



Multi-element and multi-isotopic profiles of Port and Douro wines as tracers for authenticity

Inês Coelho^{a,*}, Ana Sofia Matos^b, Ekaterina N. Epova^c, Julien Barre^c, Robin Cellier^d, Nives Ogrinc^e, Isabel Castanheira^{a,f}, João Bordado^g, Olivier F.X. Donard^d

^a Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge, INSA IP, Avenida Padre Cruz, 1649-016, Lisbon, Portugal

^b UNIDEMI, Department of Mechanical and Industrial Engineering, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

^c Advanced Isotopic Analysis, Hélioparc, Pau, France

^d Institut des Sciences Analytiques et de Physicochimie Pour l'Environnement et les Matériaux, Université de Pau et des Pays de l'Adour, Pau, France

^e Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

^f Department of Environmental Sciences and Engineering NOVA School of Science and Technology (FCT NOVA) (FCT/UNL), MARE - Marine and Environmental Sciences Centre, Caparica, Portugal

^g CERENA, Departamento de Engenharia Química, Torre Sul, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

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ABSTRACT

Port and Douro wines are produced in the Douro Demarcated Region using different wine making processes. This work aims at evaluating the influence of the geographical origin and the wine making process in their multi-element (Li, Be, B, Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Tl, Pb, Fe, Mg, P, Ca, Na, K) and isotopic compositions ($^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{11}\text{B}$, $^{206}\text{Pb}/^{207}\text{Pb}$, $\delta^{18}\text{O}$). Fifteen samples of each were analysed. ANOVA identified significant differences for Ca, Na, Rb, Zn, Cu, Cr, Se, $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $\delta^{11}\text{B}$. PCA revealed that Cr, Se and Cu are the main contributors for discrimination by type of wine. $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$ are region specific and not influenced by the winemaking process or storage. Thus, make good natural tracers for geographical origin of this region. HCA suggested a common profile in Port and Douro in white wines, for Sr and K, which should be further investigated.

1. Introduction

Port wine is very famous and appreciated worldwide (Instituto da Vinha e do Vinho, 2021) even appearing in some best selling novels like Agatha Critis's Hercule Poirot. It is produced in the Douro Demarcated Region in northeast of Portugal. In 2021, Port wine accounted for 65% of the wine production in the region and 36.4% of national wine exports (Instituto da Vinha e do Vinho, 2021; Instituto dos Vinhos do Douro e do Porto, 2021), clearly illustrating its importance in the global wine market. The main export destinations within the European Union (EU) are France, Netherlands and Belgium while outside the EU the United Kingdom, United States of America and Canada are the main consumers. Asian countries like South Korea or China represent growing markets for exportations (Instituto da Vinha e do Vinho, 2021). Its particular wine making process with the addition of grape spirit mid fermentation,

combined with the unique edapho-climatic characteristics of the region, leads to a large diversity of wines with a natural residual sweetness and an alcoholic content between 19% and 22% v/v. In addition, the blending of different batches (harvest year, vine varieties or location within the region) also contributes to the multiplicity of Port wines. Ageing can be in bottle, which is the case of Ruby and vintage Port, or in wood, like Tawny Port. However all Port wines stage a minimum of two years in wood. Within the same area and region, we can also find the production of Douro dry wines. These are table wines with Protected Designation of Origin, ageing in wood or stainless steel containers, and further referred to as Douro wine. Although being very different products, due mainly to differences in the vinification method, both wines share their geographical origin, history, agricultural practices and grapevine cultivars. In other words these wines share their terroir, defined as an interactive cultivated ecosystem, in a given place, that

* Corresponding author.

E-mail addresses: ines.coelho@insa.min-saude.pt (I. Coelho), asvm@fct.unl.pt (A.S. Matos), ekaterina.epova@ai-analysis.com (E.N. Epova), julien.barre@ai-analysis.com (J. Barre), robin.cellier@univ-pau.fr (R. Cellier), nives.ogrcin@ijs.si (N. Ogrinc), isabel.castanheira@insa.min-saude.pt (I. Castanheira), jcbordado@ist.utl.pt (J. Bordado), olivier.donard@univ-pau.fr (O.F.X. Donard).

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includes climate, soil and the vine (van Leeuwen, 2010).

Studying wine is important not only for its economic value but also because of the health effects associated with its consumption (Tariba, 2011). Mostly known for contributing to the prevention of coronary heart disease when consumed with moderation (Castaldo et al., 2019), wine can in addition contribute to mineral intake but also to exposure to toxic elements. Furthermore, while some elements positively influence the organoleptic properties of wines others may contribute to undesirable effects such as haze formation or changes in taste and aroma (Tariba, 2011). The geographical origin assumes great importance in the wine industry, as the wine's characteristics, including its chemical composition, are highly dependent on the terroir. Mislabelling compromises the authenticity of wines and can present itself in the form of misrepresentation of cultivars and/or geographical origin (Kamiloglu, 2019). The high value of origin wines supports the rising number of studies dedicated to the provenance of this beverage over the last years (Coelho et al., 2017; Kamiloglu, 2019; Kelly et al., 2005). Analytical strategies recur to multielement fingerprinting (González et al., 2009; Rodrigues et al., 2011), stable isotopes (Catarino et al., 2019; Durante et al., 2015; Horacek et al., 2021; Ogrinc et al., 2001) or more frequently a combination of two or more strategies (Epova et al., 2019; Teixeira et al., 2021), as tools for assessing the geographical origin of wines. Among the most common isotopic systems proposed to trace the geographical origin of wines are $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$.

Oxygen, $\delta^{18}\text{O}$ is used for tracing the geographical origin and for detecting watering of wines since the $\delta^{18}\text{O}$ value from tap or spring water is lower than $\delta^{18}\text{O}$ in wine water, that originates from plants (Horacek et al., 2021; Santesteban et al., 2015). In fact its value is not affected by human impact, such as pollution, but it alters with the physical processes of the hydrological cycle (Roberts et al., 2010). Studies showed that this parameter is influenced by climate and location, mainly latitude, precipitation, distance from the ocean and temperature (Camin et al., 2015; Orellana et al., 2019). The EU recognizes the determination of $\delta^{18}\text{O}$ using isotope ratio mass spectrometry (IRMS), as an official method for assessing authenticity of wines, including adulteration and conformity with the origin indicated in the label (European Commission, 2008).

Other very used tracers are Sr isotopes. The ratio $^{87}\text{Sr}/^{86}\text{Sr}$ remains constant throughout the food chain since it is not significantly altered by biological processes (Coelho et al., 2017). Studies have demonstrated that the Sr isotope ratio present in the wine is not affected by the vinification process, thus being a reflection of the soil in which the plant has grown (Coelho et al., 2017; Durante et al., 2015). This stability was demonstrated along the production starting from the bioavailable fraction of soils, passing to branches, musts and finally to wine, making $^{87}\text{Sr}/^{86}\text{Sr}$ an excellent biological marker of the terroir (Cellier et al., 2021b; Durante et al., 2013; Marchionni et al., 2016).

More recent studies approached the hypotheses of resorting to less traditional isotopic systems using various tri-isotopic systems or Pb (Cellier et al., 2021a; Epova et al., 2020; Lancellotti et al., 2020) and $\delta^{11}\text{B}$ (Lancellotti et al., 2021) as potential markers for authenticity of wines.

Although to a lesser extent than Sr or O, Pb isotopes have been used to study the geographical provenance of wines for some time (Epova et al., 2020; R. Almeida and S.D. Vasconcelos, 1999; Teixeira et al., 2021). Due to consequent health effects, lead's maximum level in wine is regulated. The contamination of wines with lead can originate from natural or more commonly anthropogenic sources. The isotopic signature of the lead is different according to the source of the contamination. Similarly, to other heavy metals Pb does not fractionate during uptake by plants (Lancellotti et al., 2020). However, studies have shown a discrepancy between soil and wine's Pb isotope ratios suggesting that Pb in wine is not originating primarily from the soil but from other sources such as atmospheric deposition (Epova et al., 2020; Kristensen et al., 2016; Lancellotti et al., 2020). While Sr isotope ratio is a geogenic tracer, Pb isotopes are indicators of environmental pollution.

One of the most recent isotopic systems applied for wine authenticity is that of B, an essential micronutrient for plants. Depending on soil and climate conditions (soil moisture, temperature, pH, salinity, organic matter, and precipitation), B is available for root absorption in the form of uncharged boric acid or borate (Shireen et al., 2018). Using hydroponic culture Coetzee et al. 2016 showed that the $^{11}\text{B}/^{10}\text{B}$ ratio in grapevine leaves depended on the cultivar/rootstock combination, although it reflected the growth medium (Coetzee et al., 2011). This suggested that B suffers fractionation during root absorption. Another study also observed B fractionation in corn and wheat, but less evident in broccoli (Marentes et al., 1997). More recently, a work combining isotope ratios and elemental concentrations showed that B isotopes, together with Sr and Pb, provided significant discrimination between Lambrusco PDO wines (Lancellotti et al., 2021).

Whether for health, economic, legal or transparency reasons, or even just for consumers' preference, it is fundamental to have reliable analytical tools for proving authenticity of wine. The analytical methods shall establish a confident and proven correlation between analytical parameters of commercialized products and their geographical region of origin. The aim of this study is to characterize two types of wine produced in the Douro Demarcated Region, in terms of their multielement (Li, Be, B, Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Tl, Pb, Fe, Mg, P, Ca, Na, K) and isotopic composition ($^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{11}\text{B}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $\delta^{18}\text{O}$). As far as we know, no work has been published with such a vast combination of elemental and isotopic systems for authenticity purposes in a broad sense (mislabelling, adulteration, geographical origin, agricultural production mode). Furthermore, the isotopic information on Portuguese wines is scarce. Comparing such information of Port and Douro wines will contribute to understanding parameters influenced by processing methods and the ones common to the region, and fundamentally dependent on the soils and climate, thus identifying suitable markers for using as tracers of authenticity for the Douro region. Our findings will be of assistance to those interested in geographical origin and food components.

2. Materials and methods

2.1. Sampling

In this study, we analysed 30 wines originating from the Douro Demarcated Region, half of which were Port wines and the other half Douro wines. To assure the authenticity and traceability of samples, these were provided by the Port and Douro Wines Institute (IVDP), which is the public entity responsible for controlling the use of the Douro geographic indication worldwide (Instituto dos Vinhos do Douro e do Porto, 2021). Samples were numbered from 1 to 15 preceded by the letter P for Port wines and the letter D for Douro wines. Supplementary Table S1 provides information regarding sample ID, year of production (when available since Port wines may be blended), colour, soil type and coordinates of the producer.

2.2. Apparatus

Elemental concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo X series II, Thermo Fisher Scientific, Waltham, USA) and inductively coupled plasma optical emission spectrometer (ICP-OES) (Thermo iCAP 6000 series, Thermo Fisher Scientific, Waltham, USA).

A high resolution MC-ICP-MS Plasma 1700 (Nu Instruments Ltd., Wrexham, UK) and a MC-ICP-MS Plasma 1 (Nu Instruments Ltd., Wrexham, UK) were used for the isotopic analysis Sr and Pb, respectively.

The isotopic analysis of B was performed on an ICP-MS instrument PlasmaQuant Elite (Analytik Jena, Jena, Germany).

The isotopic analysis of O was done on MultiFlow Bio preparation system (IsoPrime, Manchester, UK) connected to continuous flow

isotope ratio mass spectrometer (IRMS; GV instruments, Manchester, UK).

Depending on the succeeding analytical determination, sample preparation was performed using a closed vessel microwave oven (ETHOS 1 Series, Milestone, Shelton, USA), a graphite digestion block (DigiPREP, SCP Science, Montreal, Canada) and ultrasonic water bath (Elmasonic S 60 H, Elma, Singen, Germany).

2.3. Reagents and standards

For all analysis, reagents were of high purity to prevent for contaminations. Ultra-pure water grade I, as defined in EN ISO 3696, was obtained using a Milli-Q plus System (Millipore, Darmstadt, Germany). Concentrated nitric acid, analytical grade (65%; Merck, Darmstadt, Germany), was purified in the laboratory, using an acid distillation system (Milestone SubPUR, Shelton, USA), and compared to commercial ultrapure nitric acid solution (Merck, Darmstadt, Germany), on a routine basis.

Working multi-element standard solutions were prepared from mono-element high-purity ICP stock standards (SPC science, Montreal, Canada). Working multi-element standard solutions of the same elements were independently prepared from multi-element solution XVI (Merck, Darmstadt, Germany) and Mono-element ICP standards (PlasmaCAL, SCP Science, Montreal, Canada), and used for quality control. Internal standard correction for ICP-MS was made by Y and In (1000 mg/L; Merck, Darmstadt, Germany).

For isotopic analysis, NIST SRM 987 (Strontium carbonate, NIST, Gaithersburg, MD, United States), NIST SRM 981 (Common lead isotopic standard, NIST, Gaithersburg, MD, United States), NIST SRM 997 (Thallium isotopic standard, NIST, Gaithersburg, MD, United States) and NIST SRM 951a (Boric acid isotopic standard, NIST, Gaithersburg, MD, United States) were used.

2.4. Multi-elemental and isotopic analysis

Multi-elemental and isotopic analysis of different elements were performed for the samples.

2.4.1. Multielement analysis

A total of 26 elements was determined by ICP-MS (Li, Be, B, Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Tl, and Pb) and by ICP-OES (Fe, Mg, P, Ca, Na, and K). Both equipment operated under the conditions previously described and method validation, carried out in compliance with NP EN ISO/IEC 17025 and EN 13804, and followed the same protocol (Coelho et al., 2019).

Before analysis, samples were mineralized. As such, 5 ml of each wine sample was evaporated to half and left to react overnight with 5 ml of HNO₃. Afterwards, samples underwent a 4-step program using the DigiPREP: (1) 5 min up to 45 °C; (2) 25 min up to 95 °C; (3) 120 min at 95 °C; and (4) cooling down. Final volume was made up to 25 ml with ultra-pure water. Mineralized samples were kept at 5 ± 3 °C until further analysis.

Samples were analysed in triplicate. Each digestion cycle contained at least one blank, to monitor for potential contaminations, and one spiked sample to check for accuracy. Average recovery rates of spiked samples were between accepted between 95% and 111%. The first point of the calibration curve, higher than 10 *SD of digestion blanks, was considered the limit of quantification (LQ). To monitor for instrumental drift independent standards were used between each batch of five samples.

2.4.2. Sr Isotopic analysis

Sr isotopic analysis was performed on the Nu-Plasma-1700 multi-collector as described (Epova et al., 2019, Cellier et al., 2021). Briefly, before analysis, Sr in the samples was purified using Sr-Spec Eichrom resin following the microwave mineralisation of wine. The value of SRM

987 applied for data processing was 0.710255 according to Waight et al., (2002), and was measured with the precision of 55 ppm (2RSD, n = 15). For quality control, a triplicate of SRM 987 was measured after purification with precision of 32 ppm relatively the recommended value. Instrumental and procedural blanks were permanently controlled for the traces of Rb.

The levels of Sr and Rb were monitored in two critical points of the process using ICP-MS: one after mineralization and the other after purification. Recovery rates for Sr after purification were accepted above 75%. In each purification cycle at least one sample was analysed in triplicate and NIST SRM 987 was also monitored throughout the whole process. NIST SRM 987 was used for daily optimization of the MC-ICP-MS as well as bracketing standard for measurement.

2.4.3. B Isotopic analysis

The B isotopic analysis was performed on a PlasmaQuant Elite following the protocol of (Cellier, 2020). To account for mass discrimination through time, boron isotopes were measured by sample calibrator bracketing, using NIST SRM 951 (0‰ by definition), at 10 µg L⁻¹. Samples and calibration solutions were measured using the same method. The accuracy of δ¹¹B measurements was verified by measuring an in house reference material "Champagne" at 10.1‰.

Results for B are expressed in units "per mil" (‰). The δ¹¹B values were reported relative to the NIST SRM 951a boric acid. To convert results to delta notation NIST SRM 951 boric acid, with a ¹¹B/¹⁰B ratio value of 4.04367 was used.

2.4.4. Pb Isotopic analysis

Pb isotopic analysis was performed on the Nu-Plasma-1 multi-collector connected to a nebulizer DSN-100 (Nu Instruments, Wrexham, UK), under dry plasma conditions, as described (Epova et al., 2020). Briefly, before analysis, Pb in the samples was purified using Dowex 1-X8 resin following the mineralisation of wine. For data treatment, the correction using ²⁰⁴Hg, and by SRM 981 and SRM 997 were applied. An external reproducibility obtained by of SRM 981 was equivalent to 137 ppm (²⁰⁸Pb/²⁰⁴Pb) and 100 ppm (²⁰⁷Pb/²⁰⁴Pb). For quality control of complete protocol (mineralisation, Pb purification and analysis), a triplicate of a laboratory reference material "Champagne" was measured with the precision of 144 ppm (²⁰⁸Pb/²⁰⁴Pb) and 122 ppm (²⁰⁷Pb/²⁰⁴Pb), according to (Cellier et al., 2021a). Instrumental and procedural blanks were also controlled.

2.4.5. Oxygen isotopic analysis

The ¹⁸O/¹⁶O ratios were determined directly in wine after equilibration with reference gas CO₂/He (5% CO₂) at 40 °C for 6 h. Measurements were performed on a MultiFlow Bio preparation system (IsoPrime, UK) connected to a continuous flow IRMS (GV instruments, UK). Analyses were normalised against laboratory reference standard seawater with the value of δ¹⁸O = +0.36 ± 0.04‰, snow with the value of δ¹⁸O = -19.73 ± 0.02‰ and external laboratory reference material ERM with the value of δ¹⁸O = +4.83 ± 0.29‰. For independent control a laboratory reference material "Sweet wine" with δ¹⁸O = -0.73 ± 0.29‰ was used. The combined uncertainty for δ¹⁸O determination in wine was ± 0.2‰, while the expanded uncertainty was ± 0.4‰.

The stable isotope ratios of oxygen are expressed in the δ-notation. The δ¹⁸O, expressed in units "per mil" (‰), were reported relative to the V-SMOW (Vienna-Standard Mean Ocean Water) standard (Brand et al., 2014).

Data quality control charts were systematically recorded throughout the study period. To ensure the validity and comparability of the isotope results, the laboratory regularly participates in the Food analysis using Isotopic Techniques-Proficiency Testing Scheme FIT-PTS organized by EUROFINS three times per year. In this scheme, wine is also included.

2.5. Statistical analysis

Descriptive statistical data was reported as mean, maximum and minimum values, considering 15 wines for each type.

Tests were performed using Statistica® 12 software (Statsoft Ibérica, Lisboa, Portugal). Statistical significance was established at a p-value < 0.05 for all analyses. The first step consisted on testing data for normality using Kolmogorov-Smirnov test. For parameters presenting a normal distribution, the analysis continued with One-way ANOVA, to evaluate possible significant differences, and Pearson correlation coefficient, to establish relations between parameters. For the remaining Mann-Whitney and Spearman correlation were performed. Varimax rotation was used for Principal Component Analysis (PCA). This method allows evidencing the significant loadings of the elements in each principal component by rotating the axis, while maintaining the total explained variance of the model. The minimum significant factor loading was considered at 0.6. Hierarchical Cluster Analysis (HCA) used Euclidean distance and Ward's method.

3. Results and discussion

3.1. Characterization of wine samples

Port and Douro wines are produced in the Douro Demarcated Region, located in the northeast of Portugal. Agricultural practices are common to both types of wine, as is climate and soil. The major difference between the two types of wine lies in the winemaking process due to the addition of grape spirit to Port wine during the fermentation stage.

Although the geology in Portugal is quite diverse throughout the country, this region shows a relatively homogeneous geological substratum originating from old metamorphic rocks that date from the Upper Proterozoic-Cambrian Era; over 541 million years ago (Fig. 1). Wines in the region are produced in *Quintas* (farms) and the same wine may contain grapes from different vineyards within the same farm. All samples in the present study come from vineyards grown in schist soils within the Douro Valley Valley as described in Fig. 1.

Thirty wines were analysed for multielement and isotopic composition. Table 1 shows the results obtained according to the type of wine (Port vs Douro). The minimum, median and maximum values of each

element are presented. Median was chosen over mean since it better represents the variability of results within each type of wine due to the large number of independent samples within each group ($n = 15$). Elements were divided into major and minor elements depending on their median concentration being higher or lower than $500 \mu\text{g.L}^{-1}$, respectively. Major elements are expressed in mg.L^{-1} and minor elements in $\mu\text{g.L}^{-1}$. On average the %RSD for major elements was 33% and similar for both types of wine. For minor elements, the percentage was 2 times higher, reaching 66%. However, considering the high number of independent samples (30) this should not be matter of concern.

Within the major elements, the most abundant one was potassium ranging between 399 mg.L^{-1} and 1458 mg.L^{-1} . This result was also observed in previous studies (Geană et al., 2017; Hopfer et al., 2015). The order of abundance of the remaining major elements was identical for both types of wine, as follows: $\text{P} > \text{Mg} > \text{Ca} > \text{Na} > \text{B} > \text{Mn} \approx \text{Rb} > \text{Sr} > \text{Fe}$. These results are in good agreement with the existing literature, except for Mn in other Portuguese wines reported with lower values than ours (Almeida and Vasconcelos, 2003; Catarino et al., 2018; Serapinas et al., 2008). The presence of Mn in wines may come from agricultural practices due to the use of fertilizers and pesticides (Catarino et al., 2018).

Regarding the minor elements Zn and Al were the most abundant ones ranging from $106 \mu\text{g.L}^{-1}$ to $586 \mu\text{g.L}^{-1}$ and $75 \mu\text{g.L}^{-1}$ to $1043 \mu\text{g.L}^{-1}$, respectively. The least abundant were Ag, Cd and Tl with concentrations below the LQ in all samples and were removed from all further data analysis.

Unlike for major elements the order of abundance for minor elements is not the same for Port and Douro wines. In fact, while Port wine exhibits Cu ($12 \mu\text{g.L}^{-1}$ to $332 \mu\text{g.L}^{-1}$) and Cr ($31 \mu\text{g.L}^{-1}$ to $52 \mu\text{g.L}^{-1}$) as the third and fourth most abundant minor elements; in Douro wines these positions are occupied by Li and Ni.

While some elements analysed are essential for humans (Ca, Cu, Fe, K, Mg, Mn, Mo, P, Se, and Zn) and have nutritional recommendations established, others are predominantly toxic or depend on the dose or chemical species present (As, Cd, Co, Cr, Na, Ni, Pb, and Sn). Some also affect the quality of the wine (Al, Cu, Fe, Ni, and Zn) (Tariba, 2011). Several entities and/or countries have established maximum limits for elements. All analysed samples comply with the maximum acceptable limits established by the International Organization of Vine and Wine

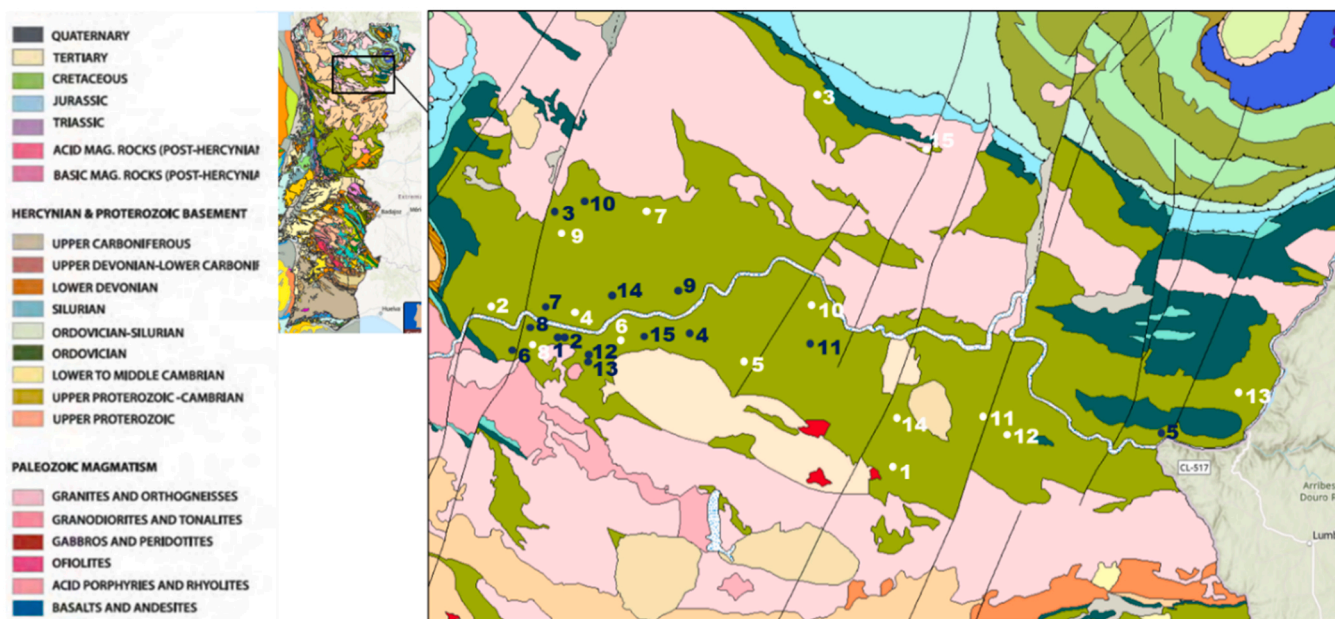


Fig. 1. Geological map of Portugal highlighting the Douro Demarcated Region with the location of the selected Quintas. Blue numbers represent samples of Port wine ($n = 15$). White numbers represent samples of Douro wine ($n = 15$). Adapted from (Carta Geológica de Portugal, Escala 1 M, 2021).

Table 1
Characterization of Port and Douro wines in terms of their a) elemental composition and b) Isotopic profile.

a)		Port wine				Douro wine			OIV limit ¹
		P10 (n = 1)	(n = 14)			(n = 15)			
Major elements mg.L ⁻¹			min	median	max	min	median	max	
	B	2.4	2.7	4.4	6.4	2.5	5.3	8.2	14
	Ca	128	26	52	72	45	63	76	n.a.
	Fe	12	0.13	0.57	3.4	0.57	0.95	1.7	n.a.
	K	954	534	872	1345	399	1090	1458	n.a.
	Mg	118	66	92	147	60	106	123	n.a.
	Mn	3.3	0.50	1.6	3.3	0.93	1.8	3.0	n.a.
	Na	17	2.6	6.4	14	6.3	11	32	80
	P	97	117	162	210	94	189	240	n.a.
	Rb	1.8	0.93	1.3	2.6	0.87	2.2	4.4	n.a.
	Sr	1.6	0.75	1.2	1.7	0.47	1.2	1.9	n.a.
Minor elements µg.L ⁻¹									
	Ag	< 2.3 *	< 2.3 *	< 2.3 *	< 2.3 *	< 2.3 *	< 2.3 *	< 2.3 *	n.a.
	Al	2697	< 113 *	164	1043	124	260	911	n.a.
	As	40	1.5	2.7	14	< 1.1 *	2.5	5.6	200
	Be	< 1.1 *	< 1.1 *	< 1.1 *	1.5	< 1.1 *	< 1.1 *	2.7	n.a.
	Cd	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	10
	Co	14	3.0	7.2	10	2.0	4.6	10	n.a.
	Cr	53	31	37	52	10	17	32	n.a.
	Cu	1716	12	91	332	4.2	31	158	2000 ² /1000
	Li	63	14	23	184	9.0	48	244	n.a.
	Mo	4.9	< 2.3 *	< 2.3 *	3.7	< 2.3 *	2.5	14	n.a.
	Ni	54	9.1	22	44	14	26	57	n.a.
	Pb	120	< 2.3 *	4.1	19	2.8	8.5	21	150 ³
	Se	6.2	< 2.3 *	< 2.3 *	4.8	< 2.3 *	< 2.3 *	2.8	n.a.
	Sn	5.7	< 1.1 *	< 1.1 *	4.7	< 1.1 *	< 1.1 *	3.6	n.a.
	Tl	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	< 1.1 *	n.a.
	Zn	1675	197	330	586	106	258	435	5000
b)		Port wine				Douro wine			
		P10 (n = 1)	(n = 14)			(n = 15)			
Isotopic Ratio			min	median	max	min	median	max	
	²⁰⁸ Pb/ ²⁰⁴ Pb	38.306	37.609	38.029	38.289	37.728	38.147	38.560	
	²⁰⁷ Pb/ ²⁰⁴ Pb	15.633	15.587	15.618	15.641	15.606	15.632	15.653	
	²⁰⁶ Pb/ ²⁰⁴ Pb	18.226	17.647	18.062	18.314	17.854	18.141	18.672	
	²⁰⁸ Pb/ ²⁰⁶ Pb	2.102	2.085	2.108	2.131	2.044	2.104	2.113	
	²⁰⁶ Pb/ ²⁰⁷ Pb	1.166	1.132	1.157	1.172	1.144	1.160	1.193	
	⁸⁷ Sr/ ⁸⁶ Sr	0.71712	0.71652	0.71985	0.72160	0.71683	0.71969	0.72290	
	δ ¹⁸ O ‰	2.67	4.23	5.38	7.27	0.25	5.24	6.91	
	δ ¹¹ B ‰	27.2	3.6	16.0	24.9	10.7	20.3	33.5	

*Limit of quantification (LD); ¹maximum limits established by the OIV at the time of harvest (International Organisation of Vine and Wine, 2015); ² for liqueur wines produced from unfermented or slightly fermented grape must; ³ for wine made, starting from the 2007 harvest year; n.a. – not applicable

(OIV) and presented in Table 1 (International Organisation of Vine and Wine, 2015). Therefore, these samples do not pose a risk for human health in regards to these toxic elements.

Among all analysed wine, the sample P10 (Port wine) presents considerably higher concentrations of the following elements – Al, As, Cu, Zn, Pb. This sample is a white Port wine from a small family production, aged in wood barrels, which had been stored for over 30 years. For this reason, it is presented in a separate column and it was removed from all statistical analysis. The high concentration of Pb in this sample is in agreement with Pb values reported in the literature for older fortified wines (International Organisation of Vine and Wine, 2020). According to the (International Organisation of Vine and Wine, 2020) environmental sources of Pb contamination in wines are factories and Pb in gasoline. The former is not a valid possibility since the region is not industrialized. However, leaded gasoline was abolished in Portugal in 1999 and still used at the time of production. To look further into this possibility it is necessary to analyse the Pb isotope ratios. Although sample P10 is over 30 years old, its Pb isotopic signature is similar to the remaining samples. In fact, its value for ²⁰⁶Pb/²⁰⁷Pb is the second highest among Port wines, and the sixth from all samples and it is reported that, in Europe, the atmospheric ²⁰⁶Pb/²⁰⁷Pb value has increased since the forbidding of leaded gasoline (Epova et al., 2020; Komárek et al., 2008). The values of Pb isotope ratios in Port and Douro wines, including P10, are consistent with previous results for the region (15.6244 ≤ ²⁰⁷Pb/²⁰⁴Pb ≤ 15.6699, n = 5) (Teixeira et al., 2021). Compared to other European wines, namely French wines, where values

for ²⁰⁶Pb/²⁰⁷Pb ranged from 1.1518 to 1.1914 (n = 43), our results are consistent although showing higher dispersion (Epova et al., 2020). Six samples presented values below the minimum French result and 1 sample was above. In fact, Fig. 2 presents a comparison between Pb isotope ratios in Port and Douro wines and Bordeaux wines. Sample B15 was removed for being an outlier according to Grubbs test. The overlapping of the two regression lines (Port and Bordeaux) is undisputable

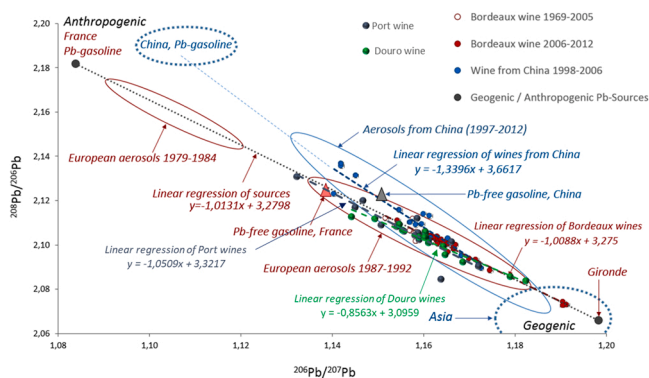


Fig. 2. ²⁰⁸Pb/²⁰⁶Pb and ²⁰⁷Pb/²⁰⁶Pb ratios in Port, Douro and Bordeaux wines in comparison with the predominant anthropogenic and geogenic lead sources
Adapted from (Epova et al., 2020).

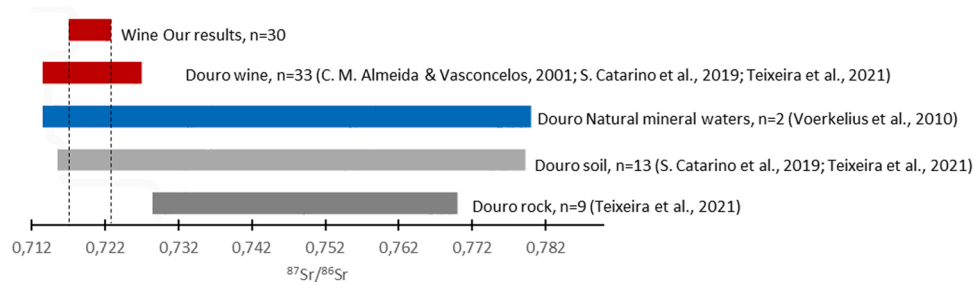


Fig. 3. Range of values of $^{87}\text{Sr}/^{86}\text{Sr}$ for wines, soil, rocks and water from Portugal, as reported in literature. References: (C. M. Almeida & Vasconcelos, 2001) (Teixeira et al., 2021) (S. Catarino et al., 2019) (Voerkelius et al., 2010) (Ribeiro et al., 2014).

and clearly illustrates a common profile for Pb isotopes in Portugal and France.

Therefore, a non-environmental source of contamination is at the origin of the higher levels of Pb found in this sample. Sample P10 also presents the highest levels of Cu and As among all samples, suggesting that Pb may be coming from vine sprays used at the time (lead arsenate, lead in copper sulphate). Another possible source is the metal material used at the time, like pipes and tubing, that came in contact with the wine during the winemaking process (International Organisation of Vine and Wine, 2020). The Pb isotope ratios are not distinctive of the year of production in the present samples.

The $\delta^{11}\text{B}$ shows a big dispersion of results within both groups with the maximum value for a Douro wine (33.5‰) being almost 10-fold higher than the lowest for a Port wine (3.60‰). Samples under study come from a limited geographical area with the same of geological formation. Such indicates a high variability of this parameter, possibly linked to anthropogenic sources like the use of fertilizers. Another hypothesis to study further is the role of the vine variety in the fractionation of B after root absorption (Coetzee et al., 2011). In the present work, only two wines are monovarietal thus it is not possible to formulate any assumptions on this matter. Some works are available for comparison in the literature regarding $\delta^{11}\text{B}$. Our results, are in line with studies on Lambrusco wines, were $\delta^{11}\text{B}$ varied between 8.49‰ and 19.94‰, and South African wines (Coetzee et al., 2011; Lancellotti et al., 2021), except for the lower results. The present results are the first ever obtained results that point to necessity of a deeper study especially for elucidating the factors behind the high variability of $\delta^{11}\text{B}$.

The isotopic profile of all 30 wine samples was analysed for 4 isotopic systems (Table 1 b). Light isotopes, B and O, are presented in delta notation while Sr and Pb show results as ratios of radiogenic and/or non radiogenic isotopes.

The value of $\delta^{18}\text{O}$ ranged between 0.25‰ and 7.27‰. The fact that all values are positive depict an enrichment in ^{18}O . The value of $\delta^{18}\text{O}$ is highly dependent on the climate and location, mainly latitude, temperature and precipitation (Camin et al., 2015). Its value in wine reflects alterations in climate conditions during harvesting period (Roßmann et al., 1999). The Douro Demarcated Region is located between latitudes 45°53' N and 45°68' N, extending itself more longitudinally than in latitude. During the years under study (2012–2016) the amount of annual precipitation across the region varied amply between its three sub regions, from 493 mm in Douro Superior to 730 mm in Cima Corgo (IPMA, 2022). However, during harvesting period, typically between September and October, the average total precipitation was similar for the three sub regions, ranging from 138 mm in Douro Superior to 182 mm in Cima Corgo. On the other hand, mean annual temperature of about 14.1 °C is relatively constant across the region, with an average of 17.2 °C reached during harvesting period (IPMA, 2022). The value $\delta^{18}\text{O}$ obtained for all wines are positive (0.25–7.27‰) and there are no apparent differences between wines produced within the region, which is coherent with the fact that the major influencing conditions are similar throughout the sampling area. Our results are higher compared to values in the literature, also when comparing to other southern

European countries like Italy (Lancellotti et al., 2021). However, as far as we know, although Portugal reports $\delta^{18}\text{O}$ values for the European Wine DataBank, there are no published data available. (Ogrinc et al., 2001) reports $\delta^{18}\text{O}$ values in Slovenian wines between - 8.24‰ and 4.31‰. In another latest work average $\delta^{18}\text{O}$ values in wines from Austria, Slovenia, Romania, Montenegro and Argentina were as listed: - 1.3‰, 1.4‰, 3.5‰, 8.0‰ and 2.8‰, respectively (Horacek et al., 2021). All samples, except one from Montenegro, were below our maximum value of 7.27‰ obtained in a sample of Douro wine. $\delta^{18}\text{O}$ values in wine in Portugal are expected to be enriched in ^{18}O since as latitude, altitude and distance from the ocean increase generally the values $\delta^{18}\text{O}$ in rain water become lower and consequently in wine (Santesteban et al., 2015). Therefore, $\delta^{18}\text{O}$ values are lower in countries from central and northern Europe compared to those in the south. In addition, hot temperatures and dry climate, as the ones typical in the Douro region during harvesting season, contribute to a higher evaporation of water from vines with further enrichment in the isotope ^{18}O (Santesteban et al., 2015).

Values for $^{87}\text{Sr}/^{86}\text{Sr}$ ranged between 0.71652 and 0.72290. These results are higher than values reported for Bordeaux wines (0.70829 – 0.71020, n = 43) or Lambrusco wines (0.70864 – 0.70911, n = 40) (Epova et al., 2019; Lancellotti et al., 2021). However, when compared to other wines from the Douro Demarcated Region our results fall within the expected range (Fig. 3). Soils from the region have been analysed for $^{87}\text{Sr}/^{86}\text{Sr}$, to study for wine authenticity, and are reported to have values ranging from 0.708 to 0.780 while the values for the bedrock are between 0.727938 and 0.770011 (Catarino et al., 2019; Teixeira et al., 2021). Several authors have observed a depletion in $^{87}\text{Sr}/^{86}\text{Sr}$ from bulk soils to wine, probably related to the texture of soils or weathering processes (Marchionni et al., 2016; Teixeira et al., 2021). Douro vineyards are cultivated mainly in soils originating from schistous rocks. These are metamorphic rocks from the Neoproterozoic Era. Together, a high initial concentration of Rb in bedrocks and age of these geological support, favours such relatively higher values of the ratio $^{87}\text{Sr}/^{86}\text{Sr}$. As far as we know, other than the Douro Demarcated Region, such high values have only been reported for Romanian wines from the Vrancea region (Geană et al., 2017).

The $\delta^{11}\text{B}$ shows a big dispersion of results within both groups with the maximum value for a Douro wine (33.5‰) being almost 10-fold higher than the lowest for a Port wine (3.60‰). Samples under study come from a limited geographical area with the same of geological formation. Such indicates a high variability of this parameter, possibly linked to anthropogenic sources like the use of fertilizers. Another hypothesis to study further is the role of the vine variety in the fractionation of B after root absorption (Coetzee et al., 2011). In the present work, only two wines are monovarietal thus it is not possible to formulate any assumptions on this matter. Some works are available for comparison in the literature regarding $\delta^{11}\text{B}$. Our results, show a higher dispersion than Lambrusco wines, were $\delta^{11}\text{B}$ varied between 8.49‰ and 19.94‰ (n = 40), (Lancellotti et al., 2021). The present results are the first ever-obtained results that point to necessity of a deeper study especially for elucidating the factors behind the high variability of $\delta^{11}\text{B}$.

Since all studied wines come from the homogeneous broad swath of land of pre-Cambrian schist and granite along the valley of the Douro River (Fig. 1), the geological signature of these wines is expected to be relatively similar. However, while this is true for most elements, Port and Douro wines presented differences in their inorganic composition for some elements and isotopes, suggesting an anthropogenic input. Statistical analysis was used to clarify some of the results obtained.

3.2. One-way analysis of variance

One-way analysis of variance (ANOVA) was applied to study further such variations and evaluate significant differences between the two types of wine. Significant differences were identified in seven elements; Ca, Na, Rb, Zn, Cu, Cr and Se and four isotope ratios; $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $\delta^{11}\text{B}$; with p-values of 0.002, 0.002, 0.001, 0.031, 0.017, 0.000, 0.000, 0.042, 0.044, 0.045 and 0.031, respectively. Fig. 4 illustrates the boxplots for elements with significant differences and the isotopic systems under study. Minerals and trace elements may come from natural or anthropogenic sources. In the particular case of this study all vineyards are grown in the same geological substratum and climate, thus the soil and climate are not

expected to be the main responsible for these differences. Agricultural practices are also common to both types of wine. Instead, differences between Port and Douro wines likely result from the winemaking process or storage conditions.

The levels of Cu and Cr were significantly higher in Port wines compared to Douro wines. Similar results had already been observed for Cu but not for Cr, between fortified and table wines of the region (Almeida and Vasconcelos, 2003). Nonetheless, results for both types of wine were in accordance with the literature (Catarino et al., 2018; Hopfer et al., 2015; Serapinas et al., 2008). Copper is present in wine because of agricultural practices, namely the use of pesticides, the winemaking process or oenological practices (Tariba, 2011). It comes from the application of fungicide in the form of copper sulphate (Cu_2SO_4) or from additions used during winemaking to control unwanted sulfidic aromas. But the levels of Cu have been reported to decrease during wine fermentation due to the formation of insoluble copper sulphides (Bica et al., 2020; Tariba, 2011). The early stopping of the fermentation in Port wine could explain the higher levels of Cu.

Regarding the concentrations of Cr, Almeida & Vasconcelos, 2003, also observed the contamination of fortified wines with Cr coming from oak barrels and the vinification system. On the other hand, the use of

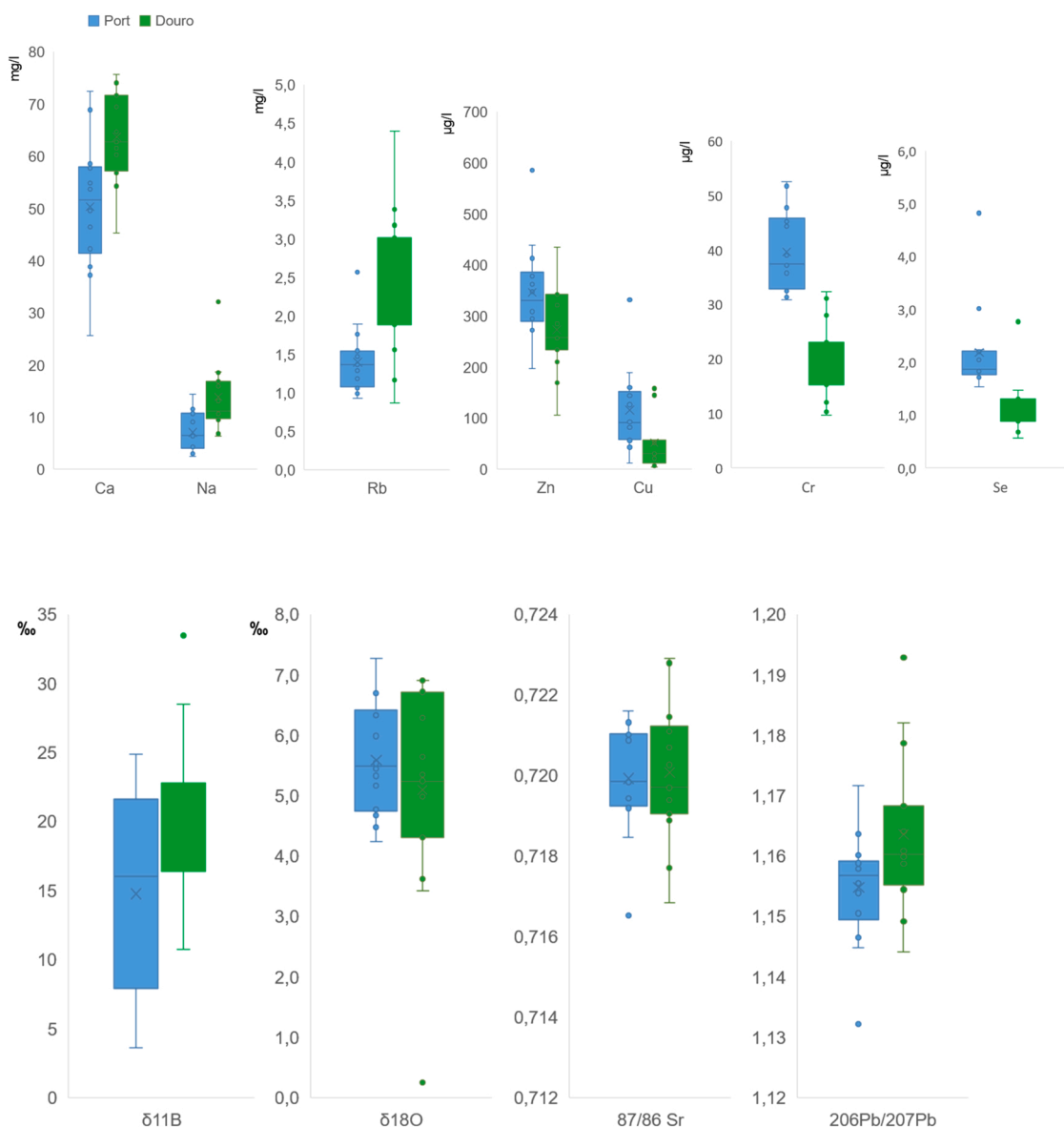


Fig. 4. Boxplots for the concentration of the inorganic elements and isotope ratios evidencing significant differences between Port and Douro wines, at $p < 0.05$.

chromium oxides for pigmentation of glass bottles contributes to an increase of Cr during storage (Cabrerá-Vique et al., 1997). According to Médina & Sudraud, 1980, the contamination of Cr is higher from glass bottles compared to stainless steel tanks. This is consistent with Douro wines presenting lower levels of Cr than Port wines. The three highest concentrations of Cr were found in sample P10 and samples P13 and P14, both bottled in 2014, while the lowest 5 are in Douro wines stored in stainless steel tanks (D9, D7, D8, D5). Since this was not the primary goal of the present study, a tailor made sampling plan is required to further elaborate on the origin of Cr in fortified wines.

The levels of Se were between the limit of detection (LD) and the LQ (Table 1) in little over half of the results, and were considered in the statistical analysis similarly to quantifiable values (Verbovše, 2011). Considering the Adequate Intake (AI) established by EFSA, of 70 µg of Se per day, no health effects, either positive or negative, are expected at this level (EFSA NDA Panel, 2014). However, the analytical technique still provided enough sensitivity to detect significant differences between Port and Douro wines in this element (p-value ≈ 0.000).

In the case of Zn, although there were significant differences in the level of this element, the range of values obtained for Douro wines is broader and covers most results for Port samples.

The levels of Ca, Na and Rb are higher in Douro wines and significantly different from Port wines. The explanations for this fact is in the dilution factor introduced by the addition of grape spirit to Port wines. Grape spirit is added to Port wines during fermentation, in the proportion of 1 L of grape spirit to 4 L of must, thereby diluting the samples and consequently lowering the elemental content. Other elements (B, Fe, K, Mg, Mn, P, Al, Li, Ni, Pb) exhibited the same behaviour although without statistically significant differences.

ANOVA also identified significant differences between the two types of wine regarding some of the Pb isotope ratios and $\delta^{11}\text{B}$. However, a visual inspection of the boxplots of $\delta^{11}\text{B}$ and $^{206}\text{Pb}/^{207}\text{Pb}$, in Fig. 4, shows large overlapping of values for both types of wine.

The remaining elements (Li, Be, B, Al, Mn, Co, Ni, As, Sr, Mo, Ag, Cd, Sn, Tl, Pb, Fe, Mg, P, and K) and isotopic systems ($\delta^{18}\text{O}$, $^{87}\text{Sr}/^{86}\text{Sr}$, $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$) did not present any significant differences between the two types of wines, suggesting they may be characteristic of the region and not influenced by anthropogenic sources. These parameters may provide good tracers for provenance traceability.

The values of $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$ are referred in the scientific literature as good geographical markers for wine (Epova et al., 2019; Horáček et al., 2021; Lancellotti et al., 2021). Although a synergistic approach, combining different data is usually more effective, both these parameters may serve as a good first indicator of geographical origin of wines from the Douro Demarcated region.

The elemental composition of wines is highly influenced by the geochemistry of the soils, and the majority of the elements under study showed a concentration range unrelated to the type of wine. However, some elements showed a variation in their content deriving from the wine production method. This is the case for Ca, Na and Rb, due to dilution effects and for Cu because of the incomplete fermentation in Port wines. In the case of Cr, multiple possible causes as the spirit added, contamination during storage or the interruption of the fermentation require further studies for clarification. Two isotopic analysis, $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$, are promising tracers of geographical authenticity for the Douro Demarcated Region since none presented statistically significant differences between the type of wine and values for both were distinctive from the literature. To clarify further the role of these elements/isotopes in the characterization of Port and Douro wines multivariate statistic will be applied to the data.

3.3. Correlation analysis

The aim of the correlation analysis is to identify possibly relationships between the 32 variables, elements and isotopes, amongst the 29 wine samples. The correlation matrix is provided in Table S2,

supplementary material. According to Kolmogorov-Smirnov test, all variables except Li follow a normal distribution, within each type of wine. Spearman's correlation was used for Li while for the remaining variables, a parametric test was applied.

All variables showed at least one correlation. This was the case of Mn and $\delta^{18}\text{O}$, each with one single significant positive correlation with Mg (0.43) and Al (0.40), respectively. Both Mo and Sn presented significant correlations with only two other variables, Mo-P (0.41), Mo-Pb (a.44), Sn-Pb (0.40) and Sn-Cu (0.64). The three strongest correlations, excluding intra lead isotope correlations, were Ni-Mg (0.70), Li-Mg (0.68) and Al-Na (0.65). Cr, Mg and Na presented the highest number of significant correlations with other variables, ten in total, followed by Li and Rb each with nine correlations. To elaborate further on the correlations between variables and the type of wine multivariate analysis was applied, namely PCA and HCA.

3.4. Multivariate analysis

3.4.1. Principal Component Analysis (PCA)

The PCA revealed six principal components explaining 85% of total variance. After Varimax rotation the explained variance by each principal component, from 1 to 6, was 18.5%, 15.2%, 13.6%, 11.7%, 11.9% and 14.1%, respectively. Only 17 of the initial 35 variables had influence on at least one principal component, considering a factor loading ≥ 0.6 , and remained on the construction of the final model. The Table with the loadings can be consulted in Table S3, supplementary material. Out of the eleven parameters identified by ANOVA with significant differences between the types of wine, only seven remained in the factorial analysis. Such indicates that the values of these seven parameters (Cr, Cu, Rb, Se, $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$ and $\delta^{11}\text{B}$) are more influenced by the wine-making process or the storage conditions than by the edaphoclimatic characteristics of the production area. All other parameters should be evaluated as potential tracers of geographical authenticity. In particular variables with no significant differences between the types of wine and no significance in the PCA like $\delta^{18}\text{O}$, $^{87}\text{Sr}/^{86}\text{Sr}$, B, Be, Co, Mo, Mn, P and Sn. These are mainly dependent on climate, soil and/or agricultural practices that are the common characteristics to Port and Douro wines.

The bi-plot between the factors PC3 and PC4 shows the formation of two groups of samples. The first on the upper quadrants identifies samples with higher levels of Sr and K (samples D2, D4, D6, D10, D14 and P13), and the other on the lower quadrant groups the samples with the lowest levels of these two elements (samples D5, D7, D8, D9 and P1). However, this the explanation for this grouping is not the wine making process (Port or Douro), since both groups are mainly composed by Douro wines and each includes one Port wine.

Looking at the PC plots in Fig. 5 it becomes evident that the factor PC5 provides the best separation between Port and Douro wine. Three elements have a higher influence here, Cr, Se and Cu. The three evidenced significant differences between the type of wine with ANOVA and a positive strong correlation between all of them, Cr-Se (0.580), Cr-Cu (0.569) and Cu-Se (0.528). Douro wines have a lower content of Cr, Se and Cu compared to Port wines and these differences permits their differentiation.

In sum PCA identified common characteristic, based on the levels of Sr and K, to a group of samples that includes Port and Douro wines (PC3 vs PC4) but it also provided the full differentiation between Port and Douro wines based on the levels of Cr, Cu and Se (PC5 vs PC6).

3.4.2. Hierarchical Cluster Analysis (HCA)

To reduce background noise HCA used only the 17 variables highlighted in the PCA (Supplementary Table S3).

HCA showed the formation of four clusters (Fig. 6). The separation between the types of wines is evident for two clusters in this statistical analysis. The first cluster contains only Douro wines, nine in total, and eleven Port wines form the third cluster. The later are characterized by showing higher levels of Cr, Se and lower Rb and Na, when compared to

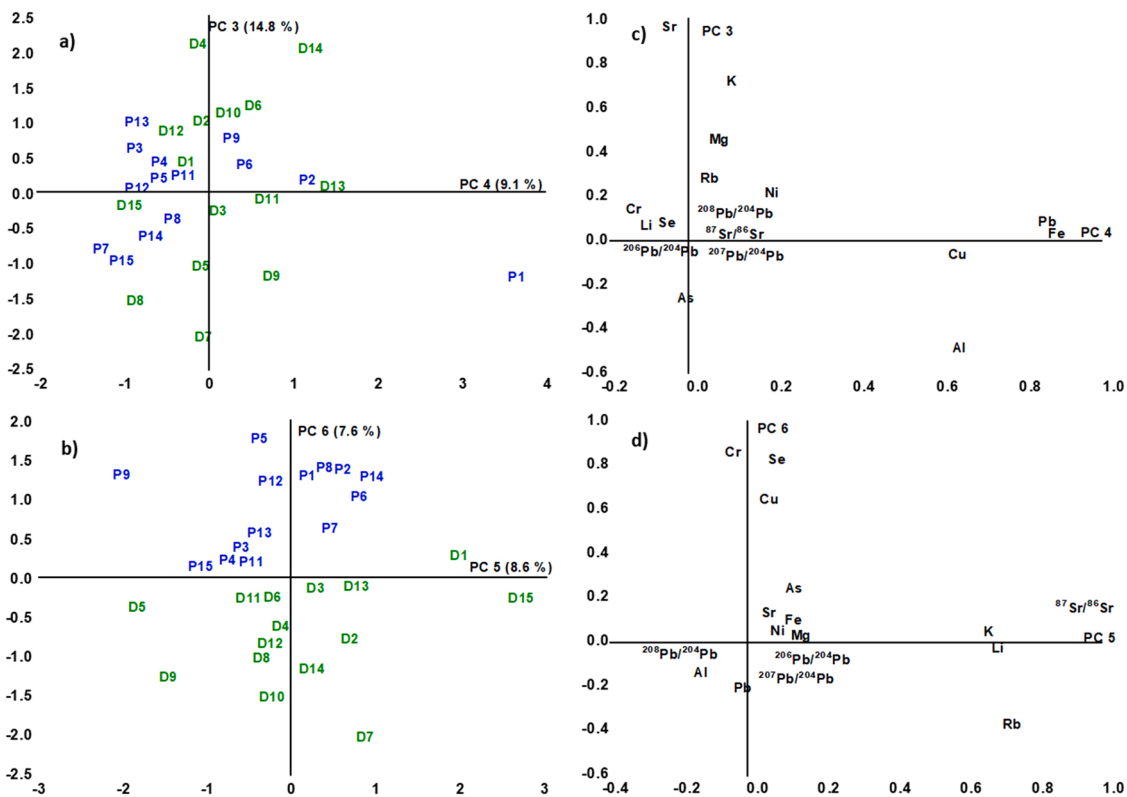


Fig. 5. PCA plots representing scores (a, b) and loadings (c, d) for wine samples. The letters “P” and “D” represent Port and Douro wines, respectively. Numbers from 1 to 15 identify the samples.

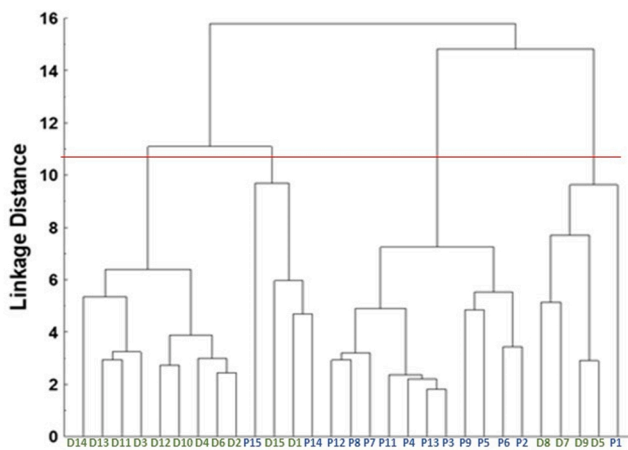


Fig. 6. Dendrogram obtained by HCA considering 29 wine samples. The letters “P” and “D” represent Port and Douro wines, respectively. Numbers from 1 to 15 identify the samples.

the global mean. On the other hand, Douro wines in the first cluster show values closer to the mean for all variables with no evident distinctive values.

The second cluster is a mixture of Port and Douro wines, containing two of each namely P14, P15, D1 and D15. On average, these wines have the highest concentrations of Li, Rb and As and the lowest of Pb, which explains the grouping of this cluster.

Four Port wines and one Douro wine form the fourth and last cluster. The common factor is that all five are white wines. In fact, the only white wine under study not classified in this cluster is P12; with the analysis assuming that, this sample presents more similarities with other Ports than with white wines and placing it in the third cluster. The wines in the fourth cluster show the highest levels of Al and Na and the lowest of Cr, Sr and K. Cr and Na presented significant differences between Port and Douro wines in ANOVA (Fig. 4). As such, the predominance of Douro wines in this cluster explains the levels of these two elements. On the other hand, Al, Sr and K did not show significant differences between the two types of wine. White wines have been reported to have higher Al and lower Sr than red wines (Gajek et al., 2021; Karaš et al., 2020). Unlike red wines, the fermentation process in white wines does not occur in contact with skin and stalk which can explain the lower levels of K. Combining this analysis with the interpretation of the PCA we verify that the plot PC3 (Al) vs PC4 (Sr, K) (Fig. 5a) provides a grouping of these wines, coherent with the HCA. In addition, Pearson’s test showed a negative correlation between Al-Sr (−0.389) and a positive one between Sr-K (0.602). Due to the reduced number of white wine samples under study, these are preliminary results and should be further investigated.

Based on the results from the statistical analysis, two 3-D Plots (Fig. 7) were constructed to represent the interaction between parameters and type of wine. Fig. 7a illustrates the characterization of wines using parameters influenced by the winemaking process or by storage (Cr, Cu, Rb). The distinction between Port and Douro wines is clear and unequivocal as there are no overlapping samples. On the other hand, parameters mainly influenced by the climate (like $\delta^{18}O$), atmospheric contamination or underlying geology ($^{87}Sr/^{86}Sr$) display a range of values common to both types of wines (Fig. 7b). This plot represents a characteristic pattern of the region. The obtained results could integrate a reference database for the Douro Demarcated Region.

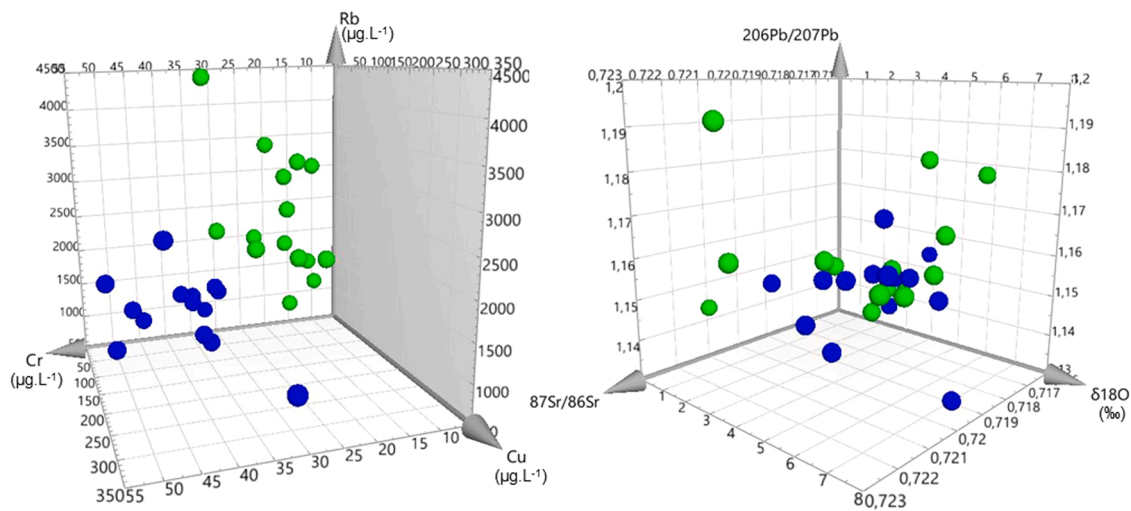


Fig. 7. 3-D plot of a) discriminant parameters process oriented (Cr,Cu,Rb) by type of wine and b) non-discriminant isotope ratios, geogenic oriented. Blue dots represent Port wines. Green dots represent Douro wines.

4. Conclusions

This work proposes, for the first time an analytical approach that combines heavy and light isotopes, as well as multielement analysis, to trace the geographical authenticity of Port and Douro wines. The analytical methods provided enough sensitivity to detect the inorganic content in the wine samples. The analytical determinations revealed that all analysed samples comply with OIV maximum limits and that K, P and Mg are the most abundant elements, in decreasing order. On the other hand, Cd, Tl and Ag were below the limit of quantification in all samples.

As a result of ANOVA seven elements (Ca, Na, Rb, Zn, Cu, Cr and Se) and four isotopic systems ($^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{207}\text{Pb}$ and $\delta^{11}\text{B}$) showed significant differences between the two types of wine, suggesting a greater influence of the wine making process or storage on these parameters rather than soil or climate. Douro wines are richer in Ca, Na and Rb, due to dilution effects coming from the addition of grape spirit to Port wine. The interruption of the fermentation leads to higher Cu values in Port wines. As for Cr further studies are required to clarify the causes behind its increased levels in Port wine.

Three elements, Cu, Cr and Se, provided a good distinction between Port and Douro wines observed in the PCA. HCA also the samples were separated, the formation of a cluster composed only by white wines showed these wines have lower levels of Sr and K but are enriched in Al. However, a larger number of samples is required to support these assumptions and further studies should be conducted.

Results obtained for both $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$ values are distinct from the scientific literature, and the type of wine did not condition either of these variables. In the case of $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}$, the addition of grape spirit to Port wines, which may come from areas outside the Douro Demarcated Region and even other countries, did not induce statistically significant differences between Port and Douro wines, nor did storage conditions. Such indicates that both parameters are specific to the region and are promising natural tracers for geographical origin of the Douro Demarcated Region, as the winemaking process or storage conditions did not influence either of them.

Authors Contribution to the Field Statement

IC contributed significantly to the conception of the paper, its design and writing, data collection, data interpretation, and analysis. AM contributed significantly to the interpretation of data and analysis, participated in the writing and critically revised the paper. EE contributed significantly to data collection and critically revised the paper. JB and RC contributed significantly to the data collection. NO, IC, JB and

OD contributed significantly to the interpretation of data and analysis, participated in the writing and critically revised the paper. All authors contributed to the article and approved the submitted version.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2022.104988](https://doi.org/10.1016/j.jfca.2022.104988).

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