Journal of Food Composition and Analysis 100 (2021) 103935

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Heavy metals assessment in the major stages of winemaking: Chemometric analysis and impacts on human health and environment

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ARTICLE INFO

Keywords:
Heavy metals
Winemaking stages
Wastewater
HR-CS AAS
Health risk assessment
Fingerprint
Heat map

ABSTRACT

Wine contamination with heavy metals may pose long-term human health risks and environmental damages. This study evaluated the frequently detected heavy metals (HMs): Cr-Chromium, Fe-Iron, Cu-Copper, Mn-Manganese, Ni-Nickel and Zn-Zinc in the winemaking stages of white (Grasă de Cotnari) and red (Fetească neagră) wine varieties. Samples from wines and wastewaters were collected at eight different winemaking stages and HMs quantification was done by high-resolution continuum source atomic absorption spectrometry. Results showed that, in white wines Pb, Cu and Cr concentrations decreased during winemaking processes, while in red wines only Pb and Cu levels were reduced. A progressive increment was observed for Ni and Fe in white wines and for Cr, Ni and Fe in red wines, during winemaking. In the final winemaking stage of white wines, Fe and Mn concentrations were 3826 and 1493 μg/L, respectively, while in red wines, 1136 and 1256 μg/L, respectively. Analysis of wines consumption safety was performed according to the estimated daily intake (EDI) and target hazard quotients (THQ). EDI values of the analysed white wines ranged from 0.0032 to 6.2217, and for red wines from 0.0004 to 2.6919. THQ values below 1 suggested that moderate wine consumption does not have negative impacts on human health.

1. Introduction

Moderate consumption of wine has shown beneficial health effects due to presence of polyphenolic compounds with antioxidant activity (Dumitriu et al., 2015). However, compounds that cause negative effects on human health such as heavy metals, pesticide residues, mycotoxins and other off-flavours may be present in commercially available wines (Cepo et al., 2018).

Contamination with heavy (toxic) metals is one of the most important problem for various environmental compartments and humans health, the biggest challenges being to reduce its related negative impacts (surface and drinking water, wastewater, ecosystems, bio-accumulation, etc). In this context, the contamination of wine with heavy metals may threaten beverage security and public health, both locally and globally. Heavy metals (HMs) are a general term which applies to the group of metals and metalloids with an atomic density of above 4000 kg/m^3 (Edelstein and Ben-Hur, 2018). Some HMs are well known for their toxicity, non-biodegradable nature and potential bio-accumulative behaviour in human tissues. It has been suggested that excessive intake of HMs could result in damage to organs and cause various diseases, characterized by renal and nerve dysfunction, carcinogenic nature, cardiovascular problems and other poisoning (World Health Organization-WHO, 2017). The main exposure route to HMs for humans is ingestion of contaminated food.

Daily consumption of wine in moderate quantities, i.e. 125 mL for women and 250 mL for men (Pomarici and Vecchio, 2014), contributes significantly to the requirements of humans for essential trace elements such as chromium (Cr), iron (Fe), copper (Cu), manganese (Mn), nickel (Ni), zinc (Zn). On the other hand, negative effects of HMs presence in wine include beverage spoilage and hazing, as well as sensorial and health consequences. Hence, the analysis of HMs in wines is of particular interest due to their toxicity in case of excessive intake and the possible impacts on the wine organoleptic properties. An example is represented by the essential trace elements copper and iron, which become potentially toxic elements for humans and impact the wine quality at high concentrations (Bekker et al., 2019).

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https://doi.org/10.1016/j.jfca.2021.103935
Received 31 January 2021; Received in revised form 11 April 2021; Accepted 13 April 2021
Available online 15 April 2021
0889-1575/

Wine contamination with HMs arises mostly from the large uses of agrochemical products due to fast economic development of industry and agriculture, responding thus to the advanced demands of food production for human consumption. HMs content in wine depends on various factors such as:

a) *Natural sources* (vineyard soil structure; grape maturity and variety), *climate* (temperature, humidity, wind), and *geography* (region, orography, presence of water resources);
b) *Viticulture management practices* (fertilizers, pesticides, chemical sprays, grape-growing approaches), which can introduce HMs during the whole winemaking process, and *surrounding industries and associated environmental pollution* (Fiket et al., 2011; Pohl, 2007);
c) *Contamination during the winemaking process*, caused by prolonged contact with different materials: pipes, casks, steel tanks, fining and clarifying substances, equipment and other operations (Fiket et al., 2011; Lara et al., 2005).

Various stages, from grape growth through harvesting, grape transport and winemaking, may be sources of metals in the final wine. The use of different materials in wineries, including stainless steel, aluminium, wood, and plastics introduce HMs into wine (Tariba et al., 2011). These elements include Cr, Fe, Mn, Ni, Pb and Zn from stainless steel and brass containers, oak barrels, tubes, fittings, and traps (Kristl et al., 2002; Rossano et al., 2007; Tariba et al., 2011). Zn and Mn are favourable for yeasts during fermentation as they are involved in enzyme activation (Pohl, 2007). Cu and Zn occur naturally in soil, but their content is influenced by agricultural practices and direct contact with tank surfaces and metallic tubing during winemaking (Marengo and Aceto, 2003). Cr and Ni contents in wines is related almost exclusively to anthropogenic sources, due to interaction with metal containers (Marengo and Aceto, 2003). Cd is considered an anthropogenic element (Hopfer et al., 2015) which may originate either from the use of fertilizers, fungicides and pesticides in the vineyard (Pohl, 2007), or from atmospheric pollution. Pb content in wines is related to fungicidal treatments, container materials and vehicular traffic, the soil contribution being minimal (Marengo and Aceto, 2003).

Winemaking is a water-intensive process that generates a significant volume of wastewater, with estimates ranging as high as 4−8 L of winery wastewater generated per litter of wine (Conradie et al., 2014). The composition of winery wastewater is highly variable between wineries because of general management practices, the amount of water used, the winery size and design and different wine-making techniques (Howell et al., 2016). Winery wastewater quantity and quality vary even within a single winery due to the various steps in winemaking and the type of wine produced (Bolzonella et al., 2019). Furthermore, the characteristics of wastewater change substantially depending on the production stage, from low-strength wastewaters used for floor, barrel, and bottle washing, to high-strength wastewaters for grape harvesting, crushing, and racking (Buelow et al., 2015). Access to safe water and sanitation, as well as climate changes and water stress are the other reasons why winemaking should facilitate less water consumption and wastewater treatment (WHO, 2017).

The quality control of consumed wines, especially in terms of heavy metals, and the assessment of their impacts on health of consumers are of particular importance. World wine consumption in 2019 was estimated at 244 million hectolitres (mhl) and Portugal leads with 56 L per capita consumption of wine per year, ahead of France (49.5 L), Italy (43 L), Spain (27.8 L) and Romania (23.9 L) (International Organization of Vine and Wine, OIV, 2020). Winemaking is a very complex process in which many chemical, physical and microbiological transformations take place and each stage involved in production has a major contribution to the final wine quality and safety. Complex chemical composition, influence of winemaking stages and high per capita consumption of wine makes imperative the analysis of potential health risks associated with heavy metals in wines.

The health risks associated with HMs via wine consumption are influenced by numerous factors that include metal concentration, daily wine consumption rate, consumer body weight, oral reference dose, among others. A limited number of studies on the concentrations of heavy metals in Romanian wines and health risk assessments of the dietary ingestion of these elements from wine are available. Agencies and organizations such as the US Environmental Protection Agency (US EPA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the OIV, have provided guidelines on the admissible intake of HMs by humans. The US EPA published target hazard quotients (THQs) for the estimation of potential health risks associated with long-term exposure to chemical pollutants.

To ensure safe wine consumption, to limit human health impacts and prevent excessive contents of HMs in wine and wastewater, winemakers should regularly monitor these contaminants in white and red wines processing. This study aims to evaluate the heavy metals contamination in different stages of winemaking and to provide new insights into safe winemaking. In this work, an integrated approach was used to assess the heavy metals contamination for white (Grasă de Cotnari) and red (Fetească neagră) wine varieties produced at pilot scale. Samples from wines and generated wastewaters were collected at eight different stages of winemaking, this being the first study that included the HMs quantification in all stages and in wastewater. High-resolution continuum source atomic absorption spectrometry was used to determine the concentrations of 6 heavy metals (Mn, Fe, Cu, Ni, Pb and Cr). This study has: (1) quantified HMs concentrations and identified their contamination sources (natural and anthropogenic) by means of statistical methods: correlation analysis, principal component analysis (PCA), multivariate analysis (MVA) and heatmap visualization, (2) investigated the HMs propagation in wines and wastewater during each important winemaking process stage, and (3) assessed human health risks caused by contaminated wine consumption.

2. Materials and methods

2.1. Winemaking process at pilot scale and samples collection

Grapes from Grasă de Cotnari and Fetească neagră varieties (*V. vinifera*) were collected in 2018 vintage from Cotnari vineyard in Romania. Grapes were harvested by hand at adequate grape maturity and in optimum sanitary conditions. Afterwards, they were placed in 15 kg plastic boxes and transported to the Oenology winery from "Ion Ionescu de la Brad" University of Agricultural and Veterinary Medicine, Iasi, Romania.

Adequate winemaking procedures (Fig. 1) were applied, depending on grapes types: for white wines-Grasă de Cotnari (GC) and for red wines-Fetească neagră (FN). For each variety, winemaking was done at pilot scale, in triplicate, in stainless steel tanks. The complete winemaking process and sample collection is presented in the Supplementary material (S2.1 and Table 1S).

2.2. Sample preparation and analytical measurement

In this study, the elements under investigation consisted of six HMs: manganese (Mn), iron (Fe), copper (Cu), nickel (Ni), lead (Pb) and chromium (Cr). To identify and quantify the metal concentrations from wines and wastewater, a ContrAA 800 G high-resolution continuum source atomic absorption spectrometer (HR-CS AAS) equipped with graphite furnace, from Analytik Jena (Germany), was used. Argon gas (purity higher than 99.99 %) was utilized as the plasmogen and carrier gas. High purity CertiPUR mono-element solutions (1000 mg/L) from Merck Millipore (Germany) were used to obtain the calibration curves for each metal. The calibration curves and metal analysis were done in presence of modifiers (Merck Millipore, Germany). The mono-element calibration curves were verified with a multi-element certified material of 1000 mg/L (Merck Millipore, Germany) and the recovery R (%)

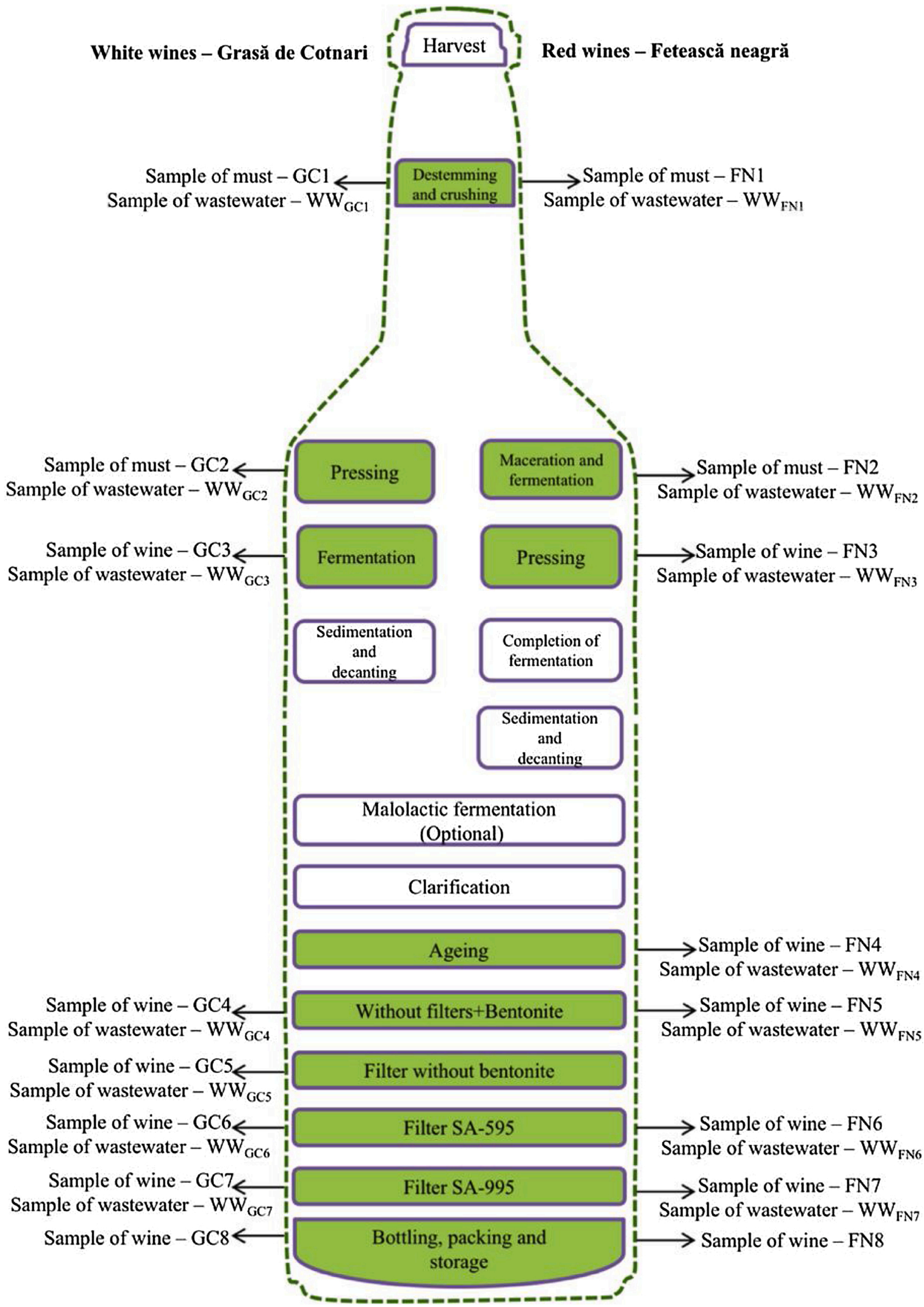

Fig. 1. Winemaking processes at pilot scale for white (Grasă de Cotnari) and red (Fetească neagră) grapes.

varied between 94.5 % and 106.1 %.

Prior to analysis, all samples were digested by using a microwave reaction system, Multiwave Digestion System, Speedwave entry (Berghof, Germany), with rotor for DAP-60 K vessel. The mineralization procedure involved that 3 mL of sample was treated with a mixture of 3 mL HNO_3 65 % (v/v) and 1 mL H_2O_2 30 % (v/v), adapted from Karataş et al. (2015). The samples were then heated in a digester block for 30 min, in a two-step temperature program at 140 °C and 160 °C. The resulting solutions were diluted to 50 mL with ultra-pure water. Reagent blanks and spiked samples in duplicate were prepared and analysed in the same conditions as the samples. The recovery for the spiked samples ranged between 84–121 %.

Nitric acid 65 % reagent grade was obtained from Merck Millipore (Germany) and hydrogen peroxide 30 % reagent grade was obtained from Chimreactiv (Romania). Ultra-pure water (Adrona Crystal EX Trace, Latvia), with a conductivity of 0.055 μS/cm (at 25 °C), was used in the preparation and dilutions of standards and digested solutions.

2.3. Statistical analysis

To establish possible differences between winemaking stages and between white and red wines on the basis of multielement data sets, data statistical processing is necessary. This approach allows distinguishing between natural and anthropogenic origin of HMs in wine. For this purpose, four different statistical methods were used: correlation analysis, principal component analysis (PCA), multivariate analysis (MVA) and heat map (or heatmap) visualization. Pearson correlation coefficients (P-values) were calculated in the statistical language R (v.1.1.453, RStudio Inc, Boston, MA, USA). P-values below 0.05 indicate statistically significant non-zero correlations at 95.0 % confidence level. Correlation maps (corrgrams) were constructed with the R package *corrplot*. The multivariate analysis and corrgram correlations are presented in Fig. 1S and Fig. 2S (Supplementary material). Correlation analysis were performed to identify possible interrelations among heavy metal concentrations from different winemaking stages and between white and red wines samples. Blue circles correspond to significant positive correlations and red circles to significant negative correlations. The size of the circle reflects the magnitude of the Pearson correlation coefficient. These correlation coefficients range between -1 and +1 and measure the strength of the association between the variables.

2.4. Transfer coefficients

Transfer coefficients were used to determine the HMs allocation between different stages of winemaking for white and red wines, respectively. In this case, four transfer coefficients were considered, in accordance to the main stages of winemaking, as described in Table 2S (Supplementary material). These translocation coefficients were interpreted as: (1) if T was close to 0, HMs allocation from one stage to another was negligible; (2) for T between 0.1 and 1.0, HMs allocation from lower to higher stage was occurring, and (3) T > 1.0 was the result of HMs accumulation in the higher stage and/or the presence of HMs external sources/inputs/contamination (Vystavna et al., 2017).

2.5. Daily intake

The estimated daily intake of HMs from wine consumption depend on the metal concentration, daily consumption rate and consumers' body weight (Qian et al., 2010). Metal concentration in wines (M_{me}), the daily wine consumption (150 mL/per person/per day) and the body weight (70 kg/per person) were used for calculations (Semla et al., 2018; Sharafi et al., 2019a; OIV, 2019):

$$EDI = M_{me} \times R/BW \tag{1}$$

where: EDI is estimated daily intake (μg/kg bw/day); M_{me} is the concentration of an individual heavy metal in the wine (μg/L); R constitutes the daily wine consumption rate for adult wine drinkers (L/day) =150 mL/per person/per day; BW represents the average body weight of assessed population (kg) =70 kg/per person.

The health risk due to wine consumption was assessed based on the target hazard quotients (THQ), which was calculated from the ratio of EDI and an oral reference dose (USEPA, 1989):

$$THQ = EDI/RfD \tag{2}$$

where RfD present the oral reference dose for each heavy metal (μg/kg bw/day). If THQ is less than 1, it means that the intake of this element from wine has no obvious adverse effects. If THQ is equal to or higher than 1, there is a health risk to humans (Wang et al., 2005). The total THQ values for the elements evaluated in wine is defined as hazard index (HI), used to evaluate the comprehensive health risks of wine (Deng et al., 2019). A value of HI > 1 indicates that the non-carcinogenesis risk is not acceptable for the target population (Sharafi et al., 2019b).

The contribution to provisional tolerable weekly intake in the Romanian wines was estimated per consumer (70 kg of body weight) according to the formula (Semla et al., 2018):

$$PTWI\ contribution = M_{me} \times \frac{0.15}{BW} \times 7days \tag{3}$$

Where PTWI contribution is the provisional tolerable weekly intake (μg/kg bw/week).

The tolerable daily intake of Pb is set at 3.6 μg /kg bw/day, while for Ni is 5 μg /kg bw/day (FAO/WHO, 1993). The recommended dietary allowance of Cr is 130 μg/day/person which is equivalent to 2.2 μg /kg bw/day (FAO/WHO, 1993). The provisional tolerable daily intake (PTDI) of Ni-based on lowest observed adverse effect level (LOAEL) is 12 μg/kg body weight (WHO, 2005). The recommended dietary allowance (RDA) for Cu ranged from 900 μg to 30 mg/day person which is equivalent to 15–500 μg/kg bw/day (FAO/WHO, 1993). The recommended dietary allowance values for Fe and Mn were 10–18 mg/day person and 2–5 mg/day person, respectively. Reference doses were obtained by conversion from United States Environmental Protection Agency (USEPA) data, and the reference doses of Pb, Cr, Mn, Cu, Ni and Fe were 3.5; 3.0; 140.0; 5.0; 20.0 and 700.0 μg/kg per day, respectively.

3. Results and discussion

Although heavy metal concentrations of wine samples have been previously studied on specific winemaking stages exclusively, there is a lack of information on the accumulation of HMs during the entire processing. The HMs concentrations in wine is known to differ in accordance to winemaking stages, as fermentation, ageing, filtration and storage (Hopfer et al., 2015; Bekker et al., 2019). Moreover, the wine industry produces important wastewater volumes during wine production processes and operations.

HMs concentrations in wine and by-products processed from white and red grapes varieties fluctuated among winemaking stages (Fig. 2 and Fig. 3). Fe and Mn showed the highest contents in white wines (3826 and 1493 μg/L) and red wines (1136 and 1256 μg/L), while for Cr, Pb and Ni low contents were quantified. For samples analysed in this study, Fe, Mn, Cr, Pb, Cu and Ni concentrations identified in white and red wines were below the limits established by the Romanian law and by the International Organisation of Vine and Wine (OIV-Law 244/2002).

3.1. Evolution of heavy metals in wine and wastewater during the main winemaking stages

3.1.1. Copper (Cu)

In the case of Grasă de Cotnari (GC) white grapes winemaking, the content of Cu has reduced from 6755 μg/L in wine (GC) and 723 μg/L in wastewater (WW) during the destemming/crushing stage to 2545 μg/L and 456 μg/L, respectively in the pressing stage. Subsequently, Cu concentration decreased dramatically during early winemaking stages, to 360 μg/L and 202 μg/L in maceration & fermentation stage. This significant decrease can be explained by co-precipitation of copper with yeast cells during fermentation and/or by forming elemental complexes with tartrates, polyphenols, proteins and polysaccharides . Also, an

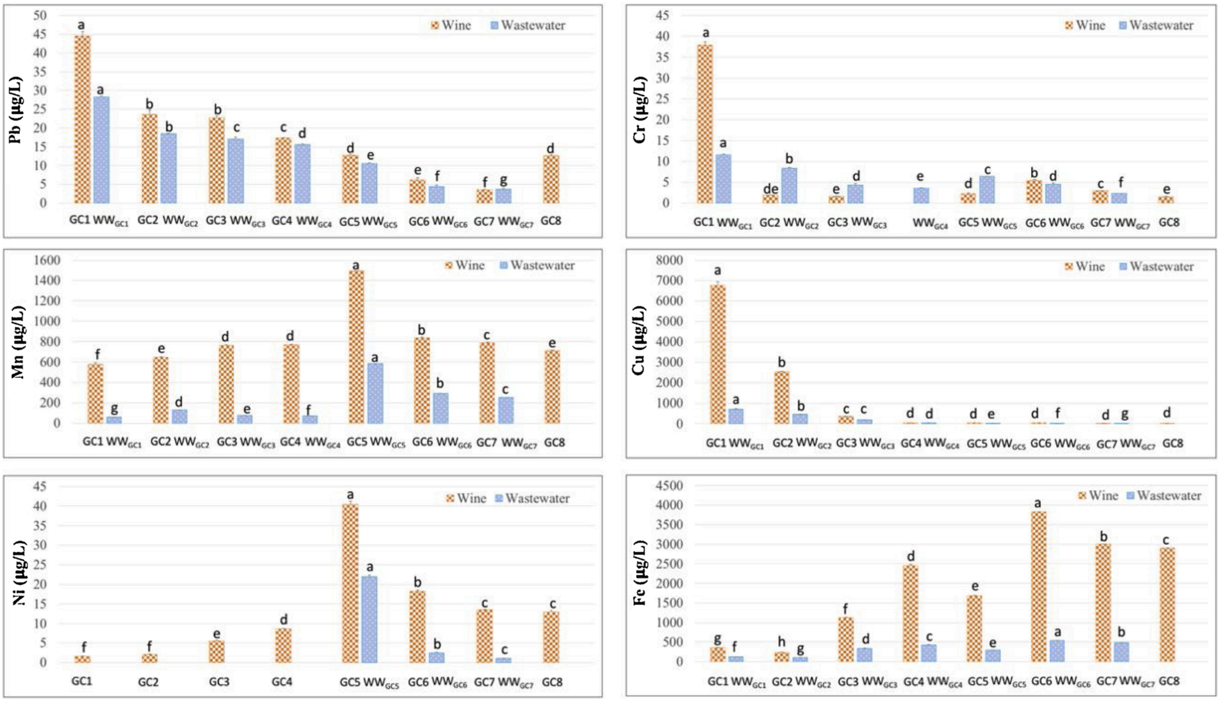

Fig. 2. Concentrations of heavy metals from winemaking stages and wastewater of white wines. Values are shown as means ± SD (n = 3). Different letters indicate significant differences at 95 % confidence level.

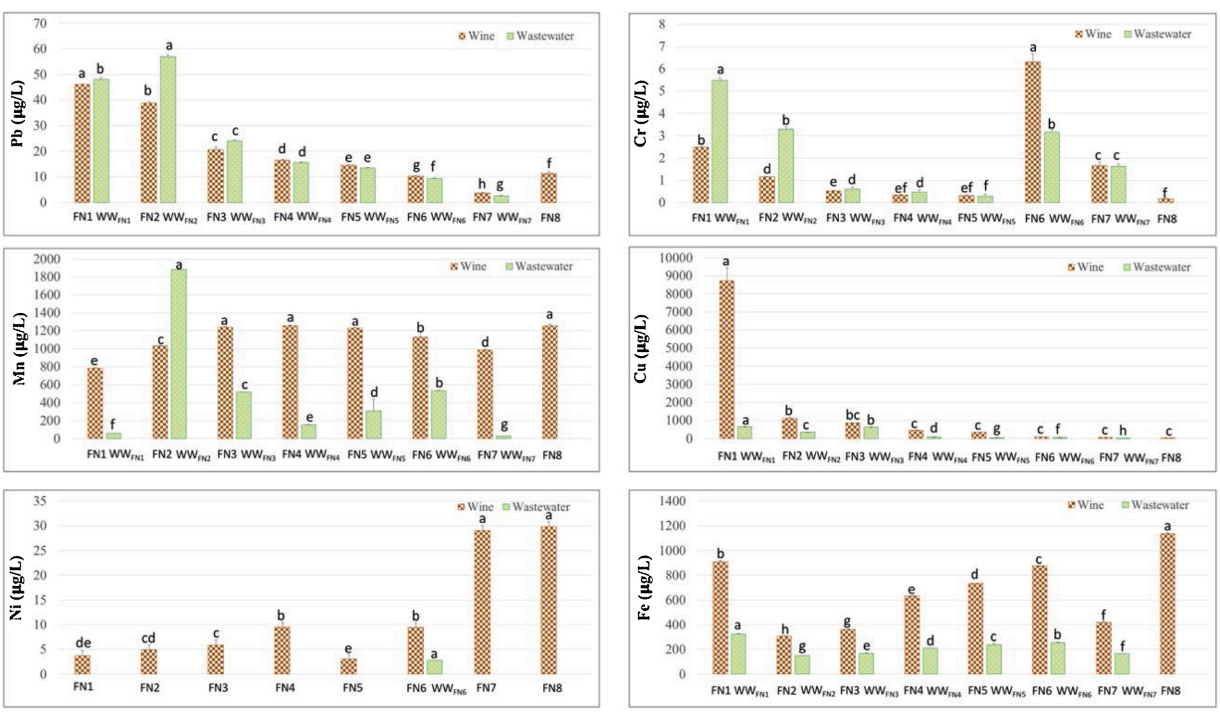

Fig. 3. Concentrations of heavy metals from winemaking stages and wastewater of red wines. Values are shown as means ± SD (n = 3). Different letters indicate significant differences at 95 % confidence level.

important concentration reduction was observed after the pressing stage due to the presence of Cu in the grapes pulp and skin. In the filtration stage, four filter variants were used (1. without filtration and with addition of bentonite; 2. without filter but with bentonite; 3. filter type SA-595; 4. filter type SA-995). In our study, the concentration of Cu ions in GC samples decreased when using the SA-995 filter and bentonite treatment (to 18 µg/L and 37 µg/L, respectively), which suggests that ions may have been sequestered by the yeast and other solid material during active fermentation and filtration. A similar trend was observed for Cu concentration in wastewater samples. Catarino et al. (2008) suggested that the concentration of various metals in wine depends on the type of bentonite used and wine pH. In case of copper, the authors reported significant decreases of its content in wine samples upon addition of six different types of bentonite at relatively high bentonite addition rates (25 g/L). In the final stage of winemaking, after a period of 7 months of storage, the Cu concentration in wines was 21 μg/L (Fig. 2).

A sharp decrease of Cu concentration was observed in the Fetească neagră (FN) red wine samples, from destemming/crushing (8712 μg/L) to maceration & fermentation stage (1102 μg/L). In case of wastewater samples, the Cu content decreased from 648 to 363 μg/L. This suggests that Cu was majorly present in the grapes pulp and skins, which have fallen down during fermentation into the deposit and thus, low metal concentrations remained in the must.

In the pressing stage, Cu concentration decreased to 864 μg/L for red wine sample and showed an increase to 630 μg/L in wastewater sample. After the filtration stage with SA-995 filter, a Cu content of 64 μg/L in red wines and 8.5 μg/L in wastewater were observed. Storage for 7 months decreased even more the total Cu concentration to 53 μg/L (Fig. 3).

According to the international regulations, the permissible limit of Cu in wines is set at 1000 μg/L (OIV, 2015). Moreover, in a study in which 72 wines were analysed, the average copper content was 180 μg/L with a maximum of 550 mg/L. National regulations allow the presence of 2000 μg/L Cu, in German wines (Wagner et al., 2010). Concentrations of around 5000 μg/L or higher can provoke a metallic taste, haze, browning and other unwanted effects on wine (Li et al., 2008).

3.1.2. Lead (Pb)

The presence of Pb in wine is a result of human activity, including environmental pollution, viticulture practices and winemaking procedures (Hopfer et al., 2015).

In this study, lead concentration in the first stage of white grapes winemaking was 45 μg/L for wine and 28 μg/L for generated wastewater (Fig. 2). After pressing, a decrease of Pb content with 46.61 % and 34.52 %, respectively was registered. After the fermentation stage, Pb concentrations in wine and wastewater remained similar to those in the pressing stage, i.e. 23 μg/L for GC3 and 17 μg/L for WW_{GC3}. Of all the filtration methods used, it was observed that the SA-995 filter resulted in the lowest Pb concentration. By using a SA-995 filter, the content of Pb was reduced drastically to 3.5 μg/L in white wines and 3.7 μg/L in wastewater.

Various winemaking practices have been identified as potential sources for heavy metal contamination of wine (Hopfer et al., 2015). For example, practices like pressing and fermentation release Pb from grape skins and seeds into the resulting liquid phase. In our study, the content of Pb after destemming/crushing stage was 46 μg/L in red wine and 48 μg/L in wastewater. During the alcoholic fermentation and the following winemaking stages, most of the Pb contained in the must was removed. This process is affected by chemical (alcohol degree, must acidity) and physical parameters (temperature). After ageing stage, the content of Pb was 17 μg/L in FN samples and 16 μg/L in wastewater samples (Fig. 3). Some authors (Nicolini et al., 2004; Rossano et al., 2007) explain that clarification with bentonites, added either to grape must or finished wine, was identified as a source for Pb, while different filtration practices (silica filters, cellulose filters, bed filtration) have been generated an increase in metal concentrations, as well as Fe and Ni levels (Eschnauer et al., 1989). On the other hand, during clarification and filtration some HMs also decrease due to settling of protein, sulphate and amino acid metal complexes (Nicolini et al., 2004; Pohl, 2007). In our research, the filtration stage (SA-995 filter) has reduced significantly the concentration of Pb in wine to 4 μg/L.

3.1.3. Chromium (Cr)

At the beginning of the winemaking process, Cr concentration was 38 μg/L for GC white wine and 11.5 μg/L for wastewater. In pressing stage, the content was reduced significantly to 1.9 μg/L in GC sample and to 8 μg/L in wastewater (Fig. 2). Among the types of filtrations, the SA-995 filter was the best for removing the Cr content from wine, lowering the concentration to 3 μg/L.

The content of Cr in destemming/crushing stage for red wines (2.5 μg/L) was clearly smaller than for the white wines in the same stage (38 μg/L). Fig. 3 illustrates Cr depletion in ageing stage, the concentration of this HM reaching values lower than the detection limit (0.569 μg/L) in red wine and corresponding wastewater. After 7 months of storage the final content in detecting Romanian red wines was 0.17 μg/L. The results indicate that the HMs loss can be due to a direct absorption of bentonite or due to an interaction of HMs with grape proteins, polyphenols, with tartrates and sugars (Eschnauer et al., 1989; Tariba et al., 2011). Bekker et al. (2019) found that addition of SO_2 had a significant effect on the Cr content, leading to its increase due to the possible extraction of Cr from the steel alloy fermentation vessels. Lastly, during ageing and storage, various studies showed that the HMs composition of wine can be changed due to the materials used. Wines stored in glass bottles had a higher content of chromium, since Cr oxides were used as colouring (Rossano et al., 2007; Tariba et al., 2011). Hopfer et al. (2015) have revealed that metal ions such as Cr and Fe present important differences associated with the winery equipment used.

3.1.4. Manganese (Mn)

The Mn content in destemming/crushing stage for white wine was 579 μg/L. A corresponding value of 61 μg/L was determined in wastewater samples (Fig. 2). During pressing (GC2)-fermentation (GC3)-clarification with bentonite (GC4) the concentrations in wine were increased to some degree, varying between 645 and 770 μg/L. As shown in Fig. 2, the filter without bentonite, (GC5) sample, presented a significant increase in Mn content (1493 μg/L). A possible contamination with Mn occurred in this stage and, as compared with GC4 sample, the absence of bentonite made the difference. Cheng and Liang (2011) discovered that separation of grape berries from the rachises during destemming/crushing increased the Cu, Fe, Zn, Mn, Al and Sr levels, while pressing increased Cu and Zn levels. Filtration stage (SA-995 filter) decreased the content of Mn down to 789 μg/L in white wines, and 254 μg/L in wastewater. Mn(II) ions cause the formation of acetaldehyde, which may polymerize phenolic compounds, leading to a change of the wine sensory quality (Benítez et al., 2002).

In destemming/crushing stage of red grapes, the Mn content presented a value of 781 μg/L in wine and 57 μg/L in wastewater (Fig. 3). Next, the maceration-fermentation stage generated higher levels of Mn. This increment in the concentration can be explained by the extraction of Mn from the seeds and grape skins into the grape must. In filtration stage, sample FN5 (without filter) presented a value of 1230 μg/L, while sample FN7 (SA-995 filter) had a decrease of Mn to 986 μg/L. Also, the content of Mn in wastewater decreased for the same stages previously mentioned from 306 μg/L to 26 μg/L.

Manganese plays an important role in the synthesis of chlorophyll and nitrogen metabolism and is present in soil as exchangeable manganese or manganese oxide (OIV Collective Expertise, 2018). The main factors of Mn concentration in vines and grapes seem to be soil bioavailability and local environmental conditions. High wine Mn concentrations consistently correspond with low soil pH values. Bentonite has also been identified as a possible source of Mn in wine (Catarino et al., 2008). The red winemaking process results in higher wine Mn contents, most likely due to the transfer of Mn during the extended grape skin and seed contact.

3.1.5. Nickel (Ni)

Regarding the white winemaking process, nickel presented a small value (1.5 μg/L) in wine from destemming/crushing stage (Fig. 2). From pressing to fermentation, Ni concentration increased from 2.08 to 8.57 μg/L. For the rest of the wine processing, values decreased from 40 μg/L for GC5 to 13 μg/L for GC8. In wastewater samples, Ni was identified in filtration stage and it varied between 22 and 1.02 μg/L.

In red wine samples, Ni content in destemming/crushing stage was 3.7 μg/L. In the next steps, maceration-fermentation, pressing and ageing stage, Ni level increased up to 9.5 μg/L (Fig. 3). In the final stages of winemaking, its content further increased up to 29 μg/L. In wastewater samples, Ni was identified only in filtration stage (SA-595 filter), registering a value of 2.7 μg/L. Ni contamination may be caused by stainless steel tanks used for fermentation and wine storing (Teissedre et al., 1998).

3.1.6. Iron (Fe)

Fe content at the start of Grasă de Cotnari winemaking process was 356 μg/L. From pressing to fermentation, the concentration of this metal increased from 237 to 1127 μg/L. Then, from the stage without filter (only with bentonite) up to bottling stage, the values ranged between 2450 and 2903 μg/L. As presented in Fig. 2, the Fe concentration in wastewater samples, varied between 122 and 481 μg/L from destemming/crushing to filter stages.

In destemming/crushing stage of red grapes, 907 μg/L of Fe was determined, which then decreased to 307 μg/L after maceration-fermentation. From pressing stage to SA-595 filter stage, the metal concentration increased from 361 to 878 μg/L. When SA-995 filter was used, the level of Fe decreased to 421 μg/L and after 7 months of storage, the final content was 1136 μg/L. In wastewater samples, the Fe concentration varied between 324 and 164 μg/L from destemming/crushing stage to filter (SA-995) stage. Fe ions in wine have the ability to form compounds with a wide variety of substances, resulting in increased turbidity and colour change. They also act as catalyst in oxidation processes involved in ageing and have an important effect on the sensory attributes of wine (Rousseva et al., 2016).

Bentonite treatment is efficient for Cu removal from wine that originates from grapes/juice. In contrast, the total Fe concentration increased during winemaking stages. These results are in agreement with previous studies by Bekker et al. (2019) and Rousseva et al. (2016) which observed that bentonite addition increased Fe content. Provenzano et al. (2010) noticed that Fe and Cu can be present in significant concentration in the grapes juice, either through general environmental contamination (dust), or due to the application of fungicides in the vineyard (particularly for Cu). Furthermore, Byrne et al. (1937) reported that Fe concentrations in juice range from 0.7 to 23.0 mg/L, with the highest concentrations from studies when contamination from cast iron equipment was more common.

The present study results are consistent with findings previously published in the literature. Lima et al. (2021) found that Fe, Cu and Mn concentrations ranged from 700 to 3200 μg/L, 23–117 μg/L and 800–2600 μg/L, respectively. In the same study, authors reported that Pb, Cr and Ni concentrations ranged between 1.1–4.5 μg/L, 7.85–20.4 μg/L, 15–115 μg/L, respectively in red wines (Pinot noir) from USA. Semla et al. (2018) found 30 μg/L Cu, 350 μg/L Fe, 40 μg/L Ni and 80 μg/L Pb, respectively, in Slovak wine. In another study on Portugal wines, Cabrita et al. (2018) reported that the concentrations for Mn, Fe, Ni, Cu, and Pb were 1375 μg/L, 3998 μg/L, 23 μg/L, 41 μg/L, 12 μg/L, respectively. Alkis et al. (2014) determined the concentration for Mn that was 935 μg/L, Ni 132 μg/L, Cu 101 μg/L and Pb 7 μg/L, respectively in Turkish wines. Moreover, Gremaud et al. (2004) reported elements such as Mn and Zn and their concentrations ranged from 270 to 600 μg/L and 340 to 1140 μg/L, respectively.

Geana et al. (2013) observed in Romanian wines that Cr, Ni, Mn, Cu and Pb concentrations were 218 μg/L, 32 μg/L, 710 μg/L, 529 μg/L, 42 μg/L, respectively. Dinca et al. (2016) reported for Romanian wines Pb, Ni, Mn and Cu values ranging from 27 to 68 μg/L, 18–85 μg/L, 397–1483 μg/L and 95–776 μg/L, respectively. Similarly, in our study, the content of Fe ranged between 1136 and 2903 μg/L, Mn was between 714 and 1256 μg/L for Romanian wines. Cu values ranged between 21 and 53 μg/L, while Ni between 13 and 29 μg/L. The observed concentrations for Ni, Mn and Cu were similar to those reported by Geana et al. (2013) and Cabrita et al. (2018). In contrast, the Fe concentration reported by Cabrita et al. (2018) was higher than our study findings. In general, differences of heavy metals content in wines can be attributed to the vine variety, pesticides type, soil characteristics, different climatic condition, winemaking techniques, material of equipment, fining agents (Sharafi et al., 2018; Claus, 2020).

3.2. Winery wastewater quality

In general, HMs concentrations in winery wastewater found in this study did not exceed the Romanian limits for discharge into the public sewerage system or in the surface river water (Table 3S, Supplementary material). Among the considered metals, the highest values of Cr, Ni and Pb were about 150, 50 and respectively, 10 times lower than the legislation requirements, as presented in Figs. 2 and 3. The highest concentration of manganese was determined in wastewater generated from red wine maceration-fermentation stage and it was close to the discharge limit.

Copper was the only element that did not meet the legislation requirements, but this was only the case of wastewater generated from winemaking stages before filtration (Anon, 2021).

3.3. Comparison of heavy metals content across major wine producing countries

Romanian wines present similar values for Pb, Ni, Mn, Cr, Cu and Fe content, as compared to other major wine processing countries (Table 4S). According to the published references, Cd levels ranged from 0.00 to 0.09 mg/L in Spanish wines, to 0.00–0.0002 mg/L in French wines. Similarly, in Czech wines, Cd was between 0.000055 and 0.0033 mg/L, whereas the Pb content fluctuated between 0.010 and 1.253 mg/L. In Slovenian wines, the Cu content was reported to be 0.11 mg/L, the Pb content 0.03 mg/L and Cr was 0.016 mg/L. In German wines, the Cu content ranged between 0.02 and 0.71 mg/L, Fe was found to be between 0.4 and 4.2 mg/L and Mn fluctuated between 0.5 and 1.3 mg/L. In Italian wines, Cu concentration varied between 0.001 and 1.340 mg/L, while Ni ranged between 0.015 and 0.210 mg/L. Also, Cd content was between 0.001 and 0.002 mg/L and Fe between 1.35 and 27.8 mg/L. At the international level, OIV has published maximum acceptable limits (MAL) for the following elements: As (0.2 mg/L); Cu (1 mg/L); Pb (0.15 mg/L); Cd (0.01 mg/L); Br (1 mg/L); Ag (0.1 mg/L) and Zn (5 mg/L) (OIV, 2015). In addition, WHO (2017) defined maximum concentration levels (MCL) for HMs in drinking-water for: Cd (3 μg/L), Cr (50 μg/L), Cu (2000 μg/L), Pb (10 μg/L) and Ni (7 μg/L).

The metal concentrations may be influenced by many factors, such as: grape varieties, climate, soil parameters, wine-growing regions, landscape environment, agricultural management practices, winemaking techniques, pH, and yeasts (van Leeuwen et al., 2004; Blotevogel et al., 2019). Moreover, pesticides applied during the growing season of vines may affect the overall content of HMs. All these factors are leading to differences among HMs concentrations specific to each wine and producing country.

3.4. Discrimination of wine varieties and winemaking stages based on heavy metal content

In this study, the discrimination between winemaking stages was based on variations of the measured HMs concentrations. Furthermore, HMs levels were used as chemical descriptors in the statistical methods, to establish differences between red and white wine samples. Principal component analysis, multivariate analysis, heatmapping and correlations were employed for the chemometric treatment of experimental data and the obtained results were compared. Chemometrics enable a complete analysis of multivariate complementary omics-data and the observation of relevant patterns in the information.

3.4.1. Principal component analysis

Generally, the PCA scores are used to group data based on overall similarities/dissimilarities by projecting the samples in 2D or 3D. In Fig. 4 we have chosen as variables the concentrations of six HMs (Mn,

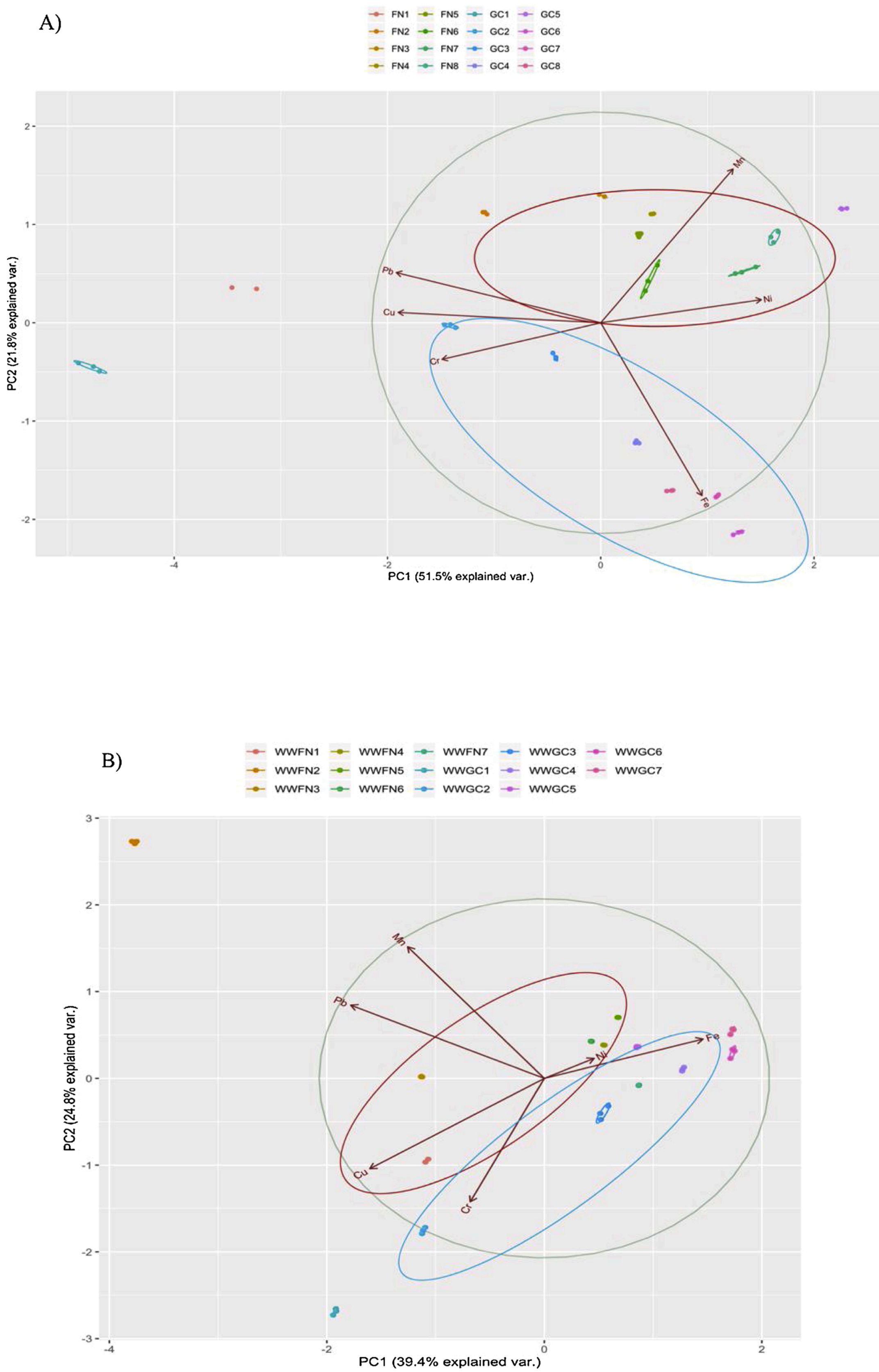

Fig. 4. Principal component analysis (PCA) using the heavy metals contents as classifying variables in white and red winemaking (A) and in wastewater (B).

Cr, Cu, Ni, Pb and Fe). The results indicated a complete separation of the two types of wine (white and red), due to the fact that the supplied data has a very distinguishable range of HMs concentration. The PCA plot in Fig. 4A clearly indicates the existence of two clusters, the first one corresponding to winemaking stage of white wines and the second one to the red wines. In fact, white wines and red wines are produced by different winemaking procedures, in particular after the crushing stage, when the contact time between the grape juice and the skins is longer for red wines. As a consequence, the absorption of HMs from the skins for red wines is more efficient than for white wines due to the differential uptake of HMs in the skins and flesh of the grapes. Such differences in HMs concentration in wines leads to the observed separation of white wines and red wines before their respective classifications according to the vine classification.

Results showed that the first principal component (PC1) explains 51.5 % of the total variance. PC1 is influenced positively by Ni, Mn and Fe, and negatively by Cr, Cu and Pb. The second principal component (PC2) explains 21.8 % of variance observed in the dataset. PC2 is positively linked with Pb and Cu and negatively linked with Fe. PC1 and PC2 variations could originate from both natural sources, such as vineyard, soil, water, and anthropogenic sources such as: pesticides, fungicides and fertilizers (Hopfer et al., 2015), or post-harvest sources, from winemaking processes (filtration, clarification, storage) (Pohl, 2007; Tariba et al., 2011). In this analysis, the negative side of the first component (PC1) comprises the first stages of winemaking, the destemming/crushing, maceration-fermentation and pressing stages, whereas the latest stages are located on the positive side, clarification, filtration and bottling stages. All the wine samples could be clearly distinguished according to the winemaking stages. On the other hand, the second component (PC2) differentiates winemaking stages of white wines from those of red wines. Thus, this component seemed to be related to the types of wines (white or red) (Fig. 4A).

Additionally, an analysis of the principal components was carried out to discriminate between the variables that separate the wastewater generated by winemaking stages of white and red wine (Fig. 4B). The PCA obtained explained 64.2 % of the total variance. PC1 (39.4 %) is positively loaded with Ni and Fe, and negatively correlated with Cu and Cr. PC2 (24.8 %) is strongly positively loaded with Pb and Mn. The positive side of PC1 is related to the last stages of winemaking as well as to wastewater generated from white and red wines production, whereas the first stages of winemaking, are on the negative side. On the other hand, PC2 is related to the stages of maceration-fermentation for both wines (Fig. 4B).

3.4.2. Multivariate analysis of heavy metal content

Multivariate analysis (MVA) is a set of techniques used for analysis of data sets that contain more than one variable, which are especially valuable when working with correlated variables. These techniques provide an empirical method for information extraction, regression, or classification. MVA was used considering as variables the concentration of the six HMs, to describe the wines, as well as the wastewater, by their HMs fingerprint. The results obtained are discussed and represented graphically in Fig. 1S (Supplementary material).

3.4.3. Heat map visualization

Matrix cluster analysis was used to generate a heat map of the concentrations of the six HMs (Mn, Cr, Cu, Ni, Pb and Fe) in wine and wastewater samples from different stages of white and red winemaking (Fig. 5A and B). The contents of each metal were clustered into different groups.

In the heat map, yellow colours represent high concentrations and blue colours indicate low contents of HMs. Along the x-axis (Fig. 5A), the six HMs are divided into two clusters. Cr, Ni, Pb, Mn and Fe are clustered in the same group, while Cu was clustered alone in a different group. On the y-axis, the heat map organized the 16 samples, in triplicate, into four clusters (cluster I—IV). Among these, the first cluster (cluster I) includes only the first winemaking stage for both white and red wines, although it distinguished destemming/crushing from all others stage of winemaking. The shades of yellow colour in this cluster indicate the high levels of Cu, Cr and Pb in white wines and of Cu and Pb in red wines. These values had also the highest concentrations for the respective metals in the winemaking process. This contamination with HMs in the first winemaking stage suggests the use of phytosanitary treatments and agrochemicals in the vineyard. Other source of contamination might include the vehicles used for grapes transportation. Cluster II includes the clarification stage (GC4) and the last stages (GC6−8) of winemaking for white wines. The yellow and bright green colours for Fe indicates its higher content when SA-595 and SA-995 filters were used and after bottling for 7 months. Therefore, a contamination of Fe occurred in these stages. For the rest of HMs in this cluster the concentrations were low. Cluster III comprises the pressing of both wine types and maceration-fermentation of red wine. In this case, the content of Cu (and partially Pb) was higher in white wine, while in red wine higher values of Pb and Mn were found. The remaining stages of white and red winemaking (GC3, GC5 and FN4−8) were grouped together in the last cluster. In cluster IV, the yellow and green colours of Mn and Ni indicated that a contamination was present in the respective stages.

The representation of heat map for wastewater samples is illustrated in Fig. 5B. The six HMs were divided into two clusters along the x-axis. Cu, Cr, Ni, Pb and Fe were clustered in the same group, while Mn was grouped alone. On the y-axis, the 14 samples by triplicate were organized into four clusters. Cluster I include only the second winemaking stage of wastewater from red wines, although the maceration-fermentation can be distinguished from others winemaking stages. The yellowish colours for Mn and Pb suggest that their contents were higher in the current stage. The second cluster includes wastewater from destemming/crushing and pressing stages of both wines. The colours for Cu and Cr had shades of yellow/green, which confirms the existence of contamination at that stages. In cluster III, which includes wastewater from GC3−4 and FN4−5 and FN7, only Fe presented a high concentration, during clarification with bentonite. Finally, cluster IV comprised the remaining winemaking stages that could generate wastewater (excepting bottling stage). The shades of yellow and green for Mn, Cr, Ni and Fe indicated that there was a contamination in those respective stages.

3.4.4. Correlation analysis

Significant correlation between two or more elements indicates similar absorption capacity by the grapes or the existence of the same source of HMs. Dependencies between HMs can be explained by their common origin in soil minerals. In this work, the Pearson correlation coefficients were calculated for the experimental data and corrgram plots are represented in Fig. 2S (Supplementary material). The coefficients of correlation between HMs concentrations in white and red wines are given in Fig. 2S (A) and Fig. 2S (B) respectively.

3.5. Transfer coefficients

The content of metals transferred into different winemaking stages is important and hence, the grape-storage of bottle transfer coefficients have been used to determine the overall metal concentrations in winemaking stages. Transfer coefficients (T1-T4) revealed different patterns of HMs accumulation and transfer between components during winemaking stages is presented in Table 5S (Supplementary material). Transfer coefficients varied between HMs.

For white wine, the transfer coefficients per stages of winemaking decreased in the following order: a) for T1: Ni > Mn > Fe > Pb > Cu > Cr; b) for T2: Fe > Ni > Mn > Pb > Cr > Cu; c) for T3: Fe > Ni > Cr > Mn > Pb > Cu; d) for T4: Ni > Fe > Mn > Pb > Cu > Cr. In the first stage of white winemaking, only Cu showed the highest accumulation in grapes, which was confirmed by T1 > T2. In contrast, Cr, Ni, Pb and Fe presented a T2 > T1 pattern, indicating the HMs weak accumulation in

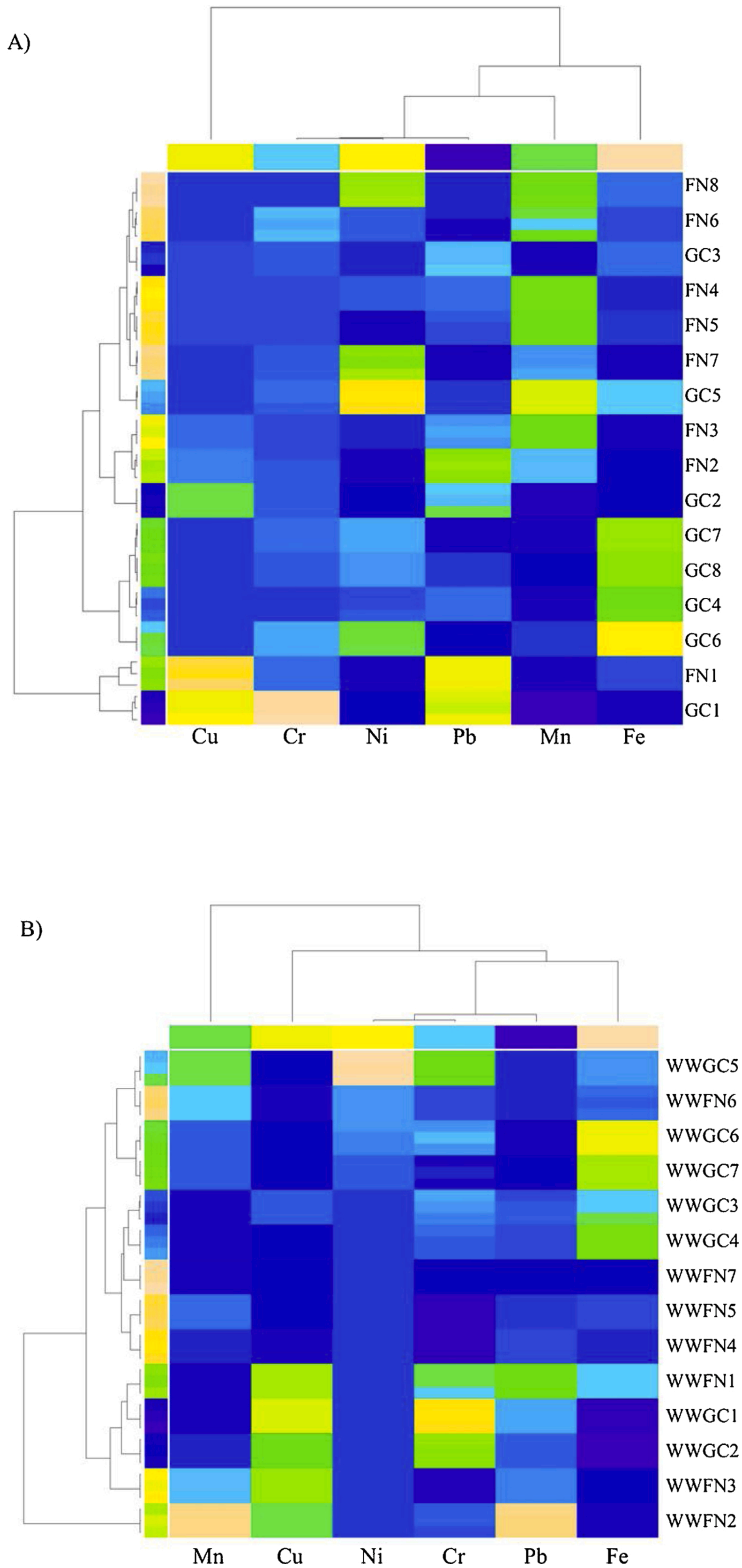

Fig. 5. Heatmap representation of heavy metals identified in the principal winemaking stages of wines (A) and wastewater (B). Each variant was done in triplicate and grouped in dendrograms (left side).

pomace and strong ability to transfer to the must, juice and wine. The same succession, T2 > T3, manifested for Pb, Mn, Cu, Ni and Fe. Afterwards, the content of the aforementioned metals decreased considerably. Cr, Mn, Ni and Fe presented T3 transfer coefficients higher than T4. In contrast, the content of Pb and Cu was higher in T4.

The following orders were found for transfer coefficients for red wines winemaking stages (Table 5S, Supplementary material): a) for T1: Mn > Ni > Pb > Cr > Fe > Cu; b) for T2: Mn > Ni > Fe > Cu > Pb > Cr; c) for T3: Fe > Ni > Mn > Pb > Cr > Cu and d) for T4: Ni > Fe > Mn > Pb > Cr > Cu. Among HMs, Pb, Cr, Mn and Ni showed the highest accumulation in grape and decreased concentration in must, which was confirmed by the T1>T2 pattern. By comparing the ratios between: (a) maceration-fermentation and destemming/crushing, (b) pressing and maceration and (c) ageing and pressing stages it may be observed that (a) stage has a higher transfer coefficient than the other stages. High concentrations of Pb and Mn in grapes can be explained by intensive accumulation of HMs in the seeds in comparison to other parts of grape, as described also by Teissedre et al. (1998). Seeds are the main components of pomace, where Pb content was found to be two-fold higher than in grapes. The sequence T3>T2 in content of Pb, Cr, Ni and Fe were noticed. For Mn and Cu the transfer coefficient was T2>T3. Moreover, T3>T4 was observed for Pb, Cr, Mn and Cu. In wine, HMs were lower due to their sedimentation and complexation. In addition, it is possible that the activity of the used yeast decreased these elements during fermentation.

3.6. *Estimated daily intake of heavy metals through the consumption of wine and its health risk assessment*

In this study, the estimated daily intake (EDI) and target hazard quotient (THQ) were evaluated based on HMs determined concentrations in white and red wines (Table 1). To analyse the potential hazards resulting from long term daily consumption of wine, we ascribed the notion of an estimated daily intake for HMs from wine.

Reference doses of Pb, Cr, Mn, Cu, Ni and Fe were extracted from the United States Environmental Protection Agency (USEPA) guide. The EDI identified in this study are lower than the Reference doses (RfD) for all heavy metals considered, thus suggesting no health risks due to HMs in wines. The EDI values for Pb, Cr, Mn, Cu, Ni and Fe from white and red wines were assessed and the results are shown in Table 1. The mean EDI values of the metals ranged in white wine from 0.0033 to 6.2217 μg/kg bw/day, following the sequence: Cr < Pb < Ni < Cu < Mn < Fe. For red wines, the EDI was between from 2.6919 and 0.0004 μg/kg bw/day, as it follows: Mn > Fe > Cu > Ni > Pb > Cr.

Health risk assessment examined the potential health effects from different contaminants delivered to humans through consumption of wines. The health risks of HMs intake from the consumption of wine were assessed based on THQ, which had been recognized as a useful parameter for the evaluation of risks associated with a contaminant. The THQ is the ratio of the estimated dose of elements from wines to a corresponding reference dose, and this approach offered an indication of the risk level due to contaminant exposure.

The THQ values estimated for each HM were below unit for both wines varieties, suggesting that the exposed population would not experience significant health risks when ingesting these metals based on a daily consumption of 150 mL of wine. In Table 1, the hazard index (HI) values of white and red were also less than 1.

The human drinking the Provisional Tolerable Weekly Intake (PTWI) value is an estimation of a heavy metal concentration that might be taken in over a lifetime without appreciable risk. PTWI admissible threshold is described by the Joint Food and Agricultural Organization for the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (WHO, World Health Organization, 2020). Currently, the values of PTWI for Cd are of 7 μg/kg bw, Cu of 3500 μg/kg bw, Fe of 5600 μg/kg bw, Ni of 35 μg/kg bw, Mn of 980 μg/kg bw and Pb of 25 μg/kg bw. For risk assessment purposes, the calculated daily intakes of Cu, Fe, Mn and Cd from white and red wine consumption were compared with their toxicity reference values. Provisional Maximal Tolerable Daily Intake (PMTDI) for Cu is regulated at 500 μg/kg bw per day, Fe at 800 μg/kg bw per day and Mn at 350 μg/kg bw per day. The provisional tolerable monthly intake (PTMI) for Cd was set at 25 μg/kg bw/month by JECFA (2020). The European Food Safety Authority (EFSA) established the tolerable weekly intake for Cd at 2.5 μg/kg of bw per week (EFSA, 2011). In the European Union, estimated mean Mn intakes of adults range from 2 to 6 mg/day, with the majority of values around 3 mg/day. Estimated mean Mn intakes range from 1.5 to 3.5 mg/day for children, and from 2 to 6 mg/day for adolescents (EFSA, 2013). EFSA has established as reference points for tolerable daily intake (TDI), 13 μg /kg bw for Ni (EFSA, 2015a). Adequate Intakes (AIs), for adults was proposed for Cu to be 1.6 mg/day for men and 1.3 mg/day for women (EFSA, 2015b).

Publications on health risk assessments of the dietary ingestion of heavy metals from wine are insufficient. In a study published by Malavolti et al. (2020), estimation of Pb daily dietary intake in white wines was 0.007 and in red wines of 0.017 μg/kg of body weight/day. Semla et al. (2018) reported the EDI for Cu, Fe and Ni at 0.0001, 0.001, 0.0001 mg/kg bw/day. Deng et al. (2019) observed EDI of Cr, Mn, Ni, Pb for wines at 0.467, 10.3, 0.124, 0.047 μg/kg bw/day, respectively. In another study, Gutierrez et al. (2017) calculated the THQ values for all metals below 1, showing a good quality of Canarian red wines.

The assessment of the risk to human health associated with the intake of HMs with food on the basis of THQ indicators has been carried out for rice (Sharafi et al., 2019a), bread (Woldetsadik et al., 2020), vegetables and fruits (Harmanescu et al., 2011; Esposito et al., 2019), fish (Moslen and Miebaka, 2017). In all these studies the THQ result for numerous metals were below 1.

Diverse chemical reactions occur in the wine during all production stages, from destemming/crushing of grapes throughout the alcoholic and malolactic fermentation, then during the clarification and filtration and in the latter stage of storage in bottles. Not all reactions are, anyway, favourable to the wine characteristics and thus may have an undesirable influence on its quality. They may lead to spoilage in the wine and special stages and actions should be taken in order to prevent, reduce or eliminate their effects.

Overall, we could state that the best strategy to reduce health risks associated with heavy metals contaminated wine is to prevent contamination of grapes and throughout the process. Practices such as the use of biological control methods and biodynamic agriculture in the vineyard, avoiding the storage of grapes after harvesting and beginning of fermentation right after removal of grapes from bunches drastically reduce the probability of HMs contamination. If contamination has already occurred, the following strategies can be used to reduce HMs content: use of selected yeast able to adsorb HMs during fermentation; use of non-contaminated pomace as an adsorbent and use of various fining agents for HMs removal; use of the filters aids.

Table 1
Estimated daily intake (EDI, μg/kg bw/day) and target hazard quotient (THQ) of each element via consumption of white and red Romanian wines.

	Pb	Cr	Mn	Cu	Ni	Fe
	EDI μg/kg bw/day					
White wines	0.0272	0.0033	1.5292	0.0454	0.0277	6.2217
Red wines	0.0244	0.0004	2.6919	0.1145	0.0639	2.4341
	THQ					
White wines	0.0078	0.0001	0.0109	0.0091	0.0014	0.0089
Red wines	0.0069	0.0001	0.0192	0.0229	0.0032	0.0035
	HI					
White wines	0.0391					
Red wines	0.0559					
	PTWI μg/kg bw/week					
White wines	0.1901	0.0228	10.7046	0.3177	0.1939	43.5519
Red wines	0.1706	0.0026	18.8438	0.8013	0.4474	17.0388

4. Conclusions

Assessment of heavy metals concentration during winemaking stages and in wastewaters is very important due to metals influence on wine quality and toxicological risks associated with wine consumption and environmental safety. Results indicated that pressing and filtration stages reduced HMs concentrations. Moreover, the effects of clarification and filtration process on HMs concentrations depended on the filter type and use of bentonite in the clarification process. The highest concentrations of Pb, Cr and Cu were found in the first winemaking stage, for both wine varieties, which means that grapes were contaminated from vineyards with phytosanitary treatments. By using PCA, the samples were clearly distinguished into winemaking stages and wine varieties, indicating that both vine management and winemaking have impact on the HMs composition of red wine.

Wastewater resulted from the winemaking process represents a relevant hotspot of heavy metals discharge into the environment. In this study, the concentration of most HMs in winery wastewater samples were significantly below the discharge limits in public sewerage with respect to the European legislation. However, elevated copper concentrations were determined for the samples before the filtration stage.

The estimated daily intake (EDI) of the analysed white and red wine samples ranged from 0.0032 to 6.2217, and 0.0004–2.6919, respectively. The THQ values estimated for each HM were below one for both wine varieties, suggesting that the exposed population should not experience significant health risks when consumption 150 mL of wine daily. The HI for the heavy metals studied was below 1, indicating that the daily intake would not be expected to cause any negative effects. Constant monitoring of heavy metals fingerprints in wines should represent an indispensable quality control tool and could acquire insights on the evolution of metal content during winemaking stages and on the final product.

Author Contributions

Georgiana-Diana Dumitriu (Gabur): Conceptualization, Investigation, Data curation, Writing original draft. Carmen Teodosiu: Conceptualization, Data curation, Visualization, Writing - review & editing, Supervision. Irina Morosanu: Methodology, Data curation, Writing - review & editing. Oana Plavan: Methodology, Formal analysis. Iulian Gabur: Writing – review & editing, Software. Valeriu V. Cotea: Writing - review & editing, Visualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-0652, and also by project number PN-III-P1-1.1-PD-2016-0325, both projects being funded within PNCDI III. We are also grateful to the Oenology Laboratory of Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2021.103935.

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