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Monitoring The Content of Heavy Metals in The Soil and Wines from Different Wine-Growing Areas of Romania

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ABSTRACT: Determination of heavy metals in soil and wine is very important for wine quality and consumer health, but at the same time it is a complementary tool for differentiating wines according to their geographical origin. In this work, soils and ten brands of wines originated from four major wine-growing areas of Romania (Iaşi, Babadag, Ştefăneşti and Blaj) were considered for performing a multielement (Cr, Ni, Cu, As, Pb, Hg, V, Sr, Mn, Zn, Fe) investigation in order to assess regional specificity. Heavy metals found in the investigated wines were below the limits imposed by European standards (O.I.V.) the elements concentrations following the trend: Zn>Fe>Mn>Sr>Ni>Cr>Cu>Pb>V>Hg>As. For the soils analyzed, the heavy metals content was found to be below the maximum permitted limit, except for Cu (135.45 mg/kg) from the Iaşi vineyard, which was at the permitted limit. The high values obtained for copper may be the result of different treatments with the Bordelaise solution for the vine. The elements CA, Mn and Hg observed from the research that they are predominantly of natural origin. Based on our results, a distinction has been made between wines according to their geographical origin, considering that the specific elements of discrimination Sr, Pb, and Ni for the Stefănesti vineyard (Muntenia region), Cr and Hg for the Babadag vineyard (Dobrogea region), Sr and V for the Iaşi vineyard (region of Moldova), respectively Fe and Zn for the Blaj vineyard (Transilvania region).

KEYWORDS: Wine, soil, heavy metals, ICP-MS, HR-CS-AAS, wine-growing area

I. INTRODUCTION

Obtaining wine was one of the oldest occupations of the Romanians, reaching the time of the dacians and romans. Wine was among the first alcoholic beverages produced and the most widespread in Romania. A special importance was reserved for the classification of wines by varieties, geographical areas and years of production. (Arvanitoyannis et al, 1999; Serrano-Lourido et al, 2012; Boschetti et al., 2013; Košir et al, 2014; Dugo et al, 2015). Most illegalities at wines are made by declaring some wines being obtained from noble and pure grape cultivars. The wines can be obtained by blending from various grape cultivars; therefore a special importance is manifested for the classification and identification of wine varieties (Huang et al, 2017).

Research into the evaluation of the authenticity of food products has increased in recent years and particular attention is paid to their quality and composition. Evaluation of these products involves the determination of food characteristics such as nutritional parameters, shelf life and healthiness (Woldemariam et al, 2011; Danezis et al, 2016; Zava et al, 2020). Due to food fraud, which can have a negative impact on human health and the economic market, producers and ordinary consumers have understood the benefits of protecting traditional or appreciated products (Everstine et al, 2013).

In order to identify the important compounds for the fingerprinting of the wine, it is done through the instrumental analysis. (Héberger et al, 2003; Franquet-Griell et al, 2004; Kiss et al, 2005; Regmi et al, 2012; De Villiers et al, 2012; Fabani et al, 2013; Huang et al, 2017) But lately, advanced analysis methods have used, such as the use of electronic tongues (Cetó et al, 2012) and chemical sensors (Gallagher et al, 2012). Various statistical analysis methods were used to characterize the wine, such as the analysis of elementary compounds in wine by PCA, the discriminant linear analysis (LDA) and the analysis of groups of elements (HCA). (Arvanitoyannis et al, 1999).

The important factors in the production and marketing of wine are authenticity and geographical origin. Various advanced analysis methods to identify the geographical origin of the wine were used, such as atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) (Almeida & Vasconcelos, 2003; Goamez et al, 2004; Coetzee et al, 2005; Geana et al, 2013).

Environmental, soil and climate factors are important in characterizing grape varieties and wines in terms of quality, quantity and flavor. (Bora, 2015). Environmental factors are the region, pedology, climate, soil type and composition (Kostic et al, 2010; Fiket et al, 2011; Huzum et al, 2012). Production factors, including: fertilizer and pesticide use, industrial pollution, wine transport and storage are anthropogenic factors leading to the pollution of vineyards (Tariba et al, 2011; Bora et al, 2015).

At present, vineyards are contaminated and still polluted, so that the heavy metal content of vineyards soils can lead to serious problems in plant productivity, food quality and endanger human health (Bora et al, 2015; Zava et al, 2020). Certain metals such as Fe, Cu and Zn are essential in the biological development of plants and other metals are extremely toxic even if they are in small quantities, such as Pb, As, Ni, Cr, Hg, V, Co, Mo and Cd (Gogoaşă et al, 2005; Tuzen, 2007; Geană et al, 2013).

For the origin of wines the most researched and determined heavy metals are: Fe, Rb, Cu, Cr, Co, Sb, Cs, As, Ag, Li, Sr, Al and Mn (Fiket et al, 2011; Geană et al, 2013; Bora et al, 2015). In Romania according to this research the most important heavy metals used to determine the geographical origin of wines are Mn and Sr (Geană et al, 2013). In this work an investigation of heavy metal content profile (Cr, Ni, Cu, As, Pb, Hg, V, Sr, Mn, Zn and Fe) of soil from four important Romanian vineyards and some wine brands of Muntenia (Stefanesti wine-growing area), Dobrogea (Babadag vineyard), Transilvania (Blaj wine-growing area) and Moldova (Iasi wine-growing area) regions is performed in an attempt to achieve an overview on the heavy metals profile and to differentiate wines by regions. For the classification of wines according to geographical origin, a multiple items analysis was applied in combination with statistical modelling of multi-output data.

II. MATERIALS AND METHODS

The study is carried out in four different geographical locations in Romania, on ten assortments of wine (Chardonnay and Fetească Neagră from Babadag vineyard - Dobrogea region; Fetească Albă and Cabernet Sauvignon from Iasi wine-growing area - Moldova region; Muscat Ottonel and Traminer Roz from Blaj wine-growing area - Transilvania region; respectively Fetească Albă, Riesling Italian, Fetească Regală, and Burgund from – and Stefanesti wine-growing area - Muntenia region) from the harvest of 2019. The coordinates for each studied geographical area are: wine centre Iasi - 47°12‹42,5”N, 27°31‹47,1”E, 120 m altitude, East exposure, the European road E 85 passes through county, at 12 km from the wine-growing area; Blaj wine-growing area - 46°18‹51,2”N, 23°92‹40,5”E, 278 m altitude, East exposure, the European road E 81 and highway A10 passes through county, at 23 km from the wine-growing area; Ştefăneşti wine-growing area - 44°51'N si 24°57' E, altitude 250 m, South exposure, the highway A1 passes through county, at 5 km from wine-growing area, and Babadag vineyard - 44°53’36”N 28°42’43”E, altitude 250 m, east exposure the European road E 87 passes through county, at 5 km from the vineyard (Figure 1).

Figure 1 .Location of the study areas for the analyzed wines

The characteristics of soils in the areas studied are: Iasi wine-growing area have salnic cernoziom soil, wine Blaj wine-growing area have soil with clay and with sarmatian marl content and Babadag vineyard have sand - clay medium clay, yellowish brown soil. The soil in Ştefăneşti wine-growing centre is of the class umbrisoils of eutricambosoil type, brown, typical eumezobasic with clay in situ; the texture of the soil is clay-sandy, clay-clay, without skeleton, and the soil tillage is done with plows, the mechanical presses at intervals, the manual hoeing in a row (Toti, 2017). In 2019, during the growing season temperatures were specific to the areas studied, a longer autumn and spring was observed. In these area autumn and winter is dry, while during winter quite high temperature were registered in recent years.

Prior to analyses by ICP-MS, the wine samples have to be carefully decomposed. The amount of 1.0 g of wine was digested using A closed iPrep vessels speed iwaveJ system MARS6 CEM One Touch (CEM Corporation, Matthews, North Carolina, USA) with concentrated acid (10 ml of HNO3 69%) according to a 2 steps temperature-controlled digestion program. Each resulted clear aliquot of the digest were quantitatively transferred to 25 ml volumetric flasks and filled to volume with ultrapure water.

For soils, an amount of 0.5 g was digested using the same closed iPrep vessels speed iwaveJ system MARS6 CEM One Touch (CEM Corporation, Matthews, North Carolina, USA) with the mixture of concentrated acids (10 ml of HNO3 69% + 1ml HCl 37%) according to a 2 steps temperature-controlled digestion program. The resulted sediment digests were quantitatively transferred to 50 ml volumetric flasks and filled to volume with ultrapure water.

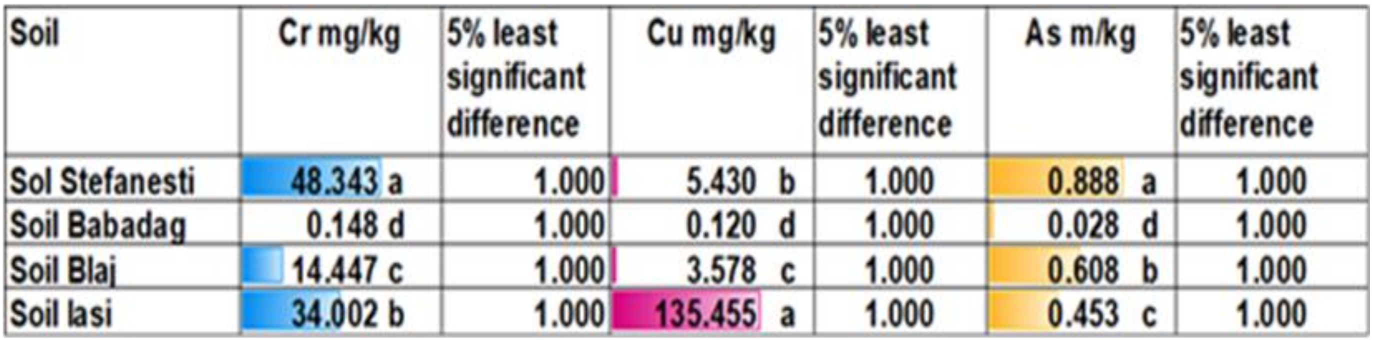
Analytical determinations of elements Cr, Ni, Cu, As, Pb, Hg, V, Sr, Mn, Zn and Fe were performed using an inductively coupled plasma mass spectrometer ICP-QMS 820-MS (Varian, Melbourne, Australia) equipped with an SPS-3 autosampler (Varian, Malgrave, Australia) and collision-reaction interface iCRI working in H2 and He modes. The optimal conditions were as follows: RF power of 1200 W, plasma gas-flow rate of 12 L/min, auxiliary gas-flow rate of 1.5 L/min, nebulizer gas-flow rate of 1.05 L/min, H2 gas flow rate of 90 mL/min, He gas flow of 90 mL/min and a dwell time of 50 ms for each isotope and 209Bi, 6Li, 45Sc, 159Tb, 89Y isotopes that served as internal standards. Blank samples were also prepared by following the analytical methodology mentioned above. These solutions were analysed by ICP–MS after appropriate dilution using external standards for calibration, considering five points on the curve and one for quality control. Calibration standard solutions and internal standards were prepared by successive dilution of a high purity ICP multi-element calibration standard (10 µg/L from twenty-nine elemental ICP-MS standards, matrix: 5% HNO3, Perkin Elmer Life and Analytical Sciences) and a mono-elemental calibration standard (10 µg/L Hg, matrix 5% HNO3, Perkin Elmer Life and Analytical Sciences).

Concentration of the compounds was evaluated by statistical methods. Authentication of the geographical origin of wines using multi-element analyses with multivariate was evaluated by statistical modelling. For the statistical interpretation of the results, the data were included in an Excel database and then statistically interpreted with the SPSS 14.0 program, and the comparison of means was performed with the Duncan`s multiple range test at 5% statistical assurance. The assessment of the degree of contamination / pollution of the soil-plant system, the results obtained were compared with the reference values provided by Order no. 756/1997 of the Minister of Waters, Forests and Environmental Protection, in the case of soil, and the values obtained in the case of wine analysis were reported to the Law on Vine and Wine but also to the regulations stipulated in O.I.V.

III. RESULTS AND DISCUSSION

Quantitative determinations for soil samples were made for 9 micro-elements Cr, Ni, Cu, As, Pb, Hg, V, Ag, Sr and 3 macro-elements Mn, Zn, Fe. Comparison of the Cr, Cu, and as level in the studied soils was performed with the Duncan’s multiple range test at significance level a = 0.05 ($P \leq 0.05$). There is a rather large difference in the soil from Iasi wine-growing area versus the other regions as concern the Cu (135,455 mg/kg), but also for Cr (34,002). Equally high values are found on the soil of Stefanesti wine-growing area for the Cr metal (48,343). The lowest values of these metals are found on the soil of Babadag vineyard. In table 1 can be seen that the values of the analyzed indicators are very different from one region to another and different statistically significantly for an insurance level of 5%. The distribution of copper from the analyzed soils is differentiated, namely: the soil from Stefănești vineyard (5,430 mg/kg) recorded the highest concentrations, at the opposite pole with the lowest concentrations were recorded in the soils of Iasi Vineyard, the differences between them being statistically assured. The variation range of the As concentration in the soils was between the maximum value of 0.888 mg/kg registered in the soils from Stefanești, followed by 0.608 mg/kg - the soils from Blaj, 0.453 mg/kg - the soils from the Iasi area, and 0.028 mg/kg in Babadag soils, the differences between them being significant for an insurance level of 5%.

Table 1. The Level of Cr, Cu, As Elements in the Soils Studied



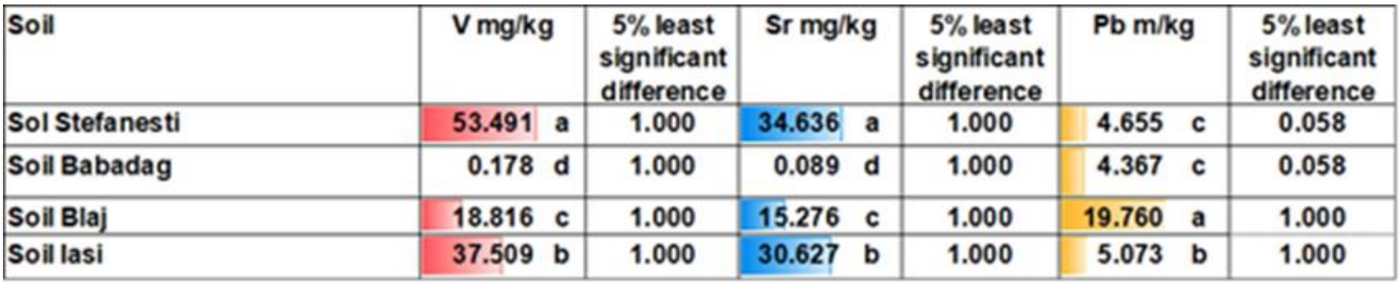
Soil	Cr mg/kg	5% least significant difference	Cu mg/kg	5% least significant difference	As m/kg	5% least significant difference
Sol Stefanesti	48.343 a	1.000	5.430 b	1.000	0.888 a	1.000
Soil Babadag	0.148 d	1.000	0.120 d	1.000	0.028 d	1.000
Soil Blaj	14.447 c	1.000	3.578 c	1.000	0.608 b	1.000
Soil Iasi	34.002 b	1.000	135.455 a	1.000	0.453 c	1.000

*Values with different letters are statistically different at 5% probability, Duncan test

In table 2 analyses the V, Sr and Pb elements using Duncan`s multiple range test. Is observed a very high value in the content of Pb in the soil of the wine centre Blaj (19,76 mg/kg), the other wine-growing centers have a lower content of Pb in the soil, the differences between the values being statistically assured. According to the analysed data the Sr content of the soil has values close in the Stefanesti and Iasi wine-growing area (with values between 30-34,636 mg/kg), but statistically different. The lowest value is found to Babadag vineyard (0,089mg/kg), followed by the wine centre Blaj (15,276 mg/kg).

The highest content of V in the soil was registred at the wine centre Ștefănești (53,491mg/kg) and the lowest in the soil of Babadag wine growing area (0,178 mg/kg).

Table 2. THE LEVEL OF V, SR AND PB ELEMENTS IN THE SOILS STUDIED

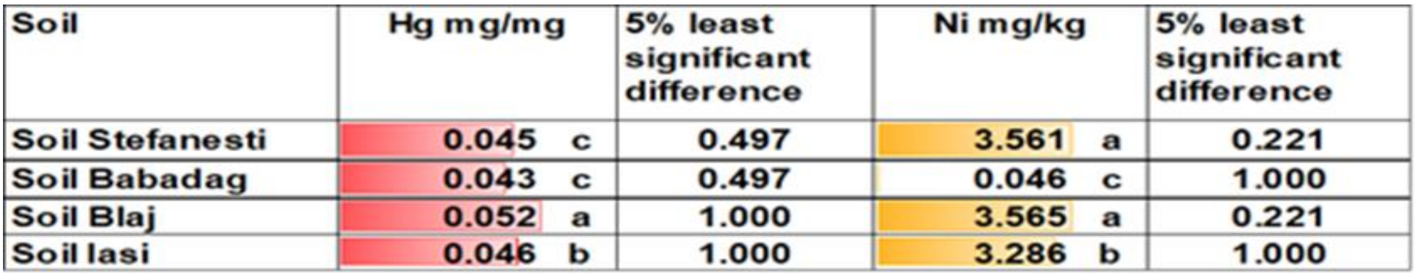


Soil	V mg/kg	5% least significant difference	Sr mg/kg	5% least significant difference	Pb m/kg	5% least significant difference
Sol Stefanesti	53.491 a	1.000	34.636 a	1.000	4.655 c	0.058
Soil Babadag	0.178 d	1.000	0.089 d	1.000	4.367 c	0.058
Soil Blaj	18.816 c	1.000	15.276 c	1.000	19.760 a	1.000
Soil Iasi	37.509 b	1.000	30.627 b	1.000	5.073 b	1.000

∗Values with different letters are statistically different at 5% probability, Duncan test

In Table 3 showed although the values of Hg content in the soil are close, but differences between them are statistically significant within the geographical areas studied, the highest value being in the Blaj region. (0,052 mg/kg Hg) and the lowest values were highlighted in the soils of Babadag and Stefănești (0.043 mg/kg, 0.045 mg/kg Hg, respectively). In the case of Ni, the lowest value is found on the soil from Babadag vineyard (0,046 mg/kg), and the highest were highlighted in Stefanesti and Blaj wine-growing area (3,561mg/kg, 3,565 mg/kg respectively, table 3), the differences between them being statistically assured. Comparing the nickel concentration to the national legislation in force, it can be seen that it is within the normal permissible limits (20 mg / kg), and it is not possible to speak in the case of these vineyards as being polluted with Ni (Bora, 2019).

Table 3. THE LEVEL OF HG AND NI METALS IN THE SOILS STUDIED



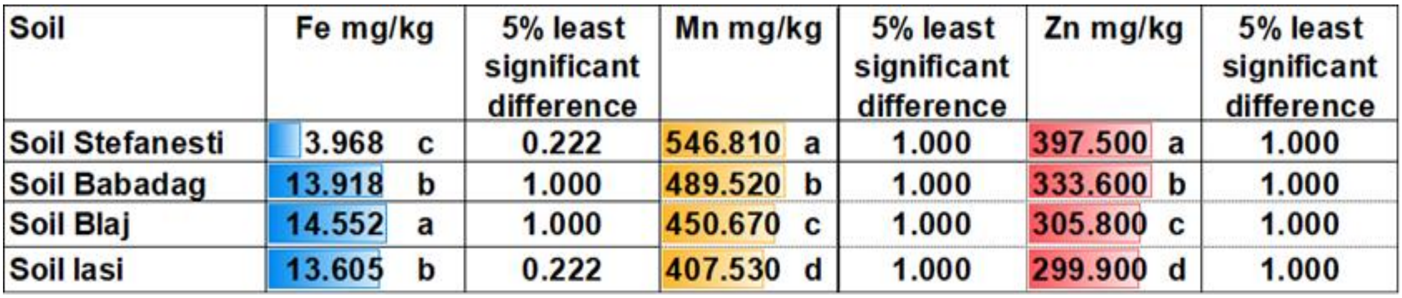
Soil	Hg mg/mg	5% least significant difference	Ni mg/kg	5% least significant difference
Soil Stefanesti	0.045 c	0.497	3.561 a	0.221
Soil Babadag	0.043 c	0.497	0.046 c	1.000
Soil Blaj	0.052 a	1.000	3.565 a	0.221
Soil Iasi	0.046 b	1.000	3.286 b	1.000

∗Values with different letters are statistically different at 5% probability, Duncan test

In the case of Zn and Mn content in the soil, their values are very small for all areas studied and differ statistically significantly between each studied geographic area. As far as Fe is concerned, the lowest content is found in the soil of Babadag vineyard, and the highest content was highlighted in the Blaj vineyard, the differences between them being statistically insured (Table 4).

Approximate values of Fe content in the soil were recorded in the Babadag and Iasi wine-growing area, including in the same class of statistical homogeneity for an insurance level of 5% (13,918 mg/kg, respectively 13,605 mg/kg).

Table 4. THE LEVEL OF FE, MN AND ZN METALS IN THE SOILS STUDIED



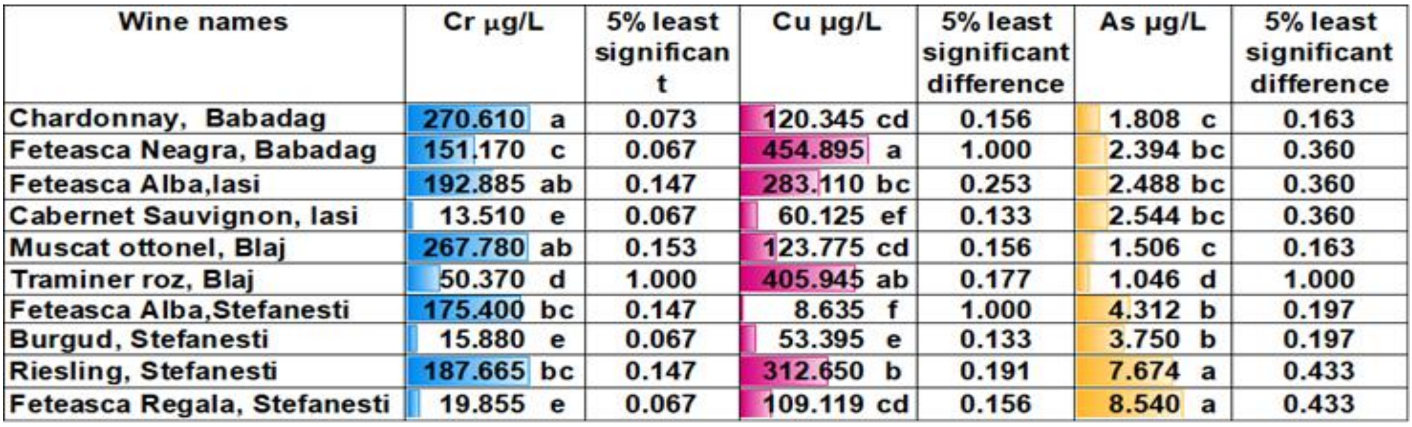
Soil	Fe mg/kg	5% least significant difference	Mn mg/kg	5% least significant difference	Zn mg/kg	5% least significant difference
Soil Stefanesti	3.968 c	0.222	546.810 a	1.000	397.500 a	1.000
Soil Babadag	13.918 b	1.000	489.520 b	1.000	333.600 b	1.000
Soil Blaj	14.552 a	1.000	450.670 c	1.000	305.800 c	1.000
Soil Iasi	13.605 b	0.222	407.530 d	1.000	299.900 d	1.000

∗Values with different letters are statistically different at 5% probability, Duncan test

In table 5 is expresses the content of Cr, Cu and As elements from 10 wine varieties, of the four geographic areas subject to the study. The highest Cr content meets in the Chardonnay wine in Babadag vineyard, and the smallest concentrations in red wine varieties in Iasi and Stefanesti wine-region (Cabernet Sauvignon – 13,15 µg/l and Burgund - 15,88 µg/l).

The content of wine may also depend on the treatments given to vines (Ivanova et al., 2013, Bora et al., 2015). The highest value of the contents meets at the Feteasca Neagra from Babadag vineyard (454,895 µg/l) and the smallest content in Feteasca Alba wine from Stefanesti vineyard (8,635 µg/l). In the case of As content, the highest values, but which do not differ statistically significantly, were highlighted in the Feteasca Regala and Riesling wines, both from the Ştefăneşti region. All other wines have registered lower values regardless of the wine-region (Table 5).

Table 5. THE LEVEL OF CR, CU AND AS METALS IN THE WINES ANALYZED

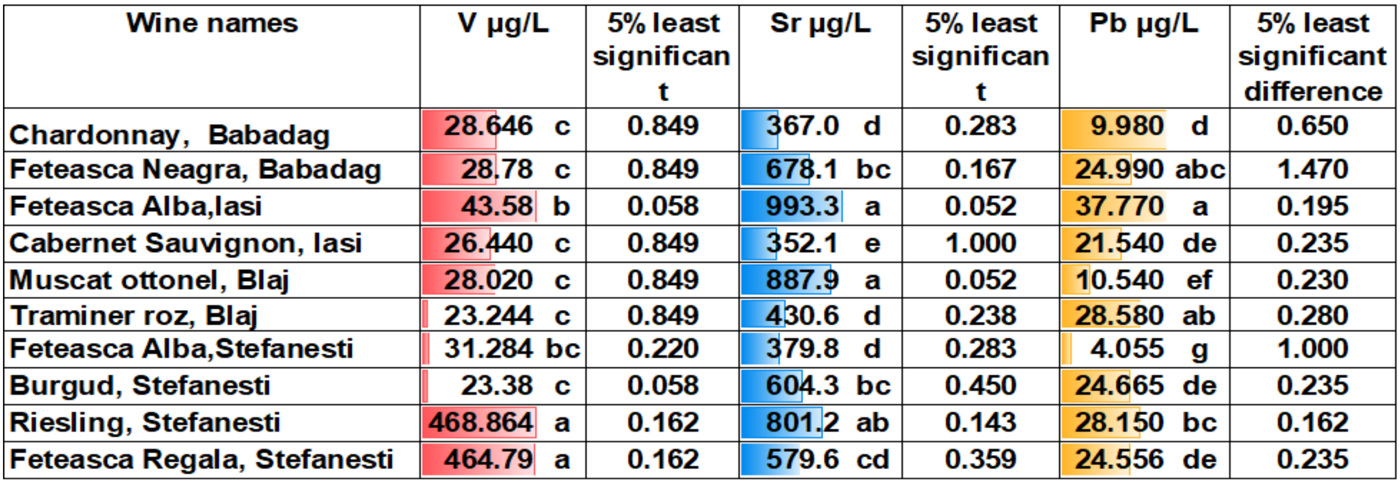


Wine names	Cr µg/L	5% least significant	Cu µg/L	5% least significant difference	As µg/L	5% least significant difference
Chardonnay, Babadag	270.610 a	0.073	120.345 cd	0.156	1.808 c	0.163
Feteasca Neagra, Babadag	151.170 c	0.067	454.895 a	1.000	2.394 bc	0.360
Feteasca Alba,Iasi	192.885 ab	0.147	283.110 bc	0.253	2.488 bc	0.360
Cabernet Sauvignon, Iasi	13.510 e	0.067	60.125 ef	0.133	2.544 bc	0.360
Muscat ottonel, Blaj	267.780 ab	0.153	123.775 cd	0.156	1.506 c	0.163
Traminer roz, Blaj	50.370 d	1.000	405.945 ab	0.177	1.046 d	1.000
Feteasca Alba,Stefanesti	175.400 bc	0.147	8.635 f	1.000	4.312 b	0.197
Burgud, Stefanesti	15.880 e	0.067	53.395 e	0.133	3.750 b	0.197
Riesling, Stefanesti	187.665 bc	0.147	312.650 b	0.191	7.674 a	0.433
Feteasca Regala, Stefanesti	19.855 e	0.067	109.119 cd	0.156	8.540 a	0.433

∗Values with different letters are statistically different at 5% probability, Duncan test

In table 6, the V element is distinguished by a very high concentration in white wine varieties in Ştefăneşti (Riesling - 468,864µg/l and Feteasca Regala - 464.79 µg/l), the other wines fall between values 23,38 µg/l Burgund from Stefanesti wine center and 43.58 µg/l for Feteasca Alba variety from Iasi wine-centre. The Sr content of the analysed wines is among the only element that determines the geographical origin of wines. The largest concentration is at the wine of Feteasca Regala from Iasi wine-center and the lowest concentration of Riesling wine from Ştefăneşti wine-center, expressed for white wines; in red wines, the highest concentration of Sr is located at the Feteasca Regala from Babadag vineyard and the lowest in Cabernet Sauvignon wine from the Iasi region.

Table 6. THE LEVEL OF V, SR AND PB METALS IN THE WINES ANALYSED



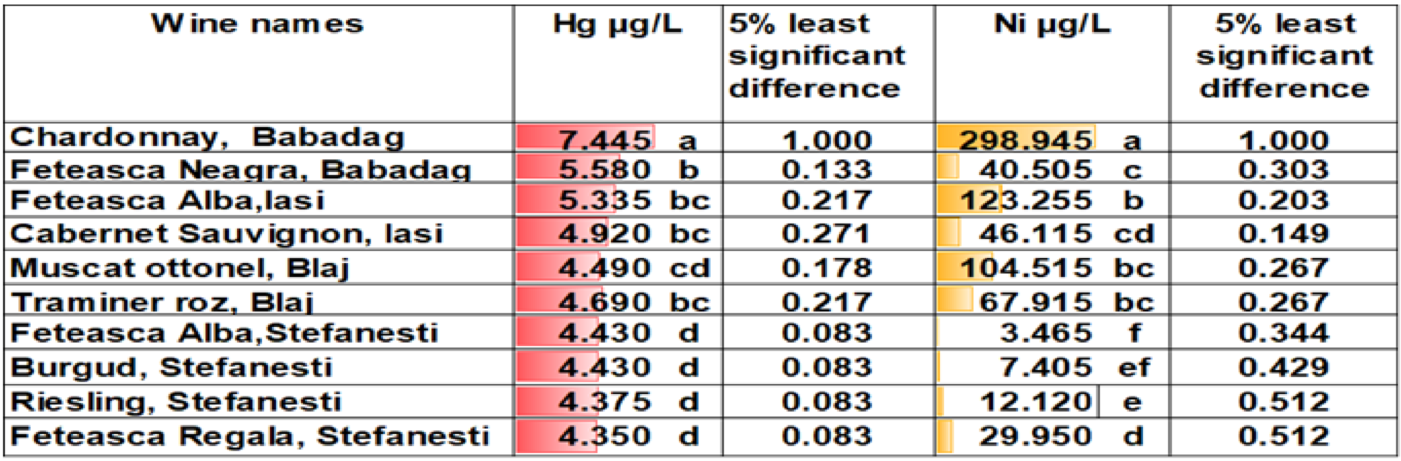
Wine names	V µg/L	5% least significant	Sr µg/L	5% least significant	Pb µg/L	5% least significant difference
Chardonnay, Babadag	28.646 c	0.849	367.0 d	0.283	9.980 d	0.650
Feteasca Neagra, Babadag	28.78 c	0.849	678.1 bc	0.167	24.990 abc	1.470
Feteasca Alba,Iasi	43.58 b	0.058	993.3 a	0.052	37.770 a	0.195
Cabernet Sauvignon, Iasi	26.440 c	0.849	352.1 e	1.000	21.540 de	0.235
Muscat ottonel, Blaj	28.020 c	0.849	887.9 a	0.052	10.540 ef	0.230
Traminer roz, Blaj	23.244 c	0.849	430.6 d	0.238	28.580 ab	0.280
Feteasca Alba,Stefanesti	31.284 bc	0.220	379.8 d	0.283	4.055 g	1.000
Burgud, Stefanesti	23.38 c	0.058	604.3 bc	0.450	24.665 de	0.235
Riesling, Stefanesti	468.864 a	0.162	801.2 ab	0.143	28.150 bc	0.162
Feteasca Regala, Stefanesti	464.79 a	0.162	579.6 cd	0.359	24.556 de	0.235

∗Values with different letters are statistically different at 5% probability, Duncan test

The Pb content of wines depends on the geographical area, pollution on national roads and highways near vineyards and also by the industrial areas in the vicinity of the studied vineyards (Ivanova et al., 2013; Bora et al., 2015). The highest value of the Pb content meets in Feteasca Alba wine from Iasi region (37,77 µg/l) and the lowest values in Chardonnay wine (9,98 µg/l) of Babadag vineyard and Feteasca Alba wine (4,055 µg/l) from the Stefanesti region. Based on the applied statistical analyzes, it can be seen that there are very significant differences between the analyzed variants (Table 6).

The Hg content of the wine is given by the diffuse, natural movement of the traces of metal elements in the soil (Catarino et al., 2006; Ivanova et al., 2013; Gheşeş et al., 2013; Bora et al., 2015; Zava and Cone., 2015; 2020), (Table 7). The Ni content of the wine is given the "natural" mineral content of the soil and the ability of vines to absorb and accumulate these elements in grape grains, products used in the agricultural practice or winemaking treatments. (Alkıs Mert et al, 2014; Bora et al, 2015). The highest content in the element is found in Cardonnay wine (298,945 µg/l) of Babadag vineyard and the lowest in Feteasca Alba (3,465 µg/l) from Ştefăneşti wine-center the differences between them being statistically assured (table 7).

Table 7. THE LEVEL OF HG AND NI METALS IN THE WINES ANALYSED



Wine names	Hg µg/L	5% least significant difference	Ni µg/L	5% least significant difference
Chardonnay, Babadag	7.445 a	1.000	298.945 a	1.000
Feteasca Neagra, Babadag	5.580 b	0.133	40.505 c	0.303
Feteasca Alba,Iasi	5.335 bc	0.217	123.255 b	0.203
Cabernet Sauvignon, Iasi	4.920 bc	0.271	46.115 cd	0.149
Muscat ottonel, Blaj	4.490 cd	0.178	104.515 bc	0.267
Traminer roz, Blaj	4.690 bc	0.217	67.915 bc	0.267
Feteasca Alba,Stefanesti	4.430 d	0.083	3.465 f	0.344
Burgud, Stefanesti	4.430 d	0.083	7.405 ef	0.429
Riesling, Stefanesti	4.375 d	0.083	12.120 e	0.512
Feteasca Regala, Stefanesti	4.350 d	0.083	29.950 d	0.512

∗Values with different letters are statistically different at 5% probability, Duncan test

The content of Fe in wine is given by wine production technology and the treatments given to vines (Jakubowski et al, 1999; di Paola-Naranjo et al, 2011; Grindlaya et al, 2011; Bora et al, 2015). The wine with the highest concentration of Fe is in Muscat Ottonel wine (12.309 mg / l) from the Blaj vineyard. According to the analysed data, the Mn content is given by the natural concentration in soil, and Zn content is given by the industrial area in the vicinity of the studied vineyards Table 8. The highest Zn content it is in Muscat Ottonel wine (10,041 mg/l) from Blaj wine region and the smallest concentration from Ştefăneşti wine-center in Feteascǎ Regala wine (2,182 mg/l).

Table 8. THE LEVEL OF HG AND NI METALS IN THE WINES ANALYSED

Wine names	Fe mg/L		5% least significant difference	Mn mg/L		5% least significant difference	Zn mg/L		5% least significant difference
Chardonnay, Babadag	0.794	e	0.468	1.006	b	0.072	5.960	d	1.000
Feteasca Neagra, Babadag	1.380	de	0.085	0.934	c	0.134	7.622	b	0.120
Feteasca Alba,Iasi	3.144	c	0.345	0.668	d	1.000	7.943	b	0.120
Cabernet Sauvignon, Iasi	0.550	e	0.468	1.288	ab	0.394	2.633	f	0.963
Muscat ottonel, Blaj	12.309	a	0.205	1.288	ab	0.394	10.041	a	1.000
Traminer roz, Blaj	6.633	b	1.000	1.346	a	0.322	7.030	c	1.000
Feteasca Alba,Stefanesti	1.39	de	0.085	1.064	b	0.072	3.722	e	0.083
Burgud, Stefanesti	0.36	cd	0.345	1.270	ab	0.394	2.642	f	0.963
Riesling, Stefanesti	0.202	f	0.205	1.206	b	0.072	3.362	e	0.083
Feteasca Regala, Stefanesti	0.288	f	0.205	0.914	c	0.134	2.182	g	1.000

∗Values with different letters are statistically different at 5% probability, Duncan test

Heavy wine metals are expressed in µg/l, except for Fe, Mn and Zn, expressed in mg/l (Figure 2).

For the latter, along with the calculated value, we have the standard error, which gives us the degree of inaccuracies (error) to the two indicators, in relation to their real values, at the level of the population at which the analysed data is assumed to be extracted. The comparative boxplot representations of Figure 2 make the relationships between the distributions analysed, reporting simultaneously at the same reference value (Figure 2) and the values of heavy metals concentrations in the analysed wines.

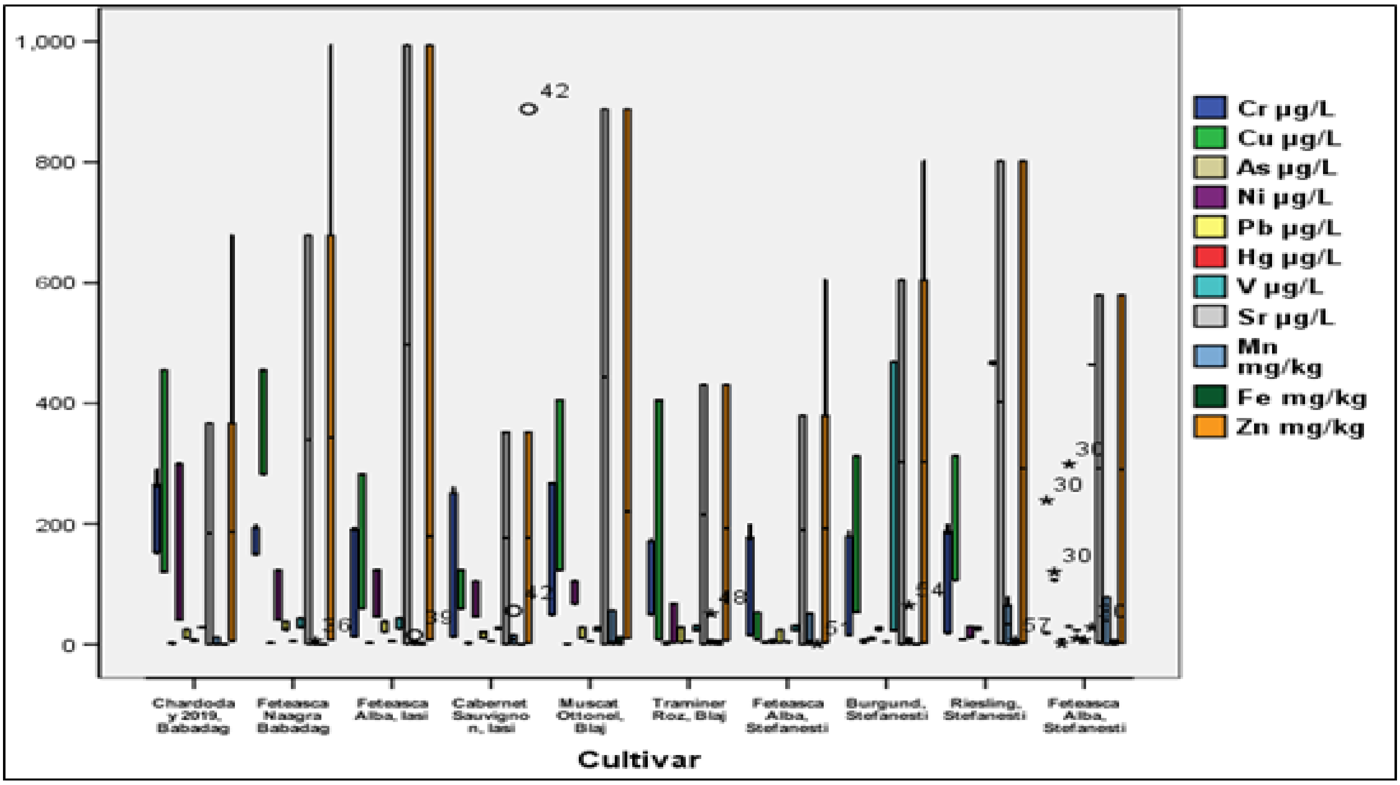


Figure 2. Heavy metals in wine

For the latter, along with the calculated value, we have the standard error, which gives us the degree of inaccuracies (error) to the two indicators, in relation to their real values, at the level of the population at which the analysed data is assumed to be extracted. The comparative boxplot representations of Figure 2 make the relationships between the distributions analysed, reporting simultaneously at the same reference value (Figure 2) and the values of heavy metals concentrations in the analysed wines.

Table 9. Results of Statistical Test to Check Normality for the Metals Analysed and the Gradations of the Wine Factor

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Cr µg/L	.209	60	.000	.854	60	.000
Cu µg/L	.276	60	.000	.860	60	.000
As µg/L	.262	60	.000	.806	60	.000
Ni µg/L	.229	60	.000	.721	60	.000
Pb µg/L	.231	60	.000	.888	60	.000
Hg µg/L	.239	60	.000	.701	60	.000
V µg/L	.454	60	.000	.531	60	.000
Sr µg/L	.310	60	.000	.804	60	.000
Mn mg/kg	.304	60	.000	.647	60	.000
Fe mg/kg	.368	60	.000	.629	60	.000
Zn mg/kg	.309	60	.000	.807	60	.000

In table 9, it is presented the results of statistical tests for normalization from the analysed metals and wine factor grades. Essentially, they test the degree of overlapping between the cumulative distribution of the variable analysed and the cumulative distribution of a variable whose distribution follows the Gaussian form.

Table 10 shows the statistical indicators of the central trend and distribution of the values of the indicators analysed for the 10 wines subjected to the tests throughout the sample: N (number of measured values and missing values); The minimum and maximum sample value, the maximum, medium, median and mode, standard deviation and asymmetry values and vault indicators (excess). For the latter, along with the calculated value, we have the standard error, which gives us the degree of inaccuracies (error) to the two indicators concerning their real values, at the level of the population at which the analysed data is assumed, is extracted. In our case, we notice that the normality test has P (sig.) Higher than 0.05 for the "heavy metal" variables, which confirms the normality hypothesis. The comparative boxplot representations of Figures 3 make the relations between the more expressive distributions, reported simultaneously at the same reference value (Figure 3), the values of heavy metals in the analysed wines.

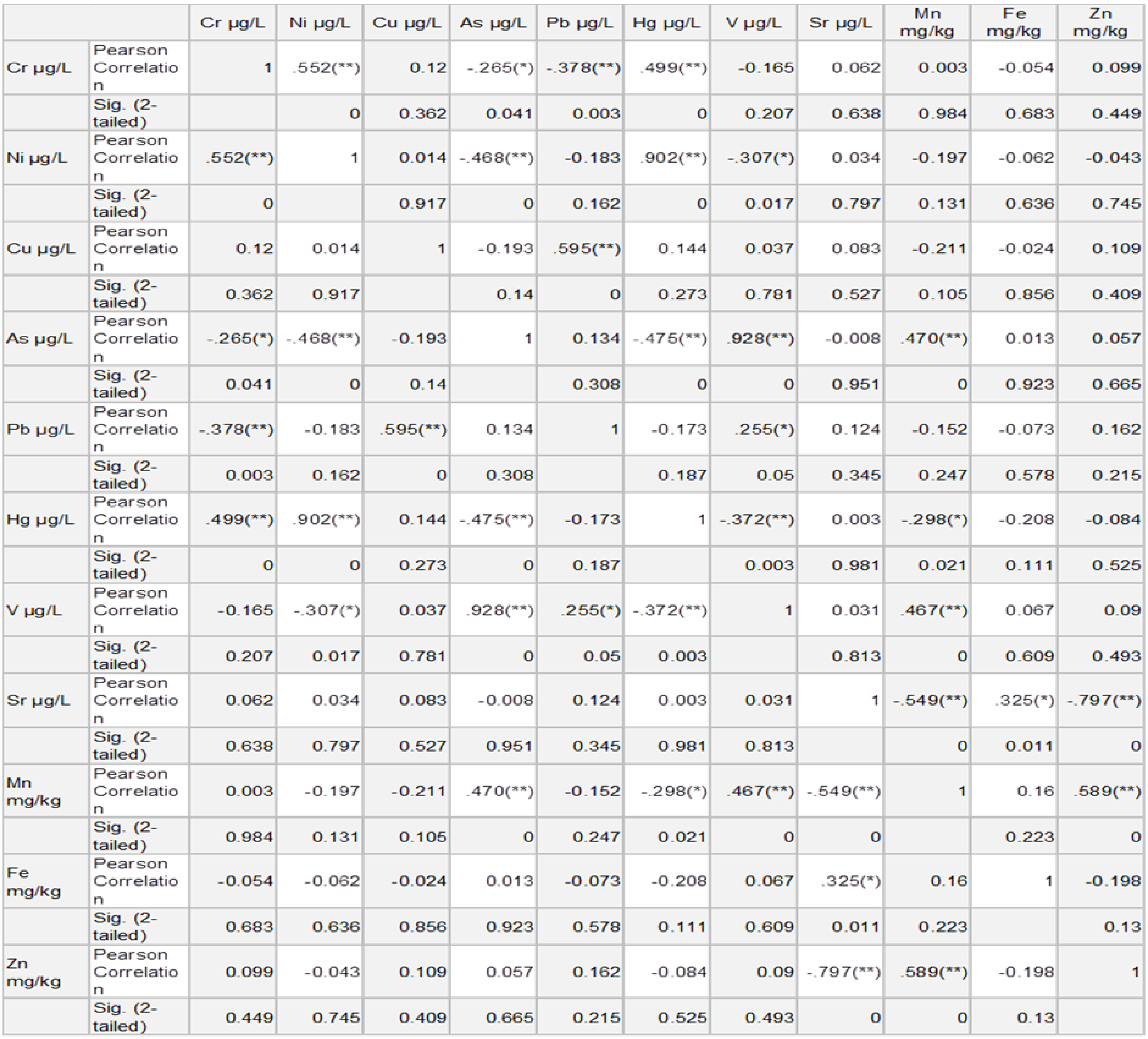
Table 10. Statistical Indicators of the Central Trend and the Spread of Heavy Metal Values Analysed From Wines Studied

	Cr µg/L	Cu µg/L	As µg/L	Ni µg/L	Pb µg/L	Hg µg/L	V µg/L	Sr µg/L	Mn mg/kg	Fe mg/kg	Zn mg/kg
Mean	129.365	192.749	3.7167	68.9686	21.725	4.971	123.9709	304.311	16.9161	2.0782	309.8845
Median	152.19	122.061	2.5385	40.5075	24.624	4.715	28.7715	177.753	1.482	0.7745	181.1225
Mode	15.88(a)	53.40(a)	1.05(a)	3.47(a)	4.06(a)	4.34(a)	23.38(a)	352.15(a)	1.29	.20(a)	2.18(a)
Std. Deviation	95.8001	151.3826	2.5263	79.9539	9.7064	0.857	181.8367	343.013	25.8851	3.23609	342.9337
Skewness	0.004	0.531	0.984	1.974	-0.399	1.997	1.408	0.616	1.47	2.162	0.59
Std. Error of Skewness	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309
Kurtosis	-1.539	-1.228	-0.492	3.466	-0.631	3.495	-0.014	-1.043	0.546	4.006	-1.094
Std. Error of Kurtosis	0.608	0.608	0.608	0.608	0.608	0.608	0.608	0.608	0.608	0.608	0.608
Range	277.09	446.27	7.51	297.49	33.71	3.11	445.63	993.22	78.33	12.63	991.11
Minimum	12.26	8.63	1.04	3.46	4.05	4.34	23.24	0.07	0.67	0.02	2.18
Maximum	289.35	454.9	8.55	300.95	37.76	7.45	468.87	993.29	79	12.65	993.29

In Table 11, the correlations between the indicators studied in wine show which we highlight below: it is noted that Cr element correlates positively with the elements: Cu, Sr, Mn and Zn (r=0.12, r=0.062, r=0.003, r=0.099), distinctly significantly positive with the elements Ni, Hg (r=0.552**, r= 0. 499**) and negative correlation with V, Fe (r=-0.165, r=-0.054), significantly negative with As (r= -0.265*) and distinctly significantly negative with Pb element (r=-0.378**), which explains that there is a balanced ratio between these indicators (Grindlay et al, 2009; Woldemariam, 2011; Bora et al, 2015; Zava et al, 2020).

The Hg element was correlated positively with Cu and Sr (r=0.144, r= 0.003), distinctly positive with Ni and Cr (r=0.902**, r= 0. 499**), and on the other hand negative correlation with Pb, Zn and Fe (r=-0.173, r=-0.208, r=-0.084), distinctly significant and significantly negative correlations with As, V and Mn (r=-0.475**, r=-0.372**, r=-0.298*). The Sr element is positively correlated with the elements: Cr, Ni, Cu, Pb, Hg, V (r=0.065, r=0.034, r=0.083, r=0.124, r=0.003, r=0.031), significantly positive with the Fe element (r=0.325*) and distinctly significant negative correlated with Mn and Zn (r=-0.549**, r=-0.797**).

Table 11. Correlations between the Elements Studied In Wine Cultivars



		Cr µg/L	Ni µg/L	Cu µg/L	As µg/L	Pb µg/L	Hg µg/L	V µg/L	Sr µg/L	Mn mg/kg	Fe mg/kg	Zn mg/kg
Cr µg/L	Pearson Correlation	1	.552(**)	0.12	-.265(*)	-.378(**)	.499(**)	-0.165	0.062	0.003	-0.054	0.099
	Sig. (2-tailed)		0	0.362	0.041	0.003	0	0.207	0.638	0.984	0.683	0.449
Ni µg/L	Pearson Correlation	.552(**)	1	0.014	-.468(**)	-0.183	.902(**)	-.307(*)	0.034	-0.197	-0.062	-0.043
	Sig. (2-tailed)	0		0.917	0	0.162	0	0.017	0.797	0.131	0.636	0.745
Cu µg/L	Pearson Correlation	0.12	0.014	1	-0.193	.595(**)	0.144	0.037	0.083	-0.211	-0.024	0.109
	Sig. (2-tailed)	0.362	0.917		0.14	0	0.273	0.781	0.527	0.105	0.856	0.409
As µg/L	Pearson Correlation	-.265(*)	-.468(**)	-0.193	1	0.134	-.475(**)	.928(**)	-0.008	.470(**)	0.013	0.057
	Sig. (2-tailed)	0.041	0	0.14		0.308	0	0	0.951	0	0.923	0.665
Pb µg/L	Pearson Correlation	-.378(**)	-0.183	.595(**)	0.134	1	-0.173	.255(*)	0.124	-0.152	-0.073	0.162
	Sig. (2-tailed)	0.003	0.162	0	0.308		0.187	0.05	0.345	0.247	0.578	0.215
Hg µg/L	Pearson Correlation	.499(**)	.902(**)	0.144	-.475(**)	-0.173	1	-.372(**)	0.003	-.298(*)	-0.208	-0.084
	Sig. (2-tailed)	0	0	0.273	0	0.187		0.003	0.981	0.021	0.111	0.525
V µg/L	Pearson Correlation	-0.165	-.307(*)	0.037	.928(**)	.255(*)	-.372(**)	1	0.031	.467(**)	0.067	0.09
	Sig. (2-tailed)	0.207	0.017	0.781	0	0.05	0.003		0.813	0	0.609	0.493
Sr µg/L	Pearson Correlation	0.062	0.034	0.083	-0.008	0.124	0.003	0.031	1	-.549(**)	.325(*)	-.797(**)
	Sig. (2-tailed)	0.638	0.797	0.527	0.951	0.345	0.981	0.813		0	0.011	0
Mn mg/kg	Pearson Correlation	0.003	-0.197	-0.211	.470(**)	-0.152	-.298(*)	.467(**)	-.549(**)	1	0.16	.589(**)
	Sig. (2-tailed)	0.984	0.131	0.105	0	0.247	0.021	0	0		0.223	0
Fe mg/kg	Pearson Correlation	-0.054	-0.062	-0.024	0.013	-0.073	-0.208	0.067	.325(*)	0.16	1	-0.198
	Sig. (2-tailed)	0.683	0.636	0.856	0.923	0.578	0.111	0.609	0.011	0.223		0.13
Zn mg/kg	Pearson Correlation	0.099	-0.043	0.109	0.057	0.162	-0.084	0.09	-.797(**)	.589(**)	-0.198	1
	Sig. (2-tailed)	0.449	0.745	0.409	0.665	0.215	0.525	0.493	0	0	0.13	

IV. CONCLUSIONS

Intensive wine production from Iasi viticulture area had like results an increase of Cu content in the soil, about tens of times larger (135,455 µg/kg) as compared to the other wine-producing geographical areas (Babadag vineyard – 0,120 µg/l), due to cultural practice on the ancestors of wine production and regarding the long term of treatments application with copper products.

The high concentration of Pb in soil (19,76 mg/kg), it is from Blaj geographic area, due to the pollution from the industry in the area and proximity to the A10 highway; this leads to a Pb content of the wine to being larger (Fetească Neagră - 24,99µ l); but the highest value is found in wine from Iasi viticulture region (Fetească Albă – 37,76 µg/l), although the Pb content in soil is lower there (5,073 mg/kg), due to the treatments performed on vines it is higher in wine. However, the other concentrations of toxic metal lead in wines were found in the range of 0,02-37,76µg/l; These values are quite low compared to the set limit of OIV as 0,2 mg/l.

The elements Cr, Ni, Sr, Cu, Pb, Hg and V were identified as indicators for the description of wines and soils for the four geographical areas studied. Concentration levels of all analysed elements in all examined wine samples were below the maximum contamination level established by O.I.V. regulations. In general, moderate wine consumption contributes to the daily nutritional requirements of many essential metals, including Fe, Zn, Mn, and Cu. The elements As, V, Pb, Hg, Cr, Ni, Mn, Zn were found to have a significant mutual correlation in analysed wines. They were also found to differ between the four wine-growing regions investigated. Since Sr, Hg, Mn are typical geological elements, their presence in wine is normally considered to reflect regional soil chemistry. The association of Pb and As with geological elements involves a predominant feature. In general, the values found are in line with the data previously reported on wines from another bibliographic source, a natural condition for these elements.

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