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Measurements of trace elements in must and wine using FAAS technique

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Abstract. An actual concern in wine production technology is the unwanted presence of heavy metals in both the stum and wine. Besides the quality issues that these heavy metals create in the wine, they also affect the health of wine consumers. Because of this situation it's necessary to improve the knowledge of the negative influence of heavy metals on wine production technologies and on human health. In this paper we wish to asses the concentration of heavy metals from samples of stum and wine, to get a wider picture on the effects of heavy metals on wine quality. Based on the presented results that the wine made in the year 2014 from the point of view of the analyzed heavy metals (Cu, Pb, Zn, Cd, Ni), the concentrations are way below the national and international legislation, an exception from this rule is the wine processed from the Fetească Regală (5.69 ± 0.46 mg Zn/L) which topped the maximal admitted limit of (5 mg/L).
Key Words: Stum, Cu, Pb, Zn, Cd, Ni, heavy metals, *Vitis vinifera* L.

Rezumat. Un subiect la oridinea zile în tehnologia de producere a vinului, este prezența nedorită a metalelor grele atât în must cât și în vin. Pe lângă defectele pe care le provoacă aceste metale grele vinului din punct de vedere calitativ, ele pun în pericol în mod direct sănătatea consumatorilor. Astfel este necesar să se înbunătățească cunoștiințele despre efectele negative ale metalelor grele asupra tehnologiei de producere a vinului și implicit asupra sănătății umane. În lucrarea de față, ne propunem evalurea concentraților unor metele grele din probe de must și vin, pentru a se obține o imagine cât mai amplă despre aceste metale și efectele lor asupra calitații vinului. Pe baza rezultatelor prezentate se poate afirma că vinul produs în anul 2014 este sigur din punct de vedere al concentraților metalelor grele analizate (Cu, Pb, Zn, Cd, Ni), aceste concentrați sunt cu mult sub limitele stabilite prin legislația națională și internațională, excepție de la această regulă face vinul obținut din soiul Fetească regală (5.69 ± 0.46 mg nZ/L) care a depășit limita maximă adimisă (5 mg/L).
Cuvinte cheie: Must, vin, trasabilitate, metale grele, *Vitis vinifera* L.

Introduction. Vines are grown for commercial use in about 50 countries with an area of about 7.51 million hectares in 2013, of which more than 60% in Europe, followed by Asia, Africa, South America, North America and Australia (Lung 2012). In 2013, Romania recorded a decrease of the total vines plantation, from 198 ha, which is the average of 2008-2009 to 178 ha in 2013 (Lădaru et al 2014).

The wine sector is an economic and strategic sector with major importance (Iațișin et al 2014) in the countries with important areas under vines (Lădaru et al 2014). Romania is considered a tradition wine producing country, he become member of the International Office of Wine and Vine since 1928 (Lădaru et al 2014).

Daily consumption, wine contributes to the requirements of essential elements, such as Ca, Fe, Mn, Mo, Co, Cr, K, Ni, Se and Zn for humans. However, the presence of significant amount of heavy metal in wine may harm the health of consumers (Dalipi et al 2015; Oroian et al 2012). For example, the application of fungicides and pesticides to vines during their growth may lead to increase in the amount of Pb, Cd and Cu in the vine or log contact of wine with the materials used to build pipes, barrels may also lead to contamination by Cd, Al, Cr, and Fe (Țârdea 2007).

Wine is a complex matrix, which beside sugar, water, alcohol, saccharides, amino acids, phenolic compounds, and other pigments contains a great variety of components, inorganic as well as organic (Voica et al 2009; Karataș et al 2015; Monaci et al 2003; Katalinic et al 2004; Roig & Thomas 2003; Nilsson et al 2004). From a chemical point of view, the wine is a complex water and ethanol mixture that contains both organic and inorganic substances (Dalipi et al 2015). The composition of wine is influenced by many factors related to the specific production as: soil and climate, grape varieties, culture, winemaking, transport and storage (Sperkova & Suchanek 2005; Catarino et al 2006; Fernandez 1988; Marini et al 2006; Núñez et al 2000). The metal content in wine is important due to their effect on the health of consumers and on the wine quality (Banović et al 2009; Karataș et al 2015). The determination of some elements is of interest for characterizing wine sample, identifying the wine origin, assessing the nutritional safety of the product, due to their toxicological, physiological properties (Fabani et al 2010; Grindlay et al 2008; Gonzalves et al 2009; Álvarez et al 2007).

There are many various reasons for which the concentrations of some major and trace elements in wines are further monitored. Some of this elements are related to the adverse influence to human health such as As, Hg, Pb, and Cd, known to be potentially toxic; others to the effects that these elements may have on the organoleptic proprieties of wines and to their ability to discriminate wines according to the geographical region in which grapes were grown, as well as to detect wine adulteration (Geana et al 2013; Lara et al 2005). It should be noted that the levels of contaminant elements, such as Cu, Zn, As, Pb and Cd at different stages of the winemaking process are of great concern because of legal requirements (O.I.V. 2005).

A number of papers have been published using FAAS methods to determine patterns of mineral and trace element in vine (Geana et al 2014; Santos et al 2009; Lemos et al 2002; Woldemariam & Chandravanshi 2011; Schiavo et al 2008; Bora et al 2015).

Only a few elements found in wine sample are directly influenced by the soil chemical composition of the vineyard of provenance and the plant uptake of elements, thus directly relating the element fingerprint to the wine origin. There are several steps of wine production such as precipitation during fermentation or filtering process, this can change elements contents in wine significantly (Thiel et al 2004).

The aim of this study was the determination of heavy metals in must, wine and reporting the results to the values allowed by Romania low. In this work we evaluated 5 elements: Cu, Pb, Zn, Cd and Ni in eighth grapevine cultivars: Pinot Gris, Rhine Riesling, Furmint, Mustoasă de Măderat, Traminer, Fetească regală, Italian Riesling and Fetească regală. Determination of heavy metal concentrations has been performed by flame atomic absorption spectrophotometry (FAAS).

Material and Method

Study area. The study area Șimleul Silvaniei is located in 47°13′48″ north, 22°48′0″ east in Salaj county, Romania. The area under study is part of the vineyard Silvaniei, and by its position is the most northern vineyard from Romania, like vineyard Cotnari, which is distinguished by the wide open air masses western and northwest which gives bio-pedo-climatic particularities of Central European type. The study area is 23 ha, and all vines were planted since 2000, and the vine plantation was organized with 2.2 x 0.9 m distances between rows and plants.

Sampling and samples preparation. Samples were collected in an average of 5 kg of grapes for each cultivar from 15-20 vines. The collected samples were placed in the lower third, middle and top of each vine and grapes expose to the shade and sun, in order to achieve better homogenization of the sample was harvest, as following: Pinot Gris (3 samples), Rhine Riesling (3 samples), Furmint (3 samples), Mustoasă de Măderat (3 samples), Traminer (3 samples), Fetească Regală (3 samples), Italian Riesling (3 samples) and Fetească Regală (3 samples). Must samples were obtained by pressing the grapes sample using manual press. In the first round each must sample (50 mL) was diluted in different proportions using ultra distilled water (Milli-Q Integral ultrapure water - Type 1).

Reagents and solutions. The must and wine were analyzed by FAAS (Perkin Elmer AAnalyst 800, Shelton, USA). FAAS is the official method of analysis for determination of trace elements with relatively high concentrations according to EU regulations. All reagents and solutions were used of analytical grade (Merck, Germany). Standard solutions were prepared every 7 days or whenever an error is suspected due to these solutions. There were used only standard solutions (Merck) at a concentration of 1000 mg/L for every assessed mineral element. All solutions were stored in polyethylene bottles, glassware was cleaned by soaking in 10% v/v HNO_3 for 24 hours and rinsing at least three times with ultra-pure water. For quality control were analyzed blanks and triplicates samples (n=3) during the procedure. The variation coefficients was under 10% and detection limits (mg/L) was determined by the calibration curve method. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the next mathematical formulas: LOD = 3 SD/s and LOQ = 10 SD/s (s - slope of the calibration curve, SD - the estimations of the standard deviations of the regression line). The results obtained are presented in Table 1.

Table 1

Instrumental conditions for the determination of each element

Element	*Correlation coefficient*	*Wave length (nm)*	*Slit (nm)*	*Flame*	*Background correction*	*LOD* (mg/L)*	*LOQ** (mg/L)*
Cu	0.999997	324.8	0.7	Air-acetylene	Deuterium	0.019	0.063
Pb	0.999993	283.3	0.2	Air-acetylene	Deuterium	0.053	0.017
Zn	0.999998	213.9	0.7	Air-acetylene	Deuterium	0.016	0.053
Cd	0.999999	228.8	0.7	Air-acetylene	Deuterium	0.028	0.093
Ni	1.000000	232.0	0.2	Air-acetylene	Deuterium	0.021	0.070

Statistical analysis. The data were expressed as mean ± standard deviation (SD) of three replications for each sample. In order to determine the significant differences among values, analysis of variance were employed (ANOVA). Significance of difference was defined at the 5% level ($p<0.05$).

Results and Discussion. Vine through its nutrition accumulates small copper quantities, with values between 0.2-0.4 mg/L stum. Through the presence of cupric fungicide residues which remain on the surface of grapes, enrich the stum with external copper source, so the copper values can reach 5-10 mg/L stum, sometimes even more (Ţârdea 2007).

The domain of Cu variation in the stum sample was between 7.48 ± 0.89 mg/L, rcorded from the stum of the Rhine Riesling variety, followed by the stum of Pinot Gris variety (5.31 ± 1.02 mg/L) and (Fetească Regală 4.51 ±0.75 mg/L). At the opposite pole, the smallest values of Cu concentration in the stum sample were found in the stum of the Italian Riesling variety (1.97±0.78 mg/L), followed by the Mustoasă de Măderat variety (2.37 ± 0.34 mg/L). The differences between variants were statistically ensured ($F = 11.118$, $p<0.000$). The medium Cu content in stum regardeless of stum variety analyzed was found 4.29 ± 0.87 mg/L. The results regarding the stum Cu content of the varieties taken into consideration, we can observe that all of them are under the legal limit (10 mg/L stum), the highest value was recorded at the stum of the Rhine Riesling variety (7.48 ± 0.89 mg/L), but this value is also way under the admitted values.

Table 2

The content of heavy metals in must from the studied area (mg/L)

Variety	*Location*	*Cu* *MLA* *10 mg/L*	*Pb* *MLA* *0.5 mg/L*	*Zn* *MLA* *10 mg/L*	*Cd* *MLA* *0.1 mg/L*	*Ni* *MLA* *-*
Pinot Gris	Şimleul Silvaniei	5.31 ± 1.02[b]	0.12 ± 0.03[cd]	4.81 ± 0.58[b]	ULD	0.31±0.03[a]
Rhine Riesling		7.48 ± 0.89[a]	0.15 ± 0.02[bc]	2.68 ± 0.55[cd]	ULD	0.31±0.05[a]
Furmint		5.06 ± 1.28[b]	0.20 ± 0.02[b]	7.05 ± 0.14[a]	ULD	0.22±0.03[bc]
Mustoasă de Măderat		2.37 ± 0.34[cd]	ULD	7.34 ± 0.96[a]	ULD	0.17±0.04[cd]
Traminer		3.97 ± 0.71[bc]	0.09 ± 0.02[d]	7.38 ± 0.32[a]	ULD	ULD
Fetească Regală		4.51 ± 0.75[b]	0.19 ± 0.01[ab]	2.70 ± 1.66[cd]	ULD	0.25±0.06[ab]
Italian Riesling		1.97 ± 0.78[d]	0.24 ± 0.08[a]	3.27 ± 1.16[c]	ULD	0.29±0.04[a]
Fetească Albă		3.62 ± 1.17[bcd]	0.11 ± 0.04[d]	1.42 ± 0.29[d]	ULD	0.13±0.01[d]
Average		4.29 ± 0.87	0.14 ± 0.02	4.58 ± 0.71	-	0.21±0.03
F (Fisher factor)		11.118	29.240	23.667	-	25.471
Statistical significance		*p<0.000*	*p<0.000*	*p<0.000*	-	*p<0.000*

Average value ± standard deviation (n=3). Different letters are significantly different for P≤0.05 between varieties. The difference between any two values, followed by at least one common letter, is insignificant. ULD - Under the limit of detection, MLA - Maximum limit allowed.

The domain of variation for the Pb concentration in grape juice (must) samples was between the maximal value 0.24±0.08 mg/L recorded in the must of the Italian Riesling variety, followed by Furmint variety 0.20±0.02 mg/L, and the minimum value 0.12 ± 0.03 mg/L recorded in the must of the Pinot Gris, followed by the Traminer variety 0.09 ± 0.02 mg/L. For the Mustoasă de Măderat variety the concentration of this heavy metal is under the detection limit of the device and of the analysis method used. The obtained results conclude that the heavy metal concentrations are below the accepted limits (0.5 mg/L must) for the stum of all the varieties taken into consideration. The differences between variants were statistically ensured ($F = 29.240$, $p<0.000$). The average value of Pb in the stum samples regardless the analyzed wine variety was found 0.14 ± 0.02 mg/L stum.

As for the concentration of Zn in the stum samples, the largest concentrations were found in the following varieties: Furmint (7.05 ± 0.14 mg/L), Mustoasă de Măderat (7.34 ± 0.96 mg/L) and Traminer (7.38 ± 0.32 mg/L), which are statistically equal (Table 2). Smaller values of Zn for the stum samples were found in the varieties Fetească Albă (1.42 ± 0.29 mg/L), followed by Fetească Regală (2.70 ± 1.66 mg/L). The average concentration of Zn in the stum samples was 4.58 ± 0.71 mg/L. The results show that these values are below the accepted limits (10 mg/L stum) for the stum of the varieties taken into consideration. The differences between variants were statistically ensured ($F = 23.667$, $p<0.000$) (Table 2).

The concentration of Ni from the analyzed stum samples ranged between wide values. The highest values were found in the stum of the Pinot Gris (0.31 ± 0.03 mg/L), Rhine Riesling (0.31 ± 0.05 mg/L) and Italian Riesling variety (0.29 ± 0.04 mg/L), these values are statistically equal. The lowest value of Ni concentration from the stum samples was found in the Fetească Albă variety (0.13 ± 0.01 mg/L), and the Traminer variety has the Ni concentration under the accepted limits. The average value of Ni in the stum samples was 0.21 ± 0.03 mg/L. For the concentration of Ni in must are not established maximal admitted values. The differences between variants were statistically ensured ($F = 25.471$, $p<0.000$).

As for the concentration of Cd from the stum samples, as it can be seen (Table 2), the concentration of this heavy metal is under the detection limit of the device and the analysis method used.

The variation domain for the concentration of Cu from the wine samples was between the maximal value of 0.54 ± 0.17 mg/L measured at the Fetească Regală, followed by the Rhine Riesling (0.16 ± 0.05 mg/L), and the lowest value was registered by Furmint (0.19 ± 0.02 mg/L), Traminer (0.29 ± 0.02 mg/L), and Italian Riesling variety (0.25 ± 0.17 mg/L), which are statistically equal (Table 3). While the Mustoasă de Măderat variety had the lowest values for the Cu concentration (0.13 ± 0.02 mg/L). The differences between variants were statistically ensured ($F = 218.358$, $p=0.001$). The average concentration of Cu regardless of the analyzed variety was 0.28 ± 0.06 mg/L, which is a low concentration, that doesn't endanger the health of wine consumers.

This concentration of Cu found in the wine sample can be explained as being caused by the usage off phyto-sanitary products based on Cu, so that this heavy metal is also found in wine, in large concentrations.

As for the Pb concentration found in the wine samples, from the data presented in Table 3 it can be seen that there are no statistical difference between variants ($F = 2.315$, $p=0.078$), in this case the significance ($p=0.078$) is much over the analyzed statistical threshold ($p\leq 0.05$).

Reporting the average concentration of Pb from the wine samples (0.14 ± 0.03 mg/L) at the maximal limit allowed (0.2 mg/L) it can be seen that the found value is much lower then this limit (Table 3).

Table 3

The content of heavy metals in wine from area studied (mg/L)

Variety	*Location*	*Cu* *MLA* *1 mg/L*	*Pb* *MLA* *0.2 mg/L*	*Zn* *MLA* *5 mg/L*	*Cd* *MLA* *0.1 mg/L*	*Ni* *MLA* *-*
Pinot Gris	Șimleul Silvaniei	0.32 ± 0.03[b]	0.13 ± 0.04[a]	2.29 ± 0.96[b]	ULD	ULD
Rhine Riesling		0.16 ± 0.05[bc]	0.08 ± 0.03[a]	3.94 ± 0.39[ab]	ULD	ULD
Furmint		0.19 ± 0.02[bc]	0.22 ± 0.10[a]	2.66 ± 1.01[b]	ULD	ULD
Mustoasă de Măderat		0.13 ± 0.02[c]	0.08 ± 0.04[a]	4.21 ± 0.91[ab]	ULD	ULD
Traminer		0.29 ± 0.02[bc]	0.15 ± 0.09[a]	3.90 ± 1.28[ab]	ULD	ULD
Fetească Regală		0.54 ± 0.17[a]	0.10 ± 0.04[a]	5.69 ± 0.46[a]	ULD	ULD
Italian Riesling		0.25 ± 0.17[bc]	0.17 ± 0.01[a]	4.28 ± 1.95[ab]	ULD	ULD
Fetească Albă		0.32 ± 0.06[b]	0.15 ± 0.03[a]	3.28 ± 0.78[b]	ULD	ULD
Average		0.28 ± 0.06	0.14 ± 0.03	3.78 ± 0.49	-	-
F (Fisher factor)		218.358	2.315	2.935	-	-
Statistical significance		*p=0.001*	*p=0.078*	*p=0.035*	-	-

Average value ± standard deviation (n=3). Different letters are significantly different for P ≤ 0.05 between varieties. The difference between any two values, followed by at least one common letter, is insignificant. MLA - Maximum limit allowed. ULD - Under the limit of detection.

The concentration of Zn in the wine samples varied between large values. The highest value was found in the wine produced by the Fetească Regală (5.69 ± 0.46 mg/L), followed by the Riesling de Rhin (3.94 ± 0.39 mg/L), Mustoasă de Măderat (4.21 ± 0.91 mg/L), Traminer (3.90 ± 1.28 mg/L), and Italian Riesling (4.28 ± 1.95 mg/L). The smallest value of Zn concentration from the wine sample was found in the varieties: Pinot Gris (2.29 ± 0.96 mg/L), Furmint (2.66 ± 1.01 mg/L) and Fetească Albă (3.28 ± 0.78 mg/L), values that are statistically equal.

The average Zn value in the wine samples was 3.78 ± 0.49 mg/L, comparing this value with the legal limit (5 mg/L), we find that it is way below the maximum value. The differenced between the variants were statistically ensured (F = 2.935, p=0.035).

Regarding the Cd and Ni heavy metals concentrations in the wine samples, these were found to be under the detection limit of the device and the analysis method used.

Conclusions. Based on the presented results we can say that the wine made in 2014 through the microvinification process is safe to use from the point of view of heavy metals traces (Cu, Pb, Zn, Cd, Ni), these concentrations are way below of the national and international legal limits, a single exception was evidenced at the Zn concentration by the wine obtained from the Fetească Regală variety (5.69 ± 0.46 mg/L) which exceeded the maximum admitted limit (5 mg/L).

A very important aspect of the wines obtained through the microvinification process, is that they can contain heavy metals in higher quantities than the wine obtained through regular processes. The wine obtained through microvinification is a wine that skipped some essential stages in the wine making process, as: bentoniztion, sulfitation, pre-filtering and filtering. All these stages which are used in the industrial wine making process enable us to reduce and even cut to half the concentration of heavy metals. So that the wine obtained from Fetească Regală variety with a Zn concentration of 5.69 ± 0.46 mg/L was over the maximum limit of 5 mg/L, but using pre-filtration, bentonization the value can reach a normal level, so that it can be consumed without concerns.

As for the concentrations of heavy metals found in the stum, comparing them to those found in the wine, based on the results we can say that these concentrations are halved, a possible explanation for the reduction of heavy metals could be the fermentation process which the stum is undertaking. After the fermentation process with the yeast of the wine some heavy metals are also eliminated and are transformed into poorly soluble compounds.

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