Investigation of the Copper Content in Vineyard Soil, Grape, Must and Wine in the Main Vineyards of Romania: a Preliminary Study

Alina DONICI[1], Claudiu Ioan BUNEA[2], Anamaria CĂLUGĂR[2], Eugenia HARSAN[3], Florin Dumitru BORA[1] *

[1] Department of Physico-Chemistry and Biochemistry, Research Station for Viticulture and Enology Târgu Bujoru, Romania
[2] Department of Horticulture and Landscaping, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[3] Department of Biochemistry University of Agricultural Sciences and Veterinary Medicine, -Horticultural Research Station, Cluj-Napoca, Romania
*corresponding author: boraflorindumitru@gmail.com

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Abstract

The long-term use of copper in viticulture has caused great copper accumulation in vineyard soils, resulting in negative effects on the environment through toxicity to aquatic and soil organisms. The aim of this study was to investigate the copper content in vineyard soils, grapes and wines from Dealu Bujorului, Murfatlar, Tarnave, Iasi and Ştefăneşti vineyards. The ICP-MS method was used for copper determination in vineyard soil, grape must and wine. Copper concentration in red wine samples was significantly higher than in the white wine samples. Values for Transfer Factor and Mobility Ratio indicates that *Vitis vinifera* L. does not allow the accumulation of copper from vineyard soil in must grape and wine. The copper concentration in grapes, must and wine has been influenced by the copper concentration in soils, by copper fungicide used to protect the vine and by other factors such as the biological specificity of cultivars during growth.

Keywords: copper, grape, wine, Romanian vineyards

Introduction

Among the processed crops, which include all agricultural and food products derived from their respective primary commodities, wine is one of the most important in the world (Brunetto *et al*., 2016). The world viticulture regions are generally located in climatic areas that are, however, favourable to the occurrence of fungal diseases, including downy mildew (*Plasmopara viticola*). The regular use of copper (Cu) based fungicides (e.g. Cu sulfate, Cu oxychloride) to protect grapevine plants from these patogene agents has led to long-term accumulation of Cu in vineyard soils (particularly in the upper layers), reaching concentration that are far higher as compared to the trace amounts that are required for healthly plant growth (Pietrzak and McPhil, 2004) and in some circumstances, even exceeding the limits imposed by the EU for agricultural soils (Komárek *et al*., 2010).

Besides Cu-containing fungicidal and bactericidal sprays, substantial Cu addition to soils can occur by the appliance of contaminated wastes (Liu *et al*., 2007) and/or mineral and organic fertilizers (Xiaorong *et al*., 2007), including organic residues (e.g. pig and poultry and organic composts) (Couto *et al*., 2015).

Copper (Cu) is one of the heavy metals of great concern in the wine industry (Jia *et al*., 2015; Sun *et al*., 2017; Sun *et al*., 2018). It should be emphasized that Cu is an essential element for plants and, by definition, it is necessary for an organism to function properly, since it plays key roles in several biochemical and physiological processes connected to plant growth and development (Yruela, 2005). Since the end of the eighteenth century, copper (mainly the Bordeaux mixture) has been used to protect grapevines from fungal diseases (Sun *et al*., 2018) which represent a large threat to winegrowers, causing significant damage and severely reducing grapes and wine quality (García-Esparza *et al*., 2006). Copper fungicides remain the most effective and most widely used fungicides for fungal diseases in viticulture (García-Esparza *et al*., 2006). Additionally, the use of copper winemaking equipment (Volpe *et al*., 2009) and copper sulphate or copper citrate additives to eliminate H_2S (García-Esparza *et al*., 2006) may increase the copper content in grape must and in wine.

Copper is an essential metal to nearly all organisms but has a very narrow optimum concentration range (Azenha *et al*., 2000; Ferreira *et al*., 2006; Sun *et al*., 2017). Above the physiological concentrations, copper can have inhibitory or even toxic effects on cell metabolism (Robinson and Winge, 2010). Copper rarely degrades or moves in arable soil layers, which causes copper enrichment in vineyard soils (Pirtzak and McPhail, 2004). As a result, the long-term use of copper in viticulture has caused great copper accumulation in vineyard soils, resulting in negative effects on the environment through toxicity to aquatic and soil organisms (García-Esparza *et al*., 2006). In the winemaking process, high copper concentration also affects the wine fermentation process and wine quality (Sun *et al*., 2017). When the concentration of copper is greater than 20 mg/L, the growth of *Saccharomyces cerevisiae* (*S. cerevisiae*) is inhibited, which delays fermentation and reduce alcohol production (Sun *et al*., 2018). Moreover, concentration above 1 mg/L in wine may cause copper casse, resulting in a turbid wine (Ferreira *et al*., 2006; Robinson, and Winge, 2010). Additionally, a high copper concentration in wine can be harmful to the health of consumers, particularly in combination with other heavy metals such as iron, manganese, zinc, nickel, lead and scandium (Araya *et al*., 2003; Turnlund*et al*., 2004; Naughton and Petróczi, 2008). Hence, the copper concentration in the wine industry has been regulated by European Community. The European Community (EC) has established a maximum permitted field dose of copper of 6 kg/ha/year (EC No. 1410/2003, 2003) and maximum residue limits (MRLs) in vineyard soils, grapes and wine of 140 mg/kg, 20 mg/L and 1 mg/L (EC regulation No. 1410 of 7/8/2003), respectively. The International Organisation of Vine and Wine (OIV) has the same MRLs for grapes and wines (OIV, 2013).

Previous studies demonstrated that the higher amounts of copper accumulates in the upper layers of vineyard soils. Komárek *et al*. (2010) observed that Cu concentration in superficial horizons often exceed 200 mg/kg and the highest Cu concentrations are present in the upper layers of soil profile. The Predicted No Effect Concentration (PNEC) of Cu in soils in the EU (estimated in the EU Risk Assessment on Cu) range from 20 to 200 mg/kg, depending on soil properties (Ruyters *et al*., 2013). In a recent research article by Tóth *et al*. (2016) on the concentration of heavy metals in agricultural soils of the EU, a threshold of 100 mg/kg was considered for Cu, with lower and higher guideline values set at 150 mg/kg and 200 mg/kg, respectively. Exceptionally high concentration of Cu in vineyard soils have been observed in France (above 1000 mg/kg Flores-Vélez *et al*. (1996)) and in Brazil (above 3000 mg/kg, Mirlean *et al*. (2007)). Toxic effects of Cu on the microbial communities in vineyard soils have been revealed for concentrations of copper that exceed 150-200 mg Cu kg (Fernández-Calviño *et al*., 2010).

The mechanisms of Cu transport in roots (micronutrient uptake) have been characterized in the last two decades (Sancenón *et al*., 2003; Wintz *et al*., 2003). In particular, Cu transport protein 1 (COPT1) is thought to mediate most of the Cu uptake into cells, whereas other members of this protein family might mediate intracellular transport of Cu (Sancenón*et al*., 2003). However, other transporters seem to be involved in this process, as for instance members of the Zn/Fe permeases (ZIPs) family, ZIP2 and ZIP4, have been shown to function as Cu-transporters in *Arabidopsis thaliana* (Wintz*et al*., 2003). Moreover, Cu-PS (Cu-mobilizing phytosiderophose (PS)) might be taken up by grass roots via yellow stripe-like (YSL) proteins (Marschner, 2011; Wintz *et al*., 2003). This family of proteins seems to be also implicated in the transport of the chelate

Cu-nicotianamine (Wintz *et al*., 2003), which is presumably involved in the translocation of this micronutrient (allocation to the shoot). Other proteins have been shown to be involved in the transport of Cu across membranes; some P1B-type ATPase (HMA) transporters can transport either Cu^{2+} or Cu^{+} (Zimmermann *et al*., 2009).

Although many regulatory limits exists, there are still numerous instances of higher Cu levels. García-Esparza *et al*. (2006) affirm that approximetely 13% of grapes and 18% of wines exceeded the maximum copper residue limit in Italy, while in southern Brazil, the maximum copper value registered in vineyard soils was as high as 3200 mg/kg, which is several times higher than the EC regulation limit of 140 mg/kg (Mirlean *et al*., 2007). These phenomena have also occurred in Jordan (Al Nasir *et al*., 2001), Italy (Marengo and Aceto, 2003), Australia (Sauvage *et al*., 2002), Poland (Pohl and Sergiel, 2009), China (Sun *et al*., 2015). In fact, in China, from 2014 to 2017, Chinese customs detained many batches of wines imported from Argentina, Spain, Chile, Cyprus, France, Ukraine and other countries due to excessive copper concentration (Sun *et al*., 2018).

The aim of this study was to investigate the copper content in vineyard soils, grapes and wines from Dealu Bujorului, Murfatlar, Tarnave, Iasi and Ştefăneşti vineyards, which are the most important vineyards in Romania. Based on these results, it can be better understood the influence of copper pollution on wine production, thereby providing a potential strategy for improvement in the wine industry.

Materials and methods

Description of the sampling area

From the bio-pedo-climatic point of view, the Dealu Bujorului vineyard is placed on the forest steppe crossed by the 45° North latitude parallel (intersected by the 28° East longitude meridian). Under the social-economic aspect, this is a region with agricultural-cereal profile and is subdivided in terms of forest and wine and fruit-growing areas, with the vines occupying between 5 and 15% of the agricultural area. Through its geographic position, this vineyard connects the Fălciului Hills in the North and the Covurlui Vineyard in the South. The soils also exhibit an altitudinal zoning plan (corresponding to the morpho-bio-climatic one), from steppe chernozemic soils to leached forest steppe chernozemic soils and to forest luvisol. The Murfatlar Vineyard is located in south-eastern Romania, namely in the South Dobrogea plateau, along the Carasu Valley, between the coordinates 44°10’-44°20’ North latitude and 22°00’-28°30’ East longitude on a VE distance of 45 km and an average NS width of 10 km. The soils are represented by steppe molisoils, namely chernozemic soils, which almost completely cover the large plateaus and the low-moderately aslope mountainsides. The Târnave Vineyard has the largest surface of the vineyards of the Transylvanian Plateau. This area corresponds almost entirely to the Târnave Plateau, crossed by Târnava Mare and Târnava Mică, continued to the West with the united Târnavas, whose affluents fragmented the landform in a multitude of hilly complexes with various orientations, exhibitions and declivities. This vineyard runs between the coordinates of 46°32 ‘ North latitude and 24°48’ Eastern longitude. The predominant soil in this vineyard is aluvisol. The Iaşi vineyard is located in the east-northeast of the Moldavian Plateau, in the contact area of the Colinar Plain of Moldova with the Central Moldavian Plateau. The vineyards are located in the Colinar Plain of Moldova and on the transitional coast of the Central Moldavian Plateau, namely the coast of Iaşi (represented by the eastern sector of the Repedea coast) and the Faţa Prutului (Tomeşti-Bohotin sector). The predominant soil in this vineyard is chernozemic soil. Ştefăneşti-Argeş Vineyard is located in the contact area of Cândeşti Plateau with the Romanian Plain, respectively on the frontal hills of this plateau and the terraces from the left of Argeş between Piteşti and Găieşti. It is shaped as a discontinuous strip in the direction of NV-SE, with a length of about 35 km and an average width of 15 km. The predominant soil in this vineyard is chernozemic soil (Cotea *et al.*, 2000). The ecoclimatic conditions of the studied areas were presented by Bora *et al.* (2016b).

Sample collection

Soil sampling was carried out on the depth of the soil profile at 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm and 50-60 cm during 2016. Soil samples were collected using stainless steel shovels and were stored in individual black plastic bags (darkness). All samples were taken in triplicates from the defined experimental plot. Soil samples have been brought first to sand-size material (< 2 mm) using a jaw crusher then

Table. 1. The program of the microwave oven Milestone START D Microwave Digestion System

Step	Target temp (°C)	Pressure max (psi)	Ram time (min.)	Hold time (min.)	Power (%)
			Soil sample		
1.	220	800	10	20	100
2.	30 min-cooling				
			Must sample		
1.	200	800	10	20	100
2.	20 min-cooling				
			Wine sample		
1.	200	800	10	20	100
2.	20 min-cooling				

mechanically split to obtain a representative samples and eventually pulverized to powder-size, grain-size smaller than 100 µ (< 400 mesh), using a ball mill. Agate ball mill is used in place of any other pulverization metal device to avoid possible trace element contamination. Soil samples before splitting and pulverization have been dried at 60 ^{0}C.

Soil sample preparation

The soil samples were dried, homogenized and then passed through a 20-mesh sieve to obtain very fine particles. The method for microwave digestion using a Milestone START D Microwave Digestion System, was optimized in a previous work (Bora *et al.*, 2018): 0.25 g soil, 9 mL of 65% nitric acid, 3 mL of 100% hydrofluoric acid and 2 mL of 37% hydrochloric acid were placed in a clean Teflon digestion vessel. The vessel was closed tightly and placed in the microwave. The digestion was carried out with the program described in Table 1.

Microvinification process

The samples of grapes was harvested in 2016, were destemmed and crushed, then transferred to a microfermentor (50 L cylindrical glass container, covered with aluminum foil to limit the effect of the light over the must) equipped with a fermentation airlock. Fermentation took place at 21-23 °C and humidity 56-60%. Afterwards wine was clarified by means of bentonite (1 g/L) and combined with SO_2 up to 100 mg/L. Then wines were allowed to cool for thirty days at -5 °C for cold stabilization (Donnini *et al.*, 2016). Then wine samples were stored in glass bottles at 5-6 °C until the analyses. Average data from three vinifications per cultivar are reported (Bora *et al.*, 2016).

Wine sample

The wine samples were taken from freshly opened bottles and prepared by a specific organic matter digestion. 2.5 mL of wine were weighed inside Teflon digestion vessels and 2.5 mL of 65% nitric acid added. Teflon digestion vessels were previously cleaned in nitric solution to avoid cross-contamination. The vessels already capped were placed in a microwave oven followed by the application of the program (Tab. 1), optimized in a previous work (Bora *et al.*, 2018). After cooling to ambient temperature, the microwave oven was opened and the content was quantitatively transferred into a 50-mL volumetric flask and brought to the volume with ultra-pure water. All the elements were measured from these extraction solutions by ICP-MS.

ICP-MS analysis

Analytical measurements were performed using an inductively coupled plasma mass spectrometer (I Cap Q ICP-MS Thermo Fisher Scientific) equipped with an ASX-520 autosampler, a micro-concentric nebulizer, nickel cones and peristaltic sample delivery pump, running a quantitative analysis mode. Each sample was analyzed in duplicate and each analysis consisted of seven replicates. The gaseous argon and helium used to form the plasma in the ICP-MS was of 6.0 purity (Messer, Austria). The heavy metals were measured by using a multi-element analysis after appropriate dilution using an external and standard calibration. The calibration was performed using XXI CertiPUR (109488/1 100

Table 2. LoD, LoQ, BEC and *r* of the calibration for each element determination

Element	*r**	LoD (μg/L) **	LoQ (μg/L) ***	BEC (μg/L) ****
Cu	0.9999	0.0402	0.1339	0.237

*r** = correlation coefficient; **LoD = detection limit; ***LoQ = Quantification limit;
****BEC = Background Equivalent Corection.

Table 3. Intsrumental (a) and data acquisition (b) parameters of ICP-MS

(a) Instrumental parameters		(b) Data acquisition parameters for quantitative mode	
RF power	1.4 kW	Measuring mode	QCell (Collision Cell)
Argon gas flow		Point per peak	3
Nebulizer	1.0 L/min	Scans/Replicate	7
Plasma	18.0 L/min	Replicate/Sample	7
Lens voltage		Dwell time (ms)	1
Mirror lens left	37 V		
Mirror lens right	32 V		
Mirror lens bottom	31 V		
Sample uptake rate	70 s	Integration time	

mL Merck, Darmstadt, Germany) multielement standard, and from individual standard solution of Hg. The working standards and the control sample were prepared daily from the intermediate standards obtained from the stock solution. The intermediate solutions stored in polyethylene bottles and glassware was cleaned by soaking in 10% v/v HNO_3 for 24 hours and rinsing at least ten times with ultrapure water (Milli-Q Integral ultrapure water-Type 1). The accuracy of the methods was evaluated by replicate analyses of fortified samples (10 μL-10 mL concentrations) and the obtained values ranged between 0.8-13.1%, depending on the element. The global recovery for each element was estimated and the obtained values were between 84.6-100.9% (Geană *et al.*, 2016).

For quality control purpose, 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 next mathematical formulas: LoD = 3SD/s and LoQ = 10 SD/s (SD = estimation of the standard deviation of the regression line; s = slope of the calibration curve) (Tab. 2). The recovery assays for the must and wine sample of 5 μg/L, for three replicates of this level of concentration (n = 3) gave the average recovery R% between 87.32% and 100.26%. The recovery for the soil and plant material samples of 5 μg/L, for three replicates of this level of concentration (n = 3) gave the average recovery R% between 83.41% and 109.02%.

The solution was sprayed into flowing argon and passed into a torch which was inductively heated, where the gas was atomized and ionized, forming plasma, which provides a rich source of both excited and ionized atoms. In ICP-MS, positive ions in the plasma were focused to a quadrupole mass spectrometer. The method was previously optimized by evaluation of the advantages and limitations of ICP-MS quantitative operational mode for multi-element analysis of wines. Optimum instrumental conditions for ICP-MS measurement are summarized (Tab. 3). The instrument was daily optimized to give maximum sensitivity for M^+ions and the double ionization and oxides monitored by the means of the ratios between $Ba^{2+}/Ba^+$and Ce^{2+}/CeO^+, respectively, these always being less than 2%. The calibration standards were prepared from the multielemental standard solution, ICP Multi Element Standard Solution XXI CertiPUR, in five concentration levels 2.5, 5, 10, 25 and 50 μL.

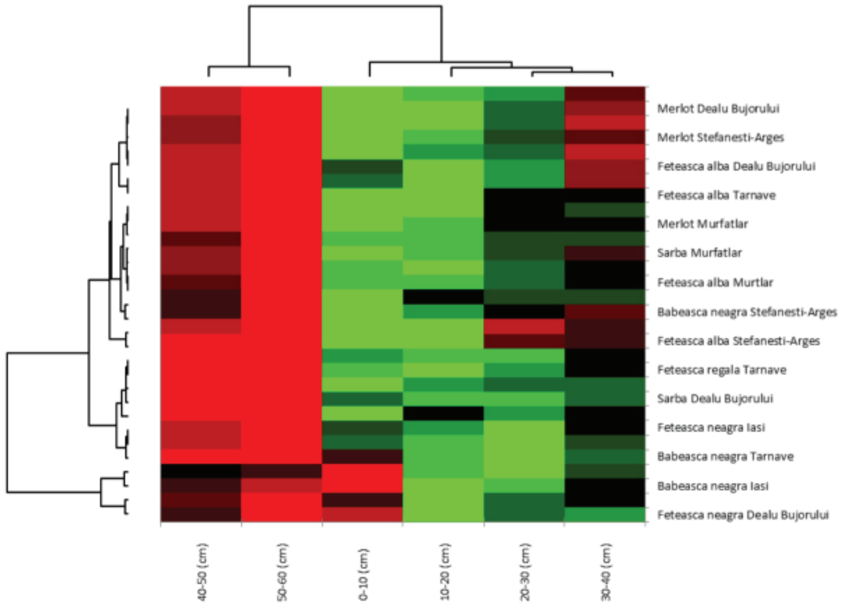

Figure 1. Heat map obtained by cluster analysis of the copper content in vineyard soils

Reagents and solutions

High purity ICP Multi Element Standard Solution XXI CertiPUR obtained from Merck (Darmstadt, Germany) was used for the calibration curve in the quantitative analysis. The ICP Multi Element Standard Solution XXI CertiPUR was mixture of 10.00 mg/L of As, Cr, Co, Cu, Sr and 10.10 mg/L U, Ni, and also 9.90 mg/L Cd, Zn, Pb, Hg. HNO_3 69% (w/v), concentrated HF and HCl, reagent grade from Merck and ultrapure water with a maximum resistivity of 18.2 MΏ cm^{-1}, obtained from Milli-Q Integral ultrapure water-Type 1 were used for sample treatment and sample dilution.

Statistical analysis

Concentration of the compounds was evaluated by statistical methods using the Duncan test, SPSS Version 24 (SPSS Inc., Chicago, IL., USA). The statistical processing of the results was primarily performed in order to calculate average and standard deviation. This data was interpreted with the analysis of variance (ANOVA) and the average separation was performed with the DUNCAN test at $p \leq 0.005$. Heat map, translocation factor and mobility ratio was performed using Microsoft Excel 2016 and XLSTAT Addinsoft version 15.5.03.3707.

Translocation factors

Trace metal translocation factor (TF) in *Vitis vinifera* was determined by the equation ($TF_{g\text{-}m} = C_{must} / C_{soil}$; $TF_{w\text{-}m} = C_{wine} / C_{must;}$ as the ratio between must-soil respectively wine-must. TF > 1 indicates that *Vitis vinifera* L. translocate metals effectively from soil to plants parts (canes, leaves and grapes) and also in must and wine (Serbula *et al.*, 2012). TF < 1 shows that *vitis vinifera* exclude metals from uptake (excluder) (Mingorance *et al.*, 2007). The mobility ratio (MR) in *Vitis vinifera* was used to determine the ratio of heavy metals concentration (C_{plant}, mg/kg) in plant parts (grapes) to the concentration level of the acid-soluble metal fraction (C_{soil-m}, mg/kg) in the top-soil $MR = C_{plant} / C_{soil\text{-}m}$. MR > 1 indicates that *Vitis vinifera* translocates metals effectively from soil to plants parts (canes, leaves and grapes) and also in must and wine (Serbula *et al.*, 2012). MR < 1 shows that *Vitis vinifera* exclude metals from uptake (excluder) (Mingorance *et al.*, 2007).

Results and discussions

Previous studies showed that copper seemed to be predominantly transported by suspended matter in runoff water (Xue *et al.*, 2000), which can be related to the affinity of copper for sorption onto some components like organic matter, clay minerals and hydrous metal oxides (Flemming and Trevors, 1989). Suspended particulate matter (SPM) transported during these events is deposited in the main channel during recession periods of discharge forming stream bed sediments (BS). BS are considered as representative as soils they originate from and the analysis of BS finest fraction (< 63 µm) gives an integrative view of SPM transported by rivers (Gaiero *et al.*, 2003). The total amount of Cu in sol, BS and SPM gives little information on its availability. Copper may exist as different chemical forms that have different chemical binding. In natural form, Cu is derived mainly from parental rock forming minerals (Jenkins and Jones, 1980) and when bound to silicates and oxides among primary minerals forming relatively immobile species.

In Romania, in addition to copper sulphate there are other copper-based fungicides in use against fungal diseases in wine-growing, such as copper (I) oxide, copper oxichloride, copper hydroxide, copper-hydroxide-potassium sulphate complex, copper-hydroxide-Ca-chloride complex, combinations of copper and organic fungicides, and combinations of copper and mineral oils.

According to information accumulated so far, long-term use of copper fungicides in wine-growing results in ingression of significant quantities of copper, which remain in the surface soil layer at 0-0.2 cm , as confirmed by numerous of researchers. Arable soil usually contain Cu 5-30 mg/kg, while concentration of total copper in treated vineyards can range between 100 and 1500 mg/kg. Besnard *et al.* (2001), determined that vineyard soils of France contain form 31 to 250 mg/kg of total copper. Pietrzak *et al*. (2004), indicated that the use of copper fungicides led to an increase of total copper (250 mg/kg) in some vineyard soils in Victoria, Australia, while Wightwick *et al*. (2008) determined that copper concentration in Australian vineyard soils were generally much lower (6-150 mg/kg) than those reported in vineyard soil in parts of Europe (130-1280 mkg/kg). High concentrations of fungicide derived copper in orchard and vineyard soils have been reported from India (29-131 mg/kg) and Australia (11-320 mg/kg).

The bulk of copper accumulated in leaves and soil after treatment of the vine with copper fungicides returns to the surface layer of soil through tillage or the biological cycle (Deluisa *et al*., 1996; Flores-Velez *et al*., 1996; Vitanović *et al*., 2010).

The average copper contents in soil, grape must and wine are presented in Table 4, Table 5 and Table 6. Firstly there can be noticed that vineyard soils at different depths had different copper contents, the surface soils (0-20) cm contained the highest copper amount, followed by medium level soils (20-40 cm), and then deep soils (40-60 cm), in accordance with a previous report (Komárek *et al*., 2010). This may be due to the fact that, the main source of copper in vineyard soils, copper fungicides are washed off from the aerial parts of grapes by rain and copper rarely degrades or sink in soils (Sun *et al*., 2016), resulting in this phenomenon of copper accumulation. Secondly, the results indicate that copper could be transported to deeper soils, which might cause deep pollution, possible even that of ground water (Mirlean *et al*., 2007). Thirdly, different vineyards showed significant differences in their copper contents, even though these vineyards were distributed in the same areas (Moldova area), with the same environmental and meteorological conditions.

In all the analyzed areas, the highest concentration of copper was recorded in soils planted with red grape varieties, especially on the surface of the soils, this shows that in the case of these varieties, more copper treatments were used than in the case of white grape varieties.

Type of plants is one of the most important influence factors on chemical composition in many food products (Li *et al*., 2015), also in grapes and in wine (Sun *et al*., 2015). Table 4 presents the copper content in the vineyard soil of the tested white an red varieties, at different depth. As can be observed the copper content of the grape varieties was different, indicating that different grape varieties and different vineyard production patterns all influence the level of copper in the soil. Previous reports have also demonstrated a varietal influence on vineyard soils content of copper (Provenzano *et al*., 2010; Vystavna *et al*., 2014, 2015).

The copper content of the studied grape varieties at different soil depths was diverse, such as Fetească neagră and Şarbă varieties from Iaşi vineyard exhibited a higher copper content than Fetească neagră and Băbească neagră in surface and medium soils. In the same vineyard, in soils planted with Merlot, the content of copper was higher in deep soils. This might be due to the translocation coefficients of copper, which was different for grape varieties (Vystavna *et al*., 2014, 2015). As it is show in Table 4, the copper content among white and red grapes soils was similar.

Heat map was used to discover sample groups, discover feature groups and also to discover related sample/feature groups. In case of copper distribution in the depth of the soil

Table. 4. Copper content in vineyard soil of different white and red varieties (mg/kg) (Mean ± standard deviation) (n = 3)

Areas	Type of soil	Depth (cm)	Fetească albă	Fetească regală	Şarba	Fetească neagră	Babească neagră	Merlot
*Normal Values M.A.L.**			20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg
Alert threshold M.A.L.		Susceptible	100 mg/kg	100 mg/kg	100 mg/kg	100 mg/kg	100 mg/kg	100 mg/kg
Less Susceptibile		250 mg/kg	250 mg/kg	250 mg/kg	250 mg/kg	250 mg/kg	250 mg/kg	
Intervention threshold M.A.L.		Susceptible	200 mg/kg	200 mg/kg	200 mg/kg	200 mg/kg	200 mg/kg	200 mg/kg
Less Susceptibile		500 mg/kg	500 mg/kg	500 mg/kg	500 mg/kg	500 mg/kg	500 mg/kg	
Dealu Bujorului	Chernozem	0-10	586.33±1.53 $^{m\gamma}$	662.24±11.31 $^{i\beta}$	607.61±3.98 $^{k\beta}$	494.09±5.94 $^{p\delta}$	583.41±5.46 $^{n\delta}$	864.25±9.76 $^{a\alpha}$
		10-20	697.55±3.63 $^{i\alpha}$	678.67±3.16 $^{h\alpha}$	632.58±8.09 $^{\text{îj}\alpha}$	716.89±5.63 $^{g\alpha}$	727.59±5.03 $^{h\alpha}$	868.29±16.51 $^{a\alpha}$
		20-30	618.86±6.46 $^{l\beta}$	676.76±6.97 $^{h\alpha}$	640.61±4.79 $^{\text{î}\alpha}$	628.98±7.14 $^{kl\beta}$	635.19±6.06 $^{k\beta}$	757.57±4.24 $^{f\beta}$
		30-40	508.12±8.31 $^{n\delta}$	595.71±8.93 $^{l\gamma}$	608.01±7.25 $^{k\beta}$	638.47±5.79 $^{k\beta}$	601.04±1.33 $^{m\gamma}$	570.88±3.24 $^{l\gamma}$
		40-50	493.06±3.99 $^{o\varepsilon}$	461.95±6.42 $^{n\delta}$	419.95±13.54 $^{p\delta}$	563.83±8.33 $^{n\gamma}$	560.54±11.60 $^{o\varepsilon}$	512.52±3.24 $^{n\delta}$
		50-60	410.14±4.25 $^{r\zeta}$	404.68±6.60 $^{o\varepsilon}$	429.76±3.70 $^{p\gamma}$	402.93±2.07 $^{q\varepsilon}$	400.55±2.13 $^{r\zeta}$	397.16±6.73 $^{q\varepsilon}$
Average			552.34±2.37	580.00±2.73	556.35±3.71	574.20±2.11	584.72±3.64	661.78±5.17
Murfatlar	Chernozem	0-10	766.98±1.99 $^{ef\beta}$	726.23±4.86 $^{f\alpha}$	784.17±5.06 $^{c\alpha}$	826.33±5.74 $^{b\alpha}$	728.59±8.66 $^{h\beta}$	825.99±4.90 $^{b\alpha}$
		10-20	779.94±5.33 $^{d\alpha}$	706.99±1.90 $^{g\beta}$	748.59±5.60 $^{e\beta}$	793.33±4.76 $^{d\beta}$	749.89±2.19 $^{g\alpha}$	779.18±12.30 $^{e\beta}$
		20-30	726.23±4.66 $^{h\gamma}$	653.46±6.00 $^{\text{iî}\gamma}$	670.24±1.38 $^{gh\gamma}$	763.14±4.33 $^{e\gamma}$	700.23±1.88 $^{i\gamma}$	688.83±2.08 $^{i\gamma}$
		30-40	670.10±10.39 $^{j\delta}$	649.13±2.01 $^{\text{îj}\gamma}$	626.74±7.71 $^{j\delta}$	651.93±2.31 $^{j\delta}$	673.87±2.23 $^{\text{î}\delta}$	681.04±5.94 $^{i\delta}$
		40-50	619.19±6.15 $^{l\varepsilon}$	589.61±4.47 $^{lm\delta}$	584.25±4.77 $^{l\varepsilon}$	599.90±1.89 $^{m\varepsilon}$	622.52±5.12 $^{l\varepsilon}$	595.58±5.53 $^{k\varepsilon}$
		50-60	425.84±9.49 $^{q\zeta}$	410.03±4.73 $^{o\varepsilon}$	400.20±1.86 $^{q\zeta}$	517.76±4.98 $^{o\zeta}$	520.87±4.99 $^{p\zeta}$	490.77±4.06 $^{o\zeta}$
Average			664.71±3.13	622.57±1.67	635.70±2.39	692.06±1.55	666.00±2.63	676.90±3.46
Tarnave	Luvosol	0-10	770.00±4.99 $^{e\beta}$	681.79±6.61 $^{h\beta}$	681.79±12.24 $^{fg\alpha}$	766.48±2.32 $^{e\beta}$	489.22±5.91 $^{q\delta}$	590.96±1.41 $^{k\gamma}$
		10-20	794.35±4.34 $^{c\alpha}$	729.36±3.72 $^{f\alpha}$	628.89±7.05 $^{\text{îj}\beta}$	788.54±2.79 $^{d\alpha}$	566.56±7.61 $^{o\beta}$	624.81±6.36 $^{j\beta}$
		20-30	630.13±6.12 $^{k\gamma}$	645.56±4.99 $^{\text{îj}\gamma}$	578.45±5.45 $^{l\delta}$	627.54±7.06 $^{l\gamma}$	591.03±4.48 $^{n\alpha}$	645.29±6.81 $^{\text{î}\alpha}$
		30-40	620.55±4.01 $^{l\delta}$	568.68±1.84 $^{m\delta}$	592.12±4.33 $^{l\gamma}$	637.47±3.09 $^{k\gamma}$	529.15±4.33 $^{p\gamma}$	567.72±7.60 $^{l\delta}$
		40-50	455.64±4.36 $^{p\varepsilon}$	330.80±7.38 $^{p\varepsilon}$	326.57±2.77 $^{r\varepsilon}$	434.62±3.75 $^{q\delta}$	409.46±4.51 $^{r\varepsilon}$	467.60±2.80 $^{p\varepsilon}$
		50-60	324.63±3.45 $^{\text{ş}\zeta}$	265.11±12.66 $^{q\zeta}$	292.24±3.01 $^{\text{ş}\zeta}$	329.26±4.20 $^{s\varepsilon}$	374.93±8.64 $^{\text{ş}\zeta}$	376.16±3.46 $^{r\zeta}$
Average			599.22±0.92	536.88±3.74	512.20±3.53	597.32±1.70	493.39±1.84	543.92±2.51
Iaşi	Chernozem	0-10	871.47±2.52 $^{a\alpha}$	879.28±6.30 $^{a\alpha}$	859.63±7.71 $^{a\alpha}$	815.47±3.44 $^{c\gamma}$	764.58±6.96 $^{f\zeta}$	732.56±4.00 $^{g\zeta}$
		10-20	878.07±3.12 $^{a\alpha}$	832.17±3.97 $^{b\beta}$	761.39±5.25 $^{d\gamma}$	828.17±4.00 $^{b\beta}$	844.31±4.29 $^{a\alpha}$	825.99±5.29 $^{b\beta}$
		20-30	827.26±4.77 $^{b\beta}$	815.18±4.28 $^{c\gamma}$	799.87±2.04 $^{b\beta}$	840.66±1.31 $^{a\alpha}$	828.86±2.52 $^{b\beta}$	831.97±3.22 $^{b\alpha}$
		30-40	741.06±5.67 $^{g\delta}$	733.47±5.16 $^{f\delta}$	766.08±5.62 $^{d\gamma}$	812.65±1.33 $^{c\gamma}$	811.21±4.50 $^{c\gamma}$	810.66±3.84 $^{c\gamma}$
		40-50	758.43±5.06 $^{f\gamma}$	730.83±5.13 $^{f\delta}$	683.35±10.22 $^{f\delta}$	787.63±1.86 $^{d\delta}$	800.96±5.30 $^{d\delta}$	800.13±1.40 $^{d\delta}$
		50-60	666.44±11.14 $^{j\varepsilon}$	674.71±3.62 $^{h\varepsilon}$	654.61±4.80 $^{i\varepsilon}$	767.10±10.22 $^{e\varepsilon}$	787.00±2.30 $^{e\varepsilon}$	792.79±2.80 $^{d\varepsilon}$
Average			790.45±3.07	777.61±0.98	757.16±2.78	808.61±3.39	806.15±1.75	799.02±1.31
Ştefaneşti-Argeş	Chernozem	0-10	768.59±2.96 $^{f\alpha}$	791.43±3.23 $^{d\alpha}$	663.80±8.48 $^{hi\beta}$	731.57±10.22 $^{f\alpha}$	764.39±5.64 $^{f\alpha}$	776.27±9.95 $^{e\alpha}$
		10-20	766.48±1.51 $^{ef\alpha}$	762.14±7.19 $^{e\beta}$	757.19±10.46 $^{de\alpha}$	679.12±6.92 $^{h\beta}$	727.86±5.75 $^{h\beta}$	736.13±5.11 $^{g\beta}$
		20-30	682.50±6.11 $^{\text{î}\beta}$	639.94±3.72 $^{j\delta}$	670.56±9.99 $^{gh\beta}$	682.90±7.76 $^{i\beta}$	680.15±7.16 $^{\text{î}\gamma}$	702.45±2.82 $^{h\gamma}$
		30-40	690.93±3.70 $^{\text{iî}\beta}$	684.47±3.05 $^{h\gamma}$	541.87±6.50 $^{m\gamma}$	678.79±9.58 $^{i\beta}$	659.27±7.76 $^{j\delta}$	645.86±10.51 $^{\text{î}\delta}$
		40-50	627.73±6.49 $^{kl\delta}$	643.47±10.99 $^{\text{îj}\delta}$	528.25±14.1 $^{n\delta}$	667.60±8.13 $^{\text{î}\gamma}$	675.80±4.76 $^{\text{î}\gamma}$	636.73±4.09 $^{\text{î}\varepsilon}$
		50-60	632.42±4.07 $^{k\gamma}$	620.09±2.59 $^{k\varepsilon}$	446.49±10.60 $^{o\varepsilon}$	569.17±5.02 $^{n\delta}$	564.59±3.54 $^{o\varepsilon}$	550.44±3.25 $^{m\zeta}$
Average			694.78±1.93	690.26±3.31	601.36±2.53	671.19±1.87	678.68±1.54	674.65±3.41
Sig			***	***	***	***	***	***
Areas		Sig	***	***	***	***	***	***
Type of soil		Sig	***	***	***	***	***	***
Depth		Sig	***	***	***	***	***	***
Varieties		Sig	***	***	***	***	***	***

profile (horizontal dendrogram), the dendrogram clearly show two cluster, the first cluster is formed between the depth of 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, and second cluster was formed from 40-50 cm, 50-60 cm depth. Based on this distribution we can say that the copper in vineyard soils recorded the highest concentration on the surface and medium soils and the lowest concentration

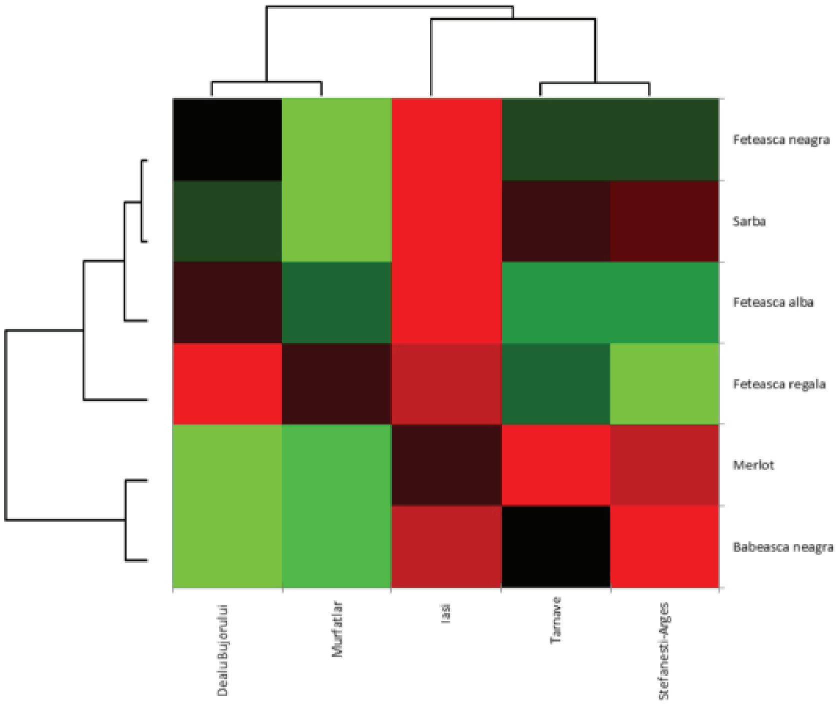

Figure 2. Heat map obtained by cluster analysis of the copper content in grape must

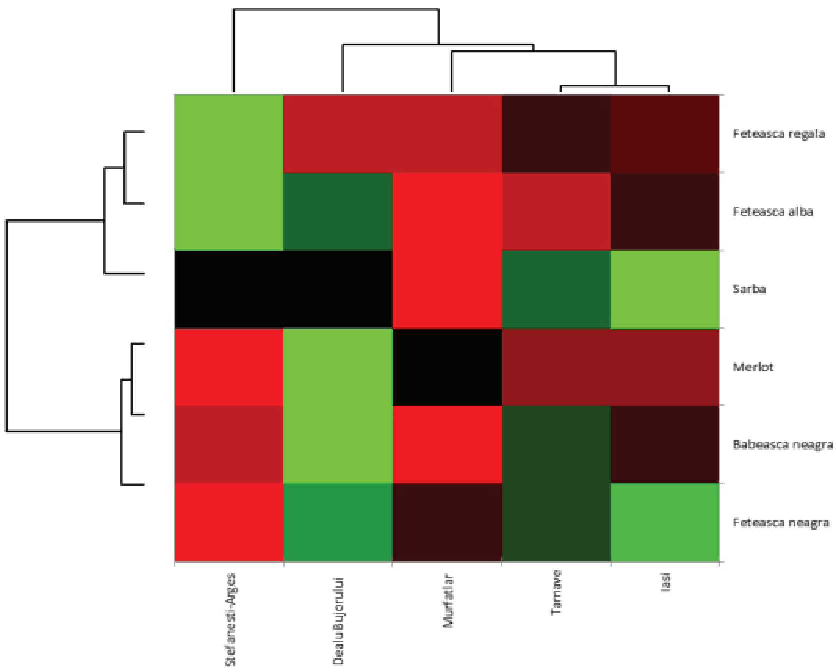

Figure 3. Heat map obtained by cluster analysis of the copper content in wine

in the depth of the soil. The vertical dendrogram shows also two clusters.

The first cluster is formed from the soil of Băbească neagră variety grown in Târnave and Iaşi vineyards and Fetească neagră from Dealu Bujorului vineyard, which were characterized by the highest concentration of copper. The second cluster was formed from the rest of the vine varieties analyzed. Băbească neagră variety from Târnave and Iaşi vineyards and Fetească neagră from Dealu Bujorului vineyard recorded the highest concentration of copper compared to the rest of the varieties. Based on these facts, it cannot be said that in all the analysed areas, the vine varieties for red wines presented the highest concentration of copper (Fig. 1).

Table. 5. Copper content in grape must of different white and red varieties (mg/L) (Mean ± standard deviation) (n = 3)

Areas	Feteasca alba	Feteasca regala	Sarba	Feteasca neagra	Babeasca neagra	Merlot
Dealu Bujorului	2.15±0.36 $^{a\,\gamma}$	1.85±0.51 $^{c\,\varepsilon}$	2.48±1.04 $^{ab\,\beta}$	2.76±0.35 $^{ab\,\gamma}$	2.38±0.25 $^{a\,\alpha}$	2.96±0.52 $^{a\,\alpha}$
Murfatlar	2.41±0.46 $^{a\,\beta}$	2.58±0.40 $^{bc\,\gamma}$	3.00±0.12 $^{a\,\alpha}$	3.24±0.34 $^{a\,\alpha}$	2.33±0.51 $^{a\,\alpha}$	2.79±0.30 $^{ab\,\beta}$
Tarnave	2.59±0.45 $^{a\,\alpha}$	3.11±0.12 $^{ab\,\beta}$	2.35±0.20 $^{ab\,\gamma}$	2.82±0.59 $^{ab\,\beta}$	2.04±0.56 $^{a\,\beta}$	1.36±0.20 $^{c\,\varepsilon}$
Iași	1.28±0.26 $^{b\,\delta}$	2.03±0.68 $^{c\,\delta}$	1.78±0.38 $^{b\,\varepsilon}$	2.01±0.67 $^{b\,\delta}$	1.68±0.84 $^{b\,\gamma}$	2.07±0.48 $^{bc\,\gamma}$
Ștefanești-Argeș	2.59±0.40 $^{a\,\alpha}$	3.89±0.55 $^{a\,\alpha}$	2.24±0.11 $^{ab\,\delta}$	2.86±0.35 $^{ab\,\beta}$	1.45±0.38 $^{c\,\delta}$	1.60±0.62 $^{c\,\delta}$
Average	2.20±0.39	2.69±0.45	2.37±0.37	2.74±0.46	1.98±0.51	2.15±0.42
Sig	**	**	ns	ns	ns	**
Areas	***	***	***	***	***	***
Varieties	***	***	***	***	***	***

Average value ± standard deviation (n = 3). Roman letters represent the significance of the variety difference (*p ≤ 0.05*). Greek letters represent the significance of the same variety cultivated in other areas (*p ≤ 0.05*). The difference between any two values, followed by at least one common letter, is insignificant.

Average value ± standard deviation (n = 12). Roman letters represent the significance of the variety difference (*p ≤ 0.05*). Greek letters represent the significance of the depth difference (*p ≤ 0.05*). The difference between any two values, followed by at least one common letter, is insignificant.

*Order of the Ministry of Waters, Forests and Environmental Protection No. 756/3 November 1997, approving the regulation on the assessment of environmental pollution, Bucharest, Romania; 1997. **M.A.L (Maximum Admissible Limit) = Normal Values.

According to the heat map obtained by cluster analysis of the copper content in vineyard soils, it can be seen a clear separation between the depth of the soil profile from 0-40 cm and between 40-60 cm. The same heat map show a clear separation between Băbească neagră variety from Târnave and Iaşi vineyards and Fetească neagră from Dealu Bujorului vineyard from the rest of the vine varieties analyzed. Băbească neagră from Târnave and Iaşi and Fetească neagră from Dealu Bujorului vineyard show higher values of copper content in the first group of soil profiles (0-40 cm), while in the second group (40-60 cm), show lower values. The same results can be seen for the rest of vine varieties.

The average copper concentration in grape must (Tab. 5) has varied between 2.20 to 2.69 mg/L in white varieties and 1.98 to 2.74 mg/L in red varieties. Firstly, between soils, the copper concentration in different vineyards was significantly different. Secondly, Murfatlar and Târnave vineyards showed the highest copper concentration in white and red grapes, while Iaşi vineyard showed the lowest copper concentration in both white and red grapes. In case of Murfatlar and Târnave vineyards, these results were similar to the soil results, indicating that the copper levels in the soil could influence the copper levels in grapes (Vystavna *et al*., 2015). In Iaşi vineyard, results showed that there are differences between soil and grape content of copper, the concentration of copper in grape must may have other sources that the vineyard soils. Thirdly, the concentration of copper in all white and red grape samples were over MRL, with an average value of 2.42 mg/L in white grapes and 2.29 mg/L in red grapes, lower than levels previously reported in Italy (García-Esparza *et al.*, 2006; Provenzano *et al.*, 2010), but higher than in Ukraine (Vystavna *et al*., 2014), possibly due to the different meteorological conditions of the cultivated region (García-Esparza *et al.*, 2006).

For grape must (Tab. 5) no overall significant differences in copper content were observed between white and red grapes. The copper concentration range varied greatly in both white and red grapes, according to the findings of Provenzano *et al*., (2010) and Sun *et al*. (2018). Additionally, as also in soils, significant varietal characteristics could be observed. Among white grapes, Fetească albă and Şarba showed the highest concentration of copper in must in Murfatlar vineyard, and lowest concentration of copper, for the same varieties in Iaşi vineyard. Among red grapes, Băbească neagră and Merlot from Dealu Bujorului vineyards registered the highest

Table. 6. Copper content in wine of different white and red varieties (mg/L) (Mean ± standard deviation) (n = 3)

Areas	Fetească albă	Fetească regală	Șarba	Fetească neagră	Babească neagră	Merlot
*Normal Values	1 mg/L	1 mg/L	1 mg/L	1 mg/L	1 mg/L	1 mg/L
Dealu Bujorului	$0.78 \pm 0.04^{ab\,\beta}$	$0.65 \pm 0.09^{a\,\delta}$	$0.76 \pm 0.12^{a\,\gamma}$	$0.86 \pm 0.08^{a\,\alpha}$	$0.85 \pm 0.09^{a\,\alpha}$	$0.87 \pm 0.08^{a\,\alpha}$
Murfatlar	$0.64 \pm 0.10^{b\,\gamma}$	$0.68 \pm 0.15^{a\,\gamma}$	$0.47 \pm 0.05^{b\,\delta}$	$0.78 \pm 0.15^{ab\,\gamma}$	$0.66 \pm 0.12^{a\,\gamma}$	$0.76 \pm 0.09^{a\,\beta}$
Târnave	$0.65 \pm 0.10^{b\,\gamma}$	$0.73 \pm 0.17^{a\,\beta}$	$0.79 \pm 0.12^{a\,\beta}$	$0.82 \pm 0.04^{ab\,\beta}$	$0.76 \pm 0.20^{a\,\beta}$	$0.72 \pm 0.21^{a\,\gamma}$
Iași	$0.73 \pm 0.05^{ab\,\delta}$	$0.71 \pm 0.03^{a\,\beta}$	$0.89 \pm 0.04^{a\,\alpha}$	$0.87 \pm 0.06^{a\,\alpha}$	$0.73 \pm 0.20^{a\,\beta}$	$0.72 \pm 0.21^{a\,\gamma}$
Ştefăneşti-Argeş	$0.86 \pm 0.09^{a\,\alpha}$	$0.88 \pm 0.09^{a\,\alpha}$	$0.75 \pm 0.14^{a\,\gamma}$	$0.65 \pm 0.09^{b\,\delta}$	$0.68 \pm 0.15^{a\,\gamma}$	$0.68 \pm 0.05^{a\,\delta}$
Average	0.73±0.07	0.73±0.10	0.73±0.07	0.80±0.08	0.74±0.15	0.75±0.13
Sig.	**	**	**	***	***	***
Areas	**	*	*	**	**	**
Varieties	**	**	**	**	**	**

Average value ± standard deviation (n = 3). Roman letters represent the significance of the variety difference (*p ≤ 0.05*). Greek letters represent the significance of the same variety cultivated in other areas (*p ≤ 0.05*). The difference between any two values, followed by at least one common letter, is insignificant. *Normal Values (OIV, 2016).

concentration of copper content in grape must. The lowest concentration of copper in must was determined in Băbească neagră (red grape must) from Ştefăneşti-Argeş vineyard and in Merlot (red grape must) from Târnave vineyard. Furthermore, the varietal characteristics observed in grapes were similar to those in soils. For example, the copper content in Merlot grapes and in soils was the same in all vineyards, indicating the transfer of copper from soil to grapes (Vystavna *et al*., 2014, 2015; Sun *et al*., 2018). However, some varietal characteristics were changed, such as Fetească albă in Dealu Bujorului, Murfatlar, Iaşi and Ştefăneşti-Argeş vineyards, Băbească neagră in Târnave vineyard and Fetească neagră in Iaşi vineyard. Finally, the copper concentration in grape must was lower than in previous reports. For example, in Italy, Provenzano *et al.* 2010, determined the copper content in Chardonnay grape must varying from 4.95 to 14.02 mg/L, while in the present study there was determined a copper content from 1.98 to 2.74 mg/L in grape must among the analyzed varieties.

In case of copper content in grape must (horizontal dendrogram), the dendrogram clearly show two clusters, the first cluster is formed from the Iasi, Tarnava and Ştefaneşti-Argeş vineyards and second cluster was formed from the Dealu Bujorului and Murfatlar vineyards. Based on this distribution we can say that the highest copper concentration was recorded in Dealu Bujorului and Murfatlar vineyards while Iasi, Tarnava and Ştefăneşti-Argeş vineyards recorded the lowest concentration of copper. The vertical dendrogram show also two clusters, the first cluster is formed from Merlot and Babească neagră, the second cluster was formed from all the rest of varieties, both white and red. Babească neagră and Merlot variety from Dealu Bujorului and Murfatlar recorded the highest concentration of copper compared to the rest of the varieties. Based on these results it cannot be concluded that in all the analyzed areas the vine varieties for red wines exhibited the highest concentration of copper (Fig. 2).

Regarding wines (Tab. 6), in contrast with soils and grapes, the copper level in the red wine samples was significantly higher than in the white wine samples. These results are in contradiction with Provenzano *et al*. (2010), Sun *et al*. (2018) which reported that white wines registered a higher copper concentration than red wines.

According to Sun *et al*. (2018), the differences might be determined by the relatively reduced number of samples and thus could not fairly reflect the results, additionally, there were significant varietal differences in wines. Furthermore, mineral composition of wines was influenced by many factors, including the chemical and physical characteristics of the soil in which the vine grew, vine variety, capacity of the grapes to absorb mineral substances, such as irrigation and application of fertilizers, pesticides, herbicides and insecticides.

Table. 7. Transfer Factor T(f) and Mobility Report M(r) of copper from vineyard soil, grape must and wine

Areas	Parameter	Fetească albă	Fetească regală	Șarba	Fetească neagră	Babească neagră	Merlot	Average	STDEV
Dealu Bujorului	T(f) grape must / soil	0.004	0.003	0.004	0.005	0.004	0.004	0.004	0.001
	T(f) wine / must	0.363	0.351	0.306	0.312	0.357	0.294	0.357	0.008
	M(r) wine / soil	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000
Murfatlar	T(f) grape must / soil	0.004	0.004	0.005	0.005	0.003	0.004	0.004	0.000
	T(f) wine / must	0.266	0.264	0.180	0.241	0.283	0.272	0.265	0.001
	M(r) wine / soil	0.001	0.001	0.323	0.001	0.001	0.001	0.001	0.000
Târnave	T(f) grape must / soil	0.004	0.006	0.005	0.005	0.004	0.003	0.005	0.001
	T(f) wine / must	0.251	0.235	0.345	0.291	0.373	0.579	0.243	0.011
	M(r) wine / soil	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Iaşi	T(f) grape must / soil	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.001
	T(f) wine / must	0.570	0.350	0.506	0.433	0.435	0.348	0.460	0.156
	M(r) wine / soil	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Ştefăneşti-Argeş	T(f) grape must / soil	0.004	0.006	0.004	0.004	0.002	0.002	0.005	0.001
	T(f) wine / must	0.332	0.226	0.348	0.227	0.469	0.425	0.279	0.075
	M(r) wine / soil	0.004	0.006	0.004	0.004	0.002	0.002	0.005	0.001

Among wine samples, the white wine samples showed the same change trend as the soils and grapes. However, some small differences have been observed in the red wine samples. These results indicate that there are many other factors that might influence the copper concentration in wines, such as the biological specificity of the cultivars in the winemaking process (Vystavna *et al.*, 2014, 2015; Sun *et al.*, 2016; 2018), the total concentration of copper fungicide applied during the production period and the number of days between the last application and the harvest (García-Esparza *et al.*, 2006).

In the case of copper content in wine (horizontal dendrogram), the dendrogram clearly show two clusters (Fig. 3). The first cluster is formed from Dealu Bujorului, Murfatlar, Târnave and Iaşi vineyards and the second cluster is formed from only the Ştefăneşti-Argeş vineyard. The vertical dendrogram show also two clusters, the first cluster is formed from Fetească regală, Fetească albă and Şarba and the second cluster is formed from Merlot, Băbească neagră and Fetească neagră.

This result indicated a clearly separation between white and red wines, as well as a separation between Ştefăneşti-Argeş and the rest of research areas. It can also be noticed that red wines registered the highest values of copper content in areas of Dealu Bujorului, Târnave and Iaşi, and lower values of copper content in wines from Ştefăneşti-Argeş area. The grapevine varieties for white wines recorded the highest concentration in Ştefăneşti-Argeş area compared to the rest of the research areas.

To characterize quantitatively the transfer of an element from soil to plant, the Partition Coefficient or Transfer Factor (TF) was used (Tab. 7). A prerequisite of the soil-plant transfer factor concept is the presence of a statistically significant relationship between the content of a given element in the soil and in the plant (Bunzl *et al.*, 2000). These values are needed for many assessment models to predict the concentration of an element for a given plant species at an anticipated contamination level in the soil (Bunzl *et al.*, 2000). TF describes the amount of an element expected to enter a plant from its substrate, under equilibrium conditions (Sheppard and Sheppard, 1985; Davis *et al*., 1998). The translocation factor (TF) in *Vitis vinifera L.* represent an essential indicator that allows assessing the mobility of copper and the danger of the metal translocation to the edible parts of the grapevine.

The mobility ratio (MR) in *Vitis vinifera L.* was used to determine the ratio of copper concentration in grapes to the concentration levels of the acid-soluble metal faction in top soil. TF > 1 indicates that *Vitis vinifera* L. translocate metals effectively from soil to plant parts (canes, leaves and grapes) also in must and wine (Serbula *et al.*, 2012). TF < 1 shows that *Vitis vinifera* exclude metals from uptake (excluder) (Mingorance *et al.*, 2007). MR > 1 indicates that *Vitis vinifera* L. translocates metals effectively from soil to plant parts (canes, leaves and grapes) also in must and wine (Serbula *et al.*, 2012). MR < 1 shows that *Vitis vinifera* exclude metals from uptake (excluder) (Mingorance *et al.*, 2007).

Mean values of translocation factor and mobility ratio for copper in *Vitis vinifera L.* for all the sampling sites are given in Table 4. Values for Transfer Factor (TF) but also for Mobility Ratio (MR) indicates that *Vitis vinifera* L. does not allow the accumulation of copper for vineyard soil, in must grape and in wine. This result indicated that there are many other factors which influence the copper concentration in wines, such as the biological specificity of the cultivars in the winemaking process (Vystavna *et al*., 2014, 2015; Sun *et al.*, 2016; 2018), the total concentration of copper fungicide applied during the production period and the number of days between the last application and harvest (García-Esparza *et al.*, 2006).

Conclusion

The copper pollution in grape must and wine from the investigated area was analysed. For all the studied areas, the values of copper pollution in vineyard soils were above the Maximum Admissible Limits (20 mg/kg (MAL)). In all grape must and wine samples, the obtained values of copper content were below the Maximum Admissible Limits (1 mg/kg (MAL)). The copper concentration in the red wine samples was significantly higher than in the white wine samples.

Values of Transfer Factor (TF) and Mobility Ratio (MR) indicate that *Vitis vinifera* L. does not allow the accumulation of copper from vineyard soil in must grape and wine. The geographical location and the variety significantly influenced the copper content in vineyard soils, grape and wine. Additionally, amongst the copper level in the vineyard soils, in grape must and in wines were highlighted certain correlations.

The copper concentration in vineyard soils and other factors such as the biological specificity of cultivars during growth, the total amount of copper fungicide applied during the production period, the number of days between the last application and harvest, and also the varietal characteristics in winemaking process codetermined the copper concentration in grape must and in wines.

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References

1. Al Nasir F, Jiries AG, Batarseh ML, Beese F (2001). Pesticides and trace metals residue in grape and home made wine in Jordan. Environmental Monitoring and Assessment, 66: 253–263.

2. Arsya M, Olivares N, Pizarro M, Gonzales M, Speisky H, Uauy R (2003). Copper exposure and potential biomarkers of copper metabolism. BioMetals, 16:199–204.
3. Azenha M, Vasconcelos M, Moradas-Ferreira J, Toit M, Toit P (2000).The influence of Cu concentration on ethanolic fermentation by *Saccharomyces cerevisiae*. Journal of Bioscience and Bioenginnering, 90: 163–137.
4. Bora FD, Donici A, Oşlobanu A, Fiţiu A, Babeş AC, Bunea CI (2016a). Qualitative assessment of the white wine varieties grown in Dealu Bujorului vineyard, Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(2): 593–602.
5. Bora FD, Dina I, Iliescu M, Zaldea G, Guşă IC (2016b). Analysis and evaluation of the main ecoclimatic conditions from Romanian vineyards. Bulletin UASMV Horticlture, 73(2): 221–223.
6. Bora FD, Donici A, Rusu T, Bunea A, Popescu D, Bunea CI (2018). Elemental profile and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ration as fingerprints for geographical traceability of Romanian wines. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46(1): 223–239.
7. Besnard E, Chenu C, Robert M (2001). Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyard soil. Environmental Pollution 112: 329-337.
8. Bunzl K, Albers BP, Schimmack W, Belli M, Ciuffo L, Menegon S (2000). Examination of a relationship between ^{137}Cs concentrations in soils and plants from alpine pastures. Journal of Environmental Radioactivity, 48: 145–158.
9. Brunetto G, Bastos de Melo GW, Terzano R, Del Buono D, Astolfi S, Tomasi N, Pii Y, Mimmo T (2016). Copper accumulation in vineyard soils: Rhizosphere processes and agronomic practices to limit its toxicity. Chemosphere, 162: 293–307.
10. Da Rosa-Couto R, Ribeiro-Lazzari CJ, Trapp T, Comin JJ, Martins SR, Belli-Filho P, Brunetto G (2015). Accumulation and distribution of copper and zinc in soils following the application of pig slurry for three to thirty years in a microwatershed of Brazil. Archives of Agronomy and Soil Science, 62(5): 593–616.
11. Davis PA, Avadhanula MR, Cancio D, Carboneras P, Coughtrey P, Johansson G, Little RH, Smith GM, Watkins BM (1998). BIOMOVS II: an international test of the performance of environmental transfer models. Journal of Environmetal Radioactivity, 42:117–130.
12. Donnini S, Tessarin P, Ribera-Fonseca A, Di Foggia M, Parpinello G P, Rombola AD (2016). Glyphosate impacts on polyphenolic composition in grapevine (*Vitis vinifera L.*) berries and wine. Food Chemistry, 213:26–30.
13. Deluisa A, Giandon P, Aichner M, Bortolami P, Bruna L, Lupetii A, Nardelli F, Stringari G (1996). Copper pollution in Italian vineyard soils. Communications in Soil Science and Plant Analysis, 27: 1537-1548.
14. Cotea DV, Grigorescu CC, Barbu N, Cotea VV (2000). Podgoriile şi vinurile României. Bucureşti: Editura Academiei Române (Chapter 1, 2, 3 and 4).
15. EC No. 1410/2003. (2003). Official Journal of the European Union. Commission Regulation (EC) No. 1410/2003 of 7 August 2003 amending Regulation (EC) No. 1622/2000 laying certain detailed rules for implementing Council Regulation (EC) No. 1493/1999 on the common organization of the markets in wine and establishing a Community code of oenological practices and processes.
16. Ferreira J, Toit M, Toit J, Toit M, Toit W (2006).The effects of copper and high sugar concentration on growth, fermentation efficiency and volatile acidity production of different commercial wine yeast strains. Australian Journal of Grape and Wine Research, 12: 50–56.
17. Flores-Vélez LM, Ducaroir J, Jaunet AM, Robert M (1996). Study of the distribution of copper in an acid sandy vineyard soil by three different methods. European Journal of Soil Science, 47: 523–532.
18. Flemming CA and Trevors JT (1989). Copper toxicity and chemistry in the environment: a review.Water. Air, & Soil Pollution, 44:143–158.
19. Fernández-Calviño D, Soler-Rovira P, Polo A, Díaz-Raviña, Aris-Estévez M, Plaza C (2010). Enzyme activities in vineyard soils long-term treated with copper-based fungicides. Soil Biology and Biochemistry, 42: 2119–2127.
20. Gaiero DM, Probst JL, Depetris PJ, Bidart SM, Leleyter L (2003). Iron and other transition metals in Patagonian riverborne and windborne materials: geochemical control and transport to the southern South Atlantic Ocean. Geochimica et Cosmochimica Acta, 67:3603–3623.
21. García-Esparza M, Capri E, Pirzadeh P, Trevisan M (2006). Cooper content of grape and wine from Italian farms. Food Additives and Contaminants, 233: 274–280.
22. Geana EI, Sandru C, Stanciu V, Ionete RE (2016). Elemental profile and $^{87}Sr/^{86}Sr$ isotope ration as fingerprints for geographical traceability of wines: an approach on Romanian wines. Food Analytical Methods, 10(1): 63–73.
23. Jenkins DA and Jones RGW (1980). Trace elements in rocks, soils, plants and animals: introduction. Chichester, (Chapther 1).
24. Jia B, Liu XY, Zhan JC, Li JY, Hung WD (2015). The effect of proanthocyanidins on growth and alcoholic fermentation of wine yeast under copper stress. Journal of Food Science, 80: 1319–1324.
25. Komárek M, Čadková E, Chrastný V, Bordas F, Bollinger J (2006). Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environment International, 36: 138–151.
26. Komárek M, Čadková E, Chrastný V, Bordas F, Bollinger JC (2010). Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environement International, 36: 138–151.
27. Li WQ, Hu QP, Xu JP (2015). Changes in physicochemical characteristics and free amino acids of hawthorn

(*Crataegus pinnatifida*) fruits during maturation. Food Chemistry, 175: 50–56.

28. Liu T, Sun C, Ta N, Hong J, Yang S, Chen C (2007). Effect of copper on the degradation of pesticides cypermethrin and cyhalathrin. Journal of Environmental Sciences, 19: 1235–1238.

29. Marengo E and Aceto M (2003). Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. Food Chemistry, 81: 621–630.

30. Marschner P (2011). Marschners mineral nutrition of higher plants. (3th ed.). London: Academic Press, (Chapter 1).

31. Mingorance MD, Valdés B, Oliva RS (2007). Strategies of heavy metal up take by plants growing under industrial emissions. Environment International, 33: 514–520.

32. Mirlean N, Roisenber A, Chies J (2007). Metal concentration of vineyard soils in wet subtropics (southern Brazil). Environmental Pollution, 149: 10–17.

33. Naughton D and Petróezi A (2008). Heavy metal ions in wines: Meta-analysis of target hazard quotients revel health risk. Chemistry Central Journal, 3: 1–7.

34. OIV (2003). International code of oenological practices (Issue 2013/01 revised). ISBN: 979-10-91799-10-2.

35. OIV (2016). Maximum acceptable limits of various substances contained in wine. In: Compendium of international methods of analysis of wine and must analysis. Paris, France.

36. Pietrzak U and McPhail D (2004). Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geodema, 122: 151–166.

37. Pohl P and Sergiel I (2009). Evaluation of the total content and the operationally defined species of copper in beers and wines. Journal of Agricultural and Food Chemistry, 57: 9378–9384.

38. Provenzano M, Bilali H, Simeone V, Baser N, Modelli D, Cesari G (2010). Copper contents in grapes and wines from Mediterranean organic vineyard. Food Chemistry, 122: 1338–1343.

39. Robinson N and Winge D (2010). Copper metalla chaperones. Annual Review of Biochemistry, 79: 537–562.

40. Ruyters S, Salaets P, Oorts K, Smolders E (2013). Copper toxicity in soils under established vineyards in Europe: a survey. Science of the Total Environmet, 443: 470–477.

41. Sancenón V, Puig S, Mira H, Thiele DJ, Peñarrubia L (2003). Identification of a copper transporter family in *Arabidopsis thaliana*. Plant Molecular Biology, 51: 577–578.

42. Sauvage L, Frank D, Stearne J, Millikan MB (2002). Trace metal studies of selected white wines: An alternative approach. AnalyticaChimica Acta, 458: 223–230.

43. Sheppard MI and Sheppard SC (1985). The plant concentration concept as applied to natural uranium. Health Physics, 48:494–500.

44. Serbula SM, Miljkovic DJD, Kovacevic MR, Ilic AA (2012). Assessment of airborne heavy metal pollution using plant parts and topsoil. Ecotoxicology and Environmental Safety, 76: 209–214.

45. Sun XY, Zhao Y, Liu LL, Jia B, Zhao F, Huang WD, Zhan JC (2015). Copper tolerance and biosorption of *Saccharomyces cerevisiae* during alcoholic fermentation. PloSOne. DOI: 10.1371/journal.pone.0128611.

46. Sun XY, Liu II, Zhao Y, Ma TT, Zhao F, Huang WD, Zhan JC (2016). Effect of copper stress on growth characteristics and fermentation properties of *Saccharomyces cerevisiae* and the pathway of copper adsorption during wine fermentation. Food Chemistry, 192: 43–52.

47. Sun XY, Ma TT, Han LY, Haung WD, Zhan JC (2017). Effects of copper pollution on the phenolic compound contents, color and antioxidant activity of wine. Molecules. Doi: 10.3390/molecules22050726.

48. Sun X, Ma T, Yu J, Huang W, Frang Y, Zhan J (2018). Investigation of copper contents in vineyard soil, grape, must and wine and the relationship among them in the Huaizhuo Basin Region, China: A preliminary study. Food Chemistry, 241: 40–50.

49. Tóth G, Herman T, Da Silva MR, Montanarella L (2016). Heavy metals in agricultural soils of the European Union with implication for food safety. Environment International, 88: 299–309.

50. Turnlund J, Jacob R, Keen J, Kelley D, Domek J, Coulter J (2004). Long-term high copper intake: Effects on indexes of copper status, antioxidant status, and immune function in young men. American Journal of Clinical Nutrition, 79: 1037–1044.

51. Vitanović E, Vidaček Ž, Katalinić K, Miloš B (2010). Copper in surface of Croatian vineyard soils. Journal of Food, Agriculture & Environment 8(1): 268-274.

52. Volpe M, Cara F, Volpe F, Mattia A, Serino V, Petitto F, Zavalloni C, Limone F, Pellecchia R, De Prisco PP, Di Stasio M (2009). Heavy metals uptake in the oenological food chain. Food Chemistry, 117: 553–560.

53. Vystavna Y, Ruskenko L, Diadin D, Klymenko O, Mykola K (2014). Trace metals in wine and vineyard environment in southern Ukraine. Food Chemistry, 146: 339–344.

54. Vystavna Y, Reelika R, Nina R, Olena D, Valentina P, Mykola K (2015). Composition of soil-to-root transfer and translocation coefficients of trace elements in wines Chardonnay and Muscat white grown in the same vinenayard. Scientia Horticulture, 192: 89–96.

55. Wightwick AM, Mollah MR, Partington DL, Allison G (2008). Cooper fungicide residue in Australian vineyard soils. Journal of Agricultural and Food Chemistry 56: 2457-2464.

56. Wintz H, Fox T, Wu YY, Feng V, Chen W, Chang HS, Zhu T, Vulpe C (2003). Expression profiles of *Arabidopsis thaliana* in mineral deficiencies reveal novel transporters involved in metal homeostasis. The Journal of Biological Chemistry, 278: 47644–47653.

57. Xue H, Sigg L, Gächter R (2000). Transport of Cu, Zn and Cd in a small agricultural catchment. Water Research, 34(9): 2558–2568.

58. Xiaorong W, Mingde H, Mingan S (2007). Copper fertilizer effects on copper distribution and vertical transport in soils. Geoderma, 138: 213–220.

59. Yruela L (2009). Copper in plants: acquisition, transport and interactions. Functional Plant Biology, 36(5): 409–430.

60. Zimmermann M, Clarke O, Gulbis JM, Keizer DW, Jarvis RS, Cobbett CS, Hinds MG, Xiao Z, Wedd AG (2009). Metal binding affinities of *Arabidopsis* Zinc and Copper transports: selectivity match the relative, but not the absolute, affinities of their amino-terminal domains. Biochemistry, 48: 11640–11654.