Assessment of Trace Elements and Human Health Risk Present in Wines Obtained in the Region of Dealurile Munteniei and Dealurile Moldovei Vineyards

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Abstract

The primary objectives of this research were to determine the concentration of some inorganic trace components, to evaluate the concentration levels of these in Romanian wines by daily consumption of wine and the health risk of inorganic trace components intake at these rates from Romanian wine. The results indicated that the concentration levels of inorganic components in red wine decreased in the order Zn>Mn>Cr>Cu>Ni>Pb>Co>Cd, in case of white wine decreased in the order Zn>Mn>Cu>Cr>Ni>Pb>Co>Cd. Based on a 60-kilogram adult person, and moderate consumption of 200 milliliters of wine/day, the estimated daily intake of these inorganic components from wines was well below the daily allowance. The target hazard quotient suggests that the exposed humans would not experience significant health risks when ingesting these individual elements from daily consumption of 200 milliliters of Romanian wines/day.

Keywords: estimated daily intake, health risk assessment, ICP-MS, multi-element analysis, Romanian wine

Introduction

Without doubt, the determination of metals in different type of food samples is of high importance for several reasons, the most important one being the nutritional, as well as toxic effects of these elements or their compounds (Płotka-Wasyłka *et al.*, 2018). Knowledge of the content of metals in alcoholic drinks such as wine is also very important. In many countries, alcoholic beverages, including wine, constitute more than 12 % of the general consumption of beverages (Goldberg and Bromberg, 1996). Therefore, they

could be an important source of several metal ions. Monitoring of certain elements in wines by oenological laboratories needs special attention due to their toxic effect on the human body, some metals must be kept under control according to the International Organization of Vine and Wine (O.I.V.) regulations.

The mineral concentration of wine is influenced by many factors. The majority part of the total metal concentration from wine is of natural origin (Kunkee and Eschnauer, 2003, Kment *et al.*, 2005, Pohl, 2007) and reflects the geochemistry

of vineyard soil on which vines are grown. The concentrations of these metals are characteristic and influenced by the type of soil, maturity of the grapes, their variety, and the climatic conditions during their growth (Pohl, 2007). The contribution of metals of a secondary origin is associated with an external concentration on the vine during growth or during the different stages in winemaking, from harvesting to bottling and cellaring (Fiket *et al.*, 2011). During the growth of grapes in a vineyard, contaminations can be classified as geogenic (originating in the vineyard soil), originating from fertilizing and plant protection practices, or from environmental pollution (Pohl, 2007). Application of fungicides, pesticides and fertilizers containing Pb, Cd, As, Mn, Cu and Zn compounds leads to accumulation of these elements in vineyard soil, grapes and wine (Galani-Nikolakaki *et al.*, 2002, Lara *et al.*, 2005, Cvetković *et al.*, 2006, Álvarez *et al.*, 2007). Finally, there is an enological source of metals, as contamination may occur at different steps of wine production. The reason for this is the long contact of wine with materials from which winemaking machinery, pipes, barrels and casks used for handling and storing of wine are made from aluminum, stainless steel, wood, brass and glass. This is the usual source of Fe, Cu, Cr, Cd, Al and Zn (Kment *et al.*, 2005, Lara *et al.*, 2005, Pohl, 2007).

Also, we must monitor permanently the metal content in wine for authenticity purposes as well as quality control (Fabani *et al.*, 2010, Kostić *et al.*, 2010). The elements can influence the winemaking process or change its taste and quality. A typical example can be Fe, which may have a strong influence on wine evolution during the fermentation process. In fact, the importance of its impact may vary due to several reasons, e.g. the coloring agents absence/presence, the ventilation of the ferments level, the extent to which this element interacts with the yeast, and the aliquots father which at the end of the process it is eliminated along with sediments (Płotka-Wasyłka *et al.*, 2018). Cu is also an important micronutrient, but at the same time, when consumed in excess, it is potentially toxic for humans (Pyrzyńska, 2007). Several elements, including Cu, Fe, Al, and Zn, contribute to haze formation and taste effects (Riganakos and Veltsistas, 2003). Monitoring and determination of other elements, e.g. Pb, As and Cd is also of high importance due to their potential

toxic effects on the human body (Elci *et al.*, 2009, Monasterio and Wuilloud, 2009). Besides, some metals content can be applied to identify its origin due to the direct relationship with soil composition (Greenough *et al.*, 2005).

Although moderate consumption of wine has proven to be very useful for human health, through the intake of a multitude of nutrients, as vitamins, mineral elements, amino acids, and polyphenols (Goldberg *et al.*, 2001, Martin *et al.*, 2017). There is an acute lack of assessment of the dangers of wine safety hazards concerning alcohol constraints. Around the world, but also in Romania, food security has always been an important point for the government. Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) reports the tolerance limits of certain pollutants for human consumption. Pb is a prevalent toxic component that impacts humans hematological, nervous, and reproductive systems, causing pathological alterations in organs, resulting in a decreased intelligent quotient (IQ) for children (Needleman, 2004). Cd is not an essential component for the human body, it is toxic to kidneys, bones and cardiovascular system also (Fang *et al.*, 2014) and the provisional tolerable monthly intake (PTMI) is 25.5 µg/kg body weight of Cd (FAO/WHO, 2011). Cr is an essential structure of the human body, depending on its valence state, it can also harm the human body. Trivalent Cr (Cr (III)) is a useful factor, but the toxicity of hexavalent Cr (Cr(VI)) is very toxic to the human body (Tuzen, 2009). Ni is not an essential component for the human body, it can cause negative lung impacts, while carbonyl nickel has acute toxicity and carcinogenicity (Forti *et al.*, 2011) and the provisional tolerable daily intake (PTDI) of Ni-based on lowest observed adverse effect level (LOAEL) is 12.00 µg/kg body weight (WHO, 2005).

The contaminants presented above, especially inorganic trace components, were also determined in previous researches on wine samples (Koreňovká and Suhaj, 2005). Although poisoning induced by consumed wine was not confirmed, these contaminants are harmful to the human body due to their chronic and long-lasting effects. Besides, some useful minerals from wine such as Mg, Co, Cu, Zn, Al, and Se can cause negative reactions if their consumption exceeds certain concentrations.

For example, Mo is a useful element for the human body, but the high levels of concentration in this mineral have a serious negative effect on the seminal material quality (Meeker *et al.*, 2008). The provisional maximum tolerable daily intake of Zn is 1.00 mg/kg body weight (FAO/WHO, 1982) and the PTWI of Al is 1000 µg/kg body weight (FAO/WHO, 2007). Compared to other ways, such as dermal contact, the consumption of some drinks or food, including inhalation, is a major source of human exposure of trace elements (Zhi-Hao *et al.*, 2019), consideration should be given to the intake of these minerals through regular consumption of wine.

To this date, only several papers were published on trace element content of Romanian wines presenting data for a rather limited number of elements (Dehelean and Voica, 2012, Geana *et al.*, 2013, Avram *et al.*, 2014, Geana *et al.*, 2016, Bora *et al.*, 2018a). Concentration levels of Cr, Cu, Fe, Mn and Zn in wine samples from the south-east of Romania were investigated by Bora *et al.* (2018a). Knowledge of the health risks associated with the ingestion of these elements from wine is insufficient. On the other hand, several agencies and organizations such as the US Environmental Protection Agency (US EPA) and JECFA have provided clear guidelines on the intake elements via food or drink by people. Based on these considerations, it is necessary to assess

the health risk of Romanian wine from different wine production areas. The main objectives of the present study were: (1) the establishment and validation of an analytical method for the determination of trace elements in wines using ICP-MS (inductively coupled plasma mass spectrometry) after microwave-assisted digestion; (2) to determine the total concentration of Pb, Cd, Ni, Co, Zn, Cr, Cu and Mn from wines and grape juice obtained from five different wine-growing areas of Romania; (3) comparing the concentration from different wine areas to the results reported in the previous literature and to analyze the distribution characteristics of this trace element in Romanian wines and grape juice samples; and (4) also to the estimation of the ingestion rates of some elements from Romanian wines by daily consumption of wine as well as the health risk caused by the intake of these elements.

Materials and methods

Study area

A total of 321 wines and grape juice samples (174 red wines and 147 white wines) were analyzed in this research. All samples (wines and grape juice) used were formed from the 2011-2016 vintage. Wines and grape juice samples were collected from five different vineyards areas: Nicoreşti, Iveşti, Panciu, Obobeshti, and Dealu Mare. The

Table 1. Wine, grape juice and production area

No.	Type	Variety	Area of production
1.	Red	Babeasca neagra	Nicoreşti; Iveşti; Panciu
2.	Red	Cabernet Sauvignon	Nicoreşti; Iveşti; Panciu; Dealu Mare
3.	Red	Feteasca neagra	Nicoreşti; Iveşti; Dealu Mare
4.	Red	Merlot	Panciu; Obobeshti; Dealu Mare
5.	Red	Pinot Noir	Dealu Mare
6.	Red	Burgund Mare	Dealu Mare
7.	White	Feteasca regala	Nicoreşti; Iveşti; Panciu
8.	White	Aligoté	Nicoreşti; Iveşti; Panciu
9.	White	Rkatiteli	Nicoreşti; Iveşti
10.	White	Italian Riesling	Panciu; Obobeshti
11.	White	Muscat Ottonel	Panciu
12.	White	Sauvignon blanc	Nicoreşti; Iveşti; Panciu; Obobeshti; Dealu Mare
13.	White	Pinot Gris	Dealu Mare
14.	White	Tamaioasa Romaneasca	Dealu Mare
15.	White	Busuioaca de Bohotin	Dealu Mare

wines and grape juice samples under this study and production areas are presented in (Tab. 1).

Sample collection and micro vinification process

Grape samples (15 kg/cultivar) were collected for each cultivar from 10-15 vines. The grapes were placed in the lower third, middle and top of each vine and grapes were exposed to shade and sun (Bora *et al.*, 2015). In this way a better homogenization of the grape samples can be achieved. The grape juice (must) samples were obtained by using the manual press. The wine samples resulted from micro-vine production. Micro-vine production was done according to the methodology described by Bora *et al.* (2016). All wines were provided from the wineries as finished wines in 750 mL glass bottles with cork stoppers and were stored at 3-4 °C before analysis. One bottle was used for each sample, and three replicates were taken. All vines were planted since 1979, and the vine plantation was organized with 2.2×1 m distance between rows and plants. Vines were pruned according to the Guyot system and were grown on speliers.

Sample preparation

To remove the appreciable amounts of deposits and colloidal suspensions present, the samples were filtrated (cellulose acetate membrane 0.45 µm pore size) and then acid-digested in a microwave oven (STARD D Microwave Digestion System, Milestone). To determine the trace elements from wine and grape juice were used an amount of 0.5 mL (sample) and adjusted 8 mL (7 mL HNO₃ 65% + 1 mL H₂O₂). The operation conditions for the applied microwave digestion system are given in (Tab. 2).

Reagents and solutions

Eight elements (Pb, Cd, Co, Ni, Cr, Zn, Mn, and Cu) were analyzed to estimate the dietary intake rates of these elements from Romanian wines by daily wine consumption as well as the health risk caused by their intake. The calibration was performed using XXICertiPUR multielement standard. 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% HNO₃ for 24 hours and rinsing

Table 2. Microwave digestion operating programmer

Step	Initial temperature (°C)	Final temperature (°C)	Ramping time (°C)	Time holds (min)	Power (W)
1	25	100	15	10	800 (75%)
2	100	150	10	5	800 (100%)
3	150	200	10	10	1600 (85%)

Table 3. ICP-MS the conditions under which the analyses were carried out

Element	Calibration coefficient	LoD ¹ (µg/L)	LoQ ² (µg/L)	BEC ³ (µg/L)
Pb	0.9999	0.013	0.028	0.019
Cd	0.9999	0.0056	0.018	0.088
Ni	0.9999	0.0016	0.0039	0.0031
Co	0.9999	0.00019	0.0006	0.00038
Zn	0.9999	0.0019	0.0052	0.0018
Cr	0.9999	0.0059	0.018	0.0053
Cu	0.9999	0.026	0.084	0.051
Mn	0.9999	0.036	0.0109	0.0058

LoD¹ = detection limit; LoQ² = quantification limits; BEC³ = background equivalent concentration.

at least ten times with ultrapure water (Milli-Q Integral ultrapure water-Type 1). The accuracy of the methods was evaluated by replicate analyses of known concentration samples (between 10 µL to 10 mL concentrations) and obtained values ranged between 0.8-13.1% (Bora *et al.*, 2018a).

For quality control, blank and triplicate 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) were calculated according to the mathematical formulas: $LoD = 3SD/s$ and $LoQ = 10 SD/s$ (SD = standard deviation; s = slope of the calibration curve) (Tab. 3).

ICP-MS Analysis

Elemental analysis was performed with an iCAP Q (Thermo Scientific, Waltham, MA, USA) X-Series II inductively coupled plasma mass spectrometer. The instrument is equipped with a quartz torch with a Plasma Screen device, a quadrupole mass analyzer, lens ion optics based on a hexapole design with a chicane ion deflector and a simultaneous detector with real-time

multichannel analyzer electronics, operating in either an analog signal or pulse counting mode. A high-efficiency ESI APEX-Q nebulizer (Eppendorf SA, Vevey, Switzerland) was used as a nebulization system. The instrument and accessories were PC-controlled by Qtegra software. All relevant instrumental conditions are given in (Tab. 4). The isotopes used were ^{53}Cr , ^{55}Mn , ^{59}Co , ^{60}Ni , ^{63}Cu , ^{66}Zn , ^{114}Cd , $^{206}Pb/^{207}Pb/^{208}Pb$. A stability test was performed before each analysis session by monitoring 7Li , ^{59}Co , ^{115}In , ^{140}Ce , and ^{238}U masses and making sure precision was better than 2%: instrumental precision was under 2% for the trace elements, while the overall uncertainty (involving both instrumental analysis and sample preparation), calculated on the basis of five genuine replicates, was better than 5%.

Instrumental drift was corrected using rhodium as an internal standard, at concentration level of 10 µg/L, added to both calibration standards and wine samples, to normalize the instrument response. Rhodium was selected as an internal standard due to very low background signal found for this element, and because it was not present in the wine samples (Ivanova-Petropulos *et al.*,

Table 4. ICP-MS operating conditions

Instrument	iCAP Q (ICP-MS Thermo Scientific)
Sampler cone	Ni (standard)
Skimmer cone	Ni (standard)
Nebulizer	MicroMist (standard)
Plasma torch	Quartz, 2.5 mm (standard)
Integration time	
Cd	0.3 s × 1 point
All other	0.1 s × 1 point
Replicates	1
Tune parameters	
RF	1500 W
Sample depth	7.6 mm
Carrier gas	1.00 L/min
Makeup gas	0.25 L/min
Extract 1	+6 V
Extract 2	-145 V
Energy discrimination	3 V
Reaction gas He	3.5 mL/min
CeO ⁺ /Ce ⁺	0.65%
Ce ⁺⁺ /Ce	2.08 %

2016). The addition of the internal standard to the wine samples was performed by the peristaltic pump.

A synthetic wine sample (Titivin), 12 % ethanol, 4 g/L tartaric acid and pH 3.4 was prepared to contain the solution to give rise to multiple interferences across a spectrum range of common analytes and test the capacity of He collision mode to remove all overlapping polyatomic species. Two sets of spectra have been obtained that demonstrate the capacity of the He collision mode to remove multiple interferences: one in "no gas" mode and the other with He added to the cell. Data correction or background correction was applied in He mode for the elements Pb, Cd, Ni, Co, Zn, Cr, Cu, and Mn.

Statistical Analysis and Calculations

All analyses were performed three times, the mean values of three duplicates were used as the result of each wine and must sample, statistical significance was calculated at 95% confidence limit using Duncan's multiple ranges ($p < 0.05$) with the help of SPSS 24.0 software for Windows (SPSS Inc., Chicago, IL, USA) software. The boxplot was created by Microsoft Office 2019 professional plus for Windows and Sigma plot 10.0.

The estimated daily intake (EDI, $\mu\text{g}/\text{kg bw}/\text{day}$) of trace elements from wine relied on the concentration of the elements in the wine, the daily intake rate of wine consumers and the bodyweight (USEPA, 1989, Zhi-Hao *et al.*, 2019) and it was calculated by the formula:

$$\text{EDI} = (\text{C} \times \text{R})/\text{BW}$$

where C – the concentration of a single element in the wine, $\mu\text{g}/\text{L}$; R – the daily rate of intake for adult wine drinkers, L/day; BW – the average body weight of the population evaluated, kg (Zhi-Hao *et al.*, 2019).

Based on the target hazard quotient (THQ) was calculated health risk due to the consumption of wine, from the ration EDI and an oral reference dose (USEPA, 1989, Zhi-Hao *et al.*, 2019).

$$\text{THQ} = \text{EDI}/\text{RfD}$$

where RfD – the oral reference dose for each element, $\mu\text{g}/\text{kg bw}/\text{day}$.

If the THQ is less than 1, it means that there are no obvious adverse effects on the intake of these elements from wine. If the THQ is equal to or above 1, there is a danger to humans health (Zhi-Hao *et al.*, 2019). The total THQ values of the elements evaluated in wine are described as the hazard index (HI) used to evaluate the comprehensive health risks of wine.

Results and discussions

The results of the measurement of Pb, Cd, Ni, Co, Zn, Cr, Cu and Mn in wine samples are shown in (Tab. 5-7) according to the type of wine, region and winemaker. The majority of elements were found at part per billion (ppb) levels so all values are reported as $\mu\text{g}/\text{L}$. In Table 5 data for maximum permissible limit (M.P.L.) of toxic elements in wine are defined as wine standards given by the Office International de la Vigne et du Vin (O.I.V., 2016).

Zn had the highest concentration levels indifferent the sample of wine analyzed (2524.69 $\mu\text{g}/\text{L}$) and Mn (1346.76 $\mu\text{g}/\text{L}$), and Pb recorded the smallest average concentration Pb (12.86 $\mu\text{g}/\text{L}$) and Cd (0.12 $\mu\text{g}/\text{L}$), other elements concentration declined in the order $\text{Cr} > \text{Cu} > \text{Co} > \text{Ni}$. The concentration of metals analyzed varied in all wine were as follows: Pb (12.86-78.36), Cd (0.12-0.79), Ni (88.45-382.39), Co (1.48-11.38), Zn (480.56-2524.69), Cr (215.18-838.87), Cu (215.37-638.53), Mn (336.85-1346.76).

If relative abundances of the elements in red wines are compared, the tendency at the ranking is as follows: $\text{Zn} > \text{Mn} > \text{Cr} > \text{Cu} > \text{Ni} > \text{Pb} > \text{Co} > \text{Cd}$. Comparison of the relative abundances of the essential elements in white wines reveals the following tendency: $\text{Zn} > \text{Mn} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Pb} > \text{Co} > \text{Cd}$. The order of the content of elements in wines is similar to the one reported by Bora *et al.* (2018a), Bora *et al.* (2018b), Geana *et al.* (2016) and approximately corresponds to the general order of the content of elements in plants (Kabata-Pendias and Pedias, 1984). This ranking is not to be taken as exact because there are many variables in wine production, such as regions, soil, and climate. For this reason, the amount of an element may be found in a wide variety of ranges for different wines even if they are produced from the same type of grapes. For example, Zn was found to be between 328.77 $\mu\text{g}/\text{L}$ and 1959.85 $\mu\text{g}/\text{L}$ in Cabernet Sauvignon red wines which is quite a wide range.

Some of the investigation elements (Pb, Cd, and Co) are present in analyzed wines in a very narrow range, whereas for other elements (Ni, Zn, Cr, Cu, and Mn) variations are much greater. For most of the analyzed elements, the obtained concentration falls into concentration ranges for Romanian wines by Avram *et al.* (2014), Dinca *et al.* (2016), Geana *et al.* (2016) and Bora *et al.* (2018a), Bora *et al.* (2018b). Only the Zn content of the studied wines is higher than Zn concentrations reported by Dinca *et al.* (2016), Geana *et al.* (2016), whereas the levels of Mn and Cr are found to be lower than those reported by Dinca *et al.* (2016), Geana *et al.* (2016). The measured concentrations are also consistent for most of the elements with findings of Kment *et al.* (2005) for Czech wines, Álvarez *et al.* (2007) for Spanish wines and Interesse *et al.* (1994) for Italian wines. Fiket *et al.* (2011) reported higher values of Cu and Pb comparing to analyzed Romanian wines. The concentration levels of Cd, Co and Mn are lower than those reported for Slovenia and Istria wines by Fiket *et al.* (2011). These differences can be explained by variations in vineyard soil geochemistry and a higher degree of anthropogenic influence of Czech and Cretan wines. Kment *et al.* (2005) and Geana *et al.* (2013) found Pb in higher concentrations in Czech and Romanian wines, which can be explained solely by differences in soil geochemistry.

Due to the significantly high concentration of Ni, Zn, Cr, and Cu in the analyzed wine samples a prediction can be made: these elements may be dissolved in the wine during the production steps in metal vessels (Preedy and Watson, 2003, Guardia and Garrigues, 2015). It sounds probable considering the acidic nature of the wine. Table 6 features the observed concentration ranges and the average of each element, according to different winemakers, to render an opportunity to compare the possible differences emerging from the wines. In terms of Cd distribution, it can be seen that this element records comparative concentrations in all the studied vines, except Odobești vineyard, and its average concentration (0.83 µg/L) was considerably greater compared to other wine regions.

Pb registered the highest concentrations in the Ivești (average 70.02 µg/L) and Nicorești area (average 33.34 µg/L) compared to other wine regions. Panciu area (average 324.93 µg/L) and Nicorești area (average 324.29 µg/L) have

recorded the highest concentrations which were significantly higher than the Ivești (average 195.36 µg/L) and Odobești area (average 210.43 µg/L) areas, in terms of Ni distribution. The Co, Zn and Cu levels have recorded significantly higher concentrations in Ivești (average 19.22 µg Co/L, 2652.74 µg Zn/L and 631.92 µg Cu/L) regions compared to other wine regions. The level concentration of Cr and Mn was the highest in the Nicorești (average 1032.19 µg Cr/L) and Dealu Mare (average 489.32 µg Mn/L) respectively. The results are given in (Tab. 6) shown that Zn amounts of Ivești and Panciu wines are relatively high which is also related to the type of wine, the mentioned wine is red.

World Health Organization (WHO) does not specify a maximum Cd in wine. But at this point, drinking water specifications are useful considering all other food samples. According to WHO, Cd concentration must be below 5.0 ppb in drinking water (Rielly, 2002a,b). The outcome of this study reveals that the concentration of Cd to be below the allowed limit for every analyzed sample. In literature, comments have been made about the effects of thermal power plants on the accumulation of non-essential metals like Cd in similar studies (Bajpai *et al.*, 2010, Kallithraka *et al.*, 2001, Dragovin *et al.*, 2013). However, such interpretation of our study would not support Kallithraka's reasoning, since the concentration of Cd is very low. The Cd values were measured to be 0.79 ± 0.09 and 0.83 ± 0.05 µg/L (Tab. 5 and 6) which are lowest than 5.0 µg/L (WHO) and much less so 10 µg/L (O.I.V., 2016).

Average value $\pm$ standard deviation ($n = 3$). Roman letters represent the significance of the difference (Duncan test, $p < 0.05$). The difference between any two values, followed by at least one common letter, is insignificant. M.P.L = maximal permissible allowed (O.I.V., 2016). LOQ = lower than the limit of quantification.

These values are mostly encountered in the wines produced from grapes grown in a highly industrialized area, with a vast number of thermal plants. On the other hand, values reported by Kallithraka *et al.* (2001) are much higher leading to the notion that thermal power plants are not the only reason for contamination.

Observed Pb concentration is very much lower than those allowed by O.I.V. The highest values identified for Pb (78.36 ± 2.62 µg/L) are

Table 5. The heavy metal content of wine by type (mean $\pm$ SD in $\mu\text{g/L}$)

Type of wine	n	Range / Average	Pb ($\mu\text{g/L}$)	Cd ($\mu\text{g/L}$)	Ni ($\mu\text{g/L}$)	Co ($\mu\text{g/L}$)	Zn ($\mu\text{g/L}$)	Cr ($\mu\text{g/L}$)	Cu ($\mu\text{g/L}$)	Mn ($\mu\text{g/L}$)
Name of grape	Color	M.P.L.	M.P.L.	M.P.L.	M.P.L.	M.P.L.	M.P.L.	M.P.L.	M.P.L.	M.P.L.
Babesca neagra	Red	5	12.17 $\pm$ 0.83	0.11 $\pm$ 0.02	222.91 $\pm$ 7.53	3.10 $\pm$ 0.79	533.31 $\pm$ 4.37	235.63 $\pm$ 4.24	217.99 $\pm$ 6.20	232.99 $\pm$ 1.53
		Range	59.21 $\pm$ 4.11	0.17 $\pm$ 0.02	325.15 $\pm$ 7.93	8.05 $\pm$ 0.77	2241.27 $\pm$ 6.21	1351.75 $\pm$ 3.08	751.57 $\pm$ 5.76	534.92 $\pm$ 3.55
		Average	32.05 $\pm$ 0.71 ^f	0.14 $\pm$ 0.02 ^g	281.75 $\pm$ 3.74 ^{cd}	4.28 $\pm$ 0.58 ^{de}	1847.31 $\pm$ 7.82 ^e	838.87 $\pm$ 7.47 ^a	587.58 $\pm$ 1.32 ^e	411.89 $\pm$ 0.32 ^f
Cabernet Sauvignon	Red	4	14.67 $\pm$ 0.84	0.10 $\pm$ 0.02	185.91 $\pm$ 1.57	2.18 $\pm$ 0.08	328.77 $\pm$ 7.58	127.07 $\pm$ 3.42	327.98 $\pm$ 6.19	125.27 $\pm$ 2.78
		Range	26.33 $\pm$ 3.03	0.23 $\pm$ 0.03	313.81 $\pm$ 5.20	5.85 $\pm$ 0.58	1959.85 $\pm$ 6.68	868.57 $\pm$ 5.94	838.69 $\pm$ 7.04	123.81 $\pm$ 5.99 ^b
		Average	17.92 $\pm$ 0.35 ^h	0.13 $\pm$ 0.01 ^g	238.34 $\pm$ 7.03 ^e	4.16 $\pm$ 0.02 ^g	1238.83 $\pm$ 22.38 ^f	527.04 $\pm$ 7.28 ^h	686.43 $\pm$ 2.73 ^a	845.42 $\pm$ 4.07 ^a
Feteasca neagra	Red	6	22.00 $\pm$ 1.09	0.08 $\pm$ 0.01	260.01 $\pm$ 5.21	10.65 $\pm$ 0.53	916.39 $\pm$ 6.73	349.89 $\pm$ 4.94	219.64 $\pm$ 5.53	230.43 $\pm$ 1.63
		Range	77.50 $\pm$ 1.36	0.13 $\pm$ 0.02	406.32 $\pm$ 7.03	7.02 $\pm$ 0.62	2537.42 $\pm$ 5.07	1252.80 $\pm$ 4.02	519.64 $\pm$ 4.28	633.13 $\pm$ 8.05
		Average	48.29 $\pm$ 1.03 ^c	0.12 $\pm$ 0.01 ^a	352.56 $\pm$ 8.12 ^b	5.52 $\pm$ 0.69 ^{de}	1528.74 $\pm$ 15.41 ^a	646.17 $\pm$ 11.53 ^f	358.08 $\pm$ 5.96 ^f	336.85 $\pm$ 13.38 ^m
Merlot	Red	5	30.02 $\pm$ 1.36	0.11 $\pm$ 0.03	93.85 $\pm$ 7.34	3.23 $\pm$ 0.34	753.16 $\pm$ 10.57	187.94 $\pm$ 1.56	264.68 $\pm$ 2.80	236.13 $\pm$ 5.12
		Range	100.73 $\pm$ 1.96	0.23 $\pm$ 0.03	278.49 $\pm$ 8.11	7.92 $\pm$ 1.08	2840.94 $\pm$ 4.49	951.99 $\pm$ 3.86	752.80 $\pm$ 6.00	1553.09 $\pm$ 1.78
		Average	78.36 $\pm$ 2.62 ^a	0.14 $\pm$ 0.02 ^g	125.66 $\pm$ 8.30 ^h	5.98 $\pm$ 0.91 ^c	1963.52 $\pm$ 11.62 ^c	748.50 $\pm$ 3.53 ^c	567.20 $\pm$ 4.08 ^f	986.75 $\pm$ 5.19 ^d
Pinot noir	Red	3	23.68 $\pm$ 2.76	-	94.44 $\pm$ 4.76	-	987.94 $\pm$ 3.57	329.18 $\pm$ 4.58	329.03 $\pm$ 4.20	395.08 $\pm$ 3.84
		Range	63.53 $\pm$ 1.47	-	426.03 $\pm$ 4.87	-	3427.16 $\pm$ 12.54	1463.76 $\pm$ 8.77	750.20 $\pm$ 5.60	1001.86 $\pm$ 5.33
		Average	38.77 $\pm$ 3.85 ^d	LOQ	175.02 $\pm$ 5.53 ^f	LOQ	2137.59 $\pm$ 4.79 ^b	750.51 $\pm$ 8.16 ^c	611.36 $\pm$ 1.50 ^d	771.06 $\pm$ 9.06 ^h
Burgund Mare	Red	6	10.70 $\pm$ 1.36	0.11 $\pm$ 0.02	125.72 $\pm$ 2.84	3.59 $\pm$ 0.35	530.64 $\pm$ 9.13	100.01 $\pm$ 2.12	428.48 $\pm$ 4.56	155.38 $\pm$ 6.24
		Range	39.36 $\pm$ 2.06	0.23 $\pm$ 0.03	551.46 $\pm$ 8.00	8.72 $\pm$ 0.85	3633.44 $\pm$ 10.73	320.76 $\pm$ 3.83	728.68 $\pm$ 3.35	886.52 $\pm$ 2.51
		Average	31.63 $\pm$ 1.86 ^f	0.13 $\pm$ 0.02 ^g	284.96 $\pm$ 6.30 ^c	4.24 $\pm$ 1.00 ^{de}	2141.72 $\pm$ 5.14 ^b	215.18 $\pm$ 2.62 ^f	627.49 $\pm$ 5.91 ^c	662.27 $\pm$ 1.05 ^f
Feteasca regala	White	6	12.08 $\pm$ 0.66	0.12 $\pm$ 0.02	73.61 $\pm$ 4.80	2.80 $\pm$ 0.51	318.24 $\pm$ 3.65	220.46 $\pm$ 2.48	129.39 $\pm$ 2.84	323.94 $\pm$ 3.61
		Range	15.70 $\pm$ 0.32	0.29 $\pm$ 0.04	142.45 $\pm$ 3.02	6.45 $\pm$ 0.62	1948.38 $\pm$ 4.08	777.10 $\pm$ 10.43	428.88 $\pm$ 3.76	524.82 $\pm$ 4.27
		Average	19.82 $\pm$ 1.66 ^h	0.20 $\pm$ 0.03 ^e	93.33 $\pm$ 3.66 ^f	4.92 $\pm$ 0.31 ^{cd}	480.56 $\pm$ 9.12 ^f	521.76 $\pm$ 9.47 ^h	367.41 $\pm$ 7.41 ^e	429.16 $\pm$ 2.66 ^k
Aligoté	White	3	9.13 $\pm$ 0.98	-	68.65 $\pm$ 3.16	2.98 $\pm$ 0.36	640.35 $\pm$ 8.02	426.95 $\pm$ 5.20	119.02 $\pm$ 4.37	330.68 $\pm$ 10.74
		Range	21.95 $\pm$ 0.73	-	122.36 $\pm$ 6.99 ^a	8.04 $\pm$ 0.55	2839.45 $\pm$ 7.44	122.76 $\pm$ 9.26	531.31 $\pm$ 2.29	1629.35 $\pm$ 5.92
		Average	12.86 $\pm$ 0.93 ⁱ	LOQ	154.34 $\pm$ 6.99 ^a	7.20 $\pm$ 1.15 ^b	1941.46 $\pm$ 5.46 ^d	766.46 $\pm$ 4.62 ^b	215.37 $\pm$ 1.11 ^m	858.42 $\pm$ 4.15 ^g
Rklatiteli	White	3	18.72 $\pm$ 1.06	0.68 $\pm$ 0.06	131.27 $\pm$ 7.46	5.95 $\pm$ 0.34	335.51 $\pm$ 8.18	216.56 $\pm$ 2.00	248.04 $\pm$ 6.07	131.92 $\pm$ 2.31
		Range	57.92 $\pm$ 4.08	1.14 $\pm$ 0.16	463.49 $\pm$ 7.57	11.56 $\pm$ 0.71	2109.87 $\pm$ 4.82	422.91 $\pm$ 9.66	635.78 $\pm$ 4.79	962.40 $\pm$ 0.94
		Average	36.92 $\pm$ 1.52 ^{de}	0.79 $\pm$ 0.09 ^a	382.39 $\pm$ 6.91 ^a	7.81 $\pm$ 0.39 ^b	1455.23 $\pm$ 1.23 ^h	358.34 $\pm$ 5.69 ^f	464.51 $\pm$ 3.19 ^g	519.11 $\pm$ 2.65 ^f
Italian Riesling	White	3	16.73 $\pm$ 1.12	0.15 $\pm$ 0.04	113.39 $\pm$ 5.10	3.88 $\pm$ 0.55	822.32 $\pm$ 8.57	564.98 $\pm$ 5.72	213.76 $\pm$ 0.64	256.78 $\pm$ 1.19
		Range	31.58 $\pm$ 0.67	0.31 $\pm$ 0.02	490.61 $\pm$ 6.22	8.82 $\pm$ 0.59	3631.51 $\pm$ 9.95	1460.09 $\pm$ 5.21	763.86 $\pm$ 2.78	1354.18 $\pm$ 2.67
		Average	26.99 $\pm$ 2.12 ^g	0.23 $\pm$ 0.02 ^{de}	272.54 $\pm$ 8.21 ^d	4.58 $\pm$ 1.05 ^{de}	1534.85 $\pm$ 9.06 ^g	723.70 $\pm$ 2.17 ^a	610.52 $\pm$ 5.53 ^d	1128.96 $\pm$ 5.97 ^c
Muscat Ottonel	White	4	33.60 $\pm$ 1.99	0.10 $\pm$ 0.03	58.58 $\pm$ 6.48	2.40 $\pm$ 0.42	522.76 $\pm$ 9.30	126.43 $\pm$ 5.61	182.46 $\pm$ 4.59	166.73 $\pm$ 1.50
		Range	111.17 $\pm$ 1.90	0.29 $\pm$ 0.02	269.37 $\pm$ 5.92	7.11 $\pm$ 0.69	1835.09 $\pm$ 9.64	919.37 $\pm$ 6.15	850.32 $\pm$ 9.47	1476.63 $\pm$ 1.86
		Average	63.83 $\pm$ 3.56 ^b	0.18 $\pm$ 0.02 ^{af}	174.92 $\pm$ 5.98 ^f	5.68 $\pm$ 0.51 ^{cd}	1227.66 $\pm$ 6.51 ^f	635.79 $\pm$ 4.82 ^f	621.44 $\pm$ 1.88 ^c	914.76 $\pm$ 2.03 ^f
Sauvignon blanc	White	6	15.07 $\pm$ 3.99	0.13 $\pm$ 0.04	64.12 $\pm$ 2.52	3.00 $\pm$ 0.77	352.89 $\pm$ 2.85	138.48 $\pm$ 2.03	218.39 $\pm$ 7.85	849.58 $\pm$ 8.53
		Range	50.67 $\pm$ 2.29	0.34 $\pm$ 0.06	259.26 $\pm$ 3.63	11.27 $\pm$ 0.93	2523.98 $\pm$ 8.88	785.06 $\pm$ 3.68	851.03 $\pm$ 5.35	1553.19 $\pm$ 8.77
		Average	34.52 $\pm$ 4.01 ^{af}	0.26 $\pm$ 0.02 ^{cd}	156.35 $\pm$ 5.14 ^g	1.48 $\pm$ 0.44 ^h	2124.96 $\pm$ 5.68 ^b	519.39 $\pm$ 7.05 ^h	418.76 $\pm$ 5.63 ^h	1239.04 $\pm$ 8.21 ^b
Pinot gris	White	3	7.21 $\pm$ 0.92	0.18 $\pm$ 0.03	69.61 $\pm$ 1.47	6.18 $\pm$ 1.70	939.78 $\pm$ 6.90	331.13 $\pm$ 6.42	123.87 $\pm$ 1.39	322.99 $\pm$ 2.83
		Range	24.33 $\pm$ 2.71	0.35 $\pm$ 0.06	218.20 $\pm$ 4.98	3.00 $\pm$ 0.51	3281.15 $\pm$ 3.00	961.35 $\pm$ 5.82	427.74 $\pm$ 4.84	1385.01 $\pm$ 1.08
		Average	37.17 $\pm$ 1.74 ^{de}	0.28 $\pm$ 0.04 ^c	184.98 $\pm$ 6.52 ^f	4.80 $\pm$ 0.40 ^{cd}	2524.69 $\pm$ 13.46 ^a	679.76 $\pm$ 4.70 ^e	312.17 $\pm$ 0.72 ^f	933.59 $\pm$ 1.50 ^e
Tamioasa Romaneasca	White	4	12.75 $\pm$ 2.74	0.27 $\pm$ 0.04	84.28 $\pm$ 2.67	7.95 $\pm$ 0.72	875.16 $\pm$ 9.51	216.26 $\pm$ 6.35	253.87 $\pm$ 1.74	240.21 $\pm$ 22.60
		Range	31.63 $\pm$ 0.40	0.83 $\pm$ 0.10	132.98 $\pm$ 4.83	15.53 $\pm$ 0.72	2840.81 $\pm$ 1.05	627.29 $\pm$ 5.44	654.23 $\pm$ 1.80	1003.42 $\pm$ 17.48
		Average	24.47 $\pm$ 2.79 ^g	0.60 $\pm$ 0.04 ^b	109.98 $\pm$ 1.91 ^f	11.38 $\pm$ 0.91 ^a	1637.85 $\pm$ 4.43 ^f	535.39 $\pm$ 5.70 ^g	411.82 $\pm$ 2.00 ^f	664.03 $\pm$ 27.74 ^f
Busuioara de Bohotin	White	4	14.27 $\pm$ 3.36	-	85.98 $\pm$ 1.31	2.48 $\pm$ 0.45	631.53 $\pm$ 10.08	134.78 $\pm$ 1.92	192.94 $\pm$ 5.45	581.38 $\pm$ 18.73
		Range	26.56 $\pm$ 2.00	-	97.21 $\pm$ 0.93	5.91 $\pm$ 0.29	1880.99 $\pm$ 16.39	327.43 $\pm$ 5.14	812.67 $\pm$ 2.69	1669.04 $\pm$ 40.26
		Average	36.45 $\pm$ 0.76 ^{de}	LOQ	88.45 $\pm$ 1.14 ^e	3.45 $\pm$ 0.76 ^{de}	1236.49 $\pm$ 7.58 ^f	215.32 $\pm$ 2.84 ^f	638.53 $\pm$ 3.00 ^a	1346.76 $\pm$ 17.82 ^a
Red Average			41.17	0.11	243.05	4.03	1809.62	621.05	577.52	669.04
White Average			32.56	0.28	179.70	5.70	1573.75	541.17	550.66	862.65

Average value $\pm$ standard deviation (n = 3). Roman letters represent the significance of the difference (Duncan test, p < 0.05). The difference between any two values, followed by at least one common letter, is insignificant. M.P.L. = maximal permissible allowed (O.I.V., 2016). LOQ = lower than the limit of quantification.

Table 6. Heavy metal content by winemaker (mean $\pm$ SD in $\mu\text{g/L}$)

Winemaker	n	Range / Average	Pb ($\mu\text{g/L}$)	Cd ($\mu\text{g/L}$)	Ni ($\mu\text{g/L}$)	Co ($\mu\text{g/L}$)	Zn ($\mu\text{g/L}$)	Cr ($\mu\text{g/L}$)	Cu ($\mu\text{g/L}$)	Mn ($\mu\text{g/L}$)
Nicorești	16	Range	13.57 $\pm$ 0.32	0.15 $\pm$ 0.02	192.19 $\pm$ 6.37	3.77 $\pm$ 0.76	640.08 $\pm$ 8.02	326.15 $\pm$ 12.60	328.97 $\pm$ 3.70	337.80 $\pm$ 11.03
		Range	50.01 $\pm$ 2.12	0.21 $\pm$ 0.02	429.64 $\pm$ 5.62	7.53 $\pm$ 1.71	2142.25 $\pm$ 2.85	1847.44 $\pm$ 6.14	627.40 $\pm$ 5.77	567.22 $\pm$ 4.16
		Average	33.34 $\pm$ 1.57 ^b	0.16 $\pm$ 0.02 ^d	324.29 $\pm$ 4.37 ^a	5.59 $\pm$ 0.70 ^c	1754.75 $\pm$ 8.73 ^c	1032.19 $\pm$ 6.29 ^a	467.82 $\pm$ 2.41 ^d	462.88 $\pm$ 3.68 ^b
Ivești	10	Range	30.45 $\pm$ 1.02	0.17 $\pm$ 0.03	101.81 $\pm$ 5.02	12.23 $\pm$ 1.35	756.98 $\pm$ 3.72	194.79 $\pm$ 5.27	428.47 $\pm$ 7.03	132.82 $\pm$ 3.11
		Range	96.36 $\pm$ 1.10	0.33 $\pm$ 0.04	237.27 $\pm$ 3.99	25.82 $\pm$ 3.04	3126.63 $\pm$ 2.53	1251.39 $\pm$ 9.10	921.74 $\pm$ 3.90	650.44 $\pm$ 7.98
		Average	70.02 $\pm$ 1.36 ^a	0.18 $\pm$ 0.06 ^{cd}	195.36 $\pm$ 8.05 ^d	19.22 $\pm$ 1.82 ^a	2652.74 $\pm$ 1.24 ^a	416.89 $\pm$ 4.03 ^e	631.92 $\pm$ 6.67 ^a	458.78 $\pm$ 3.28 ^b
Panciu	6	Range	11.51 $\pm$ 1.32	0.21 $\pm$ 0.02	134.36 $\pm$ 6.49	2.64 $\pm$ 0.49	534.64 $\pm$ 6.55	107.69 $\pm$ 3.86	328.47 $\pm$ 6.69	233.58 $\pm$ 6.66
		Range	32.59 $\pm$ 1.12	0.38 $\pm$ 0.07	636.60 $\pm$ 10.78	7.57 $\pm$ 0.59	2877.16 $\pm$ 16.09	640.48 $\pm$ 15.15	421.48 $\pm$ 1.35	534.23 $\pm$ 4.58
		Average	21.21 $\pm$ 1.23 ^d	0.29 $\pm$ 0.02 ^b	324.93 $\pm$ 3.76 ^a	3.83 $\pm$ 1.36 ^{cd}	2135.92 $\pm$ 22.73 ^b	466.31 $\pm$ 13.05 ^d	386.18 $\pm$ 10.09 ^e	381.64 $\pm$ 7.30 ^c
Obobești	10	Range	22.18 $\pm$ 0.97	0.53 $\pm$ 0.03	84.10 $\pm$ 9.58	2.68 $\pm$ 0.84	417.85 $\pm$ 5.06	218.50 $\pm$ 3.92	128.64 $\pm$ 4.10	157.18 $\pm$ 2.28
		Range	25.55 $\pm$ 0.67	1.05 $\pm$ 0.09	333.54 $\pm$ 6.95	14.02 $\pm$ 2.94	1736.56 $\pm$ 10.35	621.72 $\pm$ 10.17	627.40 $\pm$ 6.37	428.74 $\pm$ 6.57
		Average	15.51 $\pm$ 0.27 ^e	0.83 $\pm$ 0.05 ^a	210.43 $\pm$ 2.49 ^c	9.17 $\pm$ 0.34 ^b	926.38 $\pm$ 7.63 ^e	568.58 $\pm$ 2.69 ^c	488.64 $\pm$ 7.03 ^c	277.93 $\pm$ 7.33 ^d
Dealul Mare	23	Range	14.30 $\pm$ 2.38	0.21 $\pm$ 0.05	126.46 $\pm$ 8.40	2.83 $\pm$ 0.58	838.85 $\pm$ 2.48	218.90 $\pm$ 4.08	220.09 $\pm$ 8.28	358.56 $\pm$ 2.20
		Range	42.60 $\pm$ 0.51	0.32 $\pm$ 0.03	355.44 $\pm$ 3.83	3.55 $\pm$ 0.39	1622.55 $\pm$ 1.51	1131.07 $\pm$ 1.15	740.92 $\pm$ 8.03	663.78 $\pm$ 4.90
		Average	28.19 $\pm$ 1.52 ^c	0.23 $\pm$ 0.02 ^b	266.68 $\pm$ 11.59 ^b	3.15 $\pm$ 0.14 ^d	1234.97 $\pm$ 5.73 ^d	858.13 $\pm$ 3.57 ^b	527.29 $\pm$ 5.38 ^b	489.32 $\pm$ 2.52 ^a

Average value $\pm$ standard deviation (n = 3). Roman letters represent the significance of the difference (Duncan test, p < 0.05). The difference between any two values, followed by at least one common letter, is insignificant.

Table 7. Heavy metal content of the grape juice in five regions (mean $\pm$ SD in $\mu\text{g/L}$)

Winemaker	n	Range / Average	Pb ($\mu\text{g/L}$)	Cd ($\mu\text{g/L}$)	Ni ($\mu\text{g/L}$)	Co ($\mu\text{g/L}$)	Zn ($\mu\text{g/L}$)	Cr ($\mu\text{g/L}$)	Cu ($\mu\text{g/L}$)	Mn ($\mu\text{g/L}$)
Nicorești	16	Range	33.76 $\pm$ 5.11	0.13 $\pm$ 0.03	239.14 $\pm$ 2.80	2.94 $\pm$ 0.33	574.37 $\pm$ 14.13	256.48 $\pm$ 5.33	335.37 $\pm$ 8.88	222.96 $\pm$ 8.84
		Range	73.05 $\pm$ 9.98	0.18 $\pm$ 0.03	404.09 $\pm$ 4.08	5.44 $\pm$ 0.74	2149.34 $\pm$ 3.40	1245.99 $\pm$ 10.52	556.64 $\pm$ 13.59	678.39 $\pm$ 3.58
		Average	56.56 $\pm$ 3.26 ^a	0.18 $\pm$ 0.01 ^c	329.48 $\pm$ 9.11 ^d	4.06 $\pm$ 0.47 ^c	1218.24 $\pm$ 3.76 ^e	981.15 $\pm$ 6.09 ^c	513.50 $\pm$ 2.68 ^b	523.66 $\pm$ 14.16 ^b
Ivești	10	Range	31.31 $\pm$ 1.52	0.19 $\pm$ 0.07	630.06 $\pm$ 5.46	7.54 $\pm$ 0.09	748.06 $\pm$ 8.97	334.18 $\pm$ 11.39	261.35 $\pm$ 6.13	134.46 $\pm$ 7.73
		Range	53.40 $\pm$ 2.42	0.26 $\pm$ 0.04	698.66 $\pm$ 0.34	4.46 $\pm$ 0.57	1985.70 $\pm$ 2.14	875.52 $\pm$ 26.31	775.65 $\pm$ 12.55	329.50 $\pm$ 5.84
		Average	47.12 $\pm$ 2.46 ^b	0.35 $\pm$ 0.02 ^{ab}	705.76 $\pm$ 3.20 ^a	5.36 $\pm$ 0.21 ^{ab}	1360.60 $\pm$ 25.06 ^d	750.84 $\pm$ 9.23 ^d	619.22 $\pm$ 6.13 ^a	250.50 $\pm$ 4.93 ^d
Panciu	6	Range	32.57 $\pm$ 2.82	0.25 $\pm$ 0.06	125.41 $\pm$ 4.01	4.39 $\pm$ 0.45	1954.20 $\pm$ 0.96	1231.76 $\pm$ 15.87	327.89 $\pm$ 11.95	219.39 $\pm$ 4.87
		Range	40.39 $\pm$ 1.56	0.38 $\pm$ 0.15	334.05 $\pm$ 11.25	6.69 $\pm$ 0.23	3853.75 $\pm$ 10.65	1343.69 $\pm$ 19.56	517.10 $\pm$ 4.23	749.98 $\pm$ 9.26
		Average	39.26 $\pm$ 0.70 ^c	0.29 $\pm$ 0.07 ^b	531.99 $\pm$ 6.15 ^b	4.60 $\pm$ 0.08 ^{bc}	2046.71 $\pm$ 22.50 ^b	1241.05 $\pm$ 9.44 ^a	514.16 $\pm$ 8.99 ^b	580.40 $\pm$ 3.32 ^a
Obobești	10	Range	52.26 $\pm$ 3.02	0.25 $\pm$ 0.07	284.92 $\pm$ 3.01	1.80 $\pm$ 0.60	789.40 $\pm$ 5.96	357.89 $\pm$ 3.69	135.54 $\pm$ 5.81	364.60 $\pm$ 6.32
		Range	64.85 $\pm$ 5.88	0.46 $\pm$ 0.06	631.00 $\pm$ 7.62	4.97 $\pm$ 0.36	4140.58 $\pm$ 16.84	1642.54 $\pm$ 17.09	424.43 $\pm$ 10.67	457.58 $\pm$ 10.30
		Average	57.98 $\pm$ 2.05 ^a	0.39 $\pm$ 0.02 ^a	519.43 $\pm$ 7.69 ^c	5.87 $\pm$ 0.91 ^a	3156.96 $\pm$ 12.38 ^a	1037.15 $\pm$ 11.20 ^b	335.48 $\pm$ 9.01 ^c	403.38 $\pm$ 4.26 ^c
Dealul Mare	23	Range	44.29 $\pm$ 7.80	0.20 $\pm$ 0.02	171.05 $\pm$ 3.07	2.72 $\pm$ 0.47	1244.03 $\pm$ 12.19	854.35 $\pm$ 12.37	125.13 $\pm$ 6.70	159.63 $\pm$ 7.98
		Range	58.46 $\pm$ 5.71	0.40 $\pm$ 0.04	207.76 $\pm$ 2.32	3.28 $\pm$ 0.26	2395.32 $\pm$ 71.77	1255.02 $\pm$ 0.98	307.29 $\pm$ 7.66	356.58 $\pm$ 11.83
		Average	60.67 $\pm$ 8.11 ^a	0.32 $\pm$ 0.07 ^{ab}	189.28 $\pm$ 0.47 ^e	3.00 $\pm$ 0.03 ^d	1991.83 $\pm$ 9.45 ^c	994.83 $\pm$ 9.14 ^c	198.39 $\pm$ 3.90 ^d	233.51 $\pm$ 10.79 ^e

Average value $\pm$ standard deviation (n = 3). Roman letters represent the significance of the difference (Duncan test, p < 0.05). The difference between any two values, followed by at least one common letter, is insignificant.

much lower than the upper limit values given by the by International Organization of Vine and Wine of 0.15 mg/L (O.I.V., 2016). Early studies in England have reported Pb concentration as high as 1840 µg/L resulting from lead cauldrons (Sherlock *et al.*, 1986). As stainless-steel boilers are used presently this problem seems to have been resolved.

To better understand the obtained results, concerning the distribution of these elements in wine they have been compared with previous studies. In terms of the results presented by Fiket *et al.* (2001) the concentration of Cd in Croatian wines ranges from 0.175 to 1.88 µg/L, and in Bohemian wines Cr, Ni and Pb levels ranges from 18.0-32.0 µg/L, 15.0-53.0 µg/L to 11.0-48.0 µg/L, respectively according to the results presented by Sperkova and Suchanek, (2005). Marengo and Aceato (2003) who studied wine samples from Nebbiolo area, assert that the levels of concentration of Cr in this wine area range from 20.0 to 50.0 µg/L. In the research done by Gremaud *et al.* (2004) there were determined elements such as Mn and Zn and their concentration ranged from 270 to 600 µg/L and 340 to 1140 µg/L, respectively. In terms of wines obtained in Romania these were analyzed by Geana *et al.* (2013) who analyzed Mn, Cu, Co, and Zn, the average concentration of Mn, Co, Cu and Zn were 806 µg/L, 4.35 µg/L, 501 µg/L and 434 µg/L respectively.

Regarding the results obtained for Cd and Co, these are comparable with other researches, but the concentration levels of Mn from the analyzed samples in most studies were lower than in this study.

Alkiş *et al.* (2014) investigated wine samples that were obtained in Turkey focusing on determination of Mn, Co, Cu and Zn and the concentration for Mn was (average 399 µg/L), Co (average 3.37 µg/L), Cu (average 145 µg/L) and Zn (average 1244 µg/L) respectively. The highest level of concentration for Pb in this research was 78.36 µg/L, which is lower than the O.I.V. limit (150 µg/L) (O.I.V., 2016) lead content in wine, in addition, the O.I.V. issued the highest levels on Cu (1000 µg/L) and Cd (10 µg/L) in wine, the highest Cu concentration (921.74 µg/L) and Cd concentration (0.83 µg/L) in this study was lower than this restrictive limit. Similarly, the highest levels of concentration for Zn (3126.63 µg/L) in all

the wines were far less than the O.I.V. limit of Zn (5000 µg/L).

The composition of elements (metals in particular) in wine during fermentation is not stable. During fermentation, the concentration of some metals is lower than that in grapes and must be due to the precipitation with tartrate (e.g. Ca and K) or other insoluble precipitation (e.g. Al, Cu, Fe, Mn). These changes can be accentuated by the changes in pH due to alcohol production (Pohl, 2007). During classification and storage to stabilize and conserve wine, metal content also decreases, the magnitude of the decrease depending on the harvest, grape quality and alcohol content, which normally increases with decreasing solubility of salts (Hernández *et al.*, 1996).

At clarification, metals are eliminated with sediments of proteins and sulphated amino acids. During storage, they are precipitated as sulfides (e.g. Cu) in reaction with H₂S originating from free SO₂ (Rodríguez-Mozaz *et al.*, 1999). A significant part of Cu, Fe, Mn, and Zn is removed by adding K₄Fe(CN)₆ and precipitating the respective ferrocyanides to prevent wine from being susceptible to browning and formation of metal cloudiness (Benítez *et al.*, 2002).

The concentration of heavy metals in grape juice samples decreases in the order Zn > Cr > Mn > Cu > Ni > Pb > Co > Cd for the majority of wine grape cultivars studied.

The distribution of element content in juice samples from Nicoreşti and Dealu Mare is similar, while in the remaining vineyards the difference is made by Cu, Mn, and Ni concentration. Pb records the highest concentration in Nicoreşti (56.56 µg/L) and Dealu Mare (60.67 µg/L) vineyard. Iveşti vineyard records high concentration of Ni (705.76 µg/L) and Cu (619 µg/L), while the Panciu and Odobeşti vineyards record the highest values (1241.05 µg Cr/L), (580.40 µg Mn/L) in the Panciu vineyard (5.87 µg Co/L), (3156.96 µg Zn/L) in Odobeşti vineyard respectively.

To evaluate the potential hazards that may arise from the long-term consumption of wine containing these elements, we referenced the concept of an estimated daily intake (EDI). In the researches done by Towle *et al.* (2017) in order to evaluate the potential hazards from long-term daily consumption of wine uses 195 mL daily drinking volume, while Zhi-Hao *et al.* (2019) for evaluation the estimated daily intake of elements

from wine used a daily drinking volume of 200 mL. For results to be applied to as many people as possible we selected 60 kg as the adult drinker average weight for calculation of estimated daily intake (EDI). For this, we divided the measured concentration levels of each element into two parts for evaluating: the used average concentration level (average) represented the general intake,

and the 95% confidence interval upper limit (P95) of the average concentration level represented the possible highest intake. The average values for EDI of Pb, Cd, Ni, Co, Zn, Cr, Cu and Mn for all wines were 0.111, 0.001, 0.821, 0.027, 5.803, 2.228, 1.668 and 1.380 $\mu\text{g/kg bw/day}$, respectively (Tab. 8 and 9). Thus, there were no adequate toxicity studies accessible to provide a foundation for the

Table 8. Estimated daily intake (EDI, $\mu\text{g/kg bw/day}$) of each element through the consumption of wine from five wine-growing areas

Vineyard		Pb	P95	Cd	P95	Ni	P95	Co	P95
Nicoresți	Range	0.046	0.053	0.001	0.001	0.643	0.657	0.013	0.034
		0.167	0.179	0.001	0.001	1.432	1.440	0.025	0.037
	Average	0.111	0.132	0.001	0.001	1.081	1.093	0.019	0.028
Ivești	Range	0.102	0.119	0.001	0.001	0.339	0.351	0.041	0.076
		0.321	0.336	0.001	0.001	0.791	0.834	0.086	0.102
	Average	0.233	0.238	0.001	0.001	0.651	0.689	0.064	0.088
Panciu	Range	0.038	0.044	0.001	0.001	0.448	0.489	0.009	0.012
		0.109	0.118	0.001	0.001	2.122	2.345	0.025	0.034
	Average	0.071	0.089	0.001	0.001	1.083	1.121	0.013	0.023
Odobești	Range	0.074	0.083	0.002	0.002	0.280	0.298	0.009	0.016
		0.085	0.097	0.004	0.005	1.112	1.176	0.047	0.062
	Average	0.052	0.071	0.003	0.004	0.701	0.798	0.031	0.044
Dealul mare	Range	0.048	0.056	0.001	0.001	0.422	0.469	0.009	0.016
		0.142	0.152	0.001	0.001	1.185	1.199	0.012	0.029
	Average	0.094	0.103	0.001	0.001	0.889	0.925	0.011	0.035
PTDI		-		¹ 0.833		12.00		-	

¹The PTDI ($\mu\text{g/kg/day}$) of Cd was calculated from provisional tolerable monthly intake (PTMI) of 25.00 $\mu\text{g/}$ body weight based on a month of 30 days (Zhi-Hao *et al.*, 2019).

Table 9. Estimated daily intake (EDI, $\mu\text{g/kg bw/day}$) of each element through the consumption of wine from five wine-growing areas

Vineyard		Zn	P95	Cr	P95	Cu	P95	Mn	P95
Nicoresți	Range	2.134	2.456	1.087	1.701	1.097	1.345	1.126	1.561
		7.141	8.701	6.158	7.246	2.091	3.451	1.891	2.318
	Average	5.849	6.893	3.441	4.809	1.559	1.809	1.543	1.943
Ivești	Range	2.523	2.934	0.649	0.789	1.428	1.788	0.443	0.546
		10.422	13.101	4.171	5.998	3.072	4.745	2.168	3.312
	Average	8.842	9.990	1.390	1.563	2.106	2.610	1.529	1.980
Panciu	Range	1.782	1.928	0.359	0.387	1.095	1.339	0.779	0.865
		9.591	10.897	2.135	2.786	1.405	1.682	1.781	1.971
	Average	7.120	9.728	1.554	1.887	1.287	1.410	1.272	1.436
Odobești	Range	1.393	1523	0.728	0.899	0.429	0.487	0.524	0.643
		5.789	6.909	2.072	2.245	2.091	2.503	1.429	1.897
	Average	3.088	3.451	1.895	2.556	1.629	1.908	0.926	1.321
Dealul mare	Range	2.796	2.997	0.730	0.823	0.734	0.612	1.195	1.213
		5.409	5.769	3.770	4.351	2.470	2.761	2.213	2.561
	Average	4.117	4.367	2.860	3.304	1.758	1.887	1.631	1.893
PTDI		1000.00		-		¹ 500.00		² 183.00	

¹The PTDI ($\mu\text{g/kg/day}$) of Cu was calculated from provisional tolerable weekly intake (PTMI) of 3500 $\mu\text{g/}$ body weight. ² The PTDI ($\mu\text{g/kg/day}$) of Mn was calculated from a tolerable daily intake of 11.0 $\mu\text{g/}$ body weight by 60 kg adult (Zhi-Hao *et al.*, 2019).

Table 10. Target hazard quotient (THQ) of trace elements from wines of different wine-growing areas

Vineyard		Pb	P95	Cd	P95	Ni	P95	Co	P95
Nicorești	Range	0.013	0.017	0.001	0.001	0.032	0.057	0.033	0.054
		0.048	0.069	0.001	0.001	0.072	0.098	0.100	0.231
	Average	0.032	0.046	0.001	0.001	0.054	0.065	0.067	0.089
Ivești	Range	0.029	0.034	0.001	0.001	0.017	0.026	0.133	0.156
		0.092	0.106	0.001	0.001	0.040	0.063	0.300	0.467
	Average	0.067	0.079	0.001	0.001	0.033	0.045	0.200	0.309
Panciu	Range	0.011	0.014	0.001	0.001	0.023	0.039	0.033	0.058
		0.031	0.039	0.001	0.001	0.106	0.136	0.100	0.147
	Average	0.020	0.031	0.001	0.001	0.054	0.075	0.033	0.053
Odobești	Range	0.021	0.027	0.002	0.003	0.014	0.028	0.033	0.049
		0.024	0.042	0.004	0.006	0.056	0.080	0.167	0.198
	Average	0.015	0.026	0.003	0.005	0.035	0.049	0.100	0.126
Dealul mare	Range	0.014	0.018	0.001	0.001	0.021	0.034	0.033	0.043
		0.041	0.065	0.001	0.001	0.059	0.076	0.033	0.029
	Average	0.027	0.034	0.001	0.001	0.045	0.061	0.033	0.032
Total		0.032	0.043	0.001	0.018	0.044	0.060	0.087	0.121

Table 11. Target hazard quotient (THQ) of trace elements from wines of different wine-growing areas

Vineyard		Zn	P95	Cr	P95	Cu	P95	Mn	P95
Nicorești	Range	0.007	0.009	0.363	0.468	0.220	0.354	0.008	0.013
		0.024	0.033	2.053	3.756	0.418	0.689	0.014	0.019
	Average	0.020	0.028	1.147	2.698	0.312	0.451	0.011	0.013
Ivești	Range	0.008	0.015	0.217	0.365	0.286	0.356	0.003	0.005
		0.035	0.053	1.390	1.764	0.614	0.896	0.016	0.023
	Average	0.029	0.033	0.463	0.687	0.422	0.577	0.011	0.026
Panciu	Range	0.006	0.009	0.120	0.187	0.218	0.365	0.006	0.009
		0.032	0.045	0.710	0.865	0.280	0.290	0.013	0.019
	Average	0.024	0.036	0.517	0.780	0.258	0.298	0.009	0.015
Odobești	Range	0.005	0.007	0.243	0.321	0.086	0.099	0.004	0.006
		0.019	0.023	0.690	0.875	0.418	0.789	0.010	0.013
	Average	0.010	0.015	0.633	0.874	0.326	0.376	0.007	0.008
Dealul mare	Range	0.009	0.013	0.243	0.341	0.146	0.187	0.009	0.012
		0.018	0.023	1.257	1.632	0.494	0.785	0.016	0.023
	Average	0.014	0.016	0.953	1.435	0.352	0.477	0.012	0.014
Total		0.019	0.026	0.743	1.295	0.334	0.436	0.010	0.015

observed rate of adverse effect (NOAEL). Based on a 60-kilogram adult person and moderate consumption of 200 milliliters of wine/day, the estimated daily intake of these inorganic components from wines was well below the daily allowance. The target hazard quotient suggests that the exposed humans would not experience significant health risks when ingesting these individual elements from daily consumption of 200 milliliters of Romanian wines/day.

The THQ was the ratio of the estimated dose of elements from wines to a corresponding reference dose, and this approach offered an indication of the risk level due to reference doses of Pb, Cd, Ni,

Co, Zn, Cr, Cu, and Mn which were 3.50, 1.00, 20.00, 0.30, 300.00, 3.00, 5.00 and 140.00 $\mu\text{g/kg bw/day}$, respectively. An exception is made by the Nicorești vineyard, which in the case of Cr records light values above 1 (average 1.147), the higher THQ values showed a slightly potential of exposed risk. The THQ values of each estimated element did not exceed 1 in all areas, suggesting that the exposed population would not experience significant health risks when ingesting these individual elements from daily consumption of 200 mL of Romanian wines. The total THQ values used to estimate the cumulative health risk effect were calculated by the sum of the THQ values of these elements,

commonly referred to as HI. In Tables 10 and 11, the HI values of different wine areas were also less than 1, and the highest HI values were 0.743 and 1.295 (P95). It showed that these elements did not cause health problems to people through the daily consumption of 200 mL of Romanian wine, but the risks that might be presented were higher in the case of Cr level concentration. The THQ values of Cr accounted for the highest average proportions of the total THQ values 47%, and also the average of contribution proportions of Cu and Mn of the total THQ values 21% ranked second. In this research the THQ also represented the contribution of wine to contaminants in the acceptable range for daily diet, the average THQ of Cr was 0.743, which meant that the contribution of wine consumption to the tolerable daily intake of Cr was 74.3%. Risk assessment for a specific contaminant intake comprehensive consideration of all intake mechanisms, and wine consumption was just one such path, the amount of wine consumption was, therefore, more important for health risk assessment of wine in the daily diet of drinkers.

Conclusion

The concentration of Pb, Cd, Ni, Co, Zn, Cr, Cu and Mn in 321 wine and grape juice samples sourced from the five main Romanian wine-producing regions was determined. The results from this research suggested that the levels of concentration elements in red wines decreased in the order of Zn > Mn > Cr > Cu > Ni > Pb > Co > Cd, and in white wines decreased in the order of Zn > Mn > Cu > Cr > Ni > Pb > Co > Cd. The Ivești and Nicorești vineyard-areas had a higher concentration level in Pb than the other studied vineyard-areas. The measured concentration levels of Cd showed that all vineyard-areas had the same levels except Odobești area, which records high concentration in Cd. The Ni concentration was higher in the Nicorești and Panciu vineyards, Co, Zn and Cu in Ivești vineyard, Cr and Mn in the Nicorești and Dealu Mare respectively.

The daily estimated intake of these elements potentially hazardous to health for wine consumers, assuming a daily wine consumption of 200 mL wine for 60 kg drinkers, was much lower than the daily tolerable intake for this character.

The THQ values of each element did not exceed the value of 1 for all the studied areas,

which means that the population that consumes 200 mL of Romanian wine ever day not exposed to any risk, an exception is made by the wine obtained in Nicorești vineyard in case of Cr the value obtained is a slight value above 1 (average 1.147), which indicates a slight probability of exposed risk. The HI showed that consuming 200 mL daily of wine does not pose a threat to the tolerable daily intake of potential risk elements in the daily diet. High values for Cr, Cu, and Mn were obtained regarding THQ which meant that were major contaminants and needed more attention to them. On the other side, the wine consumption in the daily diet of drinkers was more important for comprehensive health risk assessment due to the presence of other intake pathways. Therefore, the cumulative impact of wine consumption on trace elements intake in the daily drinkers should not be ignored in the future.

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