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Determination of Heavy Metal Content in Wine by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

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Abstract: The determination of minerals, trace elements and heavy metals in wine has a special importance both in the qualitative characterization of wines and in terms of consumers' health. At the same time, it can be an important factor in differentiating and authenticating wines based on their geographical origin. In this work is evaluated the content in heavy metals, minerals and trace elements by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), for five assortments of wines (Muscat Ottonel, Pinot Grigio, Fetească Regală, Merlot, Italian Riesling) obtained in the north-western area of Romania, in the wine-growing year 2021. Heavy metals found in the investigated wines were under the limits imposed by European standards.

Keywords: wine, heavy metals, minerals, ICP-OES.

Introduction

A topic of interest for modern oenology is the presence in wines of contaminating metals, especially heavy metals, both for the identification of various endogenous and exogenous sources that lead to the presence of metals in wine and the identification of ways to reduce the content in these metals by using various treatments allowed by the legislation in force (Țârdea et al., 2000).

Elemental composition was used to differentiate the wines according to grape type and geographical origin of wines produced in most important Romanian vineyards. The results show that the differentiation of Romanian wines according to their provenance is based on the following main elements: Ni, Ag, Cr, Sr, Zn, and Cu for Valea Călugărească, Rb, Zn, and Mn for Murfatlar, and Pb, Co, and V for Moldova (Geana et al., 2013). Studies regarding the elemental composition of wines were also performed in Turkey (Karataş et al., 2015) and Uruguay (González et al., 2013). Grigheli et al. (2013) studied the content of trace elements in different wine samples and juices. Detailed below are the most important elements found in wine.

Iron

The factors that can influence the endogenous presence of iron are: the soil, more or less rich in Fe and in assimilable phosphoric acid, the grape variety, the must clarification technology. Factors influencing the exogenous sources of iron presence are the soil that is mixed with the grapes at harvest, the redox conditions during and after alcoholic fermentation, the oenological equipment and practices used. Among the undesirable changes that appear in wines are those of a physico-chemical nature, caused by excess iron (Cotea, 1985).

Through the process of nutrition, the vine accumulates in the grapes small amounts of iron 2-3 mg/l. The subsequent enrichment of the wine with iron is mainly due to the exogenous iron that comes from the earth residues left on the grapes during winemaking, but also from the contact with the non-insulated metal parts of the machinery used in the winemaking, thus reaching up to 20-30 mg Fe/l (Țârdeaet al. 2000; Țârdea, 2007; Onache et al., 2022).

It should be noted that during alcoholic fermentation, due to the reducing environment that is created, a large part of the iron found in the must precipitates and is eliminated together with the wine yeast. Wines with a higher iron content that exceeds 9-10 mg/l are prone to ferric scrapping (Avram et al., 2014).

In research carried out on the Fe content of some varieties of wines from the Baia Mare and Șimleul Silvaniei area, it was found that the range of variation for the Fe concentration in the wine presented maximum values of 6.76±0.08 mg/l for the Fetească albă variety and 6.64±0.09 mg/l for the Italian Riesling variety, cultivated in the Baia Mare area and minimum values regarding the concentration of Fe in the wine, was recorded by the varieties grown in the Șimleul Silvaniei area (2.05±0.04 mg/l Fetească albă;

2.35±0.06 mg /lFetească Regală and 2.26±0.02 mg/l Italian Riesling) (Bora and Bunea, 2019, Bora et al., 2015) .

Other research on the concentration of Fe in wine carried out on commercial wines from Romania showed differences depending on the cultivation area, respectively: 2407.92 μg/l Merlot from the Oltenia area and 4298.90 μg/l Merlot from the Dobrogea area (Ceptura-Colinele Dobrogei) (Avram et al.,2014).

Up to 5 - 6 mg/l, iron fulfils a positive role, participating in a series of reactions that are the basis of good maturation and natural aging. Beyond these limits, it causes processes of instability and disturbances known as "ferric breakdown". It follows that, in order not to have unwanted situations, all possible sources of accumulation of the so-called "technological" iron must be eliminated from the start, something that can be achieved especially in the case of traditional winemaking, where tools and vessels are only made of wood (Cotea, 1985; Cotea et al., 2003).

Copper

The factors that can contribute to the presence of endogenous and exogenous copper are the cupric treatments of the vineyard, rains before the picking date, the amount of SO2 applied during vinification, the materials used in different technological operations or the oxidation-reduction levels during these operations.

In grapes and respectively in must, copper is a normal component that occurs naturally, being assimilated from the soil to the plant. In this situation, the copper concentration does not exceed 0.5 mg/l. The treatments applied to the vine with different organo-copper compounds lead to the increase of the copper content of the grapes and, respectively, of the must up to 2 - 3 mg/l. Not infrequently there were situations when 5-10 mg/l or even more was found in the must (Țârdea, 2007; Onache et al., 2022).

During fermentation, the largest amount precipitates in the form of copper sulphide, being eliminated together with the yeast, so that the new wine does not contain more than 0.2 - 0.3 mg/l(Țârdea, 2007).

Regarding the concentration of Cu in wine, from the central and north-western area of Transylvania it was found that the Fetească albă variety accumulated the largest amount 1.47±0.08 mg/l, cultivated in Baia Mare, followed by the Fetească varieties royal (1.12±0.04 mg/l) and the Italian Riesling variety (1.20±0.06 mg/l). The cultivars grown in the Șimleul Silvaniei area accumulated the lowest concentration of Cu (0.12±0.02 mg/l Fetească Regală variety; 0.25±0.01 mg/l Fetească albă variety and 1.20±0.06 mg/l Italian Riesling variety) (Bora and Bunea, 2019).

Zinc

The zinc content in wines is influenced by the winemaking technology, as regards the endogenous presence (Cotea et al., 2003). The presence of an exogenous nature is influenced by the treatments applied to the vineyards and the oenological products used, as well as by the materials used in various winemaking and wine preservation operations. Normally, the zinc content of wines oscillates between 0.1 - 6 mg/l, sometimes reaching up to 19 mg/l. The maximum allowed zinc concentration is 6 mg/l (HOTĂRÂRE nr. 512 din 20 iulie 2016).

Arsenic

Arsenic, As^{3+} as an element originating from the soil is present in wine in doses that do not exceed 0.02 mg/l. Like copper, it can reach the grapes through the use of arsenic-based fungicides and insecticides (Lupa et al., 2008). Drinking wine becomes dangerous when the dose of arsenic exceeds 1 mg/l. To eliminate the excess, "red gluing" is recommended, which consists in treating the wine with ferric oxide or treatment with sodium sulphide. Because it is toxic, wine regulations and legislation limit the maximum arsenic content in wines to 0.2 mg/l (HOTĂRÂRE nr. 512 din 20 iulie 2016).

Aluminium

Aluminium, Al^{3+}, gets into the wine from the soil particles that adhere to the grapes. In wines it is found between 1 – 10 mg/l (Onache et al., 2022). Its proportion increases as a result of the contact of the wine with the aluminum in the equipment or dishes, as well as in the case of clarifying the wine with different clays. The vineyard and wine law limits the maximum aluminium content in wines to 8 mg/l.

Manganese

Manganese, Mn_{2+} is found in all wines. It comes from the grape seed where it is not evenly distributed. The seeds are relatively richer in manganese than the skins and pulp. At equal weight, the seeds are 3 times richer in manganese than the skins and 30 times more than the pulp, which is why maceration raises the manganese content of the wines. Wines from directly produced hybrids contain

5-6 times more manganese than ordinary wines from European varieties whose content rarely exceeds 2 mg/l. This difference could be used as a criterion to distinguish the two categories of wines (Onache et al., 2022). Accidentally, the manganese content of wines can reach up to 10-20 mg/l. This situation occurs when wines have been fraudulently treated with potassium permanganate in order to desulphite dry white wines. Such practices are not allowed by wine laws. The presence of manganese in wine influences the oxidation-reduction phenomena that take place during its evolution.

Lead

The endogenous presence is related to the characteristics of the soil. The exogenous presence is due to the use of lead-tin capsules, other materials used in the technological route, the use of fungicides and certain oenological practices, distillation in the case of liqueur wines, atmospheric pollution and natural conditions. There is an optimal range of concentrations for each essential element.

Lead, $Pb2+$ is found in wines between 0.1 - 0.4 mg/l (Onacheet al., 2022). Its origin in wine is due to the soil, preparations (lead arsenate) used to treat vine diseases, cisterns lined with lead silicate, tin containers, vulcanized rubber tubes with a charge based on lead salts, metal capsules and in part of plastic containers, for the manufacture of which lead salts are usually used.

The maximum limit reached is 0.4 mg/l. Excess lead can be removed by treating wines with ferric ferrocyanide, when lead is replaced by iron following ion exchange.

Materials and methods

Biological material

As biological material, 5 varieties of wine were used (Muscat Ottonel, Pinot Grigio, Fetească Regală, Merlot, Riesling Italian, see Table 1) from a vineyard located in the northwest of Romania, the wine-growing area of the Diosig vineyard that includes the wine-growing centers Diosig, Săcuieni, Siniob, Biharia and Tileagd. The lithological substratum corresponds to sand and gravel and the relief is represented by the plains and the Piedmont hills of Oradea and Barcău. The climate is, in general, continental of central European type with influences of frosty air masses in winter and tropical in summer.

The analyses are part of the research objectives and topics proposed for the doctoral thesis with the title: "Research on the evaluation and characterization of some bioactive compounds in authentic wine assortments from the north-west of Romania", respectively the analysis of some wine compounds from the category minerals, trace elements and heavy metals, the latter, potentially affecting the safety of the wine and implicitly the health of the consumer. The analysed wine samples were taken from varieties obtained from the 2021 harvest.

Procedure: *Determination of heavy metal content by inductively coupled plasma optical emission spectrometry*

Principle of the method: Mass spectrometry is a method that involves the separation of ions using electric and magnetic fields, based on the ratio between mass and electric charge.

The instrumentation used:

•Berghoff microwave digester MWS-3+ (Eningen, Germany);

•ICP-MS ELAN DRC II Perkin-Elmer; Calibration curves for some of the investigated elements are shown in Figure 1.

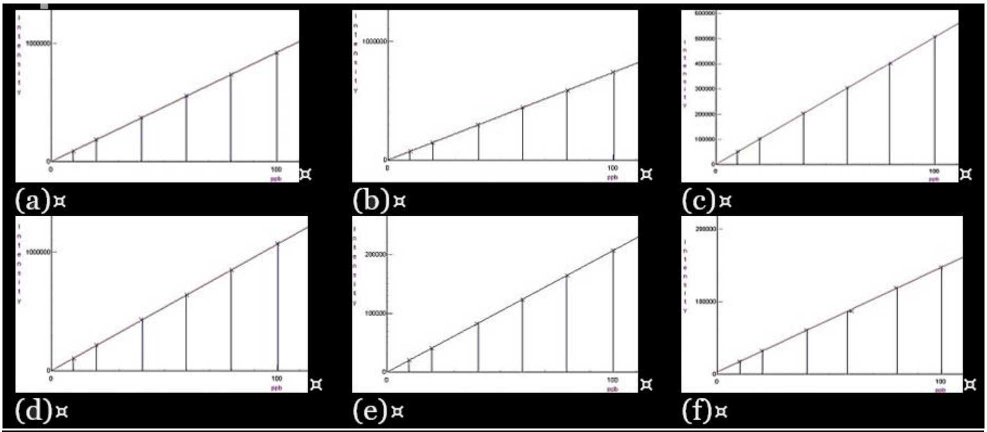

Figure 1. Calibration curves of some investigated elements (a)Cd, (b)Co, (c)Cr, (d)Cu, (e)Ni, (f)Zn using ICP-MS.

Reagents and materials used:

•HNO_3 65% (Merck, Germany)

•H_2O_2 30% of analytical purity (Merck, Germany)

•Ultrapure water, Milli-Q (Millipore, Bedford, MA, USA)

•Multi-element stock solution 1000 mg/l (Merck, Darmstadt, Germany)

Procedure:5 ml of sample was extracted by microwave digestion. 8 mL of 65% HNO_3 and 3 mL of 30% H_2O_2 were added, after which the sample was allowed to cool to room temperature and diluted with 25 mL of ultrapure water, then filtered through a 0.45 µm membrane cellulose filter. The concentration of the elements in the mineralized solutions was determined using ICP-MS. To determine the desired elements in the samples, external calibration of the equipment was used with standard multi-element solutions of different concentrations, with the help of which calibration curves were drawn. Limits of detection and quantification were determined and calculated to establish method performance.

Results and discussion

The results obtained, represent the average of three determinations (the standard deviation falls within a maximum of 5% of the average value), and are presented in Tables 1-3 and in Graphs 2-5 some of the more significant metals are presented comparatively.

According to DECISION no. 512 of July 20, 2016 for the approval of the Methodological Norms for the application of the Vine and Wine Law in the system of the common organization of the wine market no. 164/2015, maximum accepted limits of basic physico-chemical parameters are established for wines, at the time of consumption (Annex no. 4 IB to Methodological Norms). At the time of consumption, the composition of the wines must fall within the following physico-chemical parameters: citric acid, 1 g/L; methanol: 250 mg/L for white and rose wines; 400 mg/L for red wines. According to the Norms mentioned above, the Maximum Admissible Limits (MAL) for some metals and non-metals are: arsenic: 0.2 mg/L; cadmium: 0.01 mg/L; copper: 1.0 mg/L; lead: 0.2 mg/L; excess sodium: 60 mg/L; zinc: 5.0 mg/L; boron: 80 mg/L expressed in boric acid; bromine: 1.0 mg/L; It should be mentioned that all the values obtained fall within the legislation, being below the values of the Maximum Admissible Limits (LMA). In the Table 1 are presented the Heavy metals in wine assortments, production of 2021.

Table 1

Heavy metals present in wine assortments, production of 2021

Nr. crt.	U.M. µg/L	Muscat Ottonel	Pinot Grigio	Fetească Regală	Merl ot	Riesling Italian
1	Fe	778	533	685	733	390
2	Mn	424	474	434	483	409
3	Zn	79.4	191	80.8	237	123
4	Pb	42.2	10.3	9.2	2.7	6.5
5	Cu	36.5	13.4	7.6	9.0	21.9
6	Ni	16.1	27.1	14.0	23.0	19.4

The Fe content of the five varieties of wine analysed is between 778 µg/L for Muscat Ottonel and 390 µg/L for Riesling Italian (see Table 1 and Figure 2), being below the values (9 – 10 mg/L) in which the wines are prone to ferric spoilage (Avram et al., 2014).

In Table 2 we are presented other Minerals highlighted in the analysed wine assortments and in Table 3, Trace elements and other rare metals present in the wine varieties analysed, for the Year crop 2021.

Table 2

Other Minerals highlighted in the analysed wine assortments, production of 2021

Nr. crt.	U.M. µg/L	Muscat Ottonel	Pinot Grigio	Fetească Regală	Merlot	Riesling Italian
1	Mg	33640	40383	37624	49048	35428
2	Ca	38812	43510	47586	39738	41046
3	Na	37577	11113	11635	5540	8191
4	Si	4641	5378	5257	7198	5142
5	B	1765	2181	1829	2680	1770
6	Al	1117	0728	0912	00725	0415
7	Ba	114	92.5	100	47.9	79.2
8	Li	16	9.3	8.6	8.7	10.8
9	Be	3.4	2.6	2.8	0.1	1.2
10	I	0.3	0.1	0.1	0.1	0.1

Table 3

Trace elements and other rare metals present in the wine varieties analysed, production of 2021

Nr.crt.	U.M. µg/L	Muscat Ottonel	Pinot Grigio	Fetească Regală	Merlot	Riesling Italian
1	Cr	146	**264**	116	21	73
2	Co	1	14	**21**	19	1
3	Mo	**34**	16	15	7	8
4	As	**22**	14	9	5	8
5	Sn	**21**	2	15	13	17
6	Cd	1	**2**	1	1	0.0
7	Sc	3.8	**4.5**	4.1	6	3.8
8	Ti	105	**128**	111	124	126
9	V	**4.2**	3.6	**4.2**	0	1.1
10	Ga	**0.3**	0.3	0.3	0.2	0.2
11	Rb	1145	1006	937	**1598**	966
12	Sr	**290**	224	267	209	174
13	Y	**1.8**	1.7	1.3	0	0.6
14	Zr	**30.5**	22.2	22.3	1.1	10.5
15	Nb	**1.5**	1.2	1.3	0	0.5
16	Pd	1.3	**4.7**	0.6	0.9	0.3
17	Sb	**0.2**	0.1	0.1	0	0.1
18	Cs	2.6	3	2.6	**5.6**	4.2
19	La	0.6	**0.8**	0.4	0	0.2
20	Ce	1.4	**1.9**	0.9	0.1	0.3
21	Pr	**0.2**	**0.2**	0.1	0	0
22	Nd	1	**1.1**	0.6	0	0.2
23	Sm	**0.3**	**0.3**	0.1	0	0.1
24	Eu	**0.1**	**0.1**	**0.1**	0	0
25	Gd	**0.4**	**0.4**	0.2	0	0.1
26	Tb	**0.1**	**0.1**	0	0	0
27	Dy	**0.4**	**0.4**	0.3	0	0.1
28	Ho	**0.1**	**0.1**	**0.1**	0	0
29	Er	**0.4**	0.3	0.3	0	0.1
30	Tm	**0.1**	**0.1**	0	0	0
31	Yb	**0.5**	0.4	0.4	0	0.2
32	Lu	**0.1**	**0.1**	**0.1**	0	0

33	Hf	**0.9**	0.6	0.7	0	0.3
34	W	**1.2**	1	**1.2**	0.2	0.5
35	Tl	**0.8**	0.6	0.6	0.4	0.6
36	Bi	**0.8**	0.1	0.1	0	0
37	Th	**0.7**	0.3	0.2	0.1	0.1
38	U	**2.1**	1.7	1.4	0.1	0.8

Two of the varieties (Fetească Regală - 685 µg/L and Merlot - 733 µg/L) are closer to the maximum value found, while Pinot Grigio (533 µg/L) has a content close to the average between the minimum and maximum value.

Regarding trace elements and rare metals two of the varieties, namely Merlot and Pinot Grigio were found to have most of the highest values (Table 3).

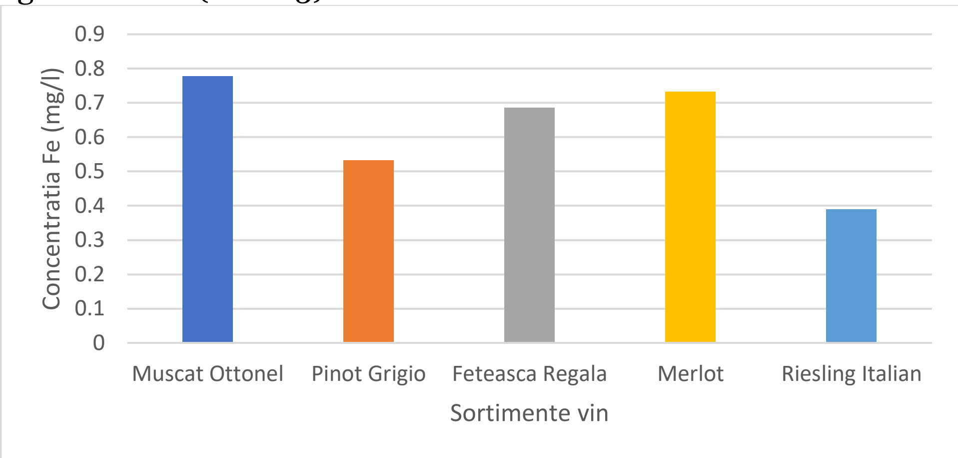

Figure 2.Fe content of wine varieties, 2021 harvest

The Zn content of the varieties analysed is between 237 µg/L for Merlot and 79.4 µg/L for Muscat Ottonel (see Table 1 and Fig, 3). Pinot Grigio (191 µg/L) has a value closer to the maximum and the other two varieties are closer to the minimum value Fetească Regală (80.8 µg/L) respectively to an average value for Italian Riesling (123 µg/L).

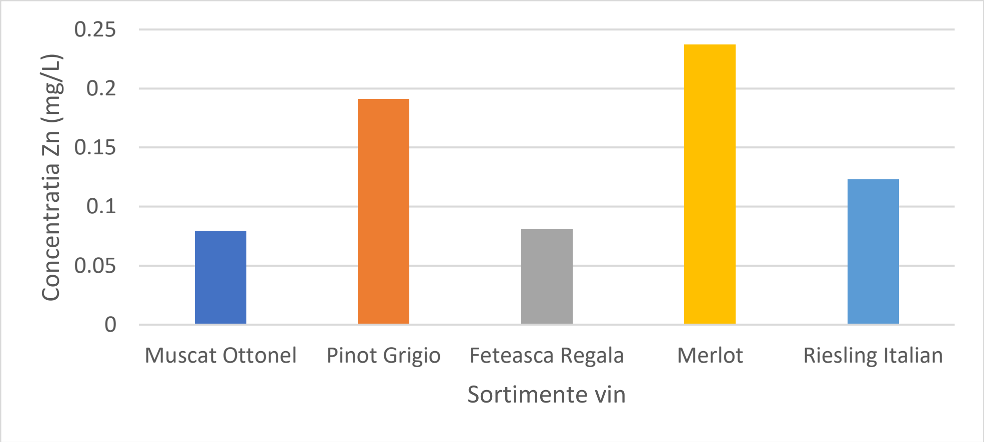

Figure 3.Zn content of wine varieties, 2021 harvest

The Pb content in the five varieties of wine analysed is between 42.2 µg/L for Muscat Ottonel and 2.7 µg/L for Merlot, being below the Maximum Admissible Limit of 200 µg/L (see Table 1 and Figure 4). The Pb content of the other varieties is much closer to the minimum value (Pinot Grigio – 10.3 µg/L; Fetească Regală – 9.2 µg/L; Riesling Italian – 6.5 µg/L).

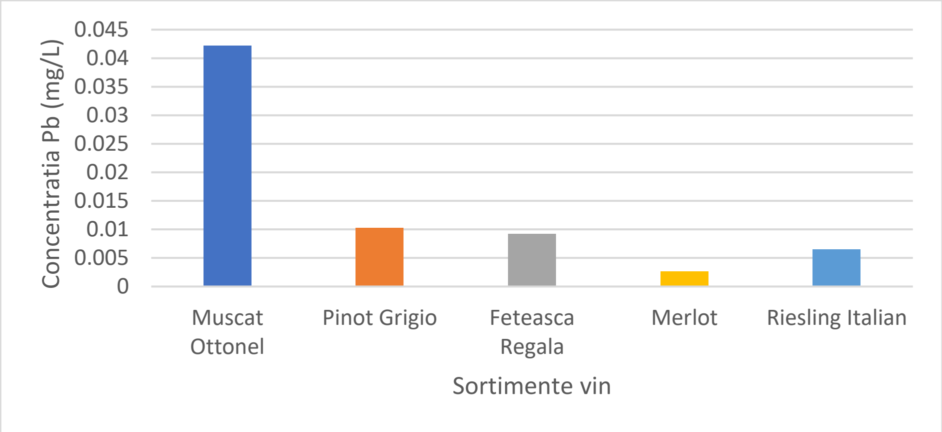

Figure 4.The Pb content of the wine varieties, 2021 harvest

The Cu content in the five varieties of wine analysed is between 36.5 µg/L for Muscat Ottonel and 7.6 µg/L for Fetească Regală, being below the Maximum Admissible Limit of 1000 µg/L (see Table 1 and Figure 5). The content found for Italian Riesling (21.9 µg/L) is closer to the maximum value and the other two varieties are closer to the minimum value (Pinot Grigio – 13.4 µg/L and Merlot – 9 µg/L).

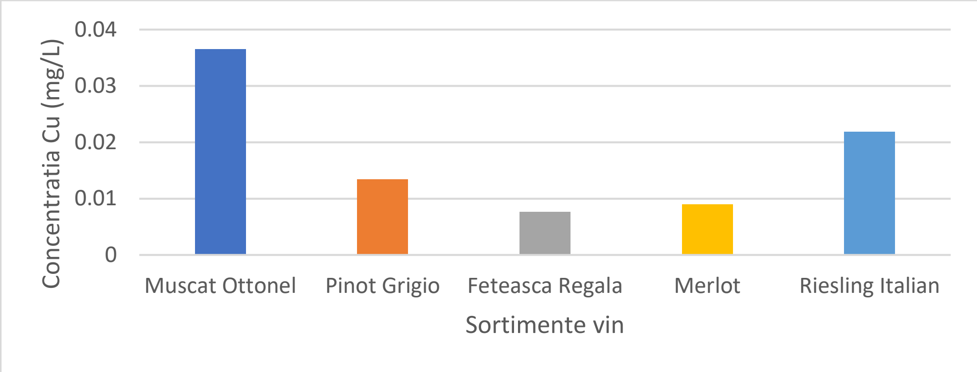

Figure 5.Cu content of wine assortments, 2021 harvest

Conclusions

➢ The mineral and heavy metal content of five varieties of wine (Muscat Ottonel, Pinot Grigio, Fetească Regală, Merlot, Riesling Italian) obtained in 2021 in the north-west area of Romania, was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

➢ The results obtained represent the average of three determinations (the standard deviation falls within a maximum of 5% of the average value) and is below the maximum limit allowed by the regulations in force regarding the content of heavy metals.

➢ A comparison was made of the content of four more relevant heavy metals of the five varieties of wines. Regarding Copper the maximum value was measured in the case of Muscat Ottonel and the lowest for Fetească Regală. For lead content the situations of similar, Muscat Ottonel presenting the highest value and Merlot the lowest. Regarding zinc content the situation is reverses, Merlot having the highest value measured and Muscat Ottonel the lowest.

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