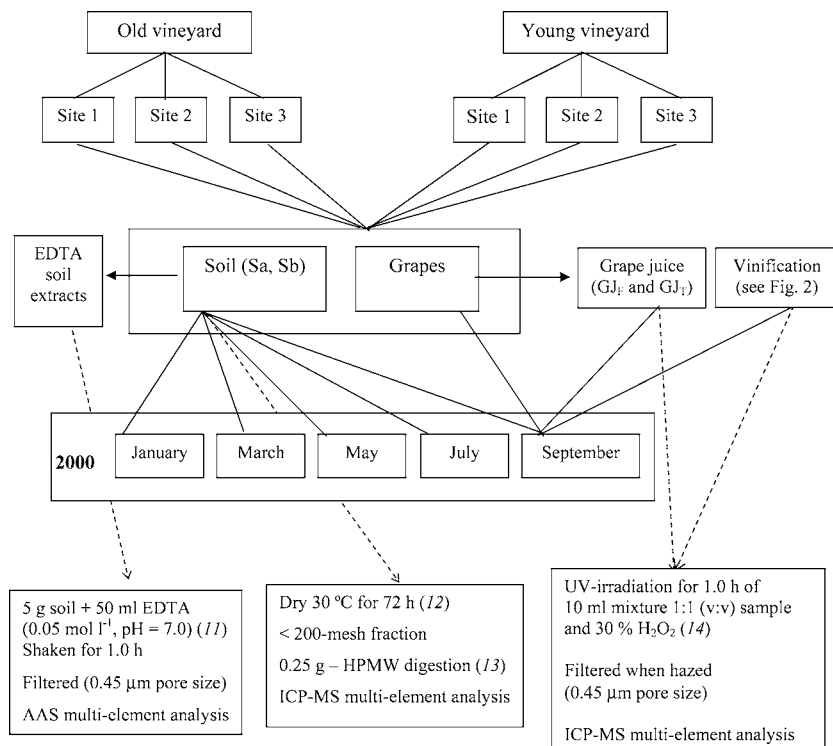


Figure 1. Schematics of the sampling strategy and analytical procedures used in this work (that took place in the year of 2000). S_a and S_b were soil samples collected at surface and at 20 cm depth, respectively. GJ_F and GJ_T were grape juices prepared in the laboratory by smashing mature grapes from the old and from the young vineyard, respectively.

Eschnauer et al. (5) showed that during fermentation and fining of wines the concentration of several elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Pb, V, and Zn) decreased. The same trend was observed by Angelova et al. (6) for Cd, Cu, Pb, and Zn and by Teissedre et al. (7) for Pb. In contrast, Kristl et al. (8) concluded that during the maturation of the wine there was a slight contamination of Cd, Cr, and Pb, which were released from wine cellar equipment (brass and stainless steel). A Pb contamination during the vinification process (fermentation and aging) was also observed by the present authors (9). In addition, Jakubowski et al. (10) reported that the concentrations of rare earth elements increased from young to finished wines, due to the use of bentonites. Therefore, because the vinification process can influence the concentration of several elements in the wine and in some regions different winemaking processes have been used, element fingerprints of the wines should be relics of soil signatures that survived metabolic and winery processing. Otherwise, the utilization of the multielement pattern as a fingerprint of wine origin has to be confined to high quality wines produced from specific vine varieties and whose influence of the vinification processes on the wine patterns had been previously studied and permanently controlled.

In this study, we were prompted to follow, from the vineyard to the final product, the production of two different wines, to investigate the relative influence of the soil pattern and the vinification process on the multielemental composition of the final product. Both selected wines were from the Douro wine district—Northeast of Portugal. However, the grapes used to produce the two wines (a monovarietal red table wine and a polyvarietal red fortified wine) were from two vineyards of very different ages; the wines were produced in two wineries according to very different procedures. A specific aim of this study constituted investigating whether there would be a significant correlation between the multielement composition of each wine and that of the provenance soil. A complementary

aim was the characterization of the wines in terms of their elemental composition as it may be of interest on nutritional and toxicological points of view.

EXPERIMENTAL PROCEDURES

Material and Reagents. Suprapure concentrated HNO₃ and a solution of 30% H₂O₂, pro analysis, both from Merck, were used without further purification. For inductively coupled plasma mass spectrometry (ICP-MS) semiquantitative mode of analysis, both a REEs (100 μg L⁻¹ from La to Lu) stock standard solution from Alfa and a multielement stock standard solution with 30 elements (1000 mg L⁻¹ of Ca; 100 mg L⁻¹ of As, B, Be, Fe, Se, and Zn; 10 mg L⁻¹ of Ag, Al, Ba, Bi, Cd, Co, Cr, Cu, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sr, Te, Tl, U, and V) from Merck, all for ICP-MS, were used for calibration. For internal standardization, a pro analysis stock standard solution of Rh (1000 mg L⁻¹) from Alfa was used. For atomic absorption spectrophotometry (AAS) measurements, standard solutions of Al, Cd, Cr, Cu, Ni, Pb, and Zn were prepared from Spectrosol stock solutions (1000 mg L⁻¹) from BDH. All of the other reagents were pro analysis or equivalent. Standard solutions were prepared daily from the stocks in polyethylene tubes by weighing. To avoid contamination, all of the material used for sampling and sample treatments was soaked in 20% v/v HNO₃ for at least 24 h, rinsed several times with deionized water, and dried in a Class 100 laminar flow hood. The sample manipulation was carried in a clean room with Class 100 filtered air.

Sampling. The sampling strategy is schematically described in **Figure 1**, which also includes summary information on sample treatments and analysis. Two vineyards, one 60–70 years old (forward called old vineyard) and the other 10 years old raised in a forest soil (young vineyard), both from the Portuguese Douro wine district, where the soil is schistous, were selected for this study. In both vineyards, only treatments with copper sulfate solutions were carried out during the study. Nevertheless, in previous years, several treatment products (pesticides and fertilizers) had been used, particularly in the old vineyard.

In **Figure 2A** is summarized the vinification process that has been used in the winery to produce the red fortified wine (similar to Port).

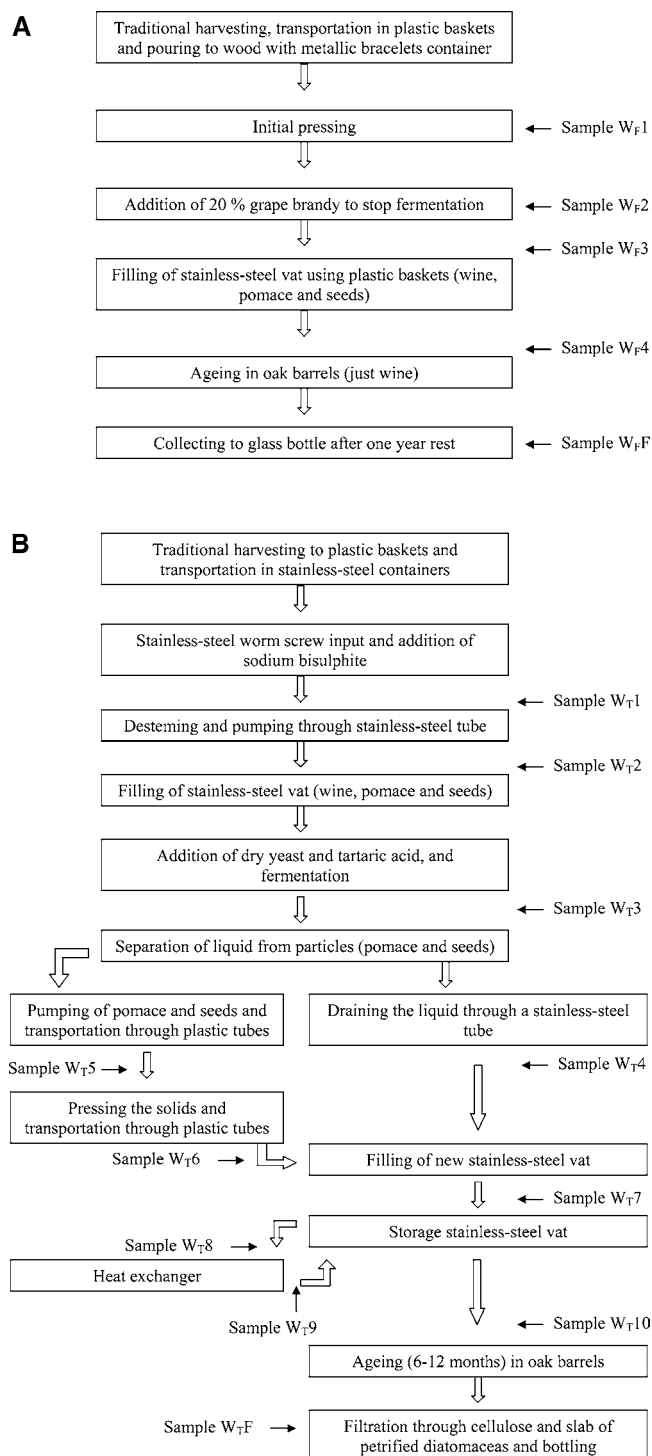


Figure 2. Schematics of the vinification processes with indication of the points where samples were collected. (A) Red fortified wine produced from polyvarietal grapes of the old vineyard (W_F1 to W_F4 and W_FF); (B) red table wine produced from monovarietal grapes (named "Touriga Nacional") of the young vineyard (W_T1 to W_T10 and W_TF).

This wine was produced with polyvarietal grapes from the old vineyard by using an old fashioned process, without automatic controls, and involving a small number of steps; the wine was poured directly from one container to the next. After vinification, the fortified wine was aged in oak barrels for periods between 2 and 20 years, depending on the desirable wine quality. By practical reasons, in the present study, the sample called "final product" was collected after only 1 year of aging.

In **Figure 2B** is summarized the vinification process that was carried out in a modern and automatic winery to produce the monovarietal

"Touriga Nacional" red table wine from grapes from the young vineyard. Stainless steel tubes and containers were used in most steps. Plastic containers and tubes (polyethylene, high-density polyethylene, and flexible poly(vinyl chloride)) were also used for the harvest and to transfer must and wine in the same steps. Aging of the wine took place in oak barrels. At the end, the wine was stored in glass bottles. In this study, the produced wine was analyzed about 1 month after bottling.

To follow the pathway of the wine production from the vineyard to the wine, vineyard soil, grapes and intermediaries, and final products were collected and analyzed. Soil and grapes were collected using plastic shovels (soil samples) and plastic gloves (grapes) and were stored in individual plastic bags, which were immediately closed. In each vineyard, samples were collected in three different sites, selected to be representative of the entire area. To differentiate any eventual change in the multielement composition as a result of atmospheric deposition, soil was collected at the surface and at 20 cm depth in each site. Throughout the vinification processes, samples were collected in triplicate in polyethylene tubes at points where a possible change in the multielement composition could be expected, for instance, as a consequence of contamination (see **Figure 2A,B**).

Samples Treatments. The samples of vineyard soil were first dried in an oven up to constant weight. The soil was fractionated and homogenized by sieving through nylon nets of 2 mm and 200 mesh. Only the fraction <200 mesh was analyzed. From each sample, three takings of about 0.25 g_{drysoil} were treated for analysis. The takings were digested with concentrated HNO₃ (13, 15) by high-pressure microwave (HPMW), using a MLS-1200 Mega system from Milestone to obtain the soil total recoverable metal levels (13). The digestion program consisted of three steps of 5 min each at 250, 400, and 500 W, respectively, and was run twice. The efficiency of the procedure was confirmed by analyzing San Joaquin Soil standard reference material SRM 2709, from the National Institute of Standard and Technology. After they were HPMW digested, the final solutions were kept at room temperature until analysis, which took place within 2 days. Before analysis by ICP-MS, suitable dilutions with a solution containing Rh were carried out. A 20 μg L⁻¹ Rh concentration was used for internal standardization, to eliminate the matrix discrepancies and to compensate for any drift occurring during the analysis.

Grape juice was prepared in the laboratory. For this purpose, identical quantities of nonwashed grapes collected in each one of the three sampling sites of each vineyard were mixed and smashed (with gloved hands) in plastic cups. The skins and seeds remained intact and were rejected. The obtained juice was transferred to polyethylene tubes. Aliquots of nonfiltered grape juice as well as nonfiltered samples from the vinification processes were pretreated by UV irradiation (14). Suitable dilutions with a solution containing HNO₃ and Rh were carried out afterward. Final solutions always contained 20 μg L⁻¹ Rh and 1% HNO₃.

ICP-MS Measurements. The analytical measurements were carried out in a Perkin-Elmer SCIEX Elan 5000 ICP-MS (Perkin-Elmer, Norwalk, CT) apparatus equipped with a cross-flow nebulizer, nickel cones, and a peristaltic sample delivery pump. The operating conditions were optimized daily as described before (16).

A procedure, using the ICP-MS semiquantitative mode of analysis, which was optimized for wine samples (16), was used for determination of Al, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Sc, Sr, Ti, Th, Tl, U, V, W, Y, Zn, Zr, and REEs in grape juices and samples collected throughout the vinification processes. In soil samples, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Hf, Li, Mn, Nb, Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn, Zr, and REEs were determined by using a similar procedure to that used for wines. The set of elements determined in both cases was not totally coincident due to technical limitations related with either limits of detection too high for the purpose or matrix interferences.

A reference soil (NIST-SRM 2709) was analyzed together with the vineyard soils every working day and several times a day during a period of about 2 months, with the purpose of controlling/detecting any ICP-MS instrumental drift over time. Daily variations (short-term precision) were between 2 and 12% for all elements and were lower than 5% in most of the cases. Exceptions were only observed for Nb,

for which the variations were between 9 and 100%, probably as a result of the relatively low concentration of this element in the reference soil. Because very low concentrations of Nb were observed in the vineyards soil, this element was excluded from the set of elements to be quantified. Similar control graphics and long-term variations, between 3 and 20% (in most cases around 10%), were observed for all of the elements measured in the reference soil, indicating that the ICP-MS instrument exhibited the same performance for low and high masses. Both short-term and long-term precisions were within those expected for the ICP-MS semiquantitative mode of analysis, since accuracy errors up to 20% for most elements have been reported in the literature (17–19).

Each working day, two red wines (one table and one Port), for which the multielement concentrations were known, were analyzed together with the grape juices and the samples from the vinification processes, also for checking any instrument drift. Daily variations between 2 and 40% were observed for all of the elements measured in both "reference" wines; most of them were lower than 10%. Variations higher than 20% were only observed for Ga (40%) and Sb (25%) in the table wine and for Dy, Ga, and Sm (25%) in the Port wine, probably as a result of their relatively low concentrations in the wines.

AAS Analysis. AAS with flame atomization (PU 9200X, Philips, Cambridge, U.K.) was used for determination of Al, in total recoverable soil solutions, and Cu and Zn, in ethylenediaminetetraacetic acid (EDTA) soil extracts. AAS with electrothermal atomization provided with a Zeeman background correction (4100 ZL, Perkin-Elmer coupled to an AS-70 autosampler) was used for determination of Cd, Cr, Ni, and Pb also in EDTA soil extracts. In both cases, external calibrations with aqueous standards were used. For Cd and Pb, a 0.2% (m/v) $(\text{NH}_4)_2\text{HPO}_4$ solution was used as a matrix modifier.

For all of these samples, three independent replicates were pretreated and analyzed, and after blank subtraction, the mean concentration and respective standard deviations were calculated.

Calculations. The statistical calculations of one way analysis of variance for comparison of several means and Pearson's correlation tests for correlation evaluation were carried out using the software package SPSS 10.0 for Windows.

RESULTS AND DISCUSSION

Relative Influence of the Soil Elemental Pattern and Vinification Process on the Wine Multielement Composition.

Vineyards Soil Content. The total recoverable metal levels (13) were measured in the soil samples collected every 2 months at three different sites of each vineyard and at two different depths (see **Figure 1**). This fraction was chosen rather than the total-metal content, because the plants cannot extract all of the ionic materials within the soil, as some are too strongly bonded within the soil structure; the ecotoxicity and mobility of metals in the soil depend strongly on specific chemical forms in which they are present.

Discussing the soil depth influence, Cu in the old vineyard soil was the only element whose levels were systematic and significantly higher (about 45%) at the surface (around $37 \mu\text{g g}^{-1}$) than at 20 cm depth. This result indicates contamination of the soil surface layer with Cu, which is compatible with the periodical application of copper sulfate in the vine for several decades. Therefore, only the Cu concentrations obtained at 20 cm depth were considered for calculation of a mean Cu concentration value in the soil, as it will be more directly related with plant uptake. Because the other elements did not display systematically significant differences in their concentrations at the two soil depths, all of the available data were accounted for the calculation of the respective mean concentration.

Systematic significant differences in the concentrations of all of the measured elements were not found among the three sampling sites of each vineyard. Therefore, all of the available data accounted for the calculation of the average concentration value of each element.

In addition, in each vineyard, the mean concentration levels of the determined elements ($n = 39$) observed in the different months were linearly correlated (correlation coefficients, $0.985 \leq R \leq 0.999$, $P < 0.01$), indicating that the concentrations obtained in any of the months would be equally suitable to establish any possible relationship between wine and soil multielement compositions. The average concentration values observed in September were chosen for further discussion, presented in **Figure 3**.

It must be noticed that the concentrations of the elements observed in the soils of both vineyards fell within typical contents of uncontaminated soils (20). Even for Cu, the relatively high concentration observed in the old vineyard soil was still within the concentration range normally found in unpolluted soils (20).

When the multielement concentrations of the two vineyard soils were compared, slight but significant differences were observed for most elements; the young vineyard soil, in general, had higher elemental concentrations (see **Figure 3**). A significant linear correlation ($R = 0.995$, $n = 39$, $P < 0.01$) was observed between the two vineyard soils multielement compositions, which was expected because the two vineyards belong to the same region.

EDTA Soil Extracts. For comparison, EDTA soil extractions were also carried out, since the composition of this fraction has been considered more directly correlated than that of the soil total recoverable with the multielement composition of the vine leaves or grapes and, therefore, with that of the wine (11). However, the determined elements were only those for which there were standardized extraction procedures (11).

Considering the three sampling sites and the two soil layers per vineyard, the percentages of extracted metals relative to the total recoverable metal contents varied between 0.10 and 0.30 for Cr, 0.80 and 1.6 for Ni, 1.0 and 5.0 for Zn, 9.0 and 48 for Cu, 15 and 21 for Pb, and 14 and 45% for Cd in the old vineyard soil. In the young vineyard, the percentages were of similar magnitude, although slightly lower: 0.03–0.15 for Cr, 0.40–0.80 for Ni, 0.40–3.0 for Zn, 3.0–12 for Cu, 8.0–16 for Pb, and 6.0–40% for Cd. These results indicated that the available metal varied with the nature of the element, as expected since it conditions both the chemical forms and the binding strengths. Among the measured metals, Cd was the most available and Cr was the least available. It is known that Cr is quite immobile in soils (20). The slightly lower percentages of extractable metals from the young vineyard soil resulted probably from the different soil utilization in the past. The young vineyard had been a forest soil and for that very reason was richer in organic matter, particularly humic substances, than the old vineyard. It is known that soil organic matter has an essential function in the accumulation and transport of metals as well as in delaying their circulation in the soil (20).

In the EDTA soil extracts, for a few elements, some statistically significant differences were observed among either the three sampling sites per vineyard or between surface and 20 cm depth soil. However, the differences were not systematic and probably resulted from heterogeneity of the soil or from a heterogeneous distribution of the different chemical forms of the metals. When the mean concentration values obtained in the different months for a given element were compared, significant differences were only observed for Cd in the old vineyard and for Cr in the young vineyard. Similar to that observed for the total recoverable metals, also for the EDTA extracts, the mean concentrations of the measured elements ($n = 6$) observed in the different months were significantly

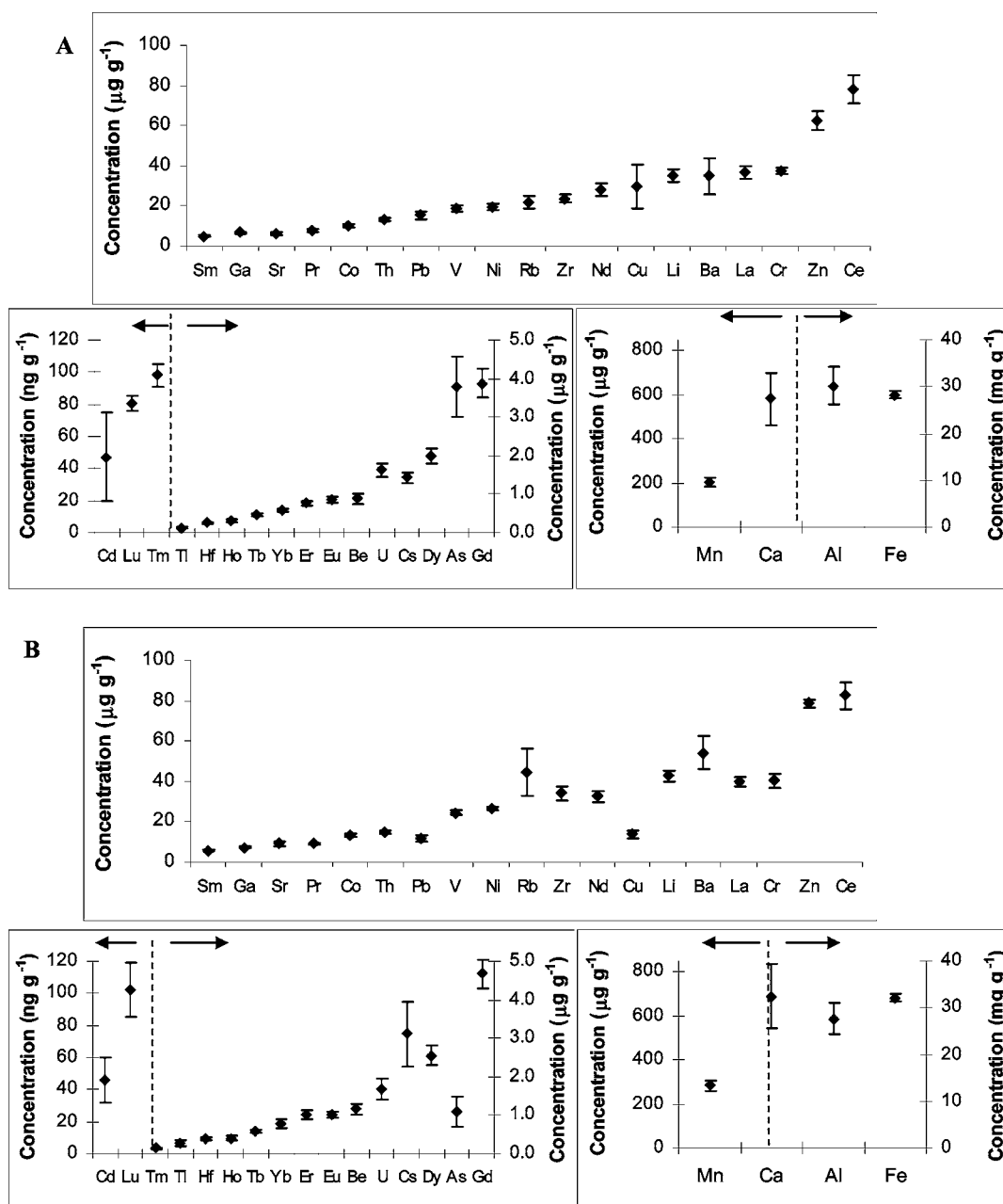


Figure 3. Mean concentration values (per g of dry soil) of Al, As, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Hf, Ho, La, Li, Lu, Mn, Nd, Ni, Pb, Pr, Rb, Sm, Sr, Tb, Th, Ti, Tm, U, V, Yb, Zn, and Zr obtained in total recoverable fraction of soil collected in September in the old (A) and young (B) vineyards.

correlated ($0.90 \leq R \leq 0.99$, $P < 0.01$), indicating that any particular month can be used to establish a possible relationship between soil and wine multielement composition. It follows therefore that for further discussion the data of September were chosen (see **Figure 4**).

Grape Juices and Samples from the Vinification Processes. **Figure 5** (A, fortified, and B, table wines) illustrates the results obtained in the grape juices prepared in the laboratory (GJ_F and GJ_T for the fortified and table wines, respectively) and in the different samples collected during and at the end of the vinification processes of both wines.

For both wines, the concentrations of most elements significantly changed during the vinification processes. The only exceptions were Ga and Sb, for which the observed changes were comparable to those of the instrument drift (daily variations). In both wines, the concentrations of most of the REEs decreased down to levels below the limit of detection in the

final product preventing the establishment of their distribution pattern and their use as tracers of wine provenance in the present case. These results are compatible with the fact that bentonites had not been added to the must during the vinification. Therefore, contamination of finished wine products by REEs, as reported for wines to which bentonites had been added (10), was not observed in this study.

In the fortified wine (W_FF), the concentrations of Al, Cu, Nb, Ti, Th, U, V, Zr, and REEs were lower than those in the GJ_F. This was probably a result of dilution, due to the addition of 20% grape brandy to stop fermentation and, eventually, metal precipitation or coprecipitation with suspended particles during fermentation and/or aging. These last phenomena have been reported for other wines (5, 6). Therefore, eventual contamination by those elements during vinification was compensated for by removing the element from the solution. The concentrations of B, Ba, Be, Co, Mo, Ni, Rb, Sc, Ti, Y, and W in W_FF were

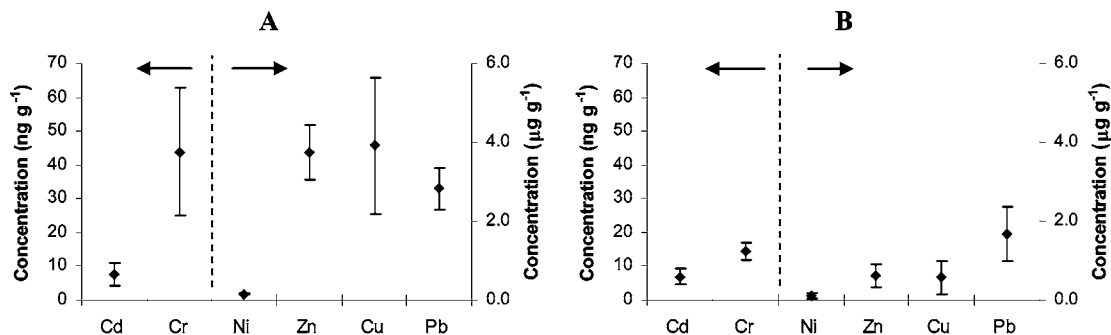


Figure 4. Mean concentration values (per g of dry soil) of Cd, Cr, Cu, Ni, Pb, and Zn observed in EDTA extract of soil collected in September in the old (A) and young (B) vineyards.

similar to those found in the GJ_F. The levels of Li, Ca, Cd, Cr, Cs, Fe, Pb, Sr, and Zn were higher in W_FF than in GJ_F. As in the step W_F2, there was a 20% dilution of the wine with brandy; these results indicate that liberation from the vinification system (contamination) of some amounts of the elements belonging to the two last sets probably occurred. Alternatively or in addition to contamination, liberation of the elements from grape skins and seeds may have happened, as they were pressed together with pomace and kept together with the must during and after fermentation (W_F2 and W_F3). In contrast, in the preparation of GJ in the laboratory, skins and seeds remained intact and were rejected. The alcohol content of the brandy facilitates the solubilization of polyphenolic compounds present in high concentration in grape skins. These compounds are strong complexing agents of heavy metals. It is also known that the distribution of Ca, Fe, K, Mg, and Na throughout the berry is not homogeneous (21). For instance, the berry pulp is richer in K and Na than the seeds or skin, while seeds and skin are richer than pulp in Ca and Mg. Berry skin is particularly rich in Fe. Teissedre et al. (22) also found significant differences in the levels of Pb in the different parts of the grape berries; the seeds had the highest content in Pb, and the pulp had the lowest one. In the present case, the enrichment observed in the Sr levels from GJ_F to W_FF was attributed to the release of the element into the must from grapes seeds and skins instead of anthropogenic contamination, as discussed in detail elsewhere (23), and was compatible with the statistically identical ⁸⁷Sr/⁸⁶Sr values observed in GJ and in the respective WF (23). In contrast, the increase of Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn concentrations in different steps of the vinification process was mainly attributed to contamination. For instance, from W_F2 to W_F3, a significant increase of Cr, Ni, Pb, and V concentrations was observed. Metallic bracelets of the wood container and its tap may be the main sources of these elements. From W_F3 to W_F4, the Cr, Fe, and V concentrations decreased significantly, suggesting that a large fraction of those elements precipitated or coprecipitated with colloidal polymeric organic compounds, such as polyphenols. On the other hand, the levels of Cd, Cu, Ni, Pb, and Zn increased from W_F3 to W_F4, which indicated a predominance of the effect of contamination during the rest in the stainless steel container. The levels of Cr and Fe increased again from W_F4 to W_FF, suggesting the presence of sources of these elements in the oak barrel where the wine rested for 1 year. Contamination of wines with Cd, Cr, and Pb released from winemaking equipment during its maturation has also been reported in the literature (8). For Pb in the present wines, the analysis of the respective isotope ratios (IRs) corroborated the evidence of contamination introduced by the different components of the vinification, as discussed elsewhere (9). Concerning

Cu, it is worthwhile to stress that the major sources of the metal were found in W_F1 (grape pressing) and W_F4 (resting in stainless steel vat) steps of the winemaking process. After grape brandy was added in W_F2, the Cu level markedly decreased, probably due to the dilution factor. Therefore, the present results did not corroborate the hypothesis frequently formulated, which attributes the relatively high levels of Cu usually found in fortified wines (in comparison with those found in table wines) to the added grape brandy, which used to be produced in copper distillation devices. The grape brandy used in the studied fortified wine was produced in a stainless steel still; therefore, it was poor in Cu.

For the table wine (Figure 5B), the results were quite similar to those observed for the fortified wine, with a few differences, for instance, in the set of elements whose concentrations decreased (Co, Ni, and Zn instead of V) or increased during the vinification (V instead of Fe, Cd, Cs, and Zn). Evidence of contamination from the material of the devices used in the winemaking process (containers, pumps, and tubes) was only observed for Al, Cr, Fe, Ni, Pb, and V. For Al, Fe, and V, the concentrations increased only during the aging period (levels higher in W_TF than in W_T10). For Pb, whose concentration was initially (W_T1) relatively low (6.3 µg L⁻¹), a more or less regular concentration increase throughout the vinification process (not compensated by precipitation phenomena) was observed, probably as a result of the presence of Pb sources in the devices used. Such as for the fortified wine, this hypothesis was corroborated by the results obtained for the Pb IRs (9). For Cr and Ni, some contamination from the containers probably also occurred, since their concentrations remained approximately constant after the solids were removed and did not decrease during aging (due to precipitation or coprecipitation with suspended colloidal matter, as was observed in the fortified wine). An increase of Cr and Ni concentrations during the aging processes in which there was contact with stainless steel utensils has been reported for other wines (6, 24).

In summary, both vinification processes influenced the multielement composition of the produced wines. Most of the elements displayed similar or even lower concentrations in the WF as compared to that observed in GJ, in which the contribution of skins and seeds did not account and contact with the winemaking system did not occur. Just for the fortified wine, a 20% dilution of the must with grape brandy constitutes a relevant additional factor. Even so, evidence of effective contamination of intermediary products induced by the vinification system was observed for a few elements: Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn in the fortified wine and Al, Cr, Fe, Ni, Pb, and V in the table wine. Nevertheless, only the levels of

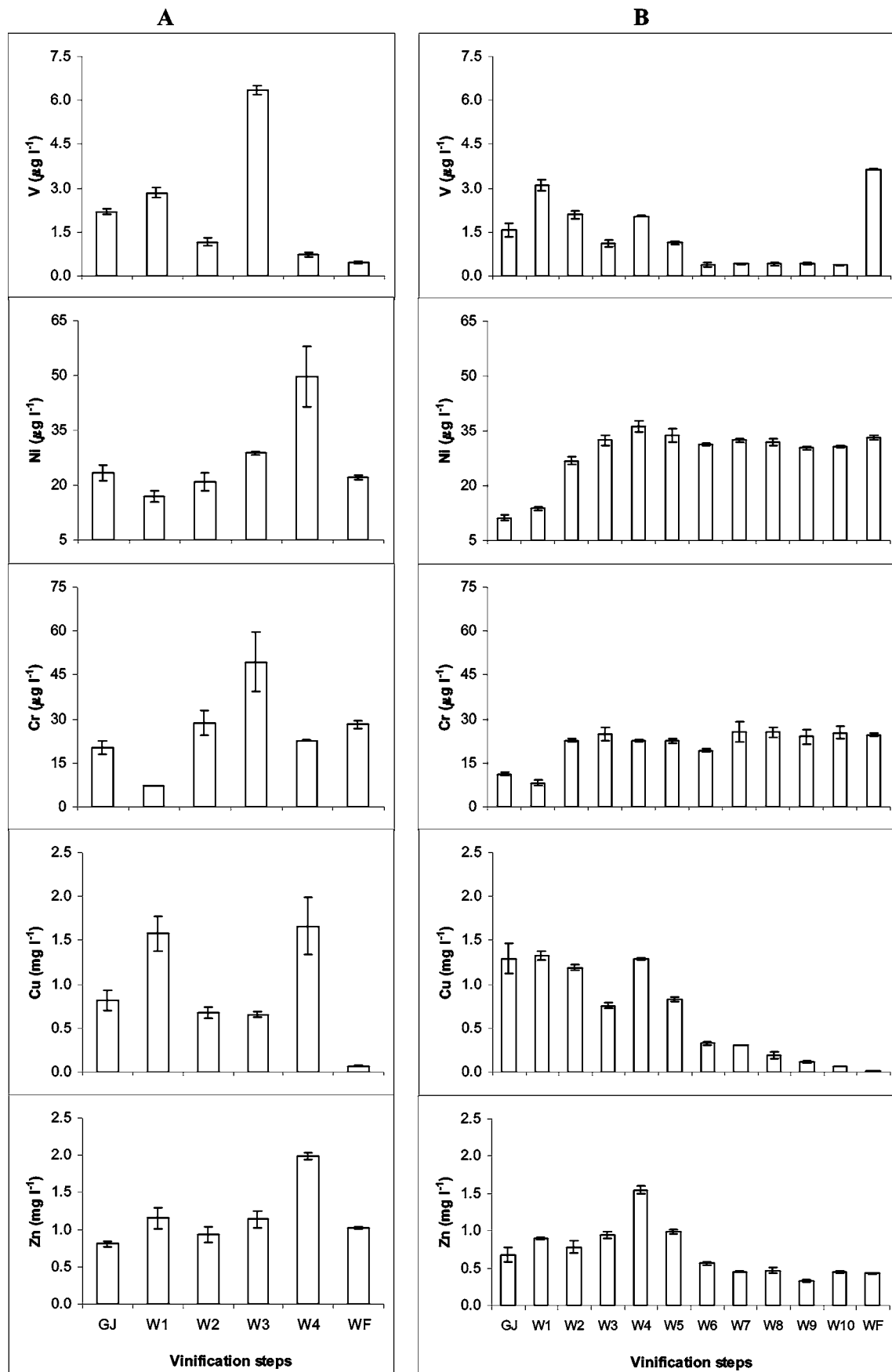


Figure 5. Total concentration of V, Ni, Cr, Cu, and Zn (in $\mu\text{g L}^{-1}$) (mean and standard deviation, $n = 3$) observed in the grape juices (GJ_F and GJ_T for the fortified and table wine, respectively), in the samples collected throughout the vinification processes (from W_F1 to W_F4 for the fortified and from W_T1 to W_T10 for the table wines) and in the final products (W_F and W_T). (A) Red fortified wine produced with polyvarietal grapes from the old vineyard; (B) red table wine produced with monovarietal grapes from the young vineyard.

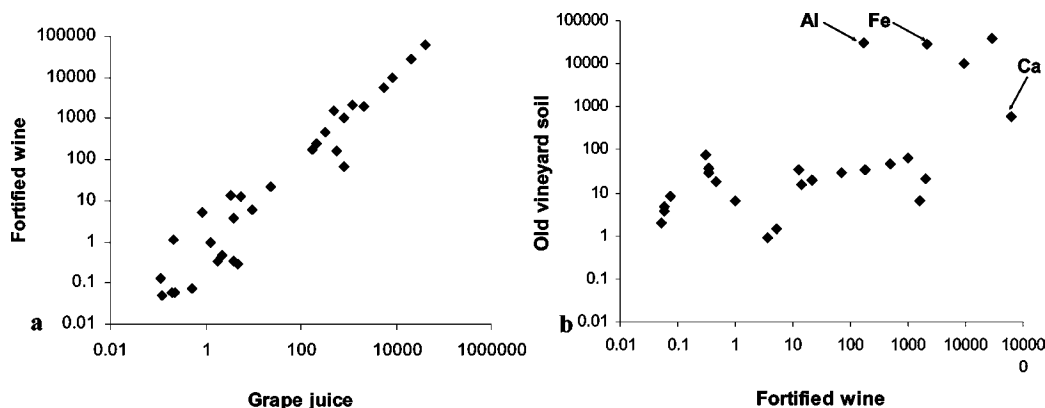


Figure 6. Examples of correlation plots, obtained for the fortified wine/old vineyard between pairs of the different types of samples. In each plot, elements excluded in order to obtain significant correlations are marked. In both axis is represented the logarithm of concentrations in order to obtain a larger scattering of the points.

Table 1. Pearson's Correlations Obtained for Average Multielement Concentrations in the Different Types of Samples

		Old Vineyard/Fortified Wine			
		soil		grape juice	wine
		total recoverable	EDTA extract		
soil	total recoverable	1			
	EDTA extract	0.988 ^a <i>n</i> = 6 ^b	1		
	grape juice	0.282/0.986 ^{a,c} <i>n</i> = 29 ^d	0.998 ^a <i>n</i> = 6 ^b	1	
	wine	0.238/0.994 ^{a,c} <i>n</i> = 24 ^e	0.989 ^a <i>n</i> = 6 ^b	0.997 ^a <i>n</i> = 31 ^f	1
		Young Vineyard/Table Wine			
		soil		grape juice	wine
		total recoverable	EDTA extract		
soil	total recoverable	1			
	EDTA extract	0.896 ^g <i>n</i> = 6 ^b	1		
	grape juice	0.225/0.904 ^{a,c} <i>n</i> = 27 ^h	0.877 ^g <i>n</i> = 6 ^b	1	
	wine	0.193/0.986 ^{a,c} <i>n</i> = 20 ⁱ	0.898 ^g <i>n</i> = 6 ^b	0.979 ^a <i>n</i> = 31 ^j	1

^a Correlation significant at $P < 0.01$. ^b Cd, Cr, Cu, Ni, Pb, and Zn. ^c Al, Ca, and Fe excluded. ^d Ba, Be, Co, Cr, Cs, Cu, Ga, Li, Mn, Ni, Pb, Rb, Sr, Th, U, V, W, Zn, Zr and La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, and Er. ^e Ba, Be, Cd, Co, Cr, Cs, Cu, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, W, Zn and La, Ce, Pr, Nd, Sm, Gd, and Dy. ^f Al, B, Ba, Be, Ca, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sb, Sc, Sr, Ti, V, W, Y, Zn and La, Ce, Pr, Nd, Sm, Gd, and Dy. ^g Correlation significant at $P < 0.05$. ^h Ba, Be, Cd, Co, Cr, Cs, Cu, Ga, Li, Mn, Ni, Pb, Rb, Sr, Th, U, V, Zn, Zr and La, Ce, Pr, Nd, Sm, Eu, Gd, and Dy. ⁱ Ba, Be, Cd, Co, Cr, Cs, Cu, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, Zn, Zr and La, Ce, and Nd. ^j Al, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sc, Sr, Ti, Ti, V, W, Y, Zn, Zr and La, Ce, and Nd.

Cd, Cr, Fe, Pb, and Zn in the fortified wine and of Cr, Ni, Pb, and V in the table wine were higher in WF than in GJ.

It is worth mentioning that despite the elemental concentration variations observed throughout the vinification of both wines, statistically significant and even linear correlations were observed between the concentrations of the elements determined in common in GJ and WF (see **Table 1**). In **Figure 6**, the results for the fortified wine are illustrated. Similar results were obtained for the table wine. Therefore, the concentration variations that occurred during the vinification do not seem to

prevent the usefulness of wine multielement composition as a fingerprint of wine origin. A linear correlation ($R = 0.996$, $n = 28$, $P < 0.01$) was also observed between the multielement composition of the two wines for the set of elements determined in common.

Soil, Grape Juice, and Wine Relationships. Pearson's correlations between soil and wine multielemental composition were carried in order to find eventual relationships (see correlation coefficients in **Table 1**). For the set of elements determined in common (see **Table 1**), significant correlations between their mean concentrations in soil and produced wine were found when only Al, Fe, and Ca, the three more abundant elements in the soil, were excluded. **Figure 6** illustrates for the fortified wine the obtained results. Similar results were obtained for the table wine and for soil vs GJ.

For the elements determined in common in soils and wines (excluding Al, Ca, and Fe), the median of the multielement concentrations of the two vineyard soils was also linearly correlated with that of the median of the two wines ($R = 0.994$, $n = 19$, $P < 0.01$).

When the multielement composition of the EDTA soil extracts was used instead of the total recoverable extracts, linear correlations were also observed (**Table 1**). Nevertheless, these results have a more limited reach, as only six elements were measured in the EDTA soil extracts.

It is worth mentioning that when the median of the determined multielement composition of the soil of the two vineyards was compared with that of a previously studied French red table wine, from the region of Bordeaux, no significant correlation was obtained even when Al, Fe, and Ca were not included. Significant correlation was not observed either between the determined multielement composition of the studied wines and that of the French wine. These results are understandable, as the composition of the wine seems to depend markedly on the vineyard soil elemental pattern.

Multielement Composition of the Studied Wines. The multielement concentrations found in the wines are shown in **Figure 7** (A, fortified, and B, table wines) as these data may have some nutritional and toxicological interest. The elemental patterns of both wines were very similar, despite the fact that the winemaking processes were markedly different. In fact, the largest differences were found for V (ca. eight times higher in the table wine), for Cu (ca. five times higher in the fortified wine), and for Cs (ca. three times higher in the table wine).

The concentrations of most of the measured elements in the two wines fell within the range of those found in wines produced in several countries as reported in ref 1. However, the

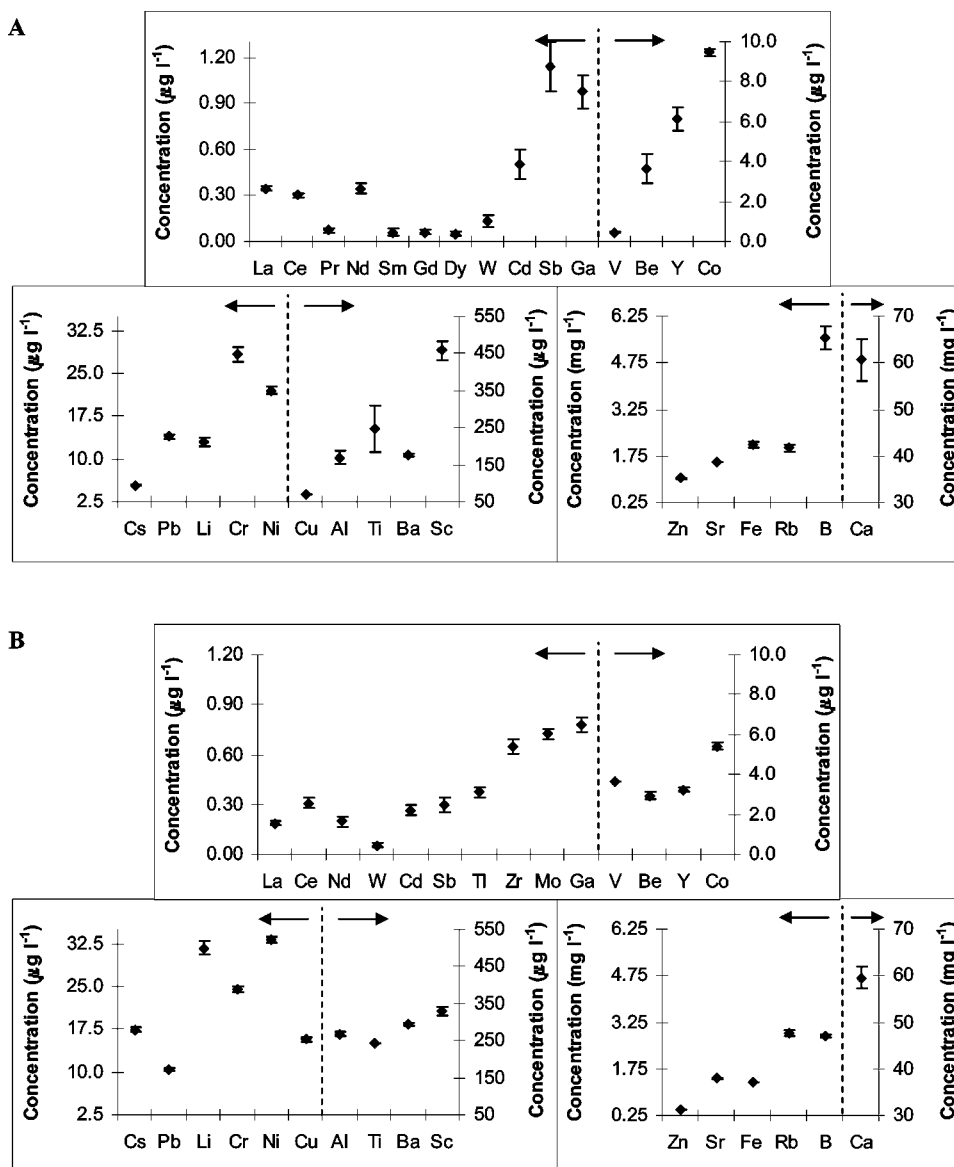


Figure 7. Total concentrations of the elements measured in both studied wines. (A) Red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard. In some cases, as follows, the concentrations of Cd, Pr, Sm, Gd, Dy (only table wine), Mo, Ti, Zr (only fortified wine) and Nb, Th, U, Eu, Tb, Ho, Er, Tm, Yb, and Lu (both wines) were below the respective limit of quantification.

concentrations of Pb in both wines and Al and Cu only in the table wine were below the respective reported range (1) (three, two, and four times lower than the respective lower limits, which were $30 \mu\text{g L}^{-1}\text{Pb}$, $500 \mu\text{g L}^{-1}\text{Al}$, and $60 \mu\text{g L}^{-1}\text{Cu}$). On the other hand, the concentrations of Be and Cs (only in the table wine) were higher (both ca. three times higher than the range upper limits (1), which were 1.3 and $5.1 \mu\text{g L}^{-1}$, respectively).

As concerns elements considered of special interest due to either their toxicity in case of excess, such as Cd, Cr, Ni, Pb, or even Cu (an essential element but toxic when consumed in excess) or the effect they seem to have on the organoleptic properties of wine, such as Al, Fe, Zn, and Cu (5, 24), relatively low concentrations were found. For instance, the Pb concentrations were $14 \mu\text{g L}^{-1}$ in the fortified wine and $11 \mu\text{g L}^{-1}$ in the table wine, both well below the threshold limit value established by the Office of Vine and Wine (OIV), which is presently $200 \mu\text{g L}^{-1}$. The relatively low levels of Pb as compared with those that were frequently found in wines in the past are not surprising. Leaded gasoline used to be a major source of atmospheric lead, which contaminated soils and vines after dry or wet deposition (25, 26). In Portugal, up to January 1996, the Pb content of

leaded gasoline was close to the legal maximum, 0.4 g L^{-1} , decreasing after that to 0.15 g L^{-1} . Nowadays, only unleaded gasoline is available and the respective Pb levels have been not higher than 0.004 g L^{-1} , much lower than the legal maximum of 0.013 g L^{-1} (27). Therefore, at present, the contribution of transportation to the lead levels in the atmospheric deposition is insignificant when compared with the past. In addition, both studied vineyards are located in an agricultural area, which is far from industrial and urban areas and heavy traffic roads. For Cu, the concentration was higher in the fortified wine, $70 \mu\text{g L}^{-1}$, than in the table wine, $16 \mu\text{g L}^{-1}$, but in both cases, it was much lower than the respective OIV threshold limit of 1 mg L^{-1} (24). The upper limit allowed for Al in wine is 10 mg L^{-1} (24) whereas the found values were only 0.17 and 0.27 mg L^{-1} in the fortified and table wines, respectively. For Cd and Zn, the levels in both wines were, respectively, 0.51 and 1.0 mg L^{-1} in the fortified wine and 0.26 and 0.43 mg L^{-1} in the table wine. These values fell in the ranges frequently found in different wines (1), which have been between 0.25 and 0.7 mg L^{-1} for Cd and between 0.5 and 3.5 mg L^{-1} for Zn. The levels of Fe, 2.1 and 1.3 mg L^{-1} in the fortified and table wines,

respectively, were within the typical range of 0.9–10 mg L⁻¹ (*I*) but much lower than the value considered problematic, 10 mg L⁻¹ (24). The levels of Cr and Ni, respectively, 28 and 22 μg L⁻¹ in the fortified wine and 25 and 33 μg L⁻¹ in the table wine, were also within the range of 30–60 μg L⁻¹ reported for these elements in different wines (*I*).

Concerning the REEs, only results for La, Ce, Nd, Sm, Gd, and Dy in the fortified wine and La, Ce, and Nd in the table wine were found in measurable amounts. Quite similar concentrations, between 0.2 and 0.3 μg L⁻¹, were observed for the REEs measured in common.

CONCLUSIONS

The multielement compositions of two different Portuguese wines of the Douro wine district and their precursors including the respective provenance soil were determined, to evaluate the suitability of those data as tools for establishing the origin of a wine.

It was found that the two studied vinification processes (one modern, used for producing the table wine, and one old fashioned, used to produce the fortified wine) influenced the multielement composition of the wine. Evidence of effective contamination during the vinification was observed for a few elements: Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn in the fortified wine and Al, Cr, Fe, Ni, Pb, and V in the table wine. The concentrations of most of the measured elements were similar or even lower in the final product than in the respective grape juice prepared in the laboratory. Precipitation or coprecipitation of a fraction of the elements with organic complexing agents present in the must, like polyphenols and tannins, as well as 20% dilution with grape brandy just in the case of the fortified wine, could account for the decrease of the concentrations.

For both studied wines, despite the elemental concentration variations observed throughout the vinification processes, significant correlations ($P < 0.01$) were observed between the concentrations of a large number of elements ($n = 31$) in the final product and in the respective grape juice. Therefore, those concentration variations should not be a preventive of the usefulness of wine multielement composition for wine provenance determination.

Significant correlations ($P < 0.01$) between the multielemental composition of wines and the provenance soil were also observed when only Al, Ca, and Fe (the most abundant elements in the soil) were excluded from the set of elements measured in common ($n = 19$) in the two types of samples. Therefore, the present results suggest that the multielement composition of the provenance soil also has potentialities to be used as a fingerprint of the origin of wines from the Portuguese Douro wine district. Nevertheless, more wines from the same and other wine districts must be analyzed in order to consolidate this conclusion.

Concerning nutritional aspects, the concentrations of most of the elements measured in the two studied wines fell within the range of those found in other wines of different characteristics and origins. As concerns the elements considered of special interest due to either their toxicity in case of excess, like Cd, Cr, Ni, Pb, or even Cu or the effect they seem to have on the organoleptic properties of wine, such as Al, Fe, Zn, and Cu, relatively low concentrations (much lower than the legal threshold limit values when available) were found.

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