ELEMENTAL CONTENT AND LEAD-STRONTIUM ISOTOPE CHARACTERIZATION OF WINE

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ABSTRACT. The ICP-MS technique was used to determine elemental composition (Pb, Sr, Cd, Ni, Co, Cu, Ni, Hg, As, Cr) of wines (Muscat Ottonel, Fetească Albă, Fetească Regală, Fetească Neagră, Merlot and Cabernet Sauvignon) produced during three consecutive years (2014-2016) in Dealu Bujorului Vineyard. The determination of $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{207}Pb$ and $^{87}Sr/^{86}Sr$ isotopes from wines was performed for establishing reliable markers for wine geographical origin. For all tested wine samples, the toxic metals contents were found in quantities below the limits imposed by legislation. The highest concentration of heavy metals (Cd and Pb) was found in red wine Cabernet Sauvignon [Cd (0.14±0.01 µg/L) (2013)], followed by the same variety of wine from 2012 [Cd (0.14±0.03 µg/L)], in case of Pb the highest concentration was recorded by Merlot [Pb (54.33±1.00 µg/L) (2011)] followed by the same variety of wine but from 2012 [Cd (51.20±1.19 µg/L). Concerning the concentration of the elements in the white wine, it can be noticed that they recorded close concentration as red wine, Muscat Ottonel [Cd (0.12±0.01 µg/L) (2011)], Feteasca Alba [Cd (0.12±0.01 µg/L) (2012)], in case of Pb from white wines the highest concentration was recorded by Feteasca Alba [Pb (52.07±0.50 µg/L) (2012)], Feteasca Regala [Cd (52.29±1.00 µg/L) (2012)]. The highest mean of $^{87}Sr/^{86}Sr$ isotopic ratio was obtained at Feteasca Neagra variety [0.726±0.004 (0.575%, 2014)] and Muscat Ottonel variety [0.723±0.003 (0.416 % 2016)]. A possible explanation for the higher mean of $^{87}Sr/^{86}Sr$ isotopic ration for wine can be

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the mineral consistency of the vineyard soil and its different eco-climatic conditions. $^{206}Pb/^{207}Pb$ isotopic ratios in the analyzed wine samples indicated traces of pollution originated in automobile emissions. ($^{206}Pb/^{207}Pb$=1.1000-1.1400). The lowest mean $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ isotopic ratios was obtained at Muscat Ottonel variety [2.141±0.007 (0.346% 2015) and 17.469±0.016 (0.094 % 2014)]. The results confirm that the elemental profile and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio can be used to track the geographical origin of wine, discriminate between wine production regions, and can be used to characterize wine *terroirs* for forensic purpose.

INTRODUCTION

Wine quality testing is made by using the state of the art of analytical techniques, because of the European community demand to establish an integrated approach on beverage control. The main purpose is a high level of wines safety within the European Union through so called *soil-to-grape-to-wine-to-glass* measuring and monitoring, ensuring the effective functioning of the European market [1].

Quality of wine is not only a gift of nature, but also the result of a wide range of technological intervention throughout the entire production process [2]. Current oenological research defines wine quality in relation to its chemical composition, pharmacological properties and health safety [3]. It is very important to monitor food products and beverage, including wines, for heavy metal contamination and assess the source of food contamination. Must and wine has a primary content of elemental composition due to natural transfer soil-root-grape-wine [4, 5] and a secondary content due to natural [6, 7] and artificial contamination [8, 9]. The natural content of elemental composition of wine and of other beverage is affected and overlapped by the artificial contamination [10].

Quantification of several elements in wines can validate the original authentication [8, 9, 11, 12], the potential toxicity and role in human diet [6, 13, 14], the effect on wine stability and organoleptic characteristics [15, 16, 17].

Wine is a source for essential elements which might improve human health, but it may also expose the consumer to undesirable levels and types of heavy metals when it is consumed regularly and in large amount over a long period of time [6]. The concern in human exposure to the metal

content of various beverages received a raising attention, since the consumption of wines in large volumes, significantly contribute to the daily dietary trace element intake by humans and so far there have not been a precise characterization of the impact of a number and the amounts of metals found in different drinks (alcoholic and non-alcoholic) on human health [13, 18]. In the production of wines, the quality control checks have to be brought to ensure that the consumer is protected and possible sources of contaminations are traced. The origin of Cu in wines is due to copper-based sprays used in vineyards, whereas the As, Cd, and Pb contents reflect the differences in grape variety, environmental factors and the wine-processing methods [11]. The Pb, Hg and Cd are undoubtedly considered toxic and should be monitored all the time, but also Cu, Ni, Cr, V, Zn, Ag, and Al, which in high concentrations may impart a certain toxicity [6, 13, 18]. The determination of Cd, Pb, Cu and Zn in wines is of great interest from oenological and toxicological points of view and correct knowledge of these parameters is required by the law. The *Organisation International de la Vigne et du Vin* (OIV) established Maximum Acceptable Limits (M.A.L.) for only a few elements in wine (Pb, Cd, Cu, Na, Zn, Ag, As, F, Br, F, and B [1]. The determination of toxic, such as Cd, As and Pb and essential trace elements, like Cu, in wines is an important and challenging analytical task, which requires multi-element methods of good selectivity, sensitivity and robustness [11, 19, 20].

Heavy metals are defined as those with a higher density than 5 mg/l, but the collective term now includes As, Cd, Cr, Cu, Fe, Pb, Mn, Hg, Mo, Ni, V and Zn [6, 21]. Elements like K, Ca, Mg, Fe, Cu, Zn and Cr are expected to be found in wines due to their high intercellular content and physiological roles and processes in plant, in wine yeasts and humans [6].

The content of elements such as Cu, Fe, Mn, and Zn were found in higher amounts in red grapes than white grapes and in lower amounts in wines than grapes [22]. Some elements may be lost through winemaking process, but the exposure to precipitants (bentonites) and containers may contribute to the quantity of some metals in wines [23].

Studies of isotopic ratios of heavy metals such Pb and Sr provide additional information on the geographical origin of wines, due to fact that plants inherit the isotopic signature of both elements from the geological and pedological environment [24].

Lead isotopic analysis of wines from France (Bordeaux) showed that the lead content in wines changed over time and reflected the dominant source of atmospheric lead pollution in southern of France [25]. Other researchers have found that lead isotopic compositions in wine may not always reflect those of leaded petrol, but reflect the isotopic signature of

local, dominant metallurgical industries [26, 27]. These studies confirm atmospheric deposition as being the dominant contributor to the lead total concentration and isotopic composition from wines. Some studies have shown that contamination from tin-lead foil capsules in the presence of corrosion and cork disintegration can dominate the source of lead from wines [28]. Other researchers have attributed the lead in wine to machinery or additives used during the winemaking process where environmental contamination in this case is low [29].

Isotopic ratios of heavy elements of geological interest, such as $^{87}Sr/^{86}Sr$, $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ have raised an interest in tracking regional provenance of wine [30, 31]. The winemaking process of red and white wines does not affect the $^{87}Sr/^{86}Sr$ ratio of wine, so this allows to link the Sr-isotopes of wine to those of the vineyard substratum [32, 33].

Inductively coupled plasma mass spectrometry (ICP-MS) is a multi-element technique frequently used for the elemental analysis of different wines (young and aged, red, rose and white, table and fortified, dry and sweet wines) in (semi)quantitative mode providing high selectivity, sensitivity and lower detection limits than other multi-element techniques [34, 35]. Also, ICP-MS was used for lead and strontium isotopic composition determination in several types of wines [36].

The purpose of this study is to determine the elemental composition (Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr) of white wines (Muscat Ottonel, Fetească albă, Fetească regală) and red wines (Fetească neagră, Merlot, Cabernet Sauvignon) from Dealu Bujorului Vineyard, using the ICP-MS technique (method approved by *L'Organisation International du Vie et Vin*). The results of this study may highlight the elemental composition of wine samples, assess the ability to discriminate wines by geographical origin and also complete the data regarding strontium ($^{87}Sr/^{86}Sr$) and lead ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$) isotope ratios in wines from Dealu Bujorului Vineyard (Galati county, Romania).

RESULTS AND DISCUSSION

Wine Mineral Content

Table 1 summarizes the total content of Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr in wine samples. In general, these elements are present in wine due to their extraction from grapes during the maceration period [37]. In grapes, these elements may occur by bioaccumulation in the vine plant from the soil, or they could originate from the pesticide agents used in plant

protection. During the maceration, extracted elements are absorbed at the cell membrane of yeast, and afterward, their declines as a result of precipitation together with the yeast cell or precipitation in complexation reactions. The contact of wine with equipment, the addition of fining agents, or the changing of filters, during post fermentation processes could increase the content of elements [37].

The mean contents of Cd, Pb and U were 0.10±0.01 µg/L, 41.51±1.05 µg/L and 0.26±0.03 µg/L, in case of Pb and U the highest concentrations were recorded in Feteasca regala [52.29±1.00 µg/L Pb (2014); 51.39±1.81 µg/L Pb (2015) and 0.38±0.03 µg/L U (2014); 0.40±0.01 µg/L U (2015)] while Cd was recorded the highest concentrations in Cabernet Sauvignon [0.14±0.01 µg/L Cd (2016); 0.14±0.03 µg/L Cd (2015) 0.13±0.02 µg/L Cd (2014)], Merlot [0.13±0.02 µg/L Cd (2014)] Feteasca Neagra [0.13±0.01 µg/L Cd (2014)]. The highest concentrations of heavy metals Cd and Pb were found in the red wine Cabernet Sauvignon [Cd (0.14±0.01 µg/L produced in 2013)], followed by the same variety of wine but produced in 2012 [Cd (0.14±0.03 µg/L)]; in the case of Pb the highest concentration was recorded in Merlot [Pb (54.33±1.00 µg/L) (2011)] followed by the same variety of wine but from 2012 [Pb (51.20±1.19 µg/L). Concerning the concentration of the elements in the white wine, it can be noticed that the recorded values are close to the red wine, *e.g.* Muscat Ottonel [Cd (0.12±0.01 µg/L) (2011)], Feteasca Alba [Cd (0.12±0.01 µg/L) (2012)]; in the case of Pb from white wines the highest concentration was recorded in Feteasca Alba [Pb (52.07±0.50 µg/L) (2012)], Feteasca Regala [Pb (52.29±1.00 µg/L) (2012)]. These results appear compatible with some other studies [37] reporting 0.25 µg/L (Cd), 46.00 µg/L (Pb), 0.11 µg/L (U), [8] 0.14 µg/L (Cd), 47.80 µg/L (Pb), but in some other reported results [38], the concentration of Cd appeared significantly higher (10.60 µg/L), while the reported values for Pb concentration appeared significantly lower (15.70 µg/L).

The concentration of Hg has recorded values between [0.58±0.04 µg/L Muscat Ottonel (2014); 0.51±0.04 µg/L Feteasca Neagra (2014)] and [0.24±0.04 µg/L Feteasca Neagra (2016); 0.19±0.02 µg/L Feteasca Neagra (2015)] with an average values of 0.28±0.03 µg/L. In this case, Fetească regală (<0.13 µg/L) and Merlot (<0.13 µg/L) have registered the values below the detection limit. Although the highest concentration was recorded in white wines, the average data shows that the red wines have the highest values 0.22 µg/L, compared to white wines 0.18 µg/L (Table 1). The results agree with those made in Romania [20] for Sauvignon Blanc 0.56 µg/L, Feteasca Alba 0.22 µg/L and Riesling 0.16 µg/L.

As and Sr content in wine ranged from [16.74±4.30 µg/L As; 6.20±0.23 µg/L As] and [277.49±3.64 µg/L Sr; 114.08±6.44 µg/L Sr] with an average values of 10.81±0.61 µg/L As and 205.29±5.63 µg/L Sr. In both

cases the maximum concentration were recorded in red wines Cabernet Sauvignon [16.74±4.30 µg/L As (2016); 14.41±2.75 µg/L As (2015)] and Feteasca Neagra [277.49±3.64 µg/L Sr (2016); 263.81±2.28 µg/L Sr (2015)] while the lowest concentrations were recorded in white wines Feteasca Alba [6.42±0.87 µg/L As (2015); 6.20±0.23 µg/L As (2014)] and Muscat Ottonel [149.12±5.50 µg/L Sr (2014); 114.08±6.44 µg/L Sr (2015)]. The average data shows that the red wines have the highest values [13.22 µg/L As and 228.44 µg/L Sr] compared to white wines values [9.25 µg/L As and 171.95 µg/L Sr] (Table 1). The results are comparable with those obtained in Serbia [8] 16.1 µg/L (As) for red wines and 9.46 (As) µg/L for white wines, in Macedonia [38] 11.7 µg/L (As), in Romania [20] 21.12 µg/L (As) and 154.90 µg/L (Sr), and higher compared to the results obtained in Italy [39] 2.91 µg/L for As, but for Sr the results obtained are significantly lower compared to the results obtained in Italy [39] 1340 µg/L (Sr).

Concentration of Co has recorded values between [6.74±0.48 µg/L Fetească regală (2015); 6.28±0.28 µg/L Feteasca Alba (2014)] and [3.55±0.39 µg/L Muscat Ottonel (2015); 3.71±0.21 µg/L Feteasca Neagra (2014)] with an average value of 4.54±0.21 µg/L. The average data shows that the red wines have the highest values [6.75 µg/L Co] compared to white wines values [2.97 µg/L Co] (Table 1). The results agree with other studies made in Macedonia [37, 38] 3.9 µg/L and 13.90 µg/L for Co, and in Serbia [8] 3.89 µg/L (Co) for red wines and 3.96 (Co) µg/L for white wines.

The mean contents of Ni and Cr were 573.11±2.85 µg/L Ni and 660.94±2.72 µg/L Cr, in case of Ni the highest concentrations were recorded in Feteasca Neagra [747.37±3.35 µg/L Ni (2014)] while Cr was recorded the highest concentrations in Muscat Ottonel [923.62±2.05 µg/L Cr (2014)]. In Fetească regală was recorded the lowest concentration of Ni [239.81±1.34 µg/L Ni (2016)] while in Feteasca Neagra was recorded the lowest concentration of Cr [323.92±4.09 µg/L Cr (2015)]. The results agree with those reported in Turkey [40] 520 µg/L Ni, in Romania [41] 147.73 µg/L Cr and significantly higher than the ones obtained in Serbia [8] 5.49 µg/L (Cr) red wines and 6.56 (Cr) µg/L white wines, in Italy [39] 54.57 µg/L (Ni) and 19.68 µg/L (Cr), in Macedonia [38] 32.10 µg/L (Cr) and 10.20 µg/L (Cr). The results indicated that Romanian wines are moderately rich in Cr (Table 1).

The concentration for Cu ranged from 0.48±0.03 mg/L to 0.91±0.05 mg/L, with average values of 0.72±0.04 mg/L, the latter value approaching the law limit (1 mg/L). The six samples [Fetească regală 0.85±0.07 mg/L (2014), Feteasca Regala 0.82±0.06 mg/L (2016), Feteasca Neagra 0.80±0.01 mg/L (2016), Cabernet Sauvignon 0.79±0.41 mg/L (2014), Feteasca Alba 0.77±0.08 mg/L (2015) and Feteasca Neagra 0.71±0.03 mg/L (2014)] showed relatively high concentration of Cu. Wide rages for Cu

concentration in wines have been previously found and reported by other researchers in Brazil [42] 0.056-0.764 mg/L Cu, in Hungary [43] 0.031-0.313 mg/L Cu, in Italy [44] 0.50-1.00 mg/L Cu and in Greece [45] 0.076-0.114 mg/L Cu (Table 1).

Examining the factors which influenced the content of metals in wines, it can be seen that the content in Cd, Pb, U, Hg, Sr, Co, Ni and Cr was significantly influenced by the Variety, Years and Variety x Years factors, while As and Cu content was significantly influenced only by the Variety factors.

Relating the obtained results [Cd average 0.10±0.01 µg/L (0.1mg/kg M.L.A. = Maximum Limit Allowed) Pb average 41.51±1.05 µg/L (0.15 mg/kg M.A.L); As average 10.81±0.61 µg/L (0.2 mg/kg M.A.L); Cu average 0.72±0.04 mg/L (1 mg/kg M.A.L) to national and international legislation we may say that the wines from Dealu Bujorului vineyard fall within the limits set by legislation (Table 1).

$^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratio from wine samples

In Central Europe, the lead isotopic ratio, as signatures of pollution sources, ranges from relatively high $^{206}Pb/^{207}Pb$ ratios (natural Pb, coals, fly ashes, $^{206}Pb/^{207}Pb$=1.1700-1.2200) to low $^{206}Pb/^{207}Pb$ values (gasoline, petrol combustion, $^{206}Pb/^{207}Pb$ = 1.0600-1.1400) [20, 27].

The Pb isotopes ratio for the selected wines (Table 2) varies in range between 1.13896-1.12545 ($^{206}Pb/^{207}Pb$), 2.13857-2.12126 ($^{208}Pb/^{206}Pb$) and 17.30857-17.44944 ($^{206}Pb/^{204}Pb$) with average 1.13269 ($^{206}Pb/^{207}Pb$), 2.12824 ($^{208}Pb/^{206}Pb$) and 17.42213. Regarding $^{206}Pb/^{207}Pb$ isotope ratios based on analyses it can be concluded that the wine obtained from wine varieties grown in the Dealu Bujorului vineyards show traces of pollution originating from automobile emissions ($^{206}Pb/^{207}Pb$=1.1000-1.1400). The obtained isotope ratio values are comparable with other Romanian wines [20] (1.1100 to 1.2000) and Brazilian wines [36] (1.1440 to 1.1820).

The highest values of $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ were recorded for wines obtained from Muscat Ottonel variety from 2012 (2.13857±0.00699 [0.32697] $^{208}Pb/^{206}Pb$) and Muscat Ottonel variety from 2011 (17.44513±0.03613 [0.14977] $^{208}Pb/^{206}Pb$). While Feteasca Regala from 2012 (2.12126±0.00106 [0.05014] $^{208}Pb/^{206}Pb$), Feteasca Alba from 2013 (17.30857±0.00560 [0.03235] $^{206}Pb/^{204}Pb$), and also Cabernet Sauvignon from 2012 (1.12545±0.00200 [0.17771] $^{206}Pb/^{207}Pb$), recorded the lowest isotope ration.

The results are comparable with Brazilian wines [36] (2.0700 to 2.1570 for $^{208}Pb/^{206}Pb$ and 16.6670 to 17.9960 for $^{204}Pb/^{206}Pb$) and also with Italian wines [46] (2.0990 to 2.1030 for $^{208}Pb/^{206}Pb$ and 17.544 to 18.3210 for $^{204}Pb/^{206}Pb$).

Table 1. Variation of the metal content of white and red wines from Dealu Bujorului Vineyard (µg/L)

Area	Variety	Year	Cd M.L.A. 0.01 mg/L	Pb M.L.A. 0.15 mg/L	U M.L.A. -	Hg M.L.A. -	As M.L.A. 0.2 mg/L	Sr M.L.A. -	Co M.L.A. -	Cu** M.L.A. 1 mg/L	Ni M.L.A. -	Cr M.L.A. -
Dealu Bujorului Vineyard	Muscat Ottonel	2011	0.12±0.01^abc α	42.35±1.00^d α	0.15±0.02^a α	0.58±0.04^a α	8.20±0.62^g β	149.12±5.50^i β	3.73±0.17^h β	0.68±0.05^ef α	517.12±4.21^k α	851.09±1.36^c γ
		2012	LOQ^g β	47.78±6.67^bc α	LOQ^f β	0.40±0.01^c β	8.31±0.37^g β	114.08±6.44 γ	3.55±0.39^i β	0.49±0.03^j β	498.01±2.33^l β	923.62±2.05^a α
		2013	LOQ^g β	27.22±2.55^gh β	LOQ^f β	0.14±0.02^e γ	10.41±0.88^d α	161.45±1.91^c α	4.73±0.47^aefg α	0.70±0.04^de α	460.89±2.14^k β	872.86±4.02^b β
	Feteasca Alba	2011	0.11±0.01^cd α	35.69±1.12^e β	0.28±0.03^b α	LOQ^f α	6.20±0.23^h β	186.11±7.36^g α	6.28±0.28^al α	0.61±0.03^fgh β	650.72±0.93^c α	867.66±3.50^b α
		2012	0.12±0.01^bcd β	52.07±0.50^ab α	LOQ^f β	LOQ^f α	6.42±0.87^h β	173.38±3.01^h β	5.09±0.09^cdef β	0.77±0.08^bcd β	535.26±1.21^gh β	734.62±6.94 γ
		2013	0.12±0.02^bcd α	29.23±3.02^fg γ	LOQ^f β	LOQ^f α	7.99±0.56^fg α	164.41±2.74^i β	4.56±0.38^efgh β	0.49±0.02^j γ	358.36±6.26^m γ	818.49±5.66^e γ
	Feteasca Regala	2011	LOQ^g β	52.29±1.00^a β	0.38±0.03^a α	0.15±0.02^e β	13.18±0.51^b β	187.22±6.69^g β	5.22±0.09^cd β	0.85±0.07^ab α	520.15±3.35^j β	761.45±5.53^e β
		2012	0.08±0.01^f α	51.39±1.81^ab α	0.40±0.01^a α	0.19±0.02^d α	12.95±0.61^bc α	202.54±6.74^ef α	6.74±0.48^a α	0.91±0.05^a α	603.50±5.38^d α	821.61±2.00^d α
		2013	0.09±0.03^e α	24.26±0.53^hi β	0.21±0.01^c β	0.19±0.01^efg α	9.62±0.54^ef β	200.89±1.07^f α	5.15±0.21^cde β	0.82±0.06^a β	239.81±1.34^n α	819.25±4.03^d α
	Feteasca Neagra	2011	0.13±0.01^ab α	23.01±1.54^i β	0.16±0.02^d α	0.51±0.04^b α	10.17±0.76^def β	254.72±4.59^c γ	3.71±0.21^i β	0.71±0.03^cde β	747.37±3.35^a α	355.90±1.74^i β
		2012	0.12±0.01^bcd αβ	20.28±1.09^i β	0.13±0.02^e β	0.19±0.02^ef β	11.98±1.28^bcg α	263.81±2.28^b β	4.48±0.23^gh α	0.60±0.05^gh γ	592.65±6.08^de γ	323.92±4.09^gv γ
		2013	0.10±0.01^e β	32.79±1.46^ef α	LOQ^f γ	0.24±0.04^d β	12.39±1.03^bcd α	277.49±3.64^a α	4.61±0.50^defg α	0.80±0.01^b α	566.51±4.60^f α	408.55±4.45^m α
	Merlot	2011	0.13±0.02^ab α	54.33±1.00^a α	0.27±0.04^b α	0.21±0.02^de α	13.46±0.76^b α	200.87±2.91^f β	4.25±0.36^gh γ	0.69±0.04^de α	523.52±0.97^h γ	478.45±2.86^k β
		2012	0.11±0.02^d β	51.20±1.19^ab β	0.13±0.02^e β	LOQ^f γ	13.83±0.68^b α	185.92±2.63^g β	5.35±0.26^c β	0.68±0.03^def β	543.26±4.28^g β	429.95±1.18 γ
		2013	LOQ^g γ	47.45±1.12^b γ	0.16±0.05^d β	0.16±0.04^fg β	12.29±1.00^bcd β	212.78±3.14^d α	6.02±0.34^b α	0.61±0.02^fgh β	636.69±5.08^c α	520.36±1.50^a
	Cabernet	2011	0.13±0.02^ab α	41.61±0.65^d β	0.30±0.05^b α	0.24±0.06^d α	13.67±1.41^b α	253.68±6.69^c α	4.04±0.13^ghi β	0.79±0.04^bc α	479.78±4.28^l γ	651.07±1.34^g α
	Sauvigno	2012	0.14±0.03^abc α	26.12±4.45^gh γ	0.27±0.01^b α	0.22±0.03^de α	14.41±2.75^a α	209.37±3.46^de β	5.08±0.50^cdef β	0.56±0.05^hi β	532.26±4.28^fh β	635.97±5.55^h β
	n	2013	0.14±0.01^a α	50.01±1.35^ab β	0.16±0.05^d β	0.23±0.03^d α	16.74±4.30^a β	189.03±1.97^g γ	5.07±0.17^cdef α	0.64±0.08^efg β	587.39±5.37^e α	530.39±1.51 γ
	Average		0.10±0.01	41.51±1.05	0.26±0.03	0.28±0.03	10.81±0.61	205.29±5.63	4.54±0.21	0.72±0.04	573.11±2.85	660.94±2.72
	Sig.		***	***	***	***	***	***	***	***	***	***
	Variety	Sig.	***	***	***	***	***	***	***	***	***	***
	Year	Sig.	***	***	***	***	ns	***	***	**	***	***
	Variety x Year	Sig.	***	***	***	***	**	***	***	***	***	***
	[1]		0.41	23.08±11.96	0.86±0.55		7.10	358.94±178.97	2.08±1.19	154.39±73.45**	18.39±1.19	146.18±70.96
	[2]		0.22		0.55	0.56	5.24	355.80	4.65		24.90	
	[3]			35.90				1600.68	2.50	2594.79**	322.73	1725.80
	[4]			235.90					42.06			

Average value ± standard deviation (n = 3). Romans letters represent the significance of the variety difference (p ≤ 0.05). Greeks letters represent the significance of the same variety cultivated in other year's difference (p ≤ 0.05). The difference between any two values, followed by at least one common letter, is insignificant. M.L.A. - maximum limit allowed (OIV, 2005); LOQ - lower than the limit of quantification; Cu* = (mg/L); Cu** = (µg/L).

The abundance of the lead isotopes ^{204}Pb (non-radiogenic), ^{206}Pb, ^{207}Pb and ^{208}Pb (radiogenic) originated from the genesis of the substrate varies with geological ages. The original composition of the rock upon its formation and consequently, with geographical areas [47], this property is useful in order to identify of the source of lead in a subjected wine sample provided that the measurements of the isotope ratio is precise and accurate.

Concerning ^{87}Sr/^{86}Sr isotope ratio, the values are between the ranges from 0.72599±0.00645 [0.89970] to 0.71288±0.00048 [0.03012] with an average value of 0.72158±0.00042 [0.05763]. The highest values were registered to wine obtained from Fetească regala variety from 2011 (0.72599±0.00422 [0.58123]), the lowest value of ^{87}Sr/^{86}Sr isotope ratio was recorded to wine obtained from 2012 Feteasca Alba variety (0.71288±0.00098 [0.13715]).

The values of ^{87}Sr/^{86}Sr isotope ratio obtained are comparable with those obtain for other Romanian wines [48] (0.71015 to 0.72311) and [20] (0.7600 to 0.9300).

Combining multielement analysis and ^{206}Pb/^{207}Pb, ^{208}Pb/^{206}Pb, ^{206}Pb/^{204}Pb, ^{87}Sr/^{86}Sr, isotope ratio from wine samples for discrimination analysis

Multivariate chemometric method was used as a supervised learning technique for the differentiation of wines intro groups on the basis of grape variety and year of production and finding markers which showed a significant discrimination value (variables with Wilk's lambda near zero, *p* value <0.05 and higher *F* coefficients). Stepwise linear discriminant analysis (LDA) was used to identify significant tracers for classification to the geographical discrimination of the wines samples. Stepwise Discriminant Analysis (LDA) was used to designate suitable variables for classification of the samples, eliminating the variables that do not contribute to discrimination of the wine. In order to validate the proposed statistic model, based on variables which showed higher significance in first LDA assessment, we performed a second Liniar Discriminant Analysis (LDA) for the test set consisting of wines used to build statistical model (training set) together with data from other wine samples that are not included in the first LDA (control-set). Cross-validation was applied to determine the optimal number of variables required to obtain robust models.

In this study, the content of certain wines showed high concentration of metals, but not exceeding the maximum recommended by International Organisation of Vine and Wine [49]; this accumulation is mostly due to agricultural practices, fertilizers and technological winemaking processes.

Table 2. The $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratios obtained from wine samples from Dealu Bujorului Vineyard

Area	Variety	Year	$^{206}Pb/^{207}Pb$	SD	RSD (%)	$^{208}Pb/^{206}Pb$	SD	RSD (%)	$^{206}Pb/^{204}Pb$	SD	RSD (%)	$^{87}Sr/^{86}Sr$	SD	RSD (%)	Pb (µg/L)	Sr (µg/L)
Dealu Bujorului Vineyard	Muscat Ottonel	2011	1.13434 abc α	0.00277	0.24458	2.12495 bcde β	0.00303	0.14269	17.44513 a α	0.03613	0.14977	0.71573 cd α	0.00573	0.80077	42.35±1.00 f α	149.12±5.50 β
		2012	1.13399 abc α	0.00168	0.14780	2.13857 f α	0.00699	0.32697	17.44443 a α	0.01981	0.11356	0.71539 cd α	0.00293	0.40990	47.78±6.67 bc α	114.08±6.44 γ
		2013	1.13203 abc f α	0.00512	0.45259	2.13259 bc αβ	0.00261	0.12254	17.43786 a α	0.03579	0.20525	0.72303 ab α	0.00302	0.41721	27.22±2.55 f β	161.45±1.91 α
	Feteasca Alba	2011	1.13456 abc α	0.00169	0.14872	2.12488 bcd α	0.00369	0.25334	17.42660 a α	0.03495	0.20089	0.72164 abc α	0.00022	0.03012	35.69±1.12 f β	186.11±7.36 α
		2012	1.13363 ae α	0.00257	0.22633	2.12390 de α	0.00039	0.01828	17.43213 a α	0.02427	0.13922	0.71288 ab β	0.00098	0.13715	52.07±0.50 b α	173.38±3.01 β
		2013	1.13106 bcd α	0.00437	0.38601	2.12362 bef α	0.00194	0.02748	17.30857 a β	0.00560	0.03235	0.72144 abc β	0.00020	0.02721	29.23±3.02 b γ	164.41±2.74 β
	Feteasca Regala	2011	1.13669 ae α	0.00102	0.08932	2.12323 bd α	0.00102	0.04781	17.42030 a α	0.09744	0.55933	0.72177 abc α	0.00078	0.10768	52.29±1.00 f β	187.22±6.69 β
		2012	1.13696 ae α	0.00642	0.56401	2.12126 f α	0.00106	0.05014	17.42944 a α	0.01932	0.11083	0.71823 acd α	0.00569	0.79181	51.39±1.81 ae α	202.54±6.74 ef α
		2013	1.13205 abc f α	0.00567	0.50055	2.12315 def α	0.00208	0.09793	17.44241 a α	0.01725	0.09890	0.72227 abc α	0.00098	0.13632	24.26±0.53 f α	200.89±1.07 α
	Feteasca Neagra	2011	1.13455 abc α	0.00185	0.16319	2.12903 bcdf α	0.00560	0.26302	17.42310 a α	0.01000	0.05737	0.72599 f α	0.00422	0.58123	23.01±1.54 f β	254.72±4.59 γ
		2012	1.13530 ae α	0.00073	0.06401	2.13308 ae α	0.00131	0.06148	17.42643 a α	0.00601	0.03450	0.71199 f α	0.00556	0.76679	20.28±1.09 β	263.81±2.29 β
		2013	1.13163 abc α	0.00299	0.26337	2.12933 bcdf α	0.00677	0.31794	17.42155 a α	0.00024	0.00135	0.72235 abc α	0.00651	0.90124	32.79±1.46 f α	277.49±3.64 α
	Merlot	2011	1.12605 d α	0.00018	0.01569	2.12223 f α	0.00591	0.27694	17.42563 a α	0.00566	0.03248	0.71985 abc α	0.00330	0.45850	54.33±1.00 f α	200.87±2.91 β
		2012	1.12769 de α	0.00713	0.63229	2.12843 bcde α	0.00716	0.33637	17.42148 a α	0.00023	0.00129	0.72370 ab α	0.00320	0.44197	51.20±1.19 ab β	185.92±2.63 β
		2013	1.13106 abcd α	0.00468	0.41419	2.12758 bcde α	0.00480	0.22537	17.41635 a α	0.00501	0.02678	0.71737 acd α	0.00045	0.89970	47.45±1.12 f γ	212.76±3.14 α
	Cabernet Sauvignon	2011	1.13502 abc α	0.00046	0.04057	2.13344 ab α	0.00196	0.09199	17.42410 a β	0.00234	0.01341	0.72346 ab α	0.00214	0.29596	41.61±0.65 f β	253.68±6.69 α
		2012	1.12545 f β	0.00200	0.17771	2.13034 bcde α	0.00625	0.29339	17.41532 a β	0.00639	0.03669	0.72262 abc α	0.00185	0.25567	26.12±4.45 f γ	209.37±3.46 ef β
		2013	1.13119 bcd αβ	0.00514	0.45450	2.12990 bcde α	0.00473	0.22186	17.44944 a α	0.01690	0.09685	0.72158 abc α	0.00042	0.05763	50.01±1.35 ab α	189.03±1.97 γ
	Average		1.13269	0.00314	0.27968	2.12824	0.00374	0.17555	17.42213	0.01794	0.10294	0.72158	0.00042	0.05763	41.51±1.05	205.29±5.63
	Minimum Values		1.12245	0.00713	0.01599	2.12126	0.00039	0.01828	17.30857	0.00023	0.00129	0.71288	0.00022	0.03012	20.28±1.09	114.08±6.44
	Maximum Values		1.13896	0.00018	0.63229	2.13857	0.00716	0.33637	17.44944	0.09744	0.20525	0.72599	0.00045	0.89970	54.33±1.00	277.49±3.64
	F		2.663			3.484			3.594			2.517			78.680	265.319
	Sig.		**			***			**			*			p≤0.000	p≤0.000
	Variety		ns			ns			ns			ns			***	***
	Year		ns			ns			ns			ns			***	***
	Variety x Year		ns			ns			ns			ns			***	***
	[5]											0.711235	0.00004			
	[6]		1.14400		1.40000	2.15700	0.15000	1.00000				0.76000	0.01000	1.30000	76.00±13.00	
	[7]		1.14000	0.10000	8.70000	2.10100	0.07000	7.10000							35.90	
	[8]		1.74000	0.00300		2.09200									11.11±5.28	171.40
	[9]											0.71000				
	[10]		1.14200	0.00200	0.14000	2.12500	0.00300	0.16000	17.60000	0.30000	1.70000					
	[11]														42.90±0.05	
	[12]		1.16620	0.00040	0.03000	2.09230	0.00060	0.03000	18.40000	0.10000	0.70000	0.71021				
	[13]b		1.10380	0.01750	1.58860	2.13420	0.01100	0.51660				0.89570	0.01930	2.15870	46.59±0.57	418.87±18.03
	[14]		1.13500	0.00100	0.01830	2.10540	0.00390	0.18430	16.66630	0.03070	0.16450	0.71510	0.00160	0.21870	55.44±1.01	202.07±8.15
	[15]											0.71000				
	[16]											0.71300				
	[17]		1.15400	0.07700											21.74	
	[18]											0.71015			52.00	905.00

Average value ± standard deviation (n = 3). Romans letters represent the significance of the variety difference ($p \le 0.05$). Greeks letters represent the significance of the same variety cultivated in other year's difference ($p \le 0.05$). The difference between any two values, followed by at least one common letter, is = insignificant.
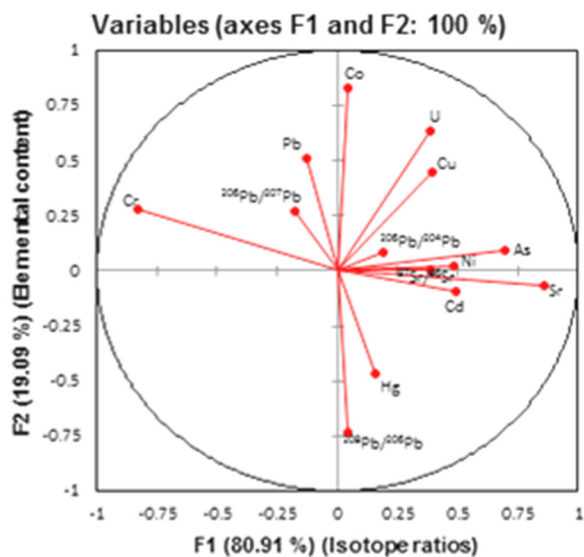
Figure 1. Correlation between analyzed parameters and the factors in discriminant analysis the origin of the wine

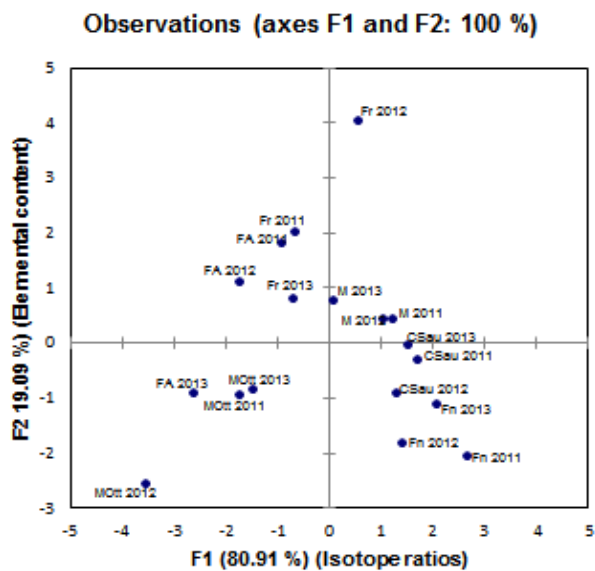**Figure 2.** Differentiation of wines based on element contents and ^{206}Pb/^{207}Pb, ^{208}Pb/^{206}Pb, ^{206}Pb/^{204}Pb, ^{87}Sr/^{86}Sr isotope ratios

Elements like Pb, Cu, Ni, Cd and Hg showed a high discriminatory power for geographic origin of Romanian, but additional new elements (Co, U, As, Sr, Cr) and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio have been investigated in order to identify new tracers for geographical traceability of Romanian wines [41].

Based on the elemental contents and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotopes ratio, the cross-validation technique provided a 100 % percentage of predicted membership according to the origin of the wine (F1 = 80.91 % and F2 = 19.09 %). The linear correlation revealed acceptable scores for the two defined discriminant factors (F1 and F2) (Figure 1). A significant differentiation of wines according to the year of wine production was carried out for wines samples, which demonstrates the importance of elemental profile for the geographical traceability of wines (Figure 2).

CONCLUSIONS

In this work the elemental composition and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio of white wines (Muscat Ottonel, Feteasca Alba, Feteasca Regala) and red wines (Feteasca Neagra, Merlot, Cabernet Sauvignon) was studied in order to highlight the elemental composition of wine samples.

Concentration of Cd, Pb, As and Cu heavy metals in analysed wine samples were under Maximum Limit Allowed (M.L.A.), respectively as published by the Organization of Vine and Wine. The content of potentially toxic elements such as Cd, Pb, U, Hg, As, Cu, Ni and Cr are lower than the recommended values found in literature, highlighting the safety and quality of the analyzed Romanian wines.

Our results confirm that the $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ and $^{87}Sr/^{86}Sr$ isotope ratio can be used to track the origin of wine, to discriminate between the wine produced in different years and can be used to characterize wine terroirs for forensic purpose. Based on the elemental profile and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio, a relevant discrimination of wines according to the production year was performed. The variation of $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio represents a strong geological marker for wines geographical traceability. The proposed methodology allowed successful classification of wines according to the region of provenance and also the production year.

EXPERIMENTAL SECTION

Study area

A total of 54 wine samples were analysed (3 white wines and 3 red wines). Samples originated from Dealu Bujorului vineyard (45°52′10″ N, 27°55′8″E). The Dealu Bujorului region is characterized by an alternate landscape, from flat to hilly areas, with altitude between 100 and 225 m and the predominant soil is levigated chernozem having a clayey sand texture with pH between values 7.4 and 8.1. Although they have moisture deficit, natural conditions (ecoclimatic and ecopedological) offer viable ecosystem for the development of vineyard. The vineyard is crossed by the parallel 46° latitude north, intersected by the 28° longitude meridian. Dealu Bujorului vineyard belongs to Galați country. The specificity of the transition area is highlighted by the predominance of deposits of clays and sands. Versants were made from clay deposits and sandy sands.

Sample collection and microvinification process

The samples used in this experiment were obtained from the wines produced from Muscat Ottonel, Feteasca Alba, Feteasca Regala, Feteasca neagra, Merlot and Cabernet Sauvignon under the conditions of 2011, 2012 and 2013. The wine samples resulted from micro-wine production. Micro-wine production was done according to the methodology describe by Bora et al. [12]. All wines were providing by the wineries as finished wines in 750 mL bottles with cork stoppers and were stored at 3-4°C before analysis. All vines were planted since 1979, and the vine plantation was organized with 2.2 x 1 m distance between rows and plants. Vines were pruned according to the Guyot system and were grown on speliers.

Reagents and solutions

Ten elements (Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr) were determined in order to assess their ability to discriminate wines by geographical origin. The analysis was made using multielement analysis and ICP-MS technique, after an appropriate dilution, using external standard calibration method. The calibration was performed using XXICertiPUR multielement standard, and from individual standard solution

of Cr and Hg. The working standards and the control sample were prepared daily from the intermediate standards that were prepared from the stock solution. The intermediate solutions stored in polyethylene bottles and glassware was cleaned by soaking in 10% v/v HNO_3 for 24 hours and rinsing at least ten rimes with ultrapure water (18.2 MΩ cm^{-1} ultrapure water-Types 1). The accuracy of the methods was evaluated by replicate analyses of fortified samples (10 µL-10 mL concentrations) and the obtained values ranged between 0.8-13.1 percent, depending on the element. The global recovery for each element was estimated and the obtained values were between 84.6-100.9% [48].

For quality control purpose, blanks and triplicates samples (n = 3) were analyzed during the procedure. The variation coefficient was under 5% and detection limits (ppb) were determined by the calibration curve method. Limit of detection (LoD) and Limit of quantification (LoQ) limits were calculated according to the next mathematical formulas: LoD = 3SD/s and LoQ = 10 SD/s (SD = estimation of the standard deviation of the regression line; s = slope of the calibration curve) (Table 3).

Table 3. Instrumental conditions for the determination of each element (ICP-MS technique)

Element	Correlation coefficient	LoD* (µg/L)	LoQ*** (µg/L)	BEC** (µg/L)
Cd	0.9999	0.0202	0.0673	0.027
U	0.9999	0.0253	0.0842	0.005
As	0.9999	0.2335	0.7776	0.538
Co	0.9999	0.0365	0.1215	0.152
Ni	0.9999	0.0591	0.1968	0.091
Pb	0.9999	0.0003	0.0010	0.002
Hg	0.9999	0.0417	0.1379	0.128
Sr	0.9999	0.1434	0.4775	0.955
Cu	0.9999	0.0402	0.1339	0.237
Cr	0.9999	1.6630	5.5378	0.636

*Detection limit; **Background equivalent concentration; ***Quantification limit.

For calibration and to verify the achieved accuracy and precision, ten NIST-SRM 987 and NIST-SRM 982 analysis results were pooled together with the calculated relative standard deviation presented in Table 4. Based on the obtained results, it was verified that, applying quadrupole ICP-MS, relative standard deviation and reproducibility of approximately 0.5% for $^{87}Sr/^{86}Sr$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ are feasible. The results were in agreement with those reported by [48, 50].

Table 4. Lead isotopic ration and Lead isotopic ration determination precision and accuracy based on the NIST SRM 982 (Lead) NIST SRM 987 (Strontium) (n=10)

Replicate	$^{207}Pb/^{206}Pb$ (a)	RSD (%)	$^{208}Pb/^{206}Pb$ (b)	RSD (%)	$^{204}Pb/^{206}Pb$ (c)	RSD (%)	$^{87}Sr/^{86}Sr$ (d)	RSD (%)
1	0.46483	0.51	0.99891	0.67	0.00271	0.32	0.70493	0.31
2	0.47891	0.48	0.99452	0.61	0.00272	0.41	0.72046	0.45
3	0.46978	0.32	0.99794	0.55	0.00275	0.28	0.70325	0.63
4	0.47123	0.64	0.99688	0.64	0.00273	0.51	0.70634	0.48
5	0.46987	0.56	0.99726	0.48	0.00246	0.14	0.71478	0.36
6	0.46154	0.37	0.99647	0.56	0.00258	0.39	0.71245	0.59
7	0.47362	0.70	0.99969	0.34	0.00279	0.47	0.70987	0.46
8	0.45641	0.43	0.99744	0.58	0.00278	0.51	0.72326	0.42
9	0.41562	0.36	0.99576	0.59	0.00273	0.49	0.70845	0.68
10	0.45612	0.45	0.99874	0.61	0.00278	0.36	0.10789	0.47
Average	0.46179	0.48	0.99736	0.56	0.00270	0.41	0.71117	0.49

[a]Certified value=$^{207}Pb/^{206}Pb$ (0.46707±0.00020); [b]Certified value=$^{208}Pb/^{206}Pb$ (1.00016±0.00036); [c]Certified value=$^{204}Pb/^{206}Pb$ (0.027219±0.00027); [d]Certified value=$^{87}Sr/^{86}Sr$ (0.71034±0.00026); RSD (%) = relative standard deviation.

Sample preparation for determination of heavy metals and isotopic ration from wine using ICP-MS.

For the determination of elements from wine samples were used an amount of 0.5 mL wine and adjust 8 mL (7 mL HNO_3 65%+1 mL H_2O_2) were placed in a clean Teflon digestion vessel, after 15-30 minutes the mineralization was performed using a microwave system Milestone START D Microwave Digestion System set in three steps: step I (time 10 min., temperature 200ºC), step II (time 15 min., temperature 200ºC) and step III (time 40 min., ventilation - temperature 32ºC). After mineralization, samples were filtered through a 0.45 mm filter and brought to a volume of 50 mL. The Pb and Sr isotope ration in the analysed wine samples ($^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$,) were determined according to the methodology indicated by Mihaljevič *et al.* [51]; Geana et al. [21]; Bora *et al.* [24].

In order to confirm the best-chosen conditions for wine digestion standard additions for checking accuracy of the microwave digestion and recoveries were calculated (Table 5). The digestion seemed visually completed in all of the combinations, but the spiked recoveries showed significant differences for total elements content (*p* - Value = *0.005*).

Table 5. Standard additions for checking accuracy of the microwave digestion ICP-MS method (n = 3)

Element	Certified Concentration (mg/L)	Measured Concentration (mg/L)
Cd	6.568±0.073	6.473±0.106
U	9.994±0.016	9.981±0.012
As	56.85±0.37	53.09±0.31
Co	27.06±0.28	24.13±0.06
Ni	62.41±0.69	61.32±0.21
Pb	19.63±0.21	19.13±0.09
Hg	0.1016±0.0017	0.1102±0.0012
Sr	314.00±19.00	314.09±09.06
Cu	21.44±0.70	21.25±0.21
Cr	18.32±0.10	19.18±0.21

Instrumentation

The determination of metals was performed on mass spectrometer with inductively coupled plasma, (ICP-MS) iCAP Q Thermo scientific model, based polyatomic species before they reach the quadrupole mass spectrometer, using a PFA micro flow concentric nebulizer. The argon used was of 99.99% purity (Messer, Austria). The instrument was daily optimized to give maximum sensitivity for M^+ ions and the double ionization and oxides monitored by the means of the rations between Ba^{2+}/Ba^+ and Ce^{2+}/CeO^+, respectively, these always being less than 2%. The experimental conditions were: argon flow on nebulizer (0.82 L/min.), auxiliary gas flow 0.80 L/min., argon flow in plasma 15 L/min., lens voltage 7.30 V; RF power in plasma 1100 W, spray chamber temperature ($2.50±1.00^{\circ}C$). Accuracy was calculated for the elements taken into consideration (0.5-5.0%).

Statistical analysis

The statistical interpretation of the results was performed using the Duncan test, SPSS Version 24 (SPSS Inc., Chicago, IL., USA). The statistical processing of the results was primarily performed in order to calculate the following statistical parameters: average and standard deviation. This data was interpreted with the analysis of variance (ANOVA) and the average separation was performed with the DUNCAN test at $p \leq 0.05$. Multivariate chemometric method was used as a supervised learning technique for the differentiation of wines intro groups on the basis of grape variety and year of production and finding markers which showed a

significant discrimination value (variables with Wilk's lambda near zero, p value <0.05 and higher F coefficients). Stepwise linear discriminant analysis (LDA) was used to identify significant tracers for classification to the geographical discrimination of the wines samples. Stepwise Discriminant Analysis (LDA) was used to designate suitable variables for classification of the samples, eliminating the variables that do not contribute to discrimination of the wine. In order to validate the proposed statistic model, based on variables which showed higher significance in first LDA assessment, we performed a second Linear Discriminant Analysis (LDA) for the test set consisting of wines used to build statistical model (training set) together with data from other wine samples that are not included in the first LDA (control-set). Cross-validation was applied to determine the optimal number of variables required to obtain robust models. Linear discriminant analysis (LDA) was performed using Microsoft Excel 2016 and XLSTAT Addinsoft version 15.5.03.3707.

ACKNOWLEDGMENTS

REFERENCES

1.   OIV, *International Code of Oenological Practices, Annex: Maximum Acceptable Limits*, **2015,** Issue 2015/01. Paris, France.
2.   F. Stoica, *Carpathian Journal of Food Science and Technology*, **2015,** *7(4)*, 139-144.
3.   G.L. La Torre, R. Rando, M. Saitta, M. Alfa, R. Malsano, G. Dugo, *Italian Journal of Food Science*, **2010**,*1*(22), 28-40.
4.   Y. Vystavna, L. Zaichenko, L. Klimenko, R. Ratsep, *Journal of Science Food and Agriculture*, **2017,** *97*, 4520–4525.
5.   J. Halili, K. Bislimi, I. Mazreku, A. Behluli, F. Osmani, A. Maloku, F. Halili, *13th SGEM GeoConference on Ecology, Economics, Education and Legislation, Conference Proceedings*, **2013**, *1*, 531– 538.

6. M.D. Blackhurst, A.D. Marais, *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, **2009**, *44*(2), 77-79.
7. I.M. Alkis, S. Oz, A. Akatol, N. Yilmaz, R. E. Anli, O. Akatol, *Journal of Food Compostion and Analysis*, **2014**, *33*, 105-110.
8. S. Đurđic, M. Pantelic, J. Trifkovic, V. Vukojevic, M. Natic, ˇZ. Tesica, J. Mutic, *RSC Advances*, **2017,** *7*, 2151-2162.
9. I. Geană, A. Iordache, R. Ionete, A. Marinescu, A. Ranca, M. Culea, *Food Chemistry*, **2013**, *138*, 1125-1134.
10. M. A. I. Chukwujindu, A. L. Ojelum, F. I. Bassey, *Food Science and Nutrition*, **2014**, *2(6):* 724–733.
11. K. Pyrzynska, *Critical Reviews in Analytical Chemistry,* **2004***, 34,* 69-83*.*
12. F. D. Bora, A. Donici, C. Voica, T. Rusu, L. Mihaly-Cozmuța, A. Mihaly-Cozmuța, C. Cimpoiu, D. E. Mihăiescu*, Advances in Agriculture and Botanics - International Journal of the Bioflux Society,* **2016***, 8(3),* 129-142*.*
13. K. M. Towle, L. C. Garnick, A. D. Monnot, *International Journal of Food Contamination,* **2017***, 4*:7.
14. P. Hajeb, J. J. Sloth, S. Shakibazadeh, N. A. Mahyudin, L. Afsah-Hejri, *Comprehensive Reviews in Food Science and Food Safety*, **2014**, *13,* 457-472.
15. J. Cacho, J. E. Castells, A. Esteban, B. Laguna, N. Sagrista, *American Journal of Enology and Viticulture,* **1995**, *46*, 381-384.
16. K. A. Riganakos, P.G. Veltsistas, *Food Chemistry*, **2003**, *82*, 637-643.
17. P. Bentez, R. Castro, J.A. S. Pazo, C.G. Barroso, *Food Research International,* **2002**, *35*, 785-791.
18. A.D. Monnot, B.E. Tvermoes, R. Gerads, H. Gürleyük, D. Paustenbach, *Food Chemistry,* **2016**, *211*, 107-113.
19. S. Ražić, A. Onjia, *American Journal of Enology and Viticulture*, **2010**, *61*(4), 506-511.
20. V. Avram, C. Voica, A. Hosu, C. Cimpoiu, C. Măruțoiu, *Revue Roumaine de Chimie*, **2014**, *59*(11-12), 1009-1019.
21. L. Järup, *British Medical Bulletin*, **2003**, *68* (1), 167-182.
22. V. Orescanin, A. Katunar, A. Kutle, V. Valkovic, *Journal of Trace and Microprobe Tehniques Trace Bioelements*, **2003**, *21*(1), 171–180.
23. S. Catarino, M. Madeira, F. Monteiro, F. Rocha, A. S. Curvelo-Garcia, R. Bruno De Sousa, *Journal of Agricultural and Food Chemistry*, **2008**, *56*, 158-165.
24. F. D. Bora, A. Donici, T. Rusu, A. Bunea, D. Popescu, C. I. Bunea, *Notulae Botanicae Horti Agrobotanicae,* **2018**, *46(1)*, 223-239.
25. B. Médina, S. Augagneur, M. Barbaste, F.E. Grousset, P.l. Buat-Ménard, *Food Additives and Contaminants*, **2000**, *17(6),* 435-445.
26. R. Larcher, G. Nicolini, P. Pangrazzi, *Journal of Agricultural and Food Chemistry*, **2003**, *51(20)*, 5956-5961.
27. M. Mihaljevic, V. Ettler, O. Sebek, L. Strnad, V. Chrastny, *Journal of Geochemistry Exploration*, **2006**, *88*, 130-133.
28. B.L. Gulson, T.H. Lee, K.J. Mizon, M.J. Korsch, H.R. Eschnauer, *American Journal of Enology and Viticulture*, **1992**, *43*, 180-190.

29. C.M.R. Almeida, M.T.S.D. Vasconcelors, *Journal of Agricultural and Food Chemistry*, **2003**, *51*, 4788-4798.
30. C.M.R. Almeida, M.T.S.D. Vasconcelors, *Food Chemistry*, **2004**, *85*, 7-12.
31. F.D. Bora, A. Donici, A. Călugăr, I.V. Petrescu Mag, E. Gál, C.I. Bunea, *Studia UBB Chemia*, **2017**, LXII, 4, Tom II, 317-332.
32. S. Marchionni, A. Buccianti, A. Bollati, E. Braschi, F. Cifelli, P. Molin, M, Paratto, M. Mattei, S. Tommasini, S. Conticelli, *Food Chemistry*, **2016**, *190*, 777-785.
33. I. Tescione, S. Marchionni, M. Mattei, F. Tassi, C. Romano, S. Conticelli, *Procedia Earth Planetary Science*, **2015**, *13*, 169-172.
34. E. Tatár, V. G. Mihucz, I. Virág, L. Rácz, G. Záray, *Microchemistry Journal*, **2007**, *85*, 132-135.
35. A. Deheleanu, C. Voica, *Romanian Journal of Physics*, **2012**, *57(7-8),* 1194-1203.
36. C. M. S. Almeida, A. C. Almeida, M. L. D. P. Godoy, T. D. Saint'Pierre, J. M. Godoy, *Journal of Brazilian Chemistry Society*, **2016**, *27(6)*, 1026-1031.
37. V. Ivanova-Petropulos, H. Wiltsche, T. Stafilov, M. Stefova, H. Motter, E. Lankmayr, *Macedonian Journal of Chemistry and Chemistry Engineering*, **2013**, *32(2),* 265-281.
38. V. Ivanova-Petropulos, B. Balavanova, S. Mitrev, D. Nedelkovski, V. Dimovska, R. Gulaboski, *Food Analytical Methods,* **2016**, *9(1)*, 48-60.
39. F. Galgano, F. Favati, M. Caruso, T. Scarpa, A. Palma, *Food Science and Technology,* **2008**, *41*, 1808-1815.
40. D. Karataş, F. Aydin, I. Aydin, H. Karataș, *Czech Journal of Food Science*, **2015**, *33(3)*, 228-236.
41. E. I. Geana, A. Marinescu, A. M. Iordache, C. Sandru, R. E. Ionete, C. Bala, *Food Analytical Methods*, **2014**, *48(2)*, 2064-2074.
42. D. Schiavo, I.Y. Neira, I.A. Nóbrega, *Talanta*, **2008**, *76*, 1113-1118.
43. Z. Ajtony, N. Szoboszlai, E.K. Suskó, P. Mezei, K. György, L. Bencs, *Talanta*, **2008**, *76*, 627-634.
44. G. Dugo, L. La Pera, T.M Pellicanó, G. Di Bella, M. D'Imperio, *Food Chemistry*, **2005**, *91*, 355-363.
45. S. Galani-Nikolakaki, N. Kallithrakas-Kontos, A.A. Katsanos, *Science of the Total Environment*, **2002**, *285*, 155-163.
46. M. Barbaste, L. Halicz, A. Galy, B. Medina, H. Emteborg, F. C. Adams, R. Lobinski, *Talanta*, **2001**, *54,* 307-317.
47. B.L. Gulson, T.H. Lee, K.J. Mizon, M.J. Korsch, H.R. Eschnauer, *American Journal of Enology and Viticulture*, **1992**, *43*, 180-190.
48. E.I. Geană, C. Sandru, V. Stanciu, R.E. Ionete, *Food Analytical Methods*, **2017**, *10(1)*, 63-73.
49. OIV. Maximum acceptable limits of various substances contained in wine. In: OIV Editor. *Compendium of international methods of analysis of wine and must analysis*. Place: Paris (Pa); **2016**.
50. M. Barbaste, K. Robinson, S. Guilfoyle, B. Medina, R. Lobinsky, *Journal of Analytical Atomic Spectrometry*, **2002**, *17*, 135-137.