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Faculty of Tropical AgriSciences



**Determination of arsenic and other potentially toxic
elements in wines grown in a risk
area by using acid digestion**

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis titled “Determination of arsenic and other potentially toxic elements in wines grown in a risk area by using acid digestion” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to citation rules of the FTA.

Prague 15/08/2025

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Abstract

Several regions affected by anthropogenic pollution and mining activity have been increasingly considered as potential area of research into the presence of potentially toxic elements in wines. The main purpose of this thesis is to evaluate the distribution of arsenic and other toxic substances in wines produced in the affected regions. in order to assess the safety of wine consumption for public health. This is relevant given the increasing demand in the world market for wine, and the need for food safety and regulatory compliance. The first objective of this research is to identify the appropriate acid digestion methodology for determination of potentially toxic elements in the wine samples. This study seeks to answer the most critical questions including incidence of toxic element contamination in wines from the risk regions compared to the set safety standards. This methodology involved evaluation of the acid digestion techniques found in literature and selecting the most suitable technique based on its compatibility with the available laboratory equipment. In the practical phase, this method was used on wine samples that were acid digested. The measurement of the concentrations of elements was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

The comparison of the concentrations of toxic elements in wine when digested with only nitric acid versus a combination of nitric acid and hydrogen peroxide revealed that there was significant difference in the concentration in Zinc ($p = 0.027$), Copper ($p = 0.014$) and Iron ($p = 0.007$). This finding highlights that digestion methods have influence on the concentration levels of toxic elements. Kutná Hora had significant difference in the mean concentration and other regions in Arsenic, Lead, Zinc, Copper and Iron. The comparison of the concentration of PTE between different wine types revealed significant difference in Arsenic in Red wine with White (mean difference = -0.04837 mg/L, $p = 0.012$) and Rose (mean difference = -0.06470 mg/L, $p = 0.040$). The result of comparison with Bulková's (2024) study and this study revealed significant difference in the mean concentration of Arsenic (MD = 0.08034 mg/L, $p < 0.001$) and Cadmium (MD = 0.00096 mg/L, $p < 0.001$).

For arsenic, wines from KH contained significantly lower concentrations compared to Mělník and Mikulov but higher than Prague. The trace element concentrations from this study were similar to Bulková's (2024), who used a direct measurement of elemental concentrations by using ICP-OES on the same set of samples. The concentrations of Ba, Ni, and Zn were significantly higher than those reported by Bulková, while Al, B, Mn, and Fe were within or below Bulková's reported

range. For Be, Co, Cr, Cu, Sb, V, and Pb similar patterns of or partial detection were found. This study revealed that digestion method, wine region and wine types have influence on the concentration of PTE in wine samples. The implication of this research indicated the need for greater action in educating customers regarding contaminated wines and tougher government approvals for human health from contaminated wines.

Key words: Wine; Acid digestion; Potentially toxic elements; Arsenic

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List of the abbreviations used in the thesis

AAS – Atomic Absorption Spectroscopy
Al – Aluminum
As – Arsenic
AsBet – Arsenobetaine
AsChol – Arsenocholine
Ba – Barium
B – Boron
Be – Beryllium
Cd – Cadmium
Co – Cobalt
CRMs – Certified Reference Materials
Cr – Chromium
Cu – Copper
ETAAS – Electrothermal Atomic Absorption Spectroscopy
EU – European Union
FAAS – Flame Atomic Absorption Spectroscopy
Fe – Iron
GF-AAS / GFAAS – Graphite Furnace Atomic Absorption Spectroscopy
HCl – Hydrochloric Acid
HClO₄ – Perchloric Acid
H₂O₂ – Hydrogen Peroxide
H₂SO₄ – Sulfuric Acid
HNO₃ – Nitric Acid
ICP-AES – Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS – Inductively Coupled Plasma Mass Spectrometry
ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy
ICP-QMS – Inductively Coupled Plasma–Quadrupole Mass Spectrometry
ICP-SF-MC-MS – Inductively Coupled Plasma Sector Field Multi-Collector Mass Spectrometry
ISO/IEC – International Organization for Standardization / International Electrotechnical Commission
Kh – Kutná Hora
LOD – Limit of Detection
Mg/L – Milligrams per liter
Mn – Manganese
NaCl – Sodium Chloride
NIST – National Institute of Standards and Technology
Ni – Nickel
NN/2 – Narodne novine Number 2 (Croatian Official Gazette regulation reference)
OIV – International Organisation of Vine and Wine
Pb – Lead
PTEs – Potentially Toxic Elements
PSA – Potentiometric Stripping Analysis
PTFE – Polytetrafluoroethylene
SWASV – Square Wave Anodic Stripping Voltammetry
TČ – Tramín Červený

TXRF – Total Reflection X-ray Fluorescence
US EPA – United States Environmental Protection Agency
USFDA – United States Food and Drug Administration
V – Vanadium
VWR – VWR International s.r.o. (a lab supply company)
Zn – Zinc
 $\mu\text{g/L}$ – Micrograms per liter
v/v – Volume per volume

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Wine Composition and Safety: A Scientific Overview of Toxic Element Presence in Wines

Fermenting grapes or another fruit into wine is a complex and culturally significant process. At the same time, it is both an artistic expression and a scientific subject (to the extent that worries are expressed about its chemical composition and its safety as a food). Red, white, rosé, and fruit wines are among several main types of wine, which also vary by grape variety and origin, fermentation and storage procedures. The variations in these also affect the final product in terms of chemical composition as well as the taste and aroma (Roxana et al., 2020; Saranraj et al., M. (2017).

On a global scale, wine consumption remains a central component of dietary and social traditions. According to recent statistics by the International Organisation of Vine and Wine (OIV), France, Italy, and Portugal lead the world in per capita wine consumption, with each citizen consuming an average of over 35 liters per year (OIV, 2021). Meanwhile, global demand continues to grow, particularly in emerging markets across Asia and Africa. In regions like, for example, Nigeria, wine consumption is steadily increasing, driven by cultural shifts and the growing middle class (Anderson and Wittwer, 2015; Ogbeide and Ele, 2015).

Wine is chemically a complex mix of water (~85%), ethanol (10–15%), trace elements, phenolic compounds, minerals, sugars, and organic acids. While all of these components are thought to be beneficial to wine's health benefits (polyphenols with antioxidant properties), trace elements, are also often present, may be toxic in excessive amounts (Nemzer et al., 2022).

Specifically concerning are elements that are potentially toxic (lead, cadmium, and arsenic), which may be found in wines at different concentrations. Their presence is influenced by multiple environmental and agricultural factors that include soil composition, fertilizer use, atmospheric deposition and industrial contamination. The scope of soil to grape to wine contamination has been demonstrated with research showing a notable pathway is the transfer of elements from soil to grape and then into the wine (Richardson & Chase, 2021; Nechita et al., 2023). Furthermore, these

fining agents and other processing aids can further change the elemental composition of wine (Griboff et al., 2021).

Although some basic minerals are also found in wines such as zinc (Zn), iron (Fe), and copper (Cu), they are only beneficial when in acceptable concentrations. Lead and arsenic are dangerous, as lead concentrations have been found in wine to be as high as the maximum permissible limit of 0.15 mg L^{-1} , especially in polluted regions. Arsenic has also been found in several wine samples from a number of geographical regions, and this calls for continued monitoring and regulatory control (Bora et al., 2022).

A moderate exposure to these elements can cause serious health hazards like cancer, neurological disorder, and kidney damage (Bora et al., 2021). Consequently, it is necessary for winemakers and the regulatory authorities to establish rigid monitoring regimes for grapes as well as environmental factors such as soil and air quality to regulate the content of toxic elements in wine (Ramić et al., 2024; Jakkielska et al., 2023). Despite the fact that moderate wine consumption has been linked to potential health benefits, including some antioxidant such as resveratrol, the pros have to be weighed against the risks of toxic element exposure. Available literature indicates that the present range of studies indicates moderate consumption of wine does not exceed safe limits of intake of toxic elements, although there are long-term health risks associated with unlimited wine consumption particularly from contaminated wine producing regions (Radeka et al., 2022; Ramić et al., 2024).

Per capita wine (L/person) consumption by country was shown in figure 1 (2007 and 2010) from the selected wine producing countries.

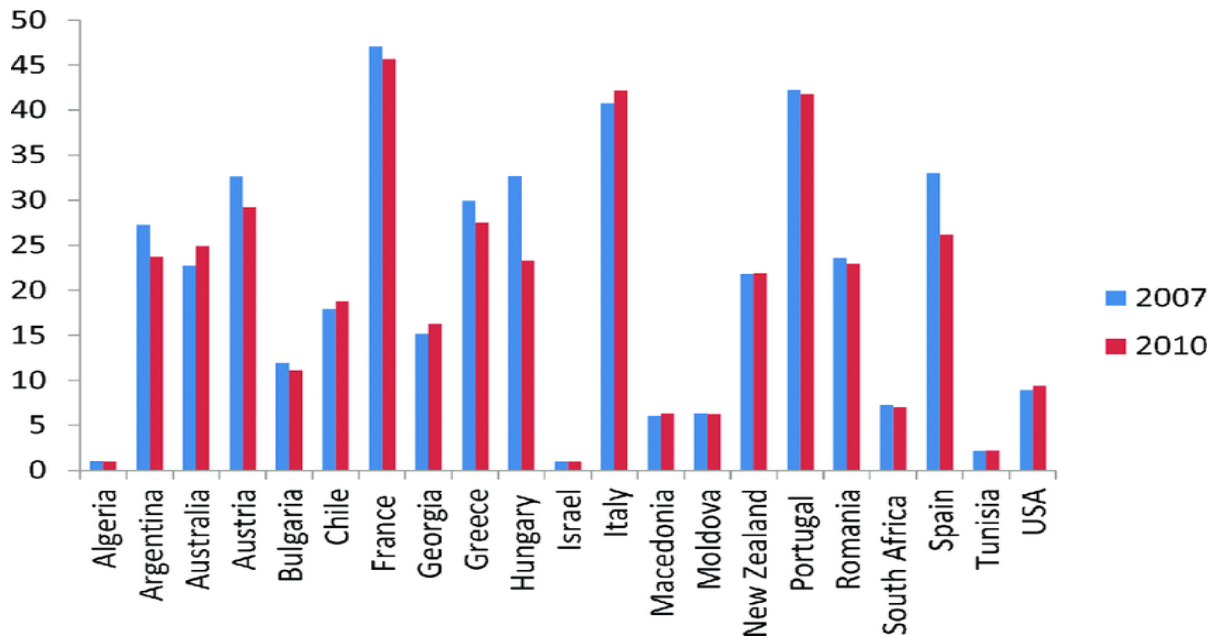


Figure 1: Per capita wine (L/person) consumption by country (2007 and 2010) for some selected countries that produce wine. Source: Ronkainen (2016).

1.2 Risk elements

Risk elements, more particularly in light of environmental health and safety, are the various potentially toxic substances that negatively impact human health and ecosystems. According to their sources of origin, these elements can be grouped into different types, anthropogenic (human made) and geogenic (natural).

Anthropogenic elements are elements introduced to the environment through industrial processes, agriculture practices and urbanization. This leads to a rise in concentration of some toxic elements in the environment (Ramić et al, 2024). Common anthropogenic risk elements are toxic elements like lead, arsenic, cadmium and mercury. They could be from many sources, such as mining and smelting operations, waste disposal, and the use of fertilisers and pesticides. Anthropogenic elements persist in soils and water bodies, and can accumulate in water bodies and soils to the extent that they become exposed via the food chain (Hao et al., 2021).

Geogenic elements refer to naturally occurring substances in the earth's crust. However, their concentrations can vastly differ depending on geological formations and environmental conditions. The availability, and uptake of these elements by plants and other organism, is a

function of the natural mineral composition of the soil and geological activities (González et al., 2021). For example, volcanic places could have some elements levels increased as a result of the mineral content of the soil which impacts the local ecosystems. Geogenic risk elements include key elements such as arsenic, uranium and selenium that can be released from natural processes of weathering and erosion (González et al., 2021).

The presence of these risk elements must be monitored and assessed to prevent them from causing negative impacts to human health and environmental safety. For the protection of public health from the negative effects caused by anthropogenic as well as geogenic risk agents, the implementation of a robust risk management framework which identifies hazards, assesses their mitigation potential and the placement of control measures, is critical (Naude & Naude, 2022).

It is important to lay out the sources and types of risk elements so as to develop proper strategies to lower exposure to and manage risks due to these hazardous substances (Masotti et al., 2022; Baiano, 2021).

1.2.1 Risk Elements of Mining-Affected Regions

As a result of mining operations, the surrounding surroundings are significantly contaminated with potentially hazardous elements (PTEs). Impact regions of mining tend to be high in PTEs, such as arsenic, lead, cadmium, and mercury, that can leach into a soil and water system, then impact agricultural products and local ecosystems (Huang et al., 2024; Al-Jumaily, 2021; Carvalho et al., 2022). These contaminants come from well-known geogenic and anthropogenic sources, with the mining operations being one of the usual causes of soil degradation and water pollution (Carvalho et al., 2022; Giao & Minh, 2022). For example, some studies found that mining cause the accumulation of toxic elements in agricultural soils that, in turn, get taken into the food chain and contribute to endangering the health of wildlife and humans (Huang et al., 2024; Al-Jumaily, 2021). In particular, this pertains to areas which have experienced intense historical mining activity, and which have affected environmental conditions of food in terms of safety and quality, such as viticulture (Vejvodová et al., 2022).

Aside from direct contamination of mining operations, weathering of mineral deposits causes toxic elements to be released in the environment, which makes this problem worse (Huang et al., 2024;

Božović et al., 2023). Highly toxic elements are common in these mineralized geological features found in mining regions such as black shale (Huang et al., 2024). In addition, the availability of these metals can differ, depending on the extent to which they are taken up into the plants, raising the likelihood of their uptake by the plants through soil making them more available to humans through food consumption (Al-Jumaily, 2021; Carvalho et al., 2022). Overall, PTE in mining industry is considered a serious environmental and health risk and should be regularly monitored and regulated as to concentrations on the areas are affected.

1.2.2 Arsenic as a Risk Element

Arsenic is an element of concern from human and environmental health standpoints. Arsenic may be present in water sourced from wells penetrating arsenic-laden geological strata or in agricultural or industrial contaminants; this may be harmful to human health (Armstrong et al., 2021). People might be exposed to arsenic through tainted mists, fumes, or dust, as well as by consuming food contaminated with pesticides, crops grown in arsenic-rich soil, or water tainted with arsenic. Saha et al. (1999) indicate that food cultivated in arsenic-contaminated regions does not contain arsenic within edible portions like fruits, pulses and cereals, but arsenic may be present on the external surfaces due to the application of arsenicated water.

On the other hand, several studies have shown that arsenic can accumulate in certain edible plants such as amaranth, cucumbers, peppers, zucchini, tomatoes, radish, spinach, beans, parsnip and black radish (Vejvodová et al. 2022). The milk of cows affected by arsenic poisoning is devoid of arsenic due to physiological barriers, although isolated cases of arsenic contamination in cow's milk have been reported (Saha et al., 1999). Arsenic has a history of being linked to criminal activities because high doses can cause severe poisoning mimicking cholera and even death. It primarily enters the body through food or water, where it is absorbed in the stomach and intestines before being released into the bloodstream (Bansal et al., 2020). In chronic poisoning, the liver converts arsenic into a less toxic form, which is mostly eliminated through urine. Inhalation and skin exposure are minor alternate routes of entry (Barrett et al., 2019). Arsenic interferes with cell respiration, mitosis, and enzymes in the sulphhydryl group, making it a protoplasmic toxin. Historically, arsenic was used medicinally in Fowler's solution for leukemia, asthma, and other conditions, as well as in topical treatments for syphilis, eosinophilia, and psoriasis (Nordenson,

2020). The use of arsenic in household, agricultural, and industrial products such as insecticides and weed killers has decreased with the availability of low-toxic pesticides. Berries contaminated with arsenic have been linked to chronic hepatitis and hepatic cirrhosis. Chronic arsenic poisoning from drinking water has been recognized at least since the mid-20th century and major cases have been reported in countries including Taiwan, Chile and India (Chiou et al., 1995; Saha et al., 1999). The first documented case of arsenical dermatosis from drinking arsenic-tainted tube well water was described by Saha et al. (1999). While inorganic arsenic species are highly toxic, organic arsenicals such as betaine (AsBet) and ascholine (AsChol) are less harmful. Identifying different arsenic forms in biological fluids, tissues, and environmental samples is crucial for accurately assessing exposure risks (Bencko et al., 2019). Marine organisms are among the highest bioaccumulators of arsenic, but certain arsenic compounds, such as algal arsenosugars, are considered harmless (Buchet, 2021).

Mining activities are strongly associated with arsenic contamination and are associated with natural geological formations and anthropogenic sources such as smelting operations (Al-Jumaily, 2021; Mara et al., 2024). Arsenic is highly toxic even at low concentrations and can lead to cancer and skin lesions (Mara et al., 2024). In mining-affected areas, the World Health Organization (WHO) notes the need for high-powered arsenic levels monitoring in drinking water (Gentscheva et al., 2021). Arsenic is found to accumulate in agricultural products, such as crops and wines, particularly in mining areas (Al-jumaily, 2021; Mara et al., 2024). It is important to understand how arsenic becomes bioavailable in soil in order to calculate its effects on food safety and public health.

1.2.3 Analytical Methods for Determining Risk Elements

1.2.3.1 Instruments Used for Measuring Concentrations of Risk Elements

Robust analytical methods are needed when the determination of risk elements in environmental samples is required, and it has carried out by different techniques (such as Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS). The minimal cost and simple nature of operation makes AAS a common method of element analysis, but the simultaneous analysis of multiple elements is limited (Bajaj et al., 2023; Upadhyay, 2024). On the

contrary, The method of choice in the fields of comprehensive biomonitoring is ICP-MS because of its great sensitivity and capacity to trace low amounts of elements. (Bajaj et al., 2023; Upadhyay, 2024).

While ICP-OES is also effective and is noted for its precision in determining specific concentrations of elements like in complex matrices like wine (Hwang et al., 2021), it's easy to see why ICP-MS is the more popular choice. Generally, the choice of analytical method is based on the required sensitivity, number of elements to be analyzed, and matrix of the sample. For example, ICP-MS is useful especially to detect low concentration of toxic elements, whereas AAS can be sufficient for analysis of samples with higher concentration (Upadhyay, 2024; Hwang et al., 2021).

Measurement of pollution concentrations of risk elements requires a careful selection of the instruments to be used. One of the best multi element analysis is ICP-MS due to its capability of determining the elements with high sensitivity in terms of mass of element divided by mass of charge (Bajaj et al., 2023; Akboğa et al., 2022). As a result, this technique can detect elements at parts per trillion levels suitable for assessment of toxic elements in food and environmental samples (Upadhyay, 2024; Bajaj et al., 2023).

Conversely, AAS is simpler and more economical than ICP analysis, which is able to be modified for practical analysis, however, it does not present outcomes of several components like ICP analysis (Upadhyay, 2024; Bajaj et al., 2023). ICP-OES has the advantage of being able to analyse multiple elements simultaneously. However, sensitivity is rather basic and the detection limits of the instrument may not reach those of ICP-MS (Hwang et al., 2021; Bajaj et al., 2023). The instrument selection depends largely on the particular analytical needs, namely the type of samples as well as the magnitude of the concentrations of elements of interest.

1.2.3.2 Direct Measurement Methods

Measurement methods for toxic elements in liquid samples such as juices and wines are carried out directly by techniques such as ICP-OES (Akboğa et al., 2022). The method enables accurate elemental concentration determination of pure samples without digestion, similar to the work carried out by Bulková in her thesis (Bulková, 2024). Another direct measurement technique is X-

ray fluorescence (XRF) on solid samples such as soil or minerals instead of liquids, although it is useful mainly for the latter and not particularly as a replacement for ICP-OES. Thus, it is a fast, non-destructive and suitable for field applications, and preliminary assessments of contaminated sites (Akboža et al., 2022). Nevertheless, it is important to know that XRF immediately gives results however the sensitivity and accuracy of the XRF is not as high as the laboratory-based methods such as ICP-OES and AAS (Akboža et al., 2022).

For example, XRF can pinpoint the presence of heavy metals in soil or sediment, but cannot be utilized for solutions such as beverages (such as wine). In this case, confirmatory analyses with more sensitive methods like ICP-OES are often needed to gain confidence in the results as well as regulatory compliance (Akboža et al., 2022). Therefore, ICP-OES is a critical method for the direct determination of toxic element concentrations in liquid matrices. Bulková's study similarly utilized a similar methodology to this one, employing ICP-OES to measure the amount of arsenic in wine samples. For the measurement, Thermo Scientific iCAP 7000 series ICP spectrometer was used and the calibration solutions were prepared with certified reference materials for obtaining accurate measurement.

1.2.3.3 Acid Digestion Methods

Success in preparing a solid matrix for elemental analysis, especially of soil and plant materials, requires acid digestion. The digestion method can greatly affect their extraction efficiency. Usually, this is done using strong acids, such as nitric acid (HNO₃) in traditional hot plate digestion, which is time consuming and may result in loss of volatile elements (Hwang et al., 2021; Akboža et al., 2022).

Recently, microwave-assisted digestion has become widely accepted because it is very quick and it is able to reach complete digestion under minimum time (Hwang et al., 2021; Akboža et al., 2022). The high temperatures and pressures are used to increase the solubility of elements thus improving the recovery rate of the elements. Furthermore, the digestion with hydrogen peroxide (H₂O₂) for digestion can also facilitate the oxidation of organic matter to further facilitate the removal of metal extraction (Hwang et al., 2021; Akboža et al., 2022).

1.2.4 Regulations and Concentration Limits

The protection of public health requires the development of regulatory frameworks for the toxic elements in food and environmental samples. Different organizations such as European Food Safety Authority (EFSA) and WHO have established permissible limits for toxic elements in food products (Gentscheva et al., 2021; Mara et al., 2024). For example, WHO recommends a maximum concentration of 0.01 mg/kg for arsenic in the food products because even at low levels this is a potential health risk (Gentscheva et al., 2021; Mara et al., 2024).

Food safety regulations are in addition to environmental standards that are set to monitor and minimize the effect of mining activities in the surrounding ecologies (Carvalho et al., 2022, Mara et al., 2024). Often such regulations demand constant checking for soil and water quality to prevent toxic elements from exceeding established concentration limits that guarantee the preservation of the health of humans and the environment (Carvalho et al, 2022; Mara et al, 2024).

In the Czech Republic, Preventive limits of hazardous substances and elements to be introduced in agricultural soil are determined and defined by Regulation No. 153/ 2016 Coll. For arsenic, the following values have been set: for common soils, 20 mg/kg dry matter (extraction with aqua regia), and for light soils, 15 mg/kg dry matter (extraction with aqua regia). The indicative value, above which the health of people and animals may be threatened, is 40 mg/kg dry matter (extraction with aqua regia) (Vácha et al., 2014; Vejvodová et al., 2022).

The ČSN ISO 17378-2 standard specifies the method (HG-AAS) for determining arsenic and antimony in water. This method is applicable for drinking, surface, groundwater, rainwater, and wastewater, NOT for seawater. Between 0.5 and 20 µg/L is the estimated concentration for As and Sb. Higher concentration samples than the specified usable range can be analyzed after appropriate dilution (Le et al., 2022).

On March 3, 2023, a new Commission Regulation (EU) 2023/465 was published, amending Regulation (EC) No. 1881/2006 concerning maximum limits of arsenic in certain foods. The tolerable weekly intake of 15 µg/kg body weight established by FAO/WHO experts for food additives (JECFA) is no longer considered adequate. It has been proven that inorganic arsenic causes lung, bladder, skin cancer, and a number of adverse effects have been recorded even at

lower exposures. The CONTAM Committee has determined a new range of lower confidence limit values for the reference dose ranging from 0.3 to 8 $\mu\text{g}/\text{kg}$ body weight per day. The most at-risk groups are people with high rice consumption and children under 3 years of age (European Commission, 2023).

Due to concerns about wine contamination with arsenic, International Organisation of Vine and Wine (OIV) has approved a limit for wine of 0.2 mg/L. Acute arsenic toxicity has been observed with ingestion of drinking water containing high concentrations of As of approximately 21.0 mg/L. Chronic toxicity has mainly been observed in children who drink arsenic-contaminated water (average concentration of 0.6 mg/L) for an average of 7 years (Stockley et al., 2021). The OIV also approved limits for other PTE including Cadmium (0.01 mg/L), Copper (1.0 mg/l), Iron (10 mg/L), Lead (0.15 mg/l) and 5mg /L limit for Zinc.

Most current legal regulations concern only total arsenic concentrations in food and drinking water. Based on the risk of cancer and other health effects from long term exposure, WHO (2019) has set a provisional tolerable weekly intake (PTWI) for inorganic arsenic at 15 $\mu\text{g}/\text{kg}$ of body weight, or about 2.1 $\mu\text{g}/\text{kg}$ per day.

1.2.5 Overview of Acid Digestion Methods Used in Wine Testing

In the field of wine testing, acid digestion methods are vital for sample preparation in determining toxic elements. The literature review shows that various acid digestion methods are used to analyze the wine (Hwang et al., 2021; Akboža et al., 2022), by using hot plate digestion, microwave assisted digestion and different acids such as HNO_3 and aqua regia.

Adjustments of digestion method can have a dramatic impact on toxic element recoveries, and accurate assessment is dependent on this. For instance, microwave assisted digestion has proven to be better than conventional methods regarding the recovery of elements in wine testing scenarios (Hwang et al., 2021; Akboža et al., 2022).

In order to support and clarify our approach, a comprehensive reference to more recent research on the methods of acid digestions in wine analysis was compiled into a summary table. The idea was to compare different methods of analysis and their chemical composition (chemical composition of digestion agents and their amount, tooling, and analytic outcome). With this

comparative overview, it was possible to understand our choice of method, which is consistent with most common practices of wine toxicology research.

A total of 54 publications were assessed in this review, with most of them sourced from references in other evaluated papers in Web of Science search engine. Ultimately, 49 publications were included in the final (Table 1). However, four publications, including Dragusha et al. (2017), were excluded because their digestion agents and methods did not align with the research criteria. For example, Dragusha et al. (2017) investigated heavy metal determination in Kosovo wines using 100 ml of wine with 1 ml of concentrated HNO₃, nebulized in an air–acetylene flame with AAS detection. Similarly, Maria (2008) optimized methods for Cu, Zn, and Pb content determination in Romanian wines using dry or microwave mineralization followed by AAS. García-Esparza et al. (2006) measured copper content in Italian grapes and wines employing digestion with HNO₃ and HClO₄ in a Büchi digestion apparatus, with AAS analysis. Wangkarn and Pergantis (1999) utilized micro flow injection ICP-MS to analyze arsenic in wines diluted and acidified with nitric acid, while Ostapczuk et al. (1997) determined cadmium, lead, and copper via potentiometric stripping analysis after acid digestion and high-pressure ashing.

Table 1: Literature overview of acid digestion methods used for analysing elemental concentrations in wine, with the amounts of reagents and analytical instrumentation used (Source: original)

	Paper reference	Digestion agent used	Digestion method used. (Microwave or Heat method)	Analytical Instrument	References
1	Multi-Elemental Analysis of Wine Samples in Relation to Their Type, Origin, and Grape Variety	4ml wine + 4ml HNO ₃	Microwave method	ICP-MS	Gajek et al., 2021

2	Geographical origin identification of Romanian wines by ICP-MS elemental analysis	2.5ml of wine + 2.5ml	Microwave method	ICP-MS	Geana, et al., 2013
3	Analysis of wines by ICP-MS: Is the pattern of the rare earth elements a reliable fingerprint for the provenance?	1) 2 mL wine + 2 mL HNO ₃ 2) 2 mL wine + 3 mL HNO ₃	1)Microwave method 2)High pressure ashing	ICP-MS	Jakubowski et al., 1999
4	Concentration levels of essential and non-essential elements in selected Ethiopian wines	Aliquots of 10 mL from the bulk sample was taken and quantitatively transferred to a 250 mL digestion flask. A freshly prepared 7 mL HNO ₃ /H ₂ O ₂ molar ratio of 2:5 was added to this flask.	Heat method	AAS	Woldemariam & Chandravanshi, 2011
5	Elemental analysis of wines from South America and their classification according to country	To obtain the aqueous phase in a single vessel, 3 mL of HNO ₃ were added to 1 mL of wine, which was	Heat method	ICP OES and ICP-MS	Bentlin et al., 2011

		transferred into PTFE flasks.			
6	Multi-element Analysis of South African Wines by ICP-MS and Their Classification According to Geographical Origin	Wine samples were diluted 1:1 with 0.14 M HNO ₃ .	Microwave method	ICP-MS	Coetzee et al., 2005.
7	Determination of metals and metalloids in wine using inductively Coupled Plasma Optical Emission Spectrometry and Mini-torch	1) 2 mL of wine sample and 8 mL of HNO ₃ 2) 2 mL of wine sample + 8 mL of concentrated suprapure nitric acid	1) Microwave method 2) Water Bath-Assisted Digestion	ICP OES	Zioła-Frankowska, & Frankowski, 2017
8	Determination of Selected Metals in Fruit Wines by Spectroscopic Techniques	10 ml of fruit wine + 5 ml of HNO ₃ + 5 ml of H ₂ O ₂	Microwave method	AES, AAS, GF-AAS	Płotka-Wasyłka, et al., 2017
9	Influence of Vintage on Cu, Fe, Zn and Pb Content in Some Croatian Red Wines	Wines were mineralized at 540C (Magnetic Roller Tube Molten Salt Furnace, SchnellVerascher). The prepared	Not defined	AAS	Banović, et al., 2009

		ashes were dissolved in 10 ml 10% HNO ₃ and dissolved up to 25 ml with bidistilled water.			
10	Study of heavy metals in Madeira wine by total reflection X-ray fluorescence analysis	2 ml aliquots were digested with 2 ml of concentrated HNO ₂ and 1 ml of H ₂ O ₂ .	Heat Method	TXRF	Carvalho, et al., 1996
11	Characterization and Classification of Turkish Wines 3 Based on Elemental Composition	10 mL HNO ₃ and 128 4 mL H ₂ O ₂	Heat method	(ICP-AES and ICP-MS)	Sen & Tokatli 2014
12	Elemental Composition of Red Wines in Southeast Turkey	5.0 ml of wine sample was added into 2.0 ml concentrated HNO ₃ and 1.0 ml H ₂ O ₂ .	Microwave method	ICP OES	Karataş, et al., 2015
13	Characterization of the geographical origin of Italian red wines based on traditional and nuclear magnetic resonance spectrometric determinations	50 ml of wine was ashed 0.5 g and dissolved in 5 ml of 70 % HNO ₃ , 2 ml of 40 % water	Microwave method	NMR, ICP-OES	Brescia et al., 2002

14	Development of a procedure for the multi-element determination of trace elements in wine by ICP-MS	1) 2.5ml of wine + 2.5ml of HNO ₃ 2) 2 ml of wine + 3 ml of HNO ₃	1) Microwave 2) High pressure ashing	ICP-MS	Castiñeira et al., 2001
15	Recent temporal variations of trace metal content in an Italian white wine	1ml of wine + 2mL ultrapure H ₂ O ₂ + 10 mL ultrapure HCl	Not defined heat was involved	Square Wave Anodic Stripping Voltammetry (SWASV)	Illuminati et al., 2014
16	Determination of heavy metals in wines by anodic stripping voltammetry with thick-film modified electrode	4 ml of H ₂ SO ₄ + 20ml wine + 30% H ₂ O ₂	Microwave method	ICP-MS	Brainina et al., 2004
17	Trace element determination of Argentine wines using ETAAS and USN-ICP-OES	5ml of wine + HClO ₃ + HNO ₃	Heat Method	ETAAS and USN-ICP-OES	Lara et al., 2005
18	Investigation of heavy metal concentrations in some Turkish wines	25 ml of wine + 5 ml of HNO ₃ + 5ML H ₂ O ₂	Heat Method	AAS	Alkiş et al., 2014.
19	Determination of lead and cadmium in wines by graphite	5 ml of wine + 5 ml of HNO ₃	Microwave method	GFAAS	Kim, 2004.

	furnace atomic absorption spectrometry				
20	Lead determination in wine by potentiometric stripping analysis	4 ml of wine + 3 ml HNO ₃	Heat Method	Potentiometric stripping analysis (PSA)	Marin & Ostapczuk, 1992
21	Classifying wine according to geographical origin via quadrupole-based ICP-mass spectrometry measurements of boron isotope ratios	Dilution of samples at 1:100 with HNO ₃ 0.65%.	Microwave method	ICP-QMS	Coetzee & Vanhaecke, 2005
22	Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry	5 ml H ₂ O ₂ + 3 ml. HNO ₃ + 10 ml wine sample	Not defined	AAS	Karadjova et al., 2002
23	An on-line system for preconcentration and determination of lead in wine samples by FAAS	25 ml wine sample + 5 ml. HNO ₃ + 5 ml H ₂ O ₂	Heat method	FAAS and ICP-MS	Lemos et al., 2002
24	On-Line Complexation/Preconcentration System for	10 ml of wine + 1 ml of HNO ₃	Heat method	ICP-AES	Wuilloud et al., 2001

	the Determination of Lead in Wine by Inductively Coupled Plasma-Atomic Emission Spectrometry with Ultrasonic Nebulization				
25	Spectrophotometric Flow Injection Determination of Lead in Port Wine Using In-line Ion-exchange Concentration	100ml of wine sample + H ₂ SO ₄ + sample was allowed to evaporate in water bath + the residue was mineralised until ash was obtained + the ash was dissolved in 1ml of 1 ml of HNO ₃ + 5 ml of H ₂ O	Heat method	ETAAS	Lopesa et al., 1996
26	On the determination of lead in wine by electrothermal atomic absorption spectrometry	1) 50 ml wine samples + 2 ml HNO ₃ + 5 ml H ₂ O ₂ 2) 10 ml of wine sample + 2ml HNO ₃ + 2 ml H ₂ O ₂	1) Heat method 2) Microwave method	AAS	Karadjova et al., 2007

27	Lead isotopic signatures of wine and vineyard soils—tracers of lead origin	1 ml conc. HNO ₃ /50 ml of wine Mineralization with H ₂ O ₂ and HNO ₃	Heat method	ICP-MS	Mihaljevič et al., 2006
28	Origin verification of French red wines with isotope and elemental analyses	Teflon digestion vessels, 5 mL HNO ₃	Microwave method	ICP-MS	Wu et al., 2021
29	Classification of Croatian wine varieties using multivariate analysis of data obtained by high resolution ICP-MS analysis	Samples are prepared by making a tenfold dilution in Milli-Q water and the addition of 2% v/v nitric acid and In as internal standard.	Not defined	ICP-MS	Kruzlicova et al., 2013
30	ICP-MS analysis of multi-elemental profile of greek wines and their classification according to variety, area and year of production	2.5ml of wine + 5 mL of HNO ₃	Microwave method	ICP-MS	Pasvanka et al., 2021
31	Chemical Elemental Composition from Vineyard Soil to Wine in Hawke's Bay	Not defined	Microwave method	ICP-MS	Lowry, 2022

	and Marlborough, New Zealand				
32	Elemental Analysis and Phenolic Profiles of Selected Italian Wines	They were diluted 10 times in water containing 2% (v/v) nitric acid.	Acid digestion	ICP-OES and ICP-MS	Fermo et al., 2021
33	Classification of wines according to several factors by ICP-MS multi-element analysis	5 ml of wine + 2 ml of nitric acid	Heat method	ICP-MS	Pérez-Álvarez et al., 2019
34	Study of mineral profile of Montilla-Moriles Bfino^ wines using inductively coupled plasma atomic emission spectrometry methods	25 mL of wines + 15 mL of hydrogen peroxide + 1 mL of nitric acid	Heat method	ICP-AES	Álvarez et al., 2007
35	Searching the Most Appropriate Sample Pretreatment for the Elemental Analysis of Wines by Inductively Coupled Plasma-Based Techniques	HNO ₃ 69% H ₂ O ₂ 35% perchloric acid 60%	Microwave method	ICP-OES and ICP MS	González et al., 2008
36	Application of a microwave based desolvation system for multi-elemental analysis of wine by inductively coupled	Chemicals used was not clearly stated	Microwave method	ICP MS	Grindlay et al., 2008

	plasma based techniques				
37	Method validation for determination of heavy metals in wine and slightly alcoholic beverages by ICP-MS	HNO ₃	Not mentioned	ICP-MS	Voica et al., 2009
38	Direct analysis of mineral components in wine by ICP-OES	50 mL volume of sample with 30 mL of perhydrol	Not defined	ICP-OES	Thiel & Danzer. 1996
39	Determination of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr and Zn in red wine samples by inductively coupled plasma optical emission spectroscopy: Evaluation of preliminary sample treatments	1) 25 ml of wine sample + 20 ml nitric acid 2) 25 mL of red wine with 15 mL of hydrogen peroxide + 1 ml of nitric acid 3) 25 ml of red wine + 5% nitric acid	1)Dry ashing 2) Wet ashing (hot plate was used) 3)Alcohol Removing technique	ICP-OES	Moreno et al., 2008
40	Application of a microwave-based desolvation system for multi-elemental analysis of wine by inductively coupled	Not defined	Microwave method	ICP-MS and ICP-AES	Grindlay et al., (2008).

	plasma based techniques				
41	Evaluation of the multi-element capabilities of collision/reaction cell inductively coupled plasma - mass spectrometry in wine analysis	Not defined	Acid digestion	ICP-MS	Grindlay et al., 2014
42	Determination of Pb and Cd in Macedonian Wines by Electrothermal Atomic Absorption Spectrometry (ETAAS)	HNO ₃ , HClO ₄ and H ₂ SO ₄	Heat method	ETAAS	Ivanova-Petropulos et al., 2015
43	Optimization and Validation of a Microwave Digestion Method for Multi-element Characterization of Vranec Wines	5 mL of wine was digested with 5 mL nitric acid	Microwave method	ICP-MS	Ivanova-Petropulos et al., 2016
44	Direct determination of iron and manganese in wine using the reference element technique and fast sequential multi-element flame atomic absorption spectrometry	10.0mL of wine + 3.0 mL of concentrated nitric acid + 5.0 mL of 30% (v/v) hydrogen peroxide	Heat method	Inductively coupled plasma optical emission spectrometry (ICP OES),	Ferreira et al., 2008

45	Inductively coupled plasma mass spectrometry analysis of wines	Not defined	Not defined	ICP-MS	Yolanda Pérez-Jordán et al., 1998
46	Determination of heavy metals in wines by anodic stripping voltammetry with thick-film modified electrode	20ml + 30% H ₂ O ₂ + 4ml of 3.5M NaCl + 100 ml with tri distilled water	Microwave method	ICP-MS	Brainina et al., 2004
47	Analytical methods for the determination of trace metals in wine	Not defined	Microwave method	AAS	Pyrzyńska, 2004
48	Determination of lithium in wines by ion chromatography	Not defined	Not defined	Ion chromatography	Zerbinati et al., 2000
49	Multi-element analysis of wines by ICP-MS and ICP-OES and their classification according to geographical origin in Slovenia	Not defined	Microwave Method	ICP-OES and ICP-MS	Šelih et al., 2014

1.2.6 Significance of the Study

Arsenic is a naturally occurring element in soil, water and air and presents a serious health risk associated with cancer, cardiovascular diseases, and neurological disorders (Shen et al., 2020). Regarding public health concerns, the presence of arsenic and other toxic elements in wine, especially for wines from high-risk regions where environmental contamination is high (Yang et al., 2020), has also been reported. Even though regulations try to curb such contaminants, little

data is recorded of the exact levels of these contaminants in wines, which represents a glaring knowledge gap (Mankash et al., 2019).

This study attempts to fill this gap by acid digesting wines from risk prone areas for arsenic and other potentially toxic substances. One justification of the approach is that acid digestion is a technique used in analytical chemistry that is well established and effective in complex wine matrix decomposing that leads to quantitative determination of trace elements with atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) (Bolas et al., 2019; Ding et al., 2020). Moreover, the study also takes into account the prospects of inductively coupled plasma optical emission spectrometry (ICP-OES) that offers huge benefits such as a high sensitivity of detection and low detection limits (Voica et al., 2020).

This research therefore informs winemakers, regulatory bodies and public health officials of the level of contamination and ways to reduce the risk of health risks from consuming wine. The outcome provides an input to the developments of standardized monitoring strategies and enhance consumer safety by the guarantee of a compliance with permissible contaminant limits (Meharg et al., 2019; Jiménez-Ballesta, 2023). This study also emphasizes the necessity of exploring further into the types of toxic constituents in wines coming from high risk regions by utilizing innovative methods of wine safety assessment.

This research builds on the work of Martina Bulková, who analyzed 20 wines — 14 from vineyards in Kutná Hora, a historically contaminated area with elevated soil arsenic, and 6 control wines from other Czech regions — using a direct sample measurement via inductively coupled plasma optical emission spectrometry (ICP-OES). Her findings revealed higher-than-expected arsenic levels in some samples, raising concerns about potential health risks and the need for more targeted investigation. To investigate the distribution and environmental sources of potentially hazardous elements (PTEs) in wine, the current study builds on this foundation by using the acid digestion method rather than the direct method from the original research in a more comprehensive analytical framework. Together, these complementary investigations provide robust, reliable data that are important for consumer safety, environmental monitoring, regulatory compliance, and the protection of regional wine reputations.

2.0 AIMS OF THE THESIS

2.1 Research Hypotheses

Hypothesis 1

Wines from anthropogenically polluted areas can contain elevated concentrations of potentially toxic elements.

Hypothesis 2

Direct analysis and digestion methods show different concentrations of potentially toxic elements.

2.2 Aims

The aims of this research are as listed below.

1. To choose the appropriate acid digestion methodology for determination of potentially toxic elements based on literature sources and available analytical equipment.
2. To compare the differences in concentrations of potentially toxic elements in wines grown in an anthropogenically contaminated region (Kutna Hora) versus the ones grown in non-contaminated areas.
3. To compare the differences in concentrations of potentially toxic elements between different wine types (white, rosé and red).
4. To compare the obtained results from the acid digestion experiment to the ones previously obtained using direct measurement by Bulková (2024) using the same set of wine samples.
5. To compare the nitric acid only digestion with nitric and peroxide acid digestion

3.0 MATERIALS & METHODS

3.1 Materials

3.1.1 Sample Collection and Preparation

Wine Samples: A total of 22 wine samples were analyzed in this experiment. The first 20 samples (samples numbered 1-20) were the same set of samples used by M. Bulková (2024) in her thesis. The samples have been properly stored in a freezer at -20° C until the start of this experiment. Within those 20 samples, 14 wines from the Kutná Hora winery were analyzed, from the Kraus winery in Mělník 3 pcs, and from the Moravíno winery in Valtice (near Mikulov) 3 pcs. The first 3 bottles are 2019 Pinot Gris. Bottles 4-6 are 2019 Pinot Noir and 7-9 are 2018 Riesling, from all three wineries. Bottles 10–20 are all from Kutná Hora.

The two remaining samples (no. 21 and 22) were recent additions, both from Troja vineyards in Prague. Sample 21 was Tramín červený, whereas sample no. 22 was Ryzlink rýnský.

The name of each wine samples and their locations are listed in table 2 below.

Table 2 : wines samples and their location (Bulkova, 2024 with little modification)

sample	Wine	Location	Type (Colour)
1	Rulandské šedé 2019	KH - Vinice nad Kapličkou	White
2	Rulandské šedé 2019	Mělník	White
3	Rulandské šedé 2019	MV - Pod svatým kopečkem, Mikulov	White
4	Rulandské modré 2019	KH - Vinice u všech svatých	Red

5	Rulandské modré 2019	Mělník	Red
6	Rulandské modré 2019	MV - Pod svatým kopečkem, Mikulov	Red
7	Ryzlink rýnský 2018	KH - Vinice u všech svatých	White
8	Ryzlink rýnský 2018	Mělník	White
9	Ryzlink rýnský 2018	MV - Viniční trať Kačisdorfské pole	White
10	Müller Thurgau 2021	KH - Vinice nad Kapličkou a u Borku	White
11	Solaris 2021	KH - Vinice nad Kapličkou a u Borku	White
12	Hibernal 2019	KH - Vinice na Příčce	White
13	Tramín Kořenný 2019	KH - Vinice u všech svatých a na Příčce	White
14	Muškat' Ottonel 2019	KH - Vinice nad Kapličkou	White
15	Regent Rosé 2019	KH - Vinice u Borku	Rose
16	Chardonnay 2019	KH - Vinice nad Kapličkou	White

17	Svatovavřínecké 2021	KH	Red
18	Svatovavřínecké 2018	KH - Vinice nad Kapličkou	Red
19	Svatovavřínecké 2017	KH - Vinice nad Kapličkou	Red
20	Mladý Punk Ryzlink rýnský 2021	KH - biodynamické víno	White
21	Tramín červený (TČ)	Prague	White
22	Ryzlink rýnský	Prague	White

3.2 Methods

Based on the literature review of acid digestion methods provided in Table 1, and based on the available laboratory equipment and instrumentation, we've opted to use a slightly modified version of the method described in Płotka-Wasyłka et al. (2017) where we would use the same amounts of reagents and cooking time, but use hot plate method with Teflon bottles (60 mL Digestion Vessels, Savillex, LLC.) heated on a hot plate under a fume hood. One of the reasons for choosing this method was what we considered to be the appropriate reagent amounts for the available digestion vessels, as well as a clearly described procedure of colour loss of the sample during digestion.

Due to the differences in digestion method (using hot plate instead of microwave extractor) sample no. 17 was chosen for the first trial of determining the appropriate heating (cooking) time, because it was visually the darkest sample of the entire series. 3 teflon digestion vessels were filled with 10 ml of sample 17 wine. To the first two, 5 ml of concentrated nitric acid (HNO₃, 65%, SELECTIPUR, Lach-Ner, s.r.o.) and 5 ml of hydrogen peroxide (H₂O₂, 30%. Unstabilized, Lach-Ner, s.r.o.) were added. The third was topped with 10 ml of concentrated nitric acid, so the amounts would be the same, but without the addition of hydrogen peroxide. This was done under the

presumption that hydrogen peroxide will speed up the colour loss observed and help dissolve the wine matrix more quickly. The samples were heated up at 130°C for a total period of 1 and a half hour, with the lids closed, but not tightened, and with periodical visual inspections every 15 minutes to observe the loss of colour. Initially, in the first 15 minutes, the sample with only the nitric acid added seemed more efficient in removing the colour from the sample, although it caused a severe, foaming reaction with the wine when added. After 30 minutes have elapsed, the samples with both the nitric acid and peroxide were more efficient, and this trend remained until the end of the test cooking. One hour seemed to be sufficient enough for a complete colour loss, but we kept the experiment going for one and a half hour to make sure. Parts of this experiment can be seen in Figures 2.

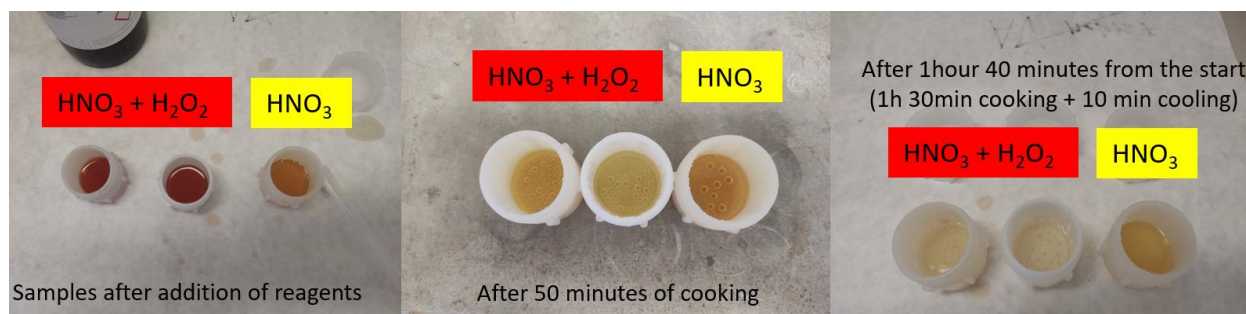


Figure 2 : Setup of the colour loss experiment

After the test experiment, it was decided to proceed with all samples digested in triplicates with 10 ml of sample, 5 ml of conc. nitric acid and 5 ml of hydrogen peroxide, with the teflon vessels fully closed, the temperature kept at 130°C and the cooking time of 2 hours (in order to ensure full digestion - this being a heating period commonly used in digestion of soil and plant samples via hot plate method), with extra caution, since this was the first time digesting wine (and liquid samples in general) in this laboratory.

The first batch, later named "Explosion batch" included samples 1-8 in triplicates (with A, B and C designations for each triplicate sample). However, due to the increased pressure in a fully sealed teflon vessel, only about 30 minutes into the experiment, one of the teflon vessels exploded under the fume hood.

The hot plate was immediately unplugged from the power source, and the laboratory was immediately evacuated and kept under strict observation due to potential further explosions until

the temperature and pressure would drop. During the next 15 minutes, two more vessels have exploded.

After a period of 2 hours from the third explosion has occurred, a thorough cleaning of the fume hood was performed, and the remaining samples were processed by moving all of the digested material to a 50 ml (Pyrex) volumetric flask and topped up to 50 ml mark with deionized water. The entire amount was filtered through a 12.5 cm filter paper and a glass funnel into a 50 ml polypropylene centrifuge tube. Approximately 10 ml of the digested solution was taken by a syringe and filtered one more time through a 0.45 μm filter membrane (VWR International s.r.o.) to a transparent polystyrene test tube to remove any remaining impurities. Samples 3C, 6A and 6C were lost in the explosions. The exploded vessels were safely stored out of reach so they wouldn't be used again. The aftermath of the explosions can be seen in Figure 3



Figure 3: Laboratory Setup Following Experimental incident

The explosions happened most likely due to increased pressure, and samples which are both liquid and highly organic, and a lack of a gas-release valve), so we had to adjust the chemical quantities for the second batch. It was assumed that the microwave system employed in the experiment also lacked a pressure release mechanism, as many microwave systems can operate in a fully sealed configuration - which was later investigated and proven false (the microwave system used in the

mentioned experiment did have a pressure release valve). Consequently, our experiment was conducted under fully sealed conditions, which would presumably keep volatile fractions of certain PTEs, mainly arsenic, inside the teflon vessel. A new laboratory rule was set up: Whenever samples are heated on the hot plate, no other chemicals should be present under the fume hood due to their potential of spillage or container breaking during a potential explosion.

For the second batch, named "Normal series" the same quantity of nitric acid and hydrogen peroxide (5 ml +5ml) were used but the Teflon bottle was only slightly closed (not fully closed) to prevent any further explosions, the heating temperature was 130°C and all samples were cooked for 1 hour and 30 minutes, then left to cool for 30 minutes. Due to already lost sample amounts in the Explosion series, samples 1-8 were tested in duplicates (A and B), whereas the remaining ones (9-22) were tested in triplicates. The experiment setup can be seen in Figure 5. The dilution and filtering were the same as previously described.

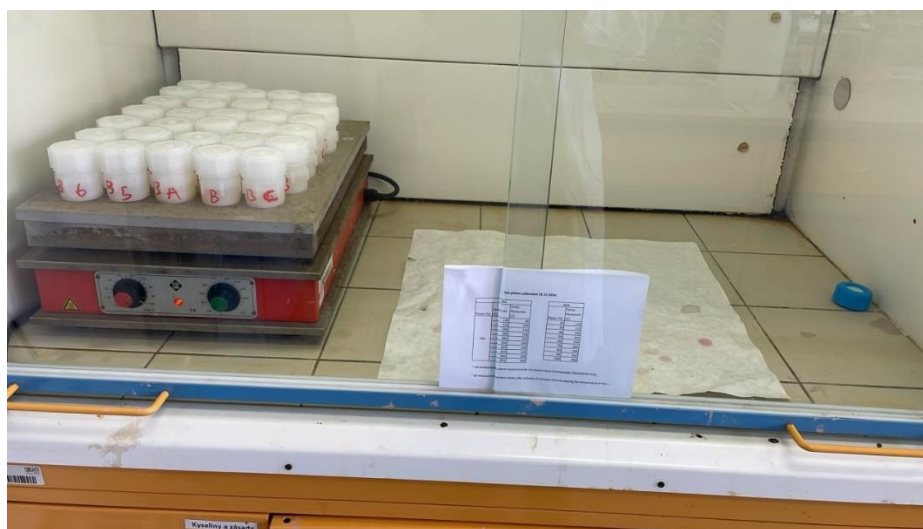


Figure 4: Setup of the Normal series

For the third batch, named "Nitric only", samples 9, 15, 17, and 18 were selected (chosen based on the sample amounts left and so that we can compare dark vs. light wines, presuming that H₂O₂ would help extract more elements - mainly from the darker wines compared to only using nitric acid). These samples were tested in duplicates, which makes it 8 samples in total. For these samples, 10 ml of wine was combined with 5 ml of nitric acid (because of the severe foaming

when using 10 ml) and no hydrogen peroxide added. All of the other parameters were the same as in the "Normal series".

After each use, the teflon vessels were washed by detergent and a soft sponge, and rinsed several times by both tap water and deionized water. The teflon vessels were later left in acid wash (10% HNO₃) for a period of at least 24 hours before using them for the next batch, before which they were again rinsed with deionized water and air-dried.

The ICP-OES iCAP 7000 of Thermo Scientific™, USA was used to analyze the samples was carried out and managed the quality assurance and control of analytical methods and data (as they were being performed) in compliance with Standard Laboratory Regulation, utilizing processing blanks and reference materials (NIST).

The immediate reaction of the wine samples following the addition of nitric acid and hydrogen peroxide is illustrated in Figure 5, where visible effervescence and colour changes can be observed.



Figure 5: Reaction of the samples after adding Nitric Acid and Hydrogen peroxide

After heating, further changes in appearance were noted, as shown in Figure 6, indicating progression of the digestion process.



Figure 6: Reaction of the samples after adding Nitric Acid and Hydrogen peroxide after cooking

Statistical Analyses

All statistical analyses were performed to assess differences in elemental concentrations between sample groups and to evaluate the influence of various experimental factors. The following procedures were applied:

1. MANOVA on Elemental Concentrations

A multivariate analysis of variance (MANOVA) was conducted on the concentrations of all measured elements in the normal series of wine samples. The analysis examined the effects of:

- Wine type (colour): Red, white and rose wines
- Wine region: Kutná Hora, Mělník, Mikulov, and Prague–Troja.

The model included both main effects and their potential interaction terms. Statistical significance was evaluated at the $\alpha = 0.05$ level, with p-values reported for each factor. Where MANOVA indicated significant effects,

2. Independent t-Test – Method Comparison

An independent-samples t-test was applied to the first 20 samples previously shared with Bulková (2024) his test compared results obtained by two analytical methods, with the goal of determining which method yielded higher precision or sensitivity in detecting elemental concentrations. Variances were checked using Levene's test, and Welch's correction was applied if homogeneity of variance was not met.

3. t-Tests Between Nitric-Only and Normal Series Samples

A direct comparison was made between four "nitric-only" samples and the corresponding four samples from the normal series:

Independent t-test alternative: Where three replicates existed in the normal series and two in the nitric-only series, an independent-samples t-test was performed instead, using all available replicates.

statistical significance was evaluated at $\alpha = 0.05$

4.0 RESULTS

4.1 Concentration of elements

A total of sixteen (16) elements (Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, V, Zn) were analyzed in replicates. The mean and standard deviation were calculated. All results are presented in Table 8a 8b 9a and 9c.

The average concentration of Aluminium (Al) ranged from 0.175 to 1.242 mg/L for the explosion series with the lowest concentration in sample 6 and highest in sample 8. The concentration of Al in normal series ranged from 0.075 to 1.709 mg/L with the lowest concentration in sample 19 & 20 and highest in sample 21 (figure 7). The concentration in Nitric acid only technique ranged from 0.333 to 0.831 mg/L for nitric only with the lowest concentration in sample 18 and highest in sample 9. The highest concentrations of Al were noted in the Normal series (nitric + peroxide).

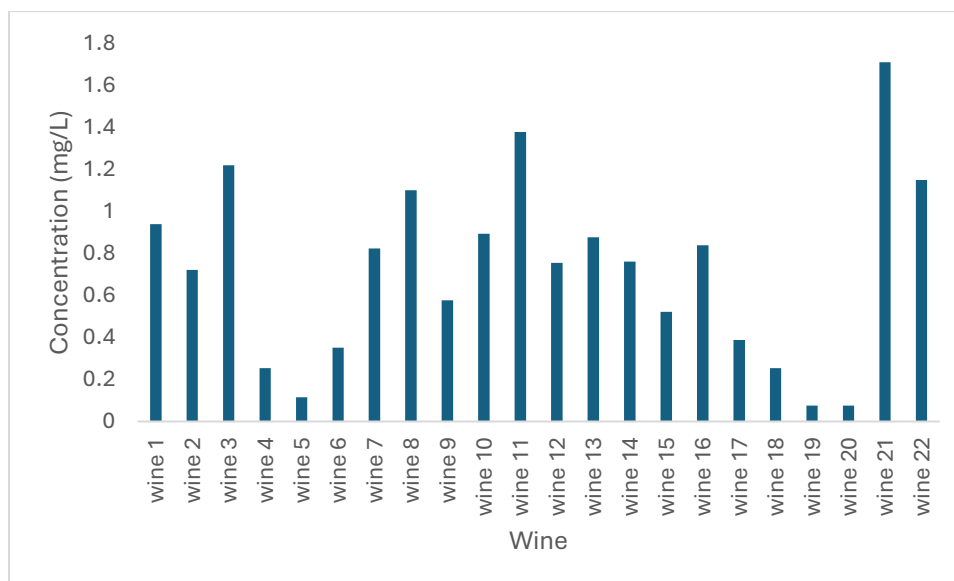


Figure 7: Concentration of Aluminium across the samples

The average concentration of Arsenic (As) ranged from 0.027–0.251 mg/L for the explosion series with the lowest concentration in sample 3 and highest in sample 5. The concentration of As in normal series ranged from 0.052–0.284 mg/L with the lowest concentration in sample 18 and highest in sample 9 (figure 8).

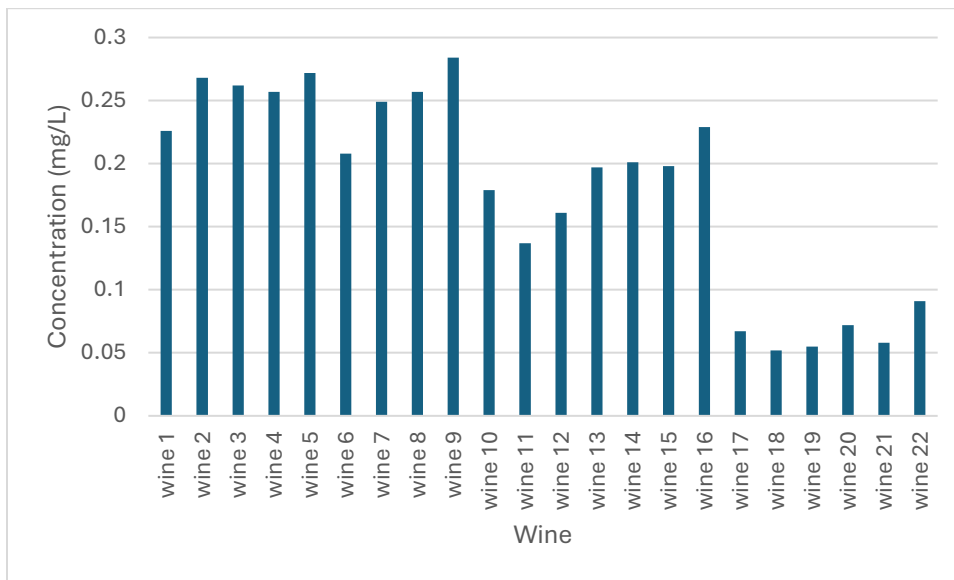


Figure 8: Concentration of Arsenic across the samples

The average concentration of Boron (B) ranged from 1.673–5.288 mg/L for the explosion series with the lowest concentration in sample 6 and highest in sample 5. The concentration of B in normal series ranged from 1.917–5.693 mg/L with the lowest concentration in sample 10 and highest in sample 5 (figure 9). The concentration in Nitric acid only series ranged from 0.050–0.073 mg/L with the lowest concentration in sample 15 and highest in sample 17.

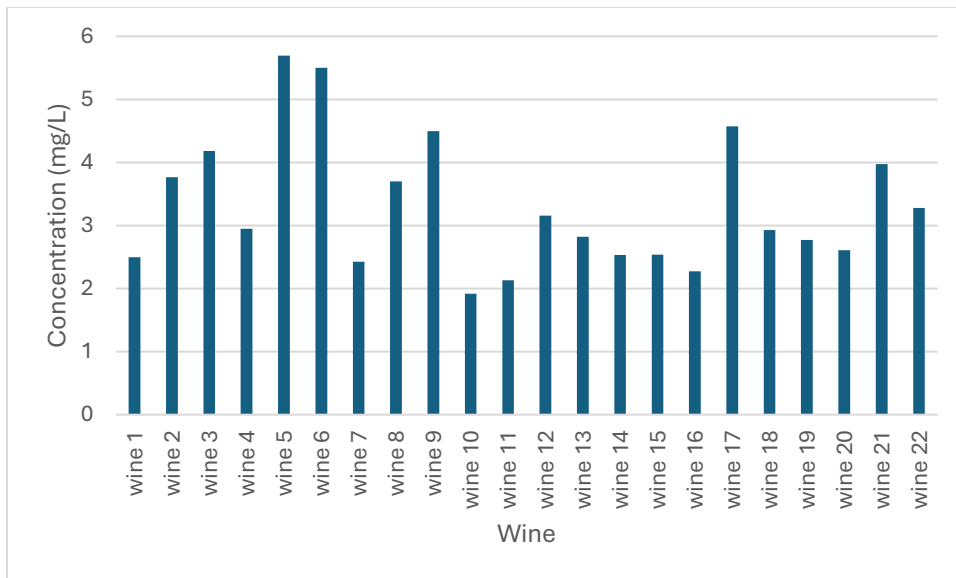


Figure 9: Concentration of Boron across the samples

The average concentration of Barium (Ba) ranged from 0.069–0.179mg/L for the explosion series with the lowest concentration in sample 6 and highest in sample 5. The concentration of Ba in normal series ranged from 0.076–0.229 mg/L with the lowest concentration in sample 2 and highest in sample 22 (figure 10).

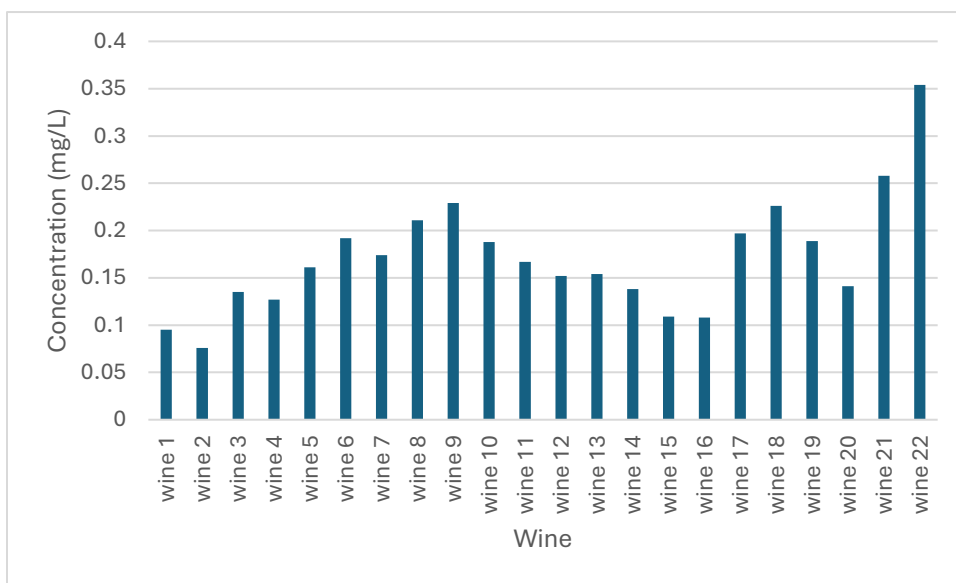


Figure 10: Concentration of Barium across the samples

The average concentration of Beryllium (Be) was nearly undetectable for the explosion and normal series, with concentrations between 0.000–0.004 mg/L and 0.000–0.006 mg/L, respectively. The Average concentration in Nitric acid only series ranged from 0.000–0.001 mg/L. Sample 4,5,6,17,18,19 and 20 were below detection, The lowest concentration is sample 3 while the highest is sample 21 in the normal series (figure 11).

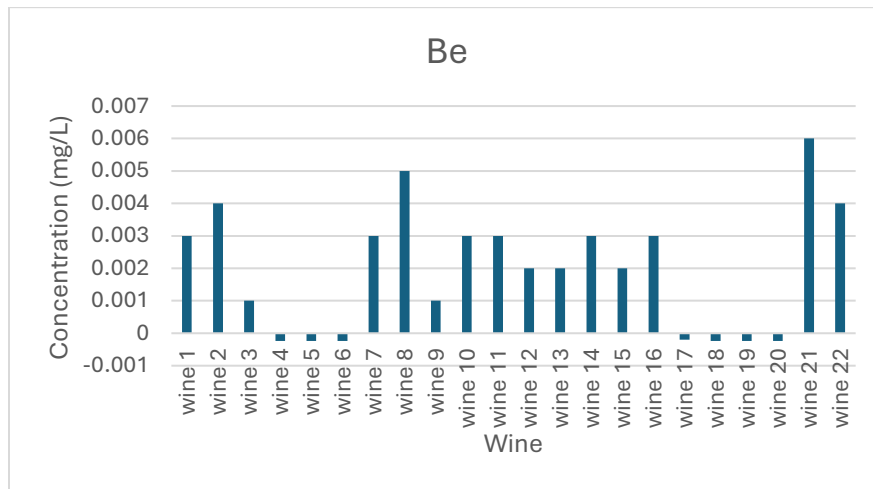


Figure 11: Concentration of Beryllium across the samples

Cadmium (Cd) average concentrations were often below the detection limits, The detected values were relatively low across all samples.

The average concentration of Cobalt (Co) ranged between 0.003–0.005 mg/L for the explosion series, the normal series ranged from 0.002–0.008 mg/L (figure 12) while the nitric acid only series ranged from 0.000–0.005 mg/L as seen in tables 8a and 9a.

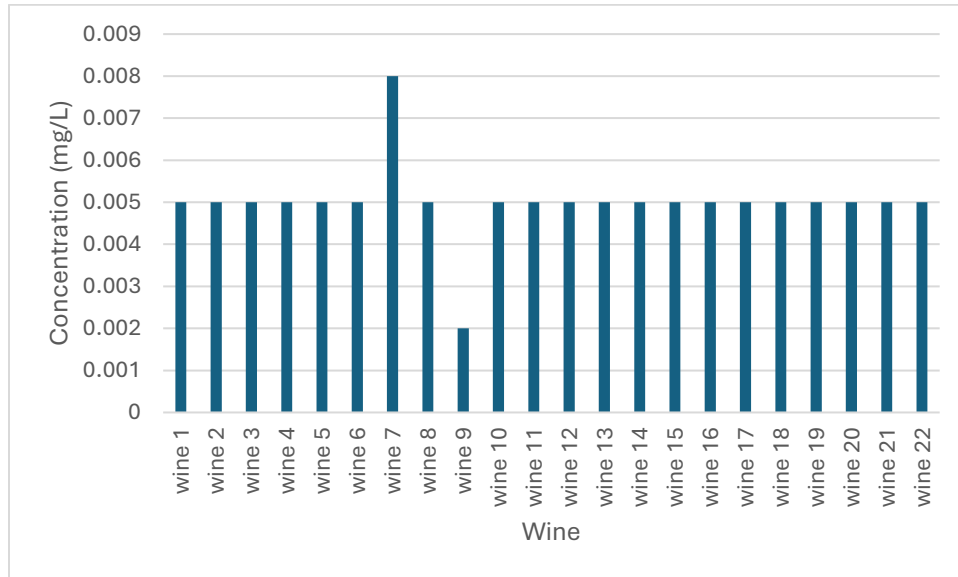


Figure 12: Concentration of Cobalt across the samples

The average concentration of Chromium (Cr) were consistently low, with values of 0.002 mg/L in both the explosion series and nitric acid only series while the normal series ranged from 0.002–0.042 mg/L. The highest concentration of Cr in the samples was recorded in sample 4 of the normal series (figure 13).

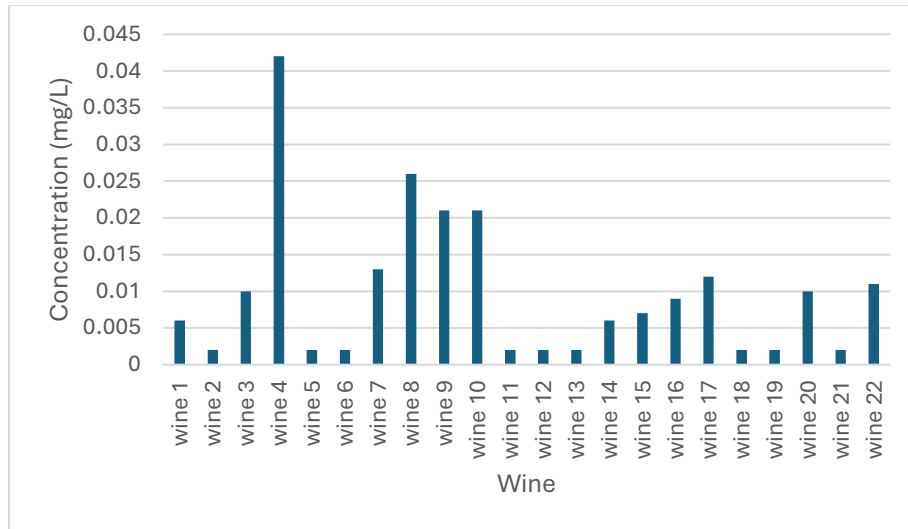


Figure 13: Concentration of Chromium across the samples

The average concentration of Copper (Cu) ranged from 0.043–0.384 mg/L in the explosion series with the lowest concentration in sample 2 and highest in sample 6. The concentration of Cu in normal series ranged from 0.020–1.065 mg/L with the lowest concentration in sample 2 and highest in sample 6 (figure 14). The concentration in Nitric acid only series ranged from 0.067–0.125 mg/L with the lowest concentration in sample 15 and highest in sample 17.

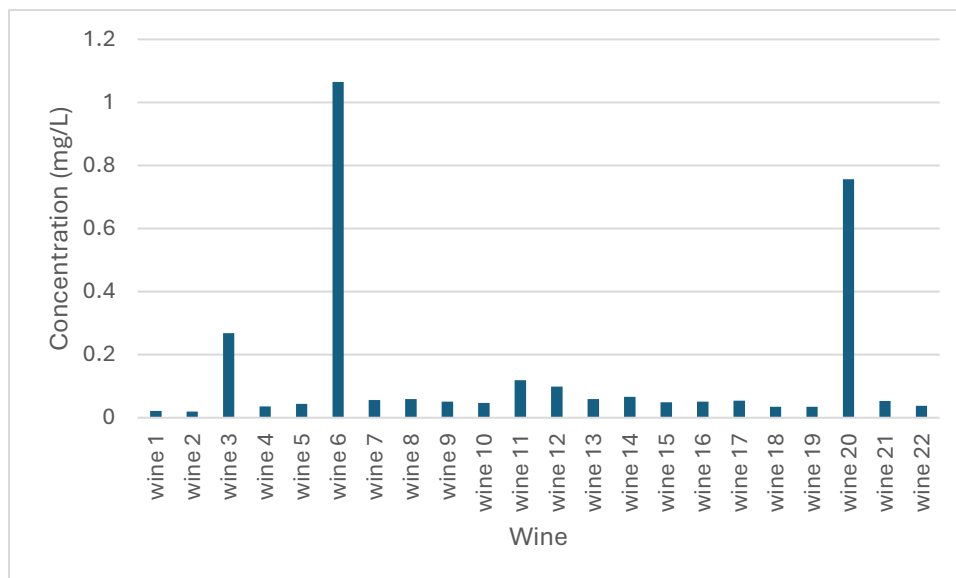


Figure 14: Concentration of Copper across the samples

The average concentration of Iron (Fe) ranged from 0.463–1.287 mg/L in the explosion series with the lowest concentration in sample 6 and highest in sample 4. The concentration of Fe in normal series ranged from 0.497–1.522 mg/L with the lowest concentration in sample 2 and highest in sample 16 (figure 15). The concentration in Nitric acid only series ranged from 1.120–2.008 mg/L with the lowest concentration in sample 18 and highest in sample 9. The highest concentration of Fe was in the Nitric only test which was in sample 9 with a concentration of 2.008 mg/L.

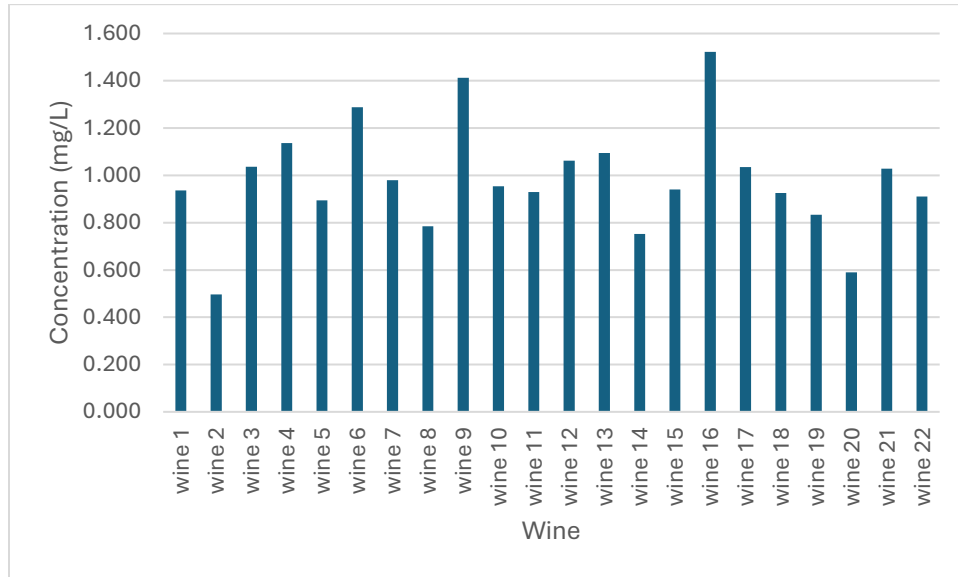


Figure 15: Concentration of Iron across the samples

The average concentration of Manganese (Mn) ranged from 0.285–0.689 mg/L for the explosion series with the lowest concentration in sample 6 and highest in sample 8. The concentration of Mn in normal series ranged from 0.419– 1.403 mg/L with the lowest concentration is sample 2 and highest in sample 22 (figure 16). The concentration in Nitric acid only series ranged from 0.471– 1.251mg/L with the lowest concentration in sample 15 and highest in sample 9. The highest concentration of Mn was in the Normal series test which was in sample 22 with a concentration of 1.403 mg/L.

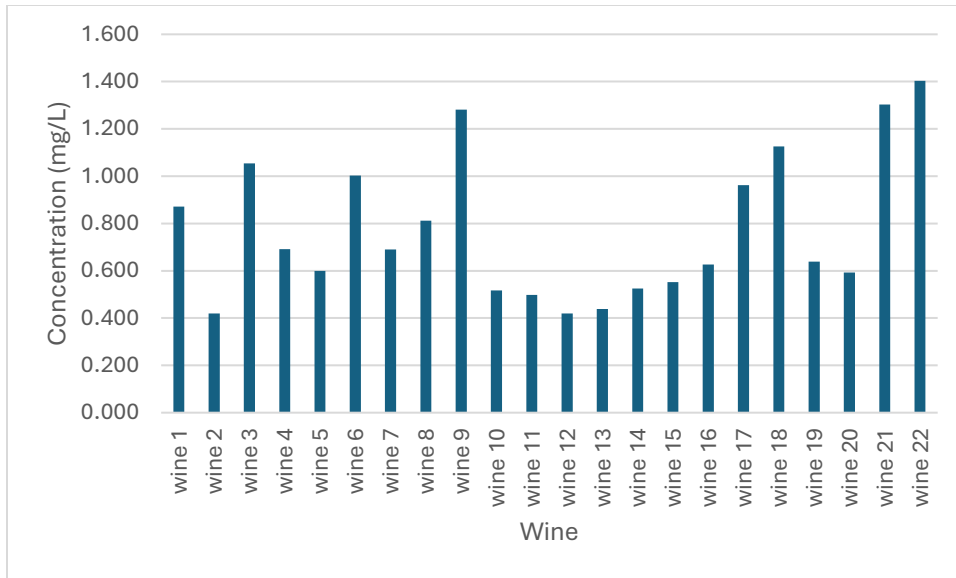


Figure 16: Concentration of Manganese across the samples

The average concentration of Nickel (Ni) ranged from 0.016–0.176mg/L for the explosion series with the lowest in sample 6 and highest in sample 4. The concentration ranged from 0.031–0.263mg/L in the normal series with highest in sample 16 and lowest in sample 14 (figure 17). it ranged from 0.034–0.061 mg/L in the nitric only series with lowest in sample 17 and highest in sample 9.

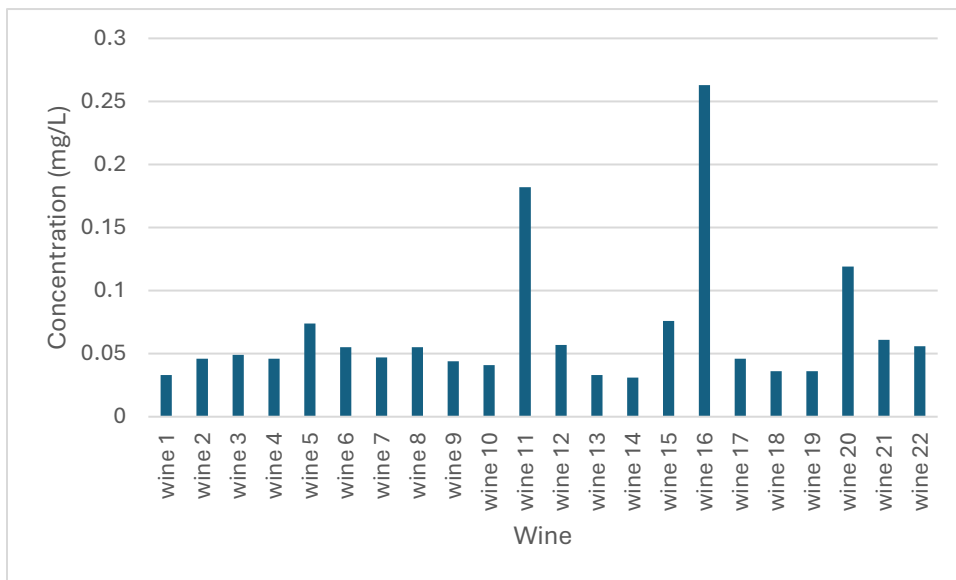


Figure 17: Concentration of Nickel across the samples

The average concentration of Lead (Pb) were low across all methods, with values ranging from 0.006–0.016 mg/L for the explosion series, 0.004–0.027 mg/L for the normal series (figure 18) and 0.006–0.015 mg/L for nitric acid only series. The highest concentration of Pb is sample 21 while the lowest is sample 13.

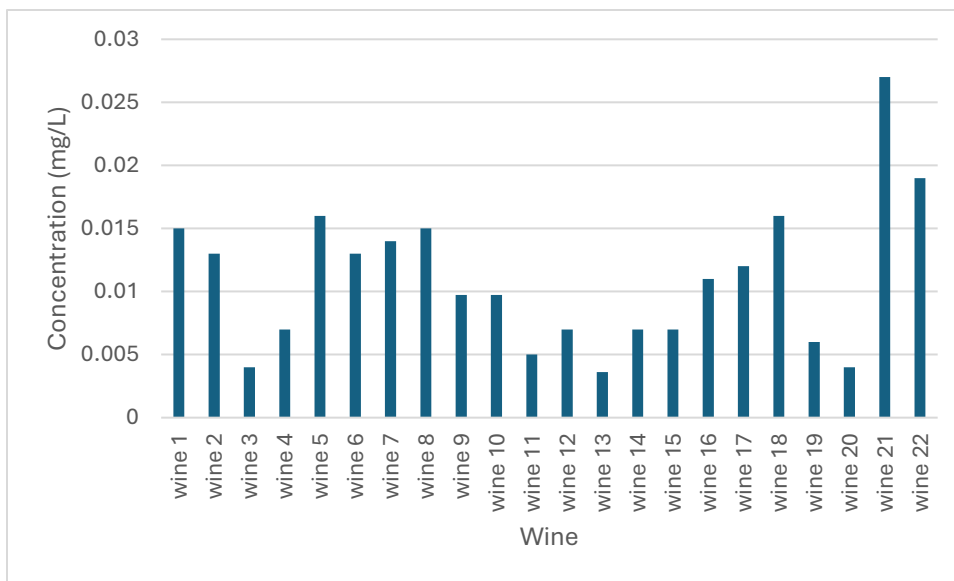


Figure 18: Concentration of Lead across the samples

The average concentration for Antimony (Sb) in the explosion series was 0.003 mg/L for all the samples. The normal series ranged from 0.003–0.007 mg/L as seen in figure 19. The concentration in Nitric acid only series ranged from <LOD–0.008 mg/L with the lowest concentration in sample 9 and highest in sample 18.

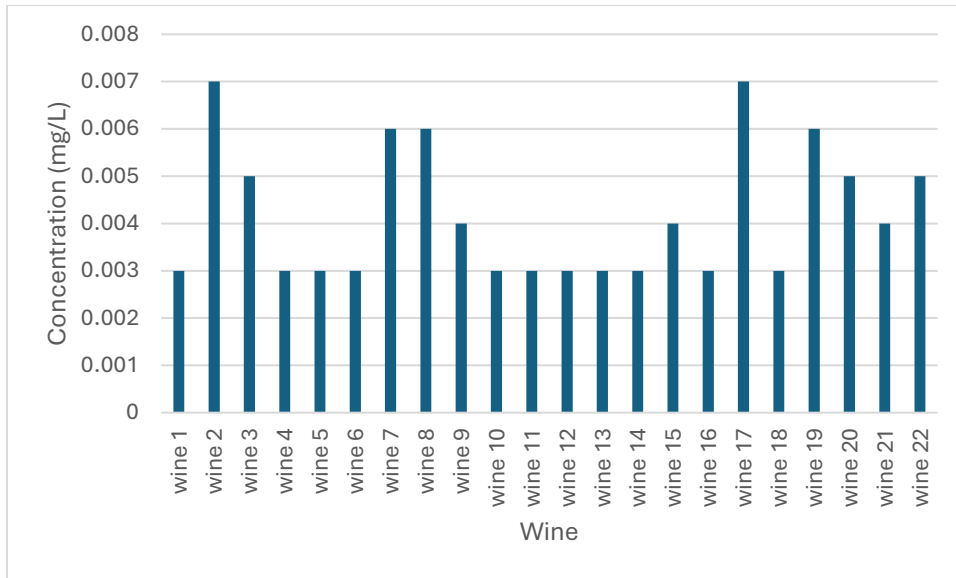


Figure 19: Concentration of Antimony across the samples

The average concentrations of Vanadium (V) for the normal series ranged from 0.002–0.366 mg/L (figure 20). The nitric only series ranged from 0.021–0.165 mg/L while it ranged from 0.002–0.401 mg/L for the explosion series as seen in table 21, with the lowest concentration in sample 5 and highest in sample 4.

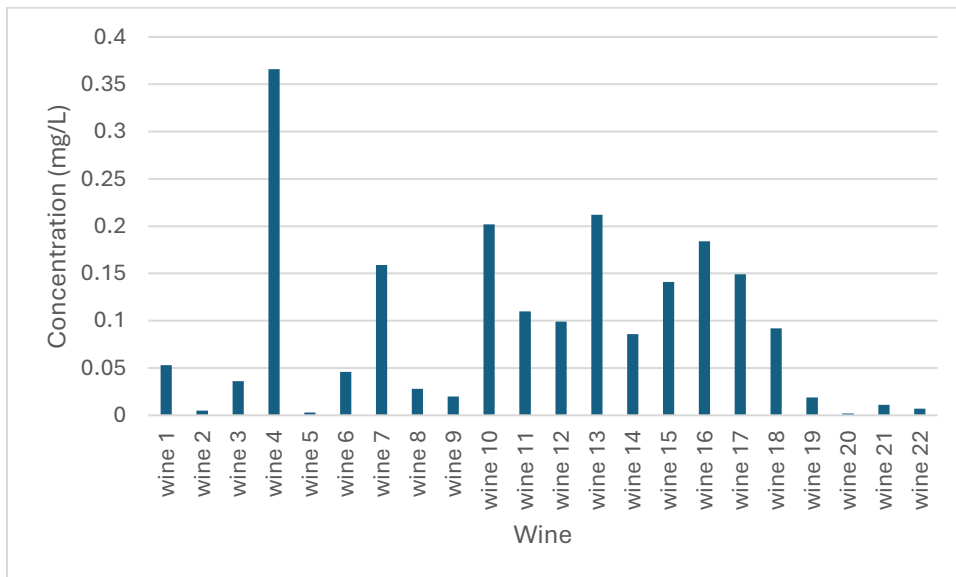


Figure 20: Concentration of Vanadium across the samples

Figure 21 reveals the average concentration of Zinc (Zn) in the wine samples in different series. The concentration of Zn in normal series ranged from 0.284–1.790 mg/L with the lowest concentration in sample 11 and highest in sample 9. The concentration of Zn ranged from 0.365–0.718 mg/L for the explosion series with the lowest concentration in sample 6 and highest in sample 5. The concentration in Nitric acid only series ranged from 0.712–2.100 mg/L with the lowest concentration in sample 15 and highest in sample 9. The highest concentration of Zn was in the Nitric acid only series test which was in sample 22 with a concentration of 1.403 mg/L.

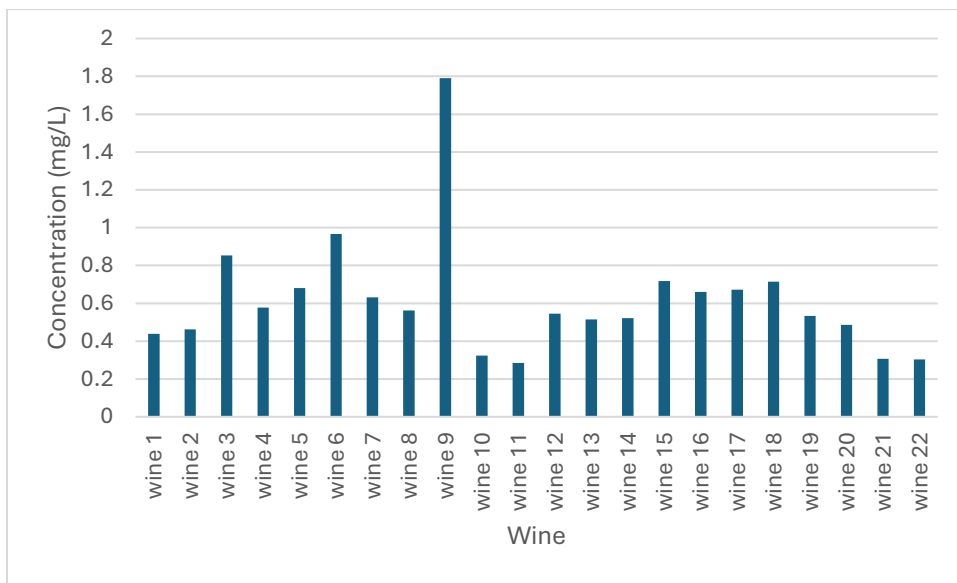


Figure 21: Concentration of Zinc across the samples

4.2. Evaluating the Effects of Wine Type and Region of Origin on Toxic Element Concentrations in Wine Samples

To achieve this objective, Multivariate Analysis of Variance (MANOVA) was used.

Table 3: Multivariate Analysis of Variance (MANOVA) Examining the Effects of Wine Type and Region of Origin across the Toxic Element Concentrations in Wine Samples

Effect	Test Statistic	Value	F Value	p-value
Region	Wilks' Lambda	0.037	16.004	0.000*
Wine Type	Wilks' Lambda	0.464	3.584	0.000*
Interaction (Region and Wine Type)	Wilks' Lambda	0.287	6.651	0.000*

Significance levels: $p < 0.05$ (*)

The findings of the multivariate analysis of variance (MANOVA) revealed statistically significant effects of both wine region and wine type, as well as their interaction, on the concentrations of toxic elements in the wine samples. These results are presented using Wilks' Lambda values as shown in Table 3 above, all of which yielded p-values < 0.05 , indicating strong multivariate differences that are worth exploring.

4.2.1. Influence of Region on Toxic Element Concentration

The significant main effect of region (Wilks' Lambda = 0.037, F = 16.004, p = 0.000) indicates that the concentration levels of toxic elements are different substantially across the wine-producing areas sampled in this study, i.e. Kutná Hora, Mělník, and Mikulov.

4.2.2. Effect of Wine Type on Toxic Element Levels

The wine type, as classified here into red, white, and rose, also had a statistically significant main effect (Wilks' Lambda = 0.464, F = 3.584, p = 0.000) on the combined levels of toxic elements.

4.2.3. Interaction of Region and Wine Type

The most compelling is the significant interaction effect observed between region and wine type (Wilks' Lambda = 0.287, F = 6.651, p = 0.000), suggesting that the influence of wine type on toxic element concentration is not uniform across regions. This interaction implies a complex relationship.

4.2.4 Influence of Wine Type and Region of Origin on individual Toxic Element Concentrations in Wine Samples

Table 4: Multivariate Analysis of Variance (MANOVA) Examining the Effects of Wine Type and Region of Origin on individual Toxic Element Concentrations in Wine Samples

Source	Element	F-Statistic	p-value
Region	As	23.418	0.000
	Pb	11.757	0.000
	Cd	0.253	0.859
	Zn	27.087	0.000
	Cu	16.868	0.000
	Fe	3.080	0.036
Wine Type	As	5.410	0.007
	Pb	3.128	0.052
	Cd	0.467	0.629
	Zn	0.438	0.647
	Cu	8.242	0.001
	Fe	0.480	0.622
Region x Wine Type	As	1.607	0.211
	Pb	0.460	0.634
	Cd	0.207	0.813
	Zn	6.509	0.003
	Cu	25.755	0.000
	Fe	0.381	0.685

The Multivariate ANOVA analysis was conducted to evaluate the impact of region, wine type, and their interaction on the concentrations of toxic elements in wine.

Arsenic concentrations were significantly influenced by region ($F = 23.418$, $p = 0.000$), wine type ($F = 5.410$, $p = 0.007$), but was not influenced by the interaction between the region and wine type ($F = 1.607$, $p = 0.211$). This indicates strong variation in arsenic levels depending on where the wine was produced and the type of wine.

Lead also showed statistically significant variation by region ($F = 11.757$, $p = 0.000$) but it was not statistically significant for wine type ($F = 3.128$, $p = 0.052$) and their interaction ($F = 0.460$, $p = 0.634$). This suggests that only wine region significantly influences lead contamination.

Cadmium concentrations did not differ significantly by region ($F = 0.253$, $p = 0.859$), wine type ($F = 0.467$, $p = 0.629$), or their interaction ($F = 0.207$, $p = 0.813$). This implies a relatively consistent level of cadmium across all wine samples, with no major variations attributable to location or interaction between region and wine type.

With the exception of wine type ($F = 0.438$, $p = 0.647$), zinc levels were strongly impacted by region ($F = 27.087$, $p = 0.000$) and their interaction ($F = 6.509$, $p = 0.003$). The notable findings imply that zinc content is influenced by the type of wine as well as the region of production.

Copper concentrations varied significantly with region ($F = 16.868$, $p = 0.000$), wine type ($F = 8.242$, $p = 0.001$) and their interaction ($F = 25.755$, $p = 0.000$). This indicates strong variation in copper levels depending on where the wine was produced, the type of wine and the combination of both factors.

Iron concentrations were significantly influenced by region ($F = 3.080$, $p = 0.036$) but did not show any statistically significant variation by wine type ($F = 0.480$, $p = 0.622$), or their interaction ($F = 0.381$, $p = 0.685$). This indicates that iron concentrations are relatively stable across different wine types as well as the combination of wine types and regions.

4.2.5 Means of Toxic Elements in Wine Types Across Regions

The OIV limit for Arsenic in wine is 0.2 mg/L. In KH region, Red wine had the lowest mean concentration at 0.058 mg/L, while White and Rosé have arsenic levels at 0.175 mg/L and 0.213 mg/L, respectively. In Mělník, Red wine exhibited the highest arsenic concentration, followed closely by White wine. In Mikulov, the arsenic concentration was relatively high across the wine types. In Prague, however, the concentration in white wine was low. Arsenic concentrations vary significantly by region and wine type. Mělník shows the highest arsenic levels, particularly in Red wine. In KH, the arsenic concentration in rosé wine exceeded the permissible limit, whereas red and white wines were within the limit. In Mělník and Mikulov, arsenic levels exceeded the OIV limit, while in Prague they remained below it.

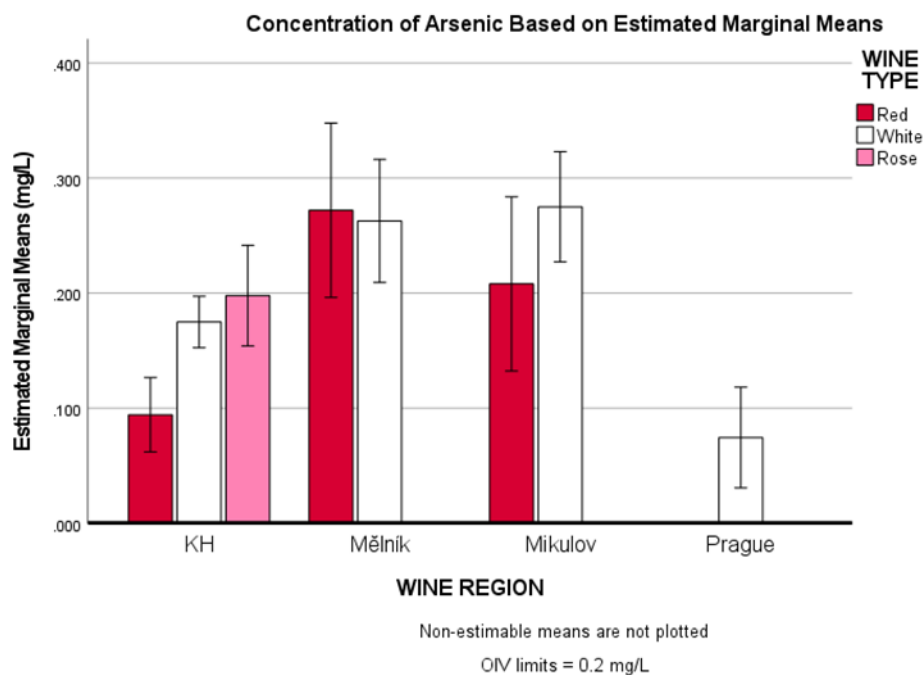


Figure 22: Concentration of Arsenic Based on Estimated Marginal Means in Wine Types Across Regions

The OIV limit for Lead in wine is 0.15 mg/L. In the KH region, Red wine shows a relatively higher concentration of lead compared to White and Rosé wines. In Mělník, lead concentrations are low across all wine types, with Red wine having the highest value, though the differences are minimal. Mikulov shows a similar trend to Mělník, with slightly lower lead levels and very small differences between wine types. In Prague, the concentration of lead was high compared to other regions. Although Lead concentrations revealed significant levels, they were below the OIV limits and do not really pose threats to health.

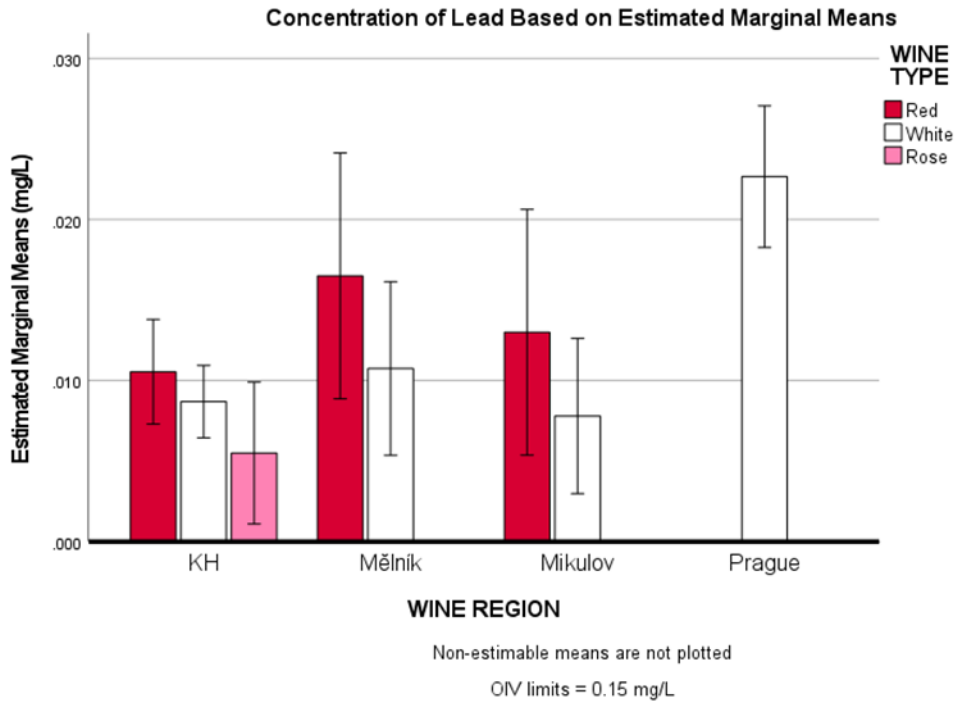


Figure 23: Concentration of Lead Based on Estimated Marginal Means in Wine Types Across Region

The OIV limit for Cadmium in wine is 0.01 mg/L. The concentrations of cadmium were very low (and on multiple occasions under the detection limit of the device), with no significant differences between the regions or wine type, and they do not pose significant health threats.

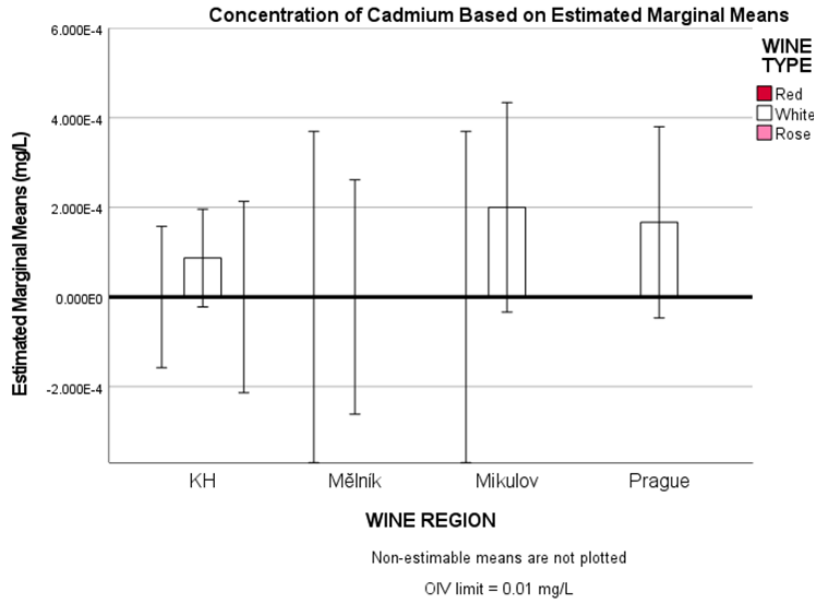


Figure 24: Concentration of Cadmium Based on Estimated Marginal Means in Wine Types Across Regions

The OIV limit for Zinc in wine is 5 mg/L. White wines display relatively consistent zinc levels in KH and Mělník while the highest concentration is in Mikulov and the lowest is in Prague. Red wines demonstrated a slightly increasing concentration of zinc across KH and Melnik, Mikulov had higher values in red wines. Rosé wine was only present in KH and had a low concentration. The concentration of Zinc across all regions and wine types were below the OIV limits and do not pose any significant health threats.

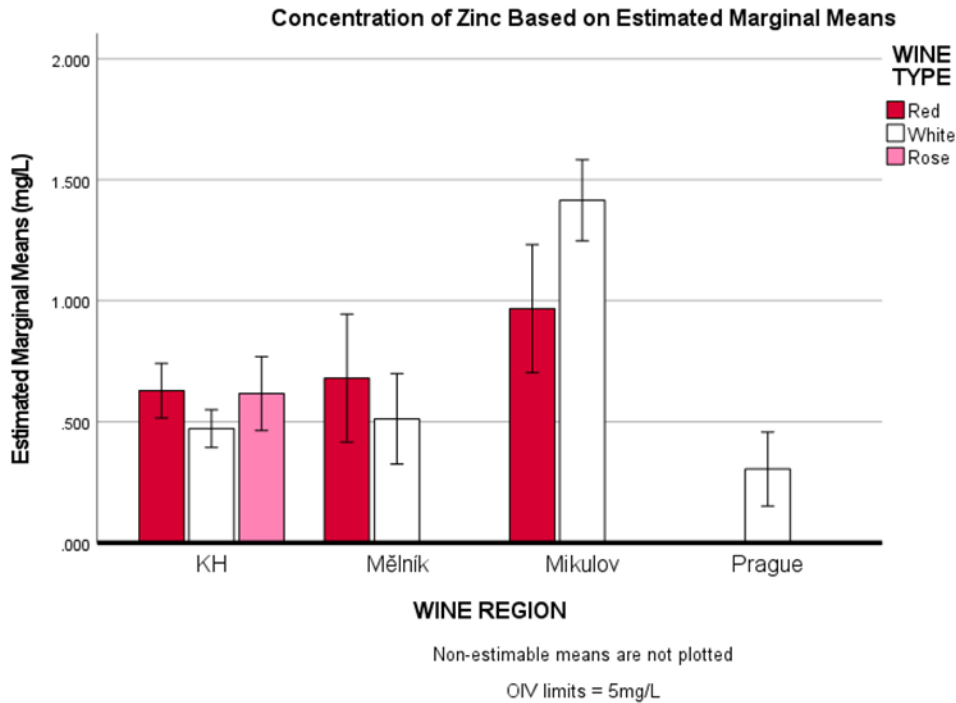


Figure 25: Concentration of Zinc Based on Estimated Marginal Means in Wine Types Across Regions

The OIV limit for copper in wine is 1.0 mg/L. In general, copper levels across all wine types and regions are relatively low. However, Red wine from the Mikulov region exhibits the highest concentration which exceeded the OIV limits of 1.0 mg/L. Red wines from KH and Mělník show similar level of concentrations. The concentration of copper in white wine from Prague is like that of Mělník. This suggests that the elevated copper in Mikulov’s red wine may be linked to local environmental conditions, grape treatment practices, or possible residue from copper-based fungicides used in viticulture. White wines across all regions demonstrate relatively low and stable copper levels.

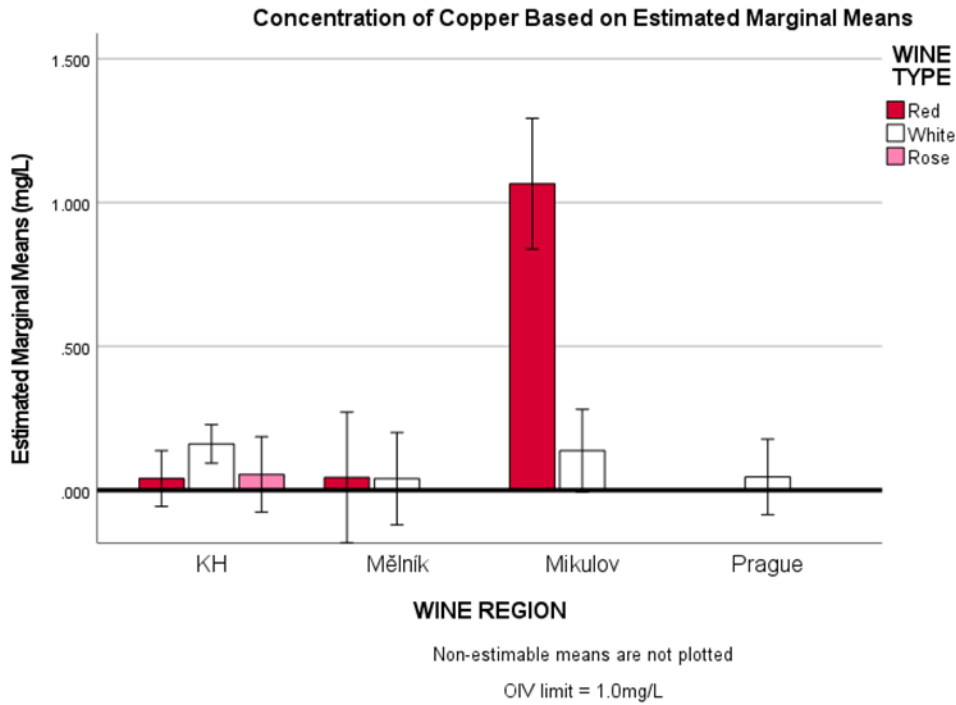


Figure 26: Concentration of Copper Based on Estimated Marginal Means in Wine Types Across Regions

In KH, the concentration of Iron increased across the wine types from white wine, to Red wines and then Rose. In Mělník, the highest concentration was in Red wine which had a slightly higher concentration than White wine. The levels of Iron in Mikulov were on the similar level which represented the highest concentration in white wine and red wine. The concentration of iron in white wine from Prague was on a similar level with that of KH. The concentration of Iron in White wine was lowest in Mělník, highest in Mikulov. The concentration across the regions and wine type were well below the OIV limits of 10 mg/L

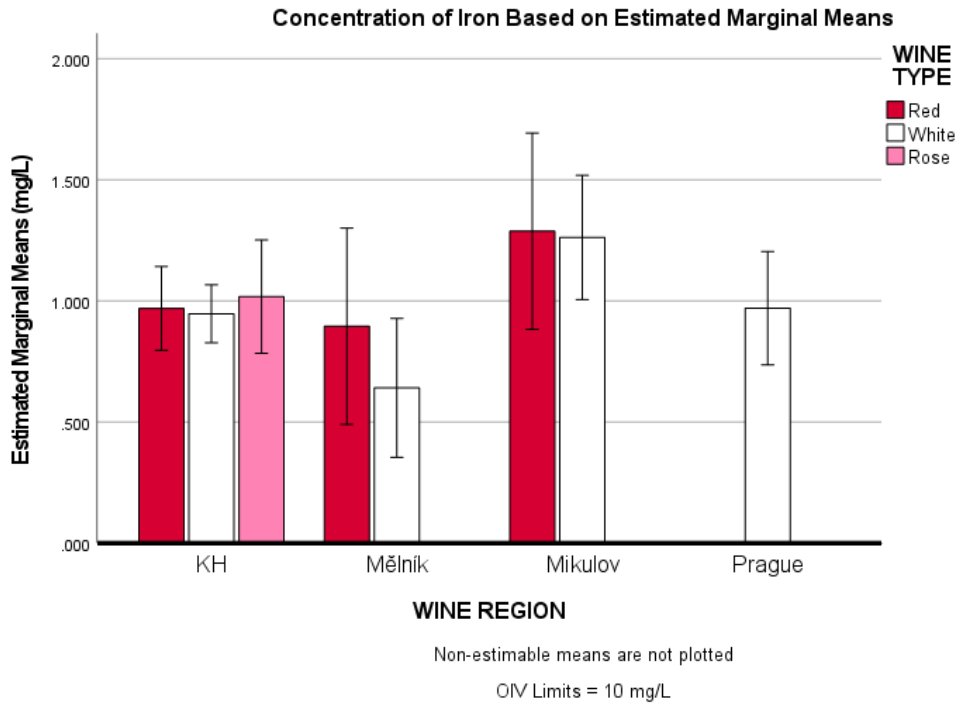


Figure 27: Concentration of Iron Based on Estimated Marginal Means in Wine Types Across Regions

Comparison between the mean of Bulková', (2024) and this study

Table 5: Comparison between the mean of Bulková's study and this study

Element	Grouping	Mean	Mean Diff	P-value
As	Bulkova	0.26212	0.08034	0.000
	This study	0.18177		
Cd	Bulkova	0.00102	0.000960	0.000
	This study	0.00006		
Cu	Bulkova	0.12812	-0.01388	0.755
	This study	0.14200		
Fe	Bulkova	0.91318	-0.06399	0.369
	This study	0.97717		
Pb	Bulkova	0.01375	0.00451	0.169
	This study	0.00925		
Zn	Bulkova	0.56892	-0.07059	0.257
	This study	0.63951		

The independent samples t-test was used to compare the concentrations of toxic elements between wines from Bulkova's study and this study as shown in Table 5

Arsenic

The mean concentration of Arsenic in Bulková's study (0.26212 mg/L) was significantly higher than in the present study (0.18177 mg/L), with a mean difference of 0.08034 mg/L ($p < 0.001$).

Cadmium

Cadmium (Cd) levels were markedly higher in Bulková's findings (0.00102 mg/L) compared to this study (0.00006 mg/L), with a mean difference of 0.00096 mg/L ($p < 0.001$). Although the absolute levels were very small, the concentrations found in Bulková's study were found to be greater

Copper

No significant difference in copper levels was found between Bulková's study ($M = 0.12812$) and this ($M = 0.14200$) study ($p = 0.755$). This suggests similar copper content across both studies.

Iron

Iron content did not differ significantly between Bulková’s study (M = 0.91318) and this (M = 0.97717) study (p = 0.369), indicating broadly comparable levels.

Lead

Lead concentrations were slightly higher in Bulkova (M = 0.01375) than in this study (M = 0.00925), but the difference was not statistically significant (p = 0.169).

Zinc

Zinc levels were higher in this study (M = 0.63951) than in Bulkova (M = 0.56892), but this difference was also not significant (p = 0.257).

The elements whose concentration differed significantly between the studies are Arsenic and Cadmium while differences in copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) were not statistically significant.

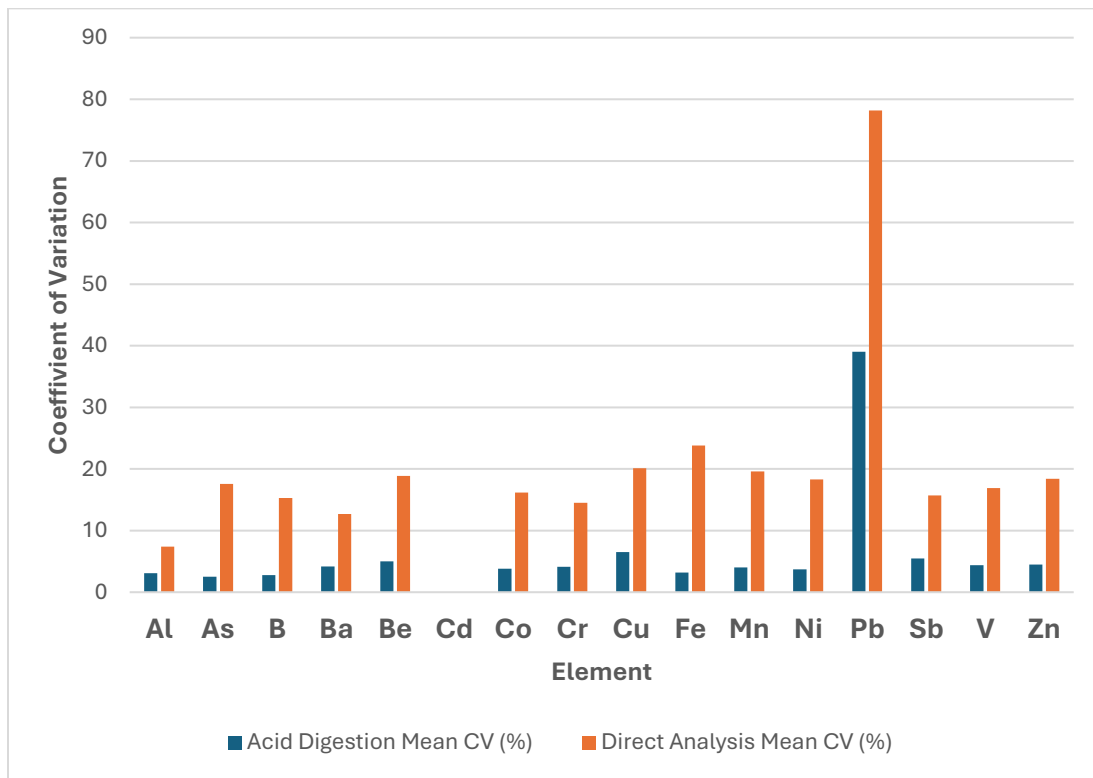


Figure 28: Coefficient of variation between the 2 studies

Comparison between nitric acid and nitric acid + peroxide

A paired samples t-test was used to compare the concentrations of toxic elements in wine when digested with only nitric acid versus a combination of nitric acid and hydrogen peroxide.

Table 6: Concentrations of toxic elements in wine digested with nitric acid and nitric acid + peroxide

Element	Mean (HNO₃)	Mean (HNO₃ + H₂O₂)	Mean Diff.	p-value
Arsenic	0.1481	0.0641	0.0840	0.062
Lead	0.0104	0.0114	-0.0010	0.731
Cadmium	0.0000	0.00025	-0.00025	0.170
Zinc	0.9813	1.1694	-0.1881	0.027
Copper	0.0493	0.0846	-0.0354	0.014
Iron	1.0825	1.3688	-0.2863	0.007

Arsenic: The mean concentration was higher with nitric acid alone (0.14813 mg/L) than with the combination of nitric acid and peroxide (0.06413 mg/L), but this difference was not statistically significant ($p = 0.062$).

Lead: The mean concentration was similar in nitric acid alone (0.0104 mg/L) with the combination of nitric acid and peroxide (0.0114 mg/L). No significant difference was found between the two methods ($p = 0.731$).

Cadmium: The small difference between nitric acid alone (0.0000 mg/L) with the combination of nitric acid and peroxide (0.00025 mg/L). The difference was not significant ($p = 0.170$).

Zinc: The mean concentration was significantly lower when nitric acid alone (0.9813) than using nitric acid and peroxide (1.1694). The difference between the means was significant ($p = 0.027$).

Copper: The mean concentrations were also significantly higher with the peroxide method (0.0846) than nitric acid alone (0.0493). The difference in the mean was significant ($p = 0.014$).

Iron: The mean concentrations were also significantly higher with the peroxide method (1.3688) than nitric acid alone (1.0825). A significant difference in the means was observed ($p = 0.007$).

Table 7a: Average concentration (mg/L) of several Elements in wine samples (normal series)

	Al	As	B	Ba	Be	Cd	Co	Cr	Cu
1	0.939±0.030	0.226±0.006	2.496±0.083	0.095±0.006	0.003±0.000	<LOD	0.005±0.000	0.006±0.006	0.022±0.003
2	0.721±0.003	0.268±0.013	3.765±0.065	0.076±0.012	0.004±0.000	<LOD	0.005±0.000	0.002±0.000	0.020±0.008
3	1.219±0.003	0.262±0.002	4.182±0.029	0.135±0.007	0.001±0.000	<LOD	0.005±0.000	0.010±0.012	0.268±0.001
4	0.254±0.009	0.257±0.004	2.949±0.005	0.127±0.008	0.000±0.000	<LOD	0.005±0.000	0.042±0.000	0.036±0.004
5	0.115±0.057	0.272±0.007	5.693±0.021	0.161±0.014	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	0.044±0.010
6	0.351±0.009	0.208±0.002	5.500±0.020	0.192±0.015	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	1.065±0.016
7	0.823±0.021	0.249±0.000	2.425±0.022	0.174±0.095	0.003±0.000	<LOD	0.008±0.005	0.013±0.016	0.056±0.012
8	1.100±0.006	0.257±0.010	3.697±0.029	0.211±0.026	0.005±0.001	<LOD	0.005±0.000	0.026±0.034	0.059±0.011
9	0.567±0.023	0.284±0.010	4.494±0.007	0.229±0.054	0.001±0.000	<LOD	0.002±0.004	0.014±0.011	0.051±0.010
10	0.903±0.018	0.179±0.004	1.917±0.038	0.188±0.020	0.003±0.000	<LOD	0.005±0.000	0.021±0.016	0.047±0.009
11	1.400±0.007	0.121±0.004	2.130±0.013	0.171±0.020	0.002±0.000	<LOD	0.005±0.000	0.002±0.000	0.125±0.002
12	0.754±0.009	0.161±0.015	3.155±0.037	0.152±0.016	0.002±0.000	<LOD	0.005±0.000	0.002±0.000	0.099±0.014
13	0.877±0.052	0.197±0.017	2.824±0.007	0.154±0.016	0.002±0.000	<LOD	0.005±0.000	0.002±0.000	0.059±0.010
14	0.761±0.025	0.201±0.006	2.531±0.019	0.138±0.035	0.003±0.000	<LOD	0.005±0.000	0.006±0.007	0.066±0.009
15	0.521±0.018	0.198±0.006	2.537±0.023	0.109±0.025	0.002±0.000	<LOD	0.005±0.000	0.007±0.009	0.049±0.021
16	0.839±0.006	0.229±0.011	2.273±0.004	0.108±0.010	0.003±0.000	<LOD	0.005±0.000	0.009±0.013	0.051±0.017

17	0.388±0.023	0.067±0.001	4.572±0.028	0.197±0.032	0.000±0.001	<LOD	0.005±0.000	0.012±0.018	0.054±0.004
18	0.253±0.011	0.052±0.010	2.927±0.105	0.226±0.019	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	0.035±0.007
19	0.075±0.000	0.055±0.012	2.772±0.012	0.189±0.052	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	0.035±0.006
20	0.075±0.000	0.072±0.035	2.610±0.022	0.141±0.023	0.000±0.000	<LOD	0.005±0.000	0.010±0.014	0.756±0.003
21	1.709±0.026	0.058±0.003	3.971±0.035	0.258±0.020	0.006±0.000	<LOD	0.005±0.000	0.002±0.000	0.053±0.017
22	1.149±0.010	0.091±0.047	3.280±0.014	0.354±0.229	0.004±0.000	<LOD	0.005±0.000	0.011±0.009	0.038±0.010

<LOD- Below detection limit yellow – Maximum concentration, Green – minimum concentration

Table 7b: Average concentration (mg/L) of several Elements in wine samples (normal series)

S/N	Fe	Mn	Ni	Pb	Sb	V	Zn
1	0.936±0.031	0.436±0.010	0.033±0.001	0.015±0.002	0.003±0.000	0.053±0.001	0.439±0.020
2	0.497±0.005	0.419±0.004	0.046±0.001	0.006±0.004	0.007±0.006	0.005±0.000	0.462±0.026
3	1.036±0.003	1.054±0.004	0.049±0.004	0.004±0.000	0.005±0.003	0.036±0.000	0.854±0.000
4	1.136±0.022	0.691±0.008	0.036±0.004	0.007±0.005	0.003±0.000	0.366±0.000	0.577±0.016
5	0.895±0.083	0.600±0.002	0.074±0.035	0.016±0.007	0.003±0.000	0.003±0.002	0.680±0.111
6	1.288±0.087	1.003±0.016	0.055±0.001	0.013±0.004	0.003±0.000	0.046±0.000	0.967±0.008
7	0.980±0.075	0.690±0.007	0.047±0.007	0.014±0.003	0.006±0.005	0.159±0.000	0.631±0.054
8	0.785±0.147	0.812±0.046	0.055±0.008	0.015±0.004	0.006±0.004	0.028±0.000	0.563±0.015
9	1.413±0.043	1.282±0.006	0.04±0.002	0.010±0.011	0.004±0.002	0.020±0.001	1.790±0.027

10	0.954±0.069	0.517±0.006	0.041±0.009	0.010±0.006	0.003±0.000	0.202±0.001	0.324±0.004
11	0.983±0.022	0.488±0.005	0.228±0.170	0.004±0.000	0.003±0.000	0.110±0.001	0.284±0.044
12	1.062±0.233	0.420±0.006	0.057±0.007	0.007±0.003	0.003±0.000	0.099±0.001	0.545±0.107
13	1.094±0.241	0.438±0.019	0.033±0.002	0.004±0.000	0.003±0.000	0.212±0.006	0.515±0.081
14	0.752±0.054	0.525±0.007	0.031±0.002	0.007±0.003	0.003±0.000	0.086±0.000	0.521±0.150
15	0.941±0.079	0.552±0.005	0.076±0.044	0.007±0.003	0.004±0.002	0.141±0.003	0.718±0.140
16	1.522±0.844	0.627±0.016	0.263±0.203	0.011±0.003	0.003±0.000	0.184±0.002	0.660±0.035
17	1.035±0.088	0.962±0.017	0.046±0.006	0.012±0.005	0.007±0.004	0.149±0.003	0.673±0.066
18	0.926±0.079	1.126±0.035	0.036±0.006	0.016±0.008	0.003±0.000	0.092±0.003	0.714±0.059
19	0.834±0.086	0.639±0.002	0.036±0.018	0.006±0.004	0.006±0.003	0.019±0.001	0.534±0.047
20	0.453±0.078	0.593±0.004	0.090±0.097	0.004±0.000	0.005±0.003	0.002±0.001	0.486±0.084
21	1.029±0.085	1.303±0.006	0.061±0.001	0.027±0.002	0.004±0.002	0.011±0.001	0.306±0.048
22	0.911±0.074	1.403±0.017	0.056±0.006	0.019±0.004	0.005±0.003	0.007±0.001	0.303±0.021

<LOD- Below detection limit yellow – Maximum concentration, Green – minimum concentration

Table 8a: Average concentration (mg/L) of several Elements in wine samples (Explosion and Nitric acid only series)

S/N	Al	As	B	Ba	Be	Cd	Co	Cr	Cu
Explosion series									
1	1.107±0.019	0.199±0.009	2.448±0.019	0.153±0.030	0.002±0.000	<LOD	0.005±0.000	0.002±0.000	0.071±0.028
2	0.882±0.009	0.224±0.009	3.654±0.050	0.140±0.005	0.004±0.000	<LOD	0.005±0.000	0.002±0.000	0.043±0.009
3	0.962±0.768	0.027±0.020	2.644±2.268	0.108±0.090	0.001±0.000	<LOD	0.003±0.003	0.002±0.000	0.196±0.166
4	0.313±0.005	0.216±0.010	2.746±0.046	0.175±0.004	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	0.059±0.013
5	0.236±0.030	0.251±0.002	5.288±0.077	0.179±0.015	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	0.060±0.004
6	0.175±0.173	0.094±0.154	1.673±2.855	0.069±0.113	0.000±0.000	<LOD	0.005±0.000	0.002±0.000	0.384±0.657
7	0.925±0.009	0.246±0.009	2.240±0.029	0.137±0.015	0.003±0.000	<LOD	0.005±0.000	0.002±0.000	0.060±0.006
8	1.242±0.018	0.235±0.005	3.317±0.054	0.141±0.001	0.00±0.000	<LOD	0.005±0.000	0.002±0.000	0.050±0.019
Nitric acid only series									
9	0.831±0.009	0.066±0.009	4.855±0.048	0.185±0.048	0.001±0.003	0.001±0.00	0.001±0.006	0.002±0.000	0.080±0.009
15	0.693±0.009	0.050±0.009	2.412±0.001	0.120±0.001	0.001±0.017	<LOD	-0.00±0.008	0.002±0.000	0.067±0.008
17	0.515±0.002	0.073±0.002	4.311±0.031	0.161±0.031	0.000±0.006	<LOD	0.005±0.000	0.002±0.000	0.125±0.048
18	0.333±0.003	0.066±0.003	2.832±0.021	0.247±0.021	0.000±0.101	<LOD	0.004±0.002	0.002±0.000	0.068±0.005

<LOD- Below detection limit Yellow – Maximum concentration, Green – minimum concentration

Table 8b: Average concentration (mg/L) of several Elements in wine samples (Explosion and Nitric acid only series)

S/N	Fe	Mn	Ni	Pb	Sb	V	Zn
Explosion series							
1	1.215±0.204	0.429±0.013	0.032±0.003	0.006±0.003	0.003±0.000	0.055±0.001	0.580±0.058
2	0.670±0.043	0.408±0.004	0.041±0.002	0.007±0.007	0.003±0.000	0.003±0.002	0.548±0.053
3	0.880±0.750	0.653±0.565	0.038±0.037	0.008±0.007	0.003±0.000	0.024±0.020	0.622±0.538
4	1.287±0.051	0.633±0.005	0.176±0.182	0.010±0.007	0.003±0.000	0.401±0.005	0.694±0.018
5	0.864±0.056	0.545±0.003	0.037±0.000	0.006±0.005	0.003±0.000	0.002±0.001	0.718±0.017
6	0.463±0.776	0.285±0.494	0.016±0.024	0.007±0.005	0.003±0.000	0.016±0.025	0.365±0.631
7	1.081±0.030	0.625±0.006	0.029±0.003	0.006±0.003	0.003±0.000	0.174±0.002	0.686±0.011
8	0.802±0.091	0.689±0.007	0.041±0.001	0.007±0.006	0.003±0.000	0.029±0.001	0.597±0.012
Nitric Only series							
9	2.008±0.017	1.251±0.010	0.061±0.028	0.010±0.001	0.00±0.003	0.021±0.000	2.100±0.002
15	1.219±0.139	0.471±0.011	0.041±0.005	0.006±0.004	0.003±0.000	0.148±0.003	0.712±0.018
17	1.128±0.075	0.829±0.011	0.034±0.008	0.015±0.005	0.003±0.000	0.165±0.003	0.918±0.217
18	1.120±0.079	0.969±0.003	0.041±0.002	0.015±0.002	0.008±0.007	0.104±0.006	0.948±0.122

<LOD- Below detection limit Yellow – Maximum concentration, Green – minimum concentration

5.0 DISCUSSION

5.1 Concentration of Toxic Elements

5.1.1 Measured Al values in wine

The concentration of Al in this study ranged from 0.075 to 1.709 mg/L. The concentration of Al reported by Bulková, (2024) ranged from 0.261 to 2.161 mg/l and the highest concentration was higher than that of this study but were within similar range.

The permissible limit of Al in Croatia is 10 mg/L while it is 8 mg/L in Germany (Aceto et al., 2002, NN/2, 2005, Tariba, 2011). This highest concentration of Al in this study is below the Croatia and Germany limit. This indicates that the presence of Al in this study will have no consequences. Bentonite use for wine fining and to a lesser extent contact with aluminum surfaces increase the concentrations of Al in wine (Stafilov et al., 2009; Tariba, 2011). The concentration of Al reported in the study carried out by Fiket et al. in 2011 was similar range of our study.

5.1.2 Measured As values in wine

The measured arsenic values ranged from 0.027 to 0.831 mg/L with the lowest in sample 3 and the sample 9 was the highest. The average arsenic concentration in this study is 0.16 mg/L. The findings of this study were statistically significantly lower than those reported by Bulková, (2024) that reported a range of 0.217 to 0.317 mg/L and average of 0.262 mg/L. were statistically significantly lower than those

Flamini & Panighel, (2006) reported that the maximum limit of As in wines was set at 0.2 mg/L by the International Organization of Vine and Wine (OIV) with the presence of few $\mu\text{g/L}$ of As widely acceptable in uncontaminated wines. This study has a significant amount of wine that exceed the limit set by OIV and may pose threat on the health of humans. Wilson, (2015) reported that the mean acceptable concentration of As in wine by the US EPA was 10 $\mu\text{g/L}$. Wilson et al., (2015), examined arsenic content in American wine. Representative wines (65) from four top states that produces wine were examined. Arsenic that exceeded the US EPA drinking water limit of 10 $\mu\text{g/L}$ was present in all samples.

The presence of As and other toxic elements in food and beverages including juices, wine, and beer have been assessed by numerous studies (Tvermoes et al., 2014, USFDA 2013, Lynch et al., 2014). Several factors such as differing environment contamination, oenological practice

and growing practices have been demonstrated to result in concentration of As content in wine and also in the differences of As content in various types of wine (Bertoldi et al. 2013, Hopfer et al. 2015).

In a study carried out by Paustenbach et al., (2016), it was reported that the overall mean As concentrations for red, white, and blush wines were 27.2 µg/L, 10.9 µg/L and 6.75 µg/L, respectively. Other reports with higher As concentration in wine have been reported by Tanabe et al. (2016) where the total As content of white, rosé, red, Port and sparkling wines was <LOQ (0.38 µg/L - 43.8 µg/L). Wilson et al. (2015) also reported that 65 wines were analysed and the average As concentration was 23.3 µg/L, with a range from 10 to 76 µg/L.

Different studies have identified that wine type has influence on the concentration of As in wines with some reporting that white and blush wines typically contain a higher total As concentration than red wines, irrespective of the origin (Fiket et al. 2011, Rodrigues et al. 2011, Paustenbach et al., 2016). Several other countries and the OIV have imposed limits of arsenic in wine ranging from 100- 200 µg/L, i.e. 10-20 times the level limit for drinking water at 10 µg/L of public water supplies (Fontcuberta et al., 2011 Huang et al., 2015).

Initially, we assumed that arsenic contamination in the study area originated primarily from historical mining and smelting activities in Kutná Hora. However, subsequent literature review revealed additional contemporary sources of arsenic and nickel emissions within the Czech Republic. According to the Czech Hydrometeorological Institute (CHMI, 2015), significant emissions of these elements are associated with coal-burning thermal power plants and heating plants, particularly in the Ústí nad Labem, Pardubice, Plzeň, and Central Bohemia regions. Facilities such as the Chvaletice and Opatovice power plants, the Teplárny ELÚ III heating plant, and the Mělník I power plant contribute substantially to atmospheric arsenic and nickel loads. These emissions occur because coal naturally contains trace amounts of metals and metalloids; during combustion, these impurities are released into the atmosphere bound to fine particulate matter, some of which escape even with modern filtration systems. This finding indicates that current industrial activities may contribute to the regional contamination pattern, and that arsenic and nickel pollution is not restricted to historical sources in Kutná Hora. Ten (10) wine samples were over the limit, 7 were white wines and 3 reds, 4 from KH, 3 from Melnik and 3 from Mikulov. Also one Rose wine from KH was close to the limit. Kutna and Melnik has a known cause but we are still not sure what may cause it in Mikulov area

Measured B values in wine

The concentration of Boron in wine samples in this study ranged from 0.050–5.288 mg/L. The concentration of B reported by Bulková, 2024 ranged from 2.550 to 6.665 mg/l.

In a study carried out by Almeida et al., (2017) on Boron Isotopic Ratio in Brazilian Red Wines, they reported that broad boron concentration range (< 0.07 to 20.4 mg/L) was observed, including a few values above the maximum allowed concentration according to the OIV (14.0 mg/L), which they indicated might be considered a Potential Tool for origin and quality studies. Other studies reported higher concentrations like Ozbek and Akman (2005) in Turkish red wines (7.94 to 10.7 mg/L) but the concentration of B in white wine (4.12 to 5.44 mg/L) was similar to that of this study.

According to Jakkielska et al., (2023), B concentration ranged from 1.5 to 9.0 mg/L which is higher than the result of this study but it is still below the OIV maximum allowed concentration.

It was reported that it is one of the essential plant nutrients such as potassium based on the highest content of B that they found in their study. Boron contribution can be accounted for in terms of the types of fertilisation practices or plant uptake (Gajek, et al., 2021).

Measured Ba values in wine

The concentration of Barium in this study ranged from 0.076–0.229 mg/L. The concentration of Barium reported by Bulková, (2024) ranged from 34 to 152 µg/l which is lower than the concentration discovered in this study.

Barium is one of the metals that are considered to be toxic to the health of humans and the environment (Zhuang, 2019; Bozorg-Haddad et al., 2021). The concentration of Ba reported in Jakkielska et al., (2023) ranged from 0.187 mg/L to 3.816 mg/L which is higher than the concentration of this study. There is no limit to compare the findings of this study with because OIV has no limit for Ba in wine same as Croatian regulative for quality control of food. The concentration of Ba reported in the Fiket et al., (2011) study was lower than that of this study as they stated that Ba concentration was present in analysed wines in very narrow range.

Measured Be values in wine

The concentration of Beryllium was nearly undetectable for majority of the samples. A few of the samples ranged from 0.120–0.247 mg/L derived from the Nitric only series with the highest concentration in sample 18 at 0.247 mg/L. Bulková (2024) reported that the measured Be values ranged from 34.0 to 152 µg/l which is similar to the range reported in this study. They also reported that Beryllium was not detected in their measurements and all values were below the detection level limit which were replaced by ½ of the detection limit – 34.0 µg/l.

Jakkielska et al., (2023) reported a mean Be concentration in wine ranging from 1 µg/L to 10 µg/L. This contradicts the finding of this study and the concentration is lower than that of this study and Bulková's (2024).

Measured Cd values in wine

The concentration of Cadmium for majority of the samples in this study were below the detection limit with the highest concentration in sample 9 at 0.001 mg/l. Bulková (2024) reported that cadmium was detected in only one sample and all other measured values were below the detection limit and were therefore replaced by ½ the detection limit - 0.1 µg/L. The limit set by the OIV is 0.01 mg/L (OIV, 2011). while that of other countries include Croatia and Germany is 0.01mg/l (NN/2, 2005 and Aceto et al., 2002) with Brazil having a limit of 0.2 mg/l (Tariba, 2011). The result of this study is below several limits, indicating that the presence of Cd does not pose any threats to human health upon consumption.

Several studies have investigated the concentration of Cd in wine. Mean concentrations of Cd in the white and red wines from continental and coastal Croatia are reported to be 0.7 µg/L as the Cd concentrations are similar to Croatian and Polish wines (Leder et al., 2021). In comparison the result was lower than that of this study and Bulková, (2024). Deng et al. (2019) reported that the element with the lowest average concentration was Cd (0.568 µg/L) ranging from 0.076–2.36 µg/L while Fiket et al. (2011) reported that the concentration Cd in wines from eastern Croatia was 0.175–1.88 µg/L.

Measured Co values in wine

The Cobalt concentrations ranged between 0.000–0.008 mg/L with the highest concentration was 0.008 mg/l in sample 7. Bulková (2024) reported that Cobalt was present in only 7 wines

out of 20 with the other thirteen (13) below detection limit. This is similar to the findings of this study.

Several studies have investigated the concentration of Cobalt in wines. Jakkielska et al., (2023) reported a mean concentration of Co in wine ranging from 1 µg/L to 10 µg/L which is similar to the finding of this study having similar range of Co concentration. Deng et al. (2019) analyzed trace element content in 315 Chinese wines from eight (8) different regions and reported that the concentration of Co ranged from 1.15 to 15.3 µg/L.

Leder et al. (2021) performed a multielement composition analysis of white and red wines from continental and coastal Croatia, and Alkis et al., (2014) investigated the concentrations of Co (average 3.37 µg/L) in Turkish wines. The mean concentrations of Co (5.9 µg/l) were higher in Croatian wines than in Polish wines. Geana et al. (2013) determined the geographical origins of Mn, Co, Cu and Zn in Romanian wines and the average Co concentration was 4.35 µg/L.

Measured Cr values in wine

Chromium concentrations were consistently low in this study with values of 0.002 mg/L in most of the samples while the highest concentration was in sample 4 with 0.042 mg/L. Bulková (2024) reported that chromium values ranged from 15.4 to 46.5 µg/l which is similar to the findings of this study.

The limit set by the Croatian regulative for quality control of food is 0.01 mg/L (NN/2, 2005) with no limit set by OIV for Cr. Few samples from this study exceeded the Croatian Cr permissible limit. The mean concentration of Cr reported by Leder et al. (2021) that conducted a multielement composition analysis of white and red wines from continental and coastal Croatia was 19 µg/L which were higher in Croatian wines than in Polish wines while Fiket et al. (2011) reported that the concentration of Cr in wines from eastern Croatia ranged were 6.50–31.1 µg/L.

According to Sperkova and Suchanek (2005), the Cr concentrations in Bohemian wines ranged from 18.0 to 32.0 µg/L. Marengo and Aceto (2003) studied the concentration of Cr in Nebbiolo-based wines, varying from 20.0 to 50.0 µg L⁻¹. Additionally, Cr, Pb and Al also render wine unstable, and their presence can cause astringency, turbidity and browning that adversely affect the quality of the wine, and the organoleptic properties of the wine (Cepo et al. 2022). The

presence of these impurities is related to the use of chromium oxides added to wine after bottling and the fermentation method.

The increase of Cr content with increasing age of wine was found for different vintage wines from the same vineyard and winery (Cabrera-Vique et al., 1997, Tariba, 2011). These features might be due to contamination when in storage by stainless steel or after bottling by chromium oxide used in pigmentation of glass bottle during the ageing as opposed to from natural sources (Volpe et al 2009).

Measured Cu values in wine

For Cu, the highest concentration was 1.065 mg/l while Bulková (2024) reported that the highest copper value was up to 1034 µg/l which was higher than the concentration of a majority of the samples and in the same range with the highest concentration of Cu in this study. European Union (EU) has sets limit regarding the copper concentration in the wine, which is less than 0.5 mg/L (Durguti et al., 2020), while according to OIV it should be less than 1.0 mg/l. Only two samples from this study exceeded the limit of copper in wine according to the EU, and only one sample exceeded the OIV limit.

This study revealed that the concentration of Cu found in some wine samples were exceeded the maximum acceptable limit set by the OIV for copper in wine which is 1 mg/l. Wine sample no. 6 showed similar copper concentrations to those reported in Bulková's thesis, indicating that the values are relatively consistent and attributable to the sample itself rather than the analytical equipment, methods, or other errors. The consequence of copper as either a citrate or a sulfate used in removing sulfidic odors is the increased concentration of copper in wine (Clark et al., 2015). Durguti et al., (2020) reported in their study a higher concentration of Cu at 5.668 mg/l which exceeds the OIV limit while Fiket et al., 2011 reported values lower than the OIV limit.

In the study carried out by Deng et al. (2019) on trace element content in 315 Chinese wines from eight (8) different regions, they reported that the concentration of Cu ranged from 13.7 to 543 µg/L with the highest concentration below the OIV limit on Cu in wine (1000 µg/L).

Measured Fe values in wine

The highest concentration of Iron (Fe) in wines in this study is 2.008 mg/L. The limit set by OIV < 10 mg/L, or according to the EU for Fe in the wine is < 30 mg/L (Durguti et al., 2020,

OIV, 2011). Bulková (2024) reported concentrations of Fe similar to that of this study with values ranging from 148 to 1416 µg/l.

In the study carried out by Fiket et al., (2011) on Arsenic and other trace elements in wines of eastern Croatia, the concentration of Fe reported were a little higher than the result of this study. The result of the study was within the Croatia limit of Fe in wine which is 10,000 µg/L (10 mg/L) (NN/2, 2005). In the study carried out by Durguti et al., (2020) on the Determination of Iron, Copper and Zinc in the Wine by FAAS reported a concentration of Fe similar to the findings of this study which was below the OIV limit.

Nevertheless, based on the results, it can be deduced that none of the analyzed wine samples is a risk for future significant instability after it has been bottled and undergoing aging. These wines do not create a risk to human health either.

Measured Mn values in wine

The concentration of Mn in this study ranged from 0.285–1.403 mg/L with the highest concentration of Mn was in sample 22 with a concentration of 1.403 mg/L. Bulková (2024) reported that the measured manganese values ranged from 325 to 1138 µg/l (0.325 to 1.138 mg/l). In comparison, the findings of Bulková was within the same range with that of this study. The OIV does not have a limit for Mn, so there isn't any limit to compare this study with.

According to Obhodaš et al. (2021), mean concentrations of Mn (0.985 mg/L) in Croatian and Austrian red and white red wines are lower than those from Poland. In white and red wines from coastal and continental Croatia, Leder et al. (2021) investigate the multielement composition. The study found that wines from Croatia had lower mean concentrations of Mn (0.96 mg/L) than Polish wines. Also, Gremaud et al. (2004) quantified elements like Al, Zn and Mn ranged from 270 to 600 µg/L, while Geana et al. (2013) analyzed the geographical origins of Zn, Cu, Co and Mn in Romanian wines and had average Mn concentrations of 806 µg/L. In comparison, the findings of Gremaud et al. (2004), Geana et al. (2013) Obhodaš et al. (2021) and Leder et al. (2021) are within the same range as this study.

Measured Ni values in wine

The concentration of Nickel in this study ranged from 0.016–0.263 mg/L with the highest concentration of Ni was in sample 16 with a concentration of 0.263 mg/L. Bulková, (2024)

reported that the measured nickel values ranged from 17.7 to 40.8 $\mu\text{g/l}$. (0.0177 to 0.0408 mg/l). A few samples from this study were higher than majority of the samples from Bulková, (2024) study.

The limit set by the Croatian regulative for quality control of food is 0.1 mg/L (NN/2, 2005) with no limit set by OIV for Ni. A few samples from this study exceeds the Croatian regulative control of food. Only samples 11, 16 and 20 (all 3 originating from Kutna Hora) have had values in at least one of the replicates that exceeded the mentioned limit. However, the concentrations between the replicates themselves were not very stable, and probably caused by some instrumentation malfunction), having in mind the high differences between Bulková's results and these ones. Nickel was not initially included in the statistical processing because like for many of the other elements, we didn't have a clear limit set by OIV for this element.

The presence of Ni in wines is due to use of Ni containing stainless steel containers in modern cellar technology for wine fermentation and storage (Tariba, 2011). The contamination has been reported to have likely come from stainless steel storage tanks and Ni containing pigments in bottles due to report of Ni concentration increases during storage (Teissedre et al. 1998). As reported by Galani-Nikolakaki et al. (2002), the (determined) Ni concentrations for the analyzed Greek wines were independent of the type of the container used and did not vary with the storage time of the wine in the container.

Several studies have been conducted regarding the Ni concentration in wines. The concentration of Ni in Bohemian wines ranged from 15.0 – 53 $\mu\text{g/L}$ while Fiket et al. (2011) reported that the concentration ranges of Ni in wines from eastern Croatia is from 15.3 – 50.0 $\mu\text{g/L}$ (Sperkova and Suchanek 2005). In a study by Deng et al. (2019) on trace element content in 315 Chinese wines from 8 different regions shows that Ni concentration varied from 9.67–189 $\mu\text{g/l}$. The concentration of this study was in line with these studies.

Measured Pb values in wine

The concentration of Pb in this study ranged from 0.004 to 0.027mg/L with the highest concentration of lead in this study in sample 21 which is lower than some of the samples in Bulková's (2024). The awareness of the Pb concentration in wine is crucial due to its toxic impact on consumer's health which led to the setting of the maximum acceptable limit to 0.15 mg/l (OIV, 2011). The concentration of Pb in this study was below the OIV permissible limit indicating the consumers were safe.

Galani-Nikolakaki et al. (2002) and Tariba, (2011) reported that Lead contamination in wines might be due to contamination in the wineries where the wine was produced or pollution from the environment (car exhausts). The Pb concentration in a few homemade Croatian wines was higher than the allowable limit reported by Tariba et al., (2011) whereas, the Pb concentration in commercial wines in Croatia is much below that limit. Higher Pb concentrations reported in Tariba (2011) could be induced by the pollution from the container, infraction in technological norms, smaller dilution, or mixture of different kinds of grapes.

Measured Sb values in wine

The concentration for Antimony (Sb) in this study ranged from below detection limit (<LOD) to 0.008 mg/L. Bulková, (2024) reported that all measured antimony values were below the detection limit and were replaced by $\frac{1}{2}$ of the detection limit – 41.0 $\mu\text{g/l}$ which is similar to the result of this study. The limit set by the Croatian regulative for quality control of food is 0.2 mg/L (NN/2, 2005) with no limit set by OIV for Sb.

In the study carried out by Gajek et al., (2021) on Multi-Elemental Analysis of Wine Samples in Relation to Their Type, Origin, and Grape Variety it was reported that the range of Sb in the study was 0.006 to 69.95 $\mu\text{g/l}$ with the mean value reached 1.469 $\mu\text{g/L}$. They obtained values comparable to study of Bentlin et al. (2011) (mean value of 0.249 $\mu\text{g/L}$) and Płotka-Wasyłka et al. (2018) (mean value for red wines (0.4 $\mu\text{g/L}$) and white wines (2.3 $\mu\text{g/L}$)). In comparison, the findings of the studies align with the result of this study.

Jakkielska et al., (2023) reported that the mean concentration in their study was below 1 $\mu\text{g/L}$. Antimony is one of the metals that have high toxicity alongside, Cd, Cr, Hg, Ni and Pb (Zhuang, 2019; Bozorg-Haddad et al., 2021). Antimony levels below detection limits were most often observed in studies on wine samples, while the use of other methods in some European wines show Sb levels close to 10 $\mu\text{g}\cdot\text{L}^{-1}$ (Periferakis et al., 2022).

Measured V values in wine

Vanadium (V) mean concentrations from this study ranged from 0.002–0.401 mg/L with highest in seen in sample 4. Bulková, (2024) reported that the concentration of vanadium in their study ranged from 3.00 $\mu\text{g/}$ to 356 $\mu\text{g/l}$. the two study have similar results when compared to each other.

Jakkielska et al., (2023) in their study on reported Vanadium is an essential mineral and it has the mean concentration of V in their study ranged from 10 µg/L to 100 µg/L. Teissèdre et al., (1998) in their study on Vanadium levels in wines from California and France reported that the V levels ranged from 6.6 to 43.9 mg/l in white and from 7.0 to 90.0 mg/l in red wines.

The grape variety and environmental factors (soil or climate) in vineyards will determine final levels of vanadium in wine (Teissèdre et al., 1998). The wine's V content may also relate to the nature of the metal oxides used in the bottle pigmentation, which affects their shelf life (Teissèdre et al., 1998). Mean concentrations of V (83.6 µg/L) were higher in Croatian wines, compared to Polish wines according to the report by Leder et al. (2021) who analyzed the multielement composition of red and white wines from coastal and continental Croatia.

Measured Zn values in wine

The concentration of Zn in this study ranged from 0.284 to 2.100 mg/L with the highest concentration of Zn in this study in sample 9 at 2.100 mg/L. The result of this study was higher than the range reported by Bulková, (2024). The highest concentration reported was 1635 µg/l which was in similar range to that of this study. According to OIV, the values of zinc concentration in the wine should be less than 5 mg /L while the European Union reported that it should be lower than 30 mg /L. This study therefore resulted in a Zn concentration in wines below the OIV and EU permissible limit for Zn.

The concentrations of the analyzed samples of Zn do not threaten the instability of wine, nor the threat of human health as every concentration of zinc studied (Durguti et al., 2020) which aligns with the study of NN/2, 2005, a Croatia limit for Zn in wine was set at 5 mg / L. Their report stated that zinc concentration was found within the permissible limits. The phytosanitary treatments with Zn fungicides or Bordeaux mixture are widely used in vineyards and usually increase the content of soil Cu and Zn (Ronkainen, 2016; Korchagin et al., 2020).

5.2 Effects of Wine Type and Region of Origin on Toxic Element Concentrations in Wine Samples

5.2.1. Influence of Region on Toxic Element Concentration

The significant main effect of region (Wilks' Lambda = 0.037, F = 16.004, p = 0.000) indicates that the concentration levels of toxic elements are different substantially across the wine-producing areas sampled in this study, i.e. Kutná Hora, Mělník, Mikulov and Prague . This

finding is consistent with previous research suggesting that the geographical origin of wine contributes meaningfully to its elemental composition due to differences in soil geochemistry, agricultural practices, and exposure to environmental contaminants (Danezis et al., 2016; Hao et al, 2021).

Kutná Hora in particular, which is known to have elevated levels of historical industrial pollution, especially from mining activities, may explain higher concentrations of heavy metals such as lead (Pb), cadmium (Cd), and arsenic (As) in some samples Bulková,2024).

Moreover, the regional effect also reflects agricultural inputs such as the use of phosphate fertilisers and copper-based fungicides, which have been associated with residual levels of Cd, Cu, and Zn in vine leaves and grapes (Liang et al., 2015; Mahlungulu et al, 2023). The soil pH, organic matter, and cation exchange capacity in different terroirs also influence the bioavailability of metals for uptake by vines, as described by Bravo et al. (2015).

5.2.2. Effect of Wine Type on Toxic Element Levels

The wine type, as classified here into red, white, and rose, also had a statistically significant main effect (Wilks' Lambda = 0.464, F = 3.584, p = 0.000) on the combined levels of toxic elements. For Mělník, this result is consistent with earlier studies showing that red wines often contain higher levels of certain metals such as iron (Fe), manganese (Mn), and zinc (Zn), compared to white and rosé wines (Gajek et al., 2021).

This is primarily due to the longer maceration periods and fermentation with skins and seeds typical of red wine production, which facilitate greater extraction of both beneficial and harmful minerals from the grape matrix (Shimizu et al., 2020; Watrelot & Delchier, 2025). Furthermore, red wines are more likely to accumulate metal residues from production equipment contacts (e.g., stainless steel tanks, filters), and these contributions can vary depending on the production scale and quality assurance protocols (Scutarașu, & Trincă, 2023). Rosé wines, on the other hand, often have shorter contact with skins and are subject to different clarification processes, which may limit the extent of elemental absorption. White wines, which are usually fermented without skin contact, showed relatively lower concentrations of most metals, confirming previous findings by Kment et al. (2005) and Roig-Puscama et al., (2025).

5.2.3. Interaction of Region and Wine Type

The most compelling is the significant interaction effect observed between region and wine type (Wilks' Lambda = 0.287, F = 6.651, p = 0.000), suggesting that the influence of wine type on toxic element concentration is not uniform across regions. This interaction implies a complex relationship. Such regional-specific differences underscore the terroir-dependent

nature of metal accumulation, influenced by local environmental exposure and winemaking traditions (Van Leeuwen et al., 2020; Van Leeuwen et al., 2022).

The implication of this interaction is profound for traceability and food safety assessments, where it becomes crucial to analyse not just the wine type but also its regional origin to determine potential risks of metal contamination. Studies by Catarino et al. (2008) emphasised that understanding regional variations helps build effective risk mitigation strategies and can inform targeted policies on acceptable levels of heavy metals in wines.

5.3 Influence of Wine Type and Region of Origin on individual Toxic Element Concentrations in Wine Samples

Arsenic concentrations were significantly influenced by region ($F = 23.418$, $p = 0.000$), wine type ($F = 5.410$, $p = 0.007$), but was not influenced by the interaction between the region and wine type ($F = 1.607$, $p = 0.211$). This indicates strong variation in arsenic levels depending on where the wine was produced, the type of wine. Arsenic is a known toxicant, and these results suggest a need for careful monitoring of its levels in certain wine groups. The significant influence of region suggests that environmental and agricultural conditions unique to each region may contribute to the differences observed in arsenic concentrations (Huzum & Sirbu-Radasanu, 2021; Jiménez-Ballesta et al., 2023). The influence of wine type also points to variations in arsenic uptake and retention between red, white, and rosé wines. Tanabe et al., (2019) reported that white wines may accumulate more arsenic due to limited filtration processes or the use of certain clarifying agents that may introduce or fail to remove arsenic.

Lead also showed statistically significant variation by region ($F = 11.757$, $p = 0.000$) but it was not statistically significant for wine type ($F = 3.128$, $p = 0.052$) and their interaction ($F = 0.460$, $p = 0.634$). This suggests that only type of wine significantly influence lead contamination. The presence of lead in wine is a public health concern, especially with chronic exposure. This result underscores the potential risk associated with environmental and processing factors in different regions that may contribute to lead contamination—such as soil composition, use of pesticides or fertilizers, and the materials used in wine storage or bottling. Red wines, for example, have previously been reported to exhibit higher heavy metal concentrations, possibly due to longer contact with grape skins during fermentation (Dumitriu et al., 2021)

Cadmium concentrations did not differ significantly by region ($F = 0.253$, $p = 0.859$), wine type ($F = 0.467$, $p = 0.629$), or their interaction ($F = 0.207$, $p = 0.813$). This implies a relatively consistent level of cadmium across all wine samples, with no major variations attributable to location or interaction between region and wine type. Although cadmium is a toxic heavy metal known for its potential to cause kidney damage, bone demineralization, and carcinogenic

effects with prolonged exposure (Genchi et al., 2020; Charkiewicz et al., 2023), the lack of significant variation in this study may reflect consistent agricultural practices, similar soil cadmium content, or standardized winemaking processes across the sampled regions.

Zinc levels were significantly influenced by region ($F = 27.087$, $p = 0.000$) and their interaction ($F = 6.509$, $p = 0.003$) except for wine type ($F = 0.438$, $p = 0.647$). Zinc is an essential trace element but can be toxic at high levels. The significant results suggest that the region where the wine is produced as well as the combination of region and type of wine can influence zinc content. Zinc is an essential micronutrient that plays a vital role in numerous biological processes but excessive zinc intake can lead to gastrointestinal disturbances, immune dysfunction, and interference with the absorption of other trace elements like copper and iron (Maywald & Rink, 2022; Kiouri et al., 2023).

Copper concentrations varied significantly with region ($F = 16.868$, $p = 0.000$), wine type ($F = 8.242$, $p = 0.001$) and their interaction ($F = 25.755$, $p = 0.000$). Copper may be introduced through fungicide use or equipment, and this result highlights the importance of managing winemaking practices to control copper content.

Iron concentrations were significantly influenced by region ($F = 3.080$, $p = 0.036$) but did not show any statistically significant variation by wine type ($F = 0.480$, $p = 0.622$), or their interaction ($F = 0.381$, $p = 0.685$). This indicates that iron concentrations are relatively stable across different wine types and regions.

5.4 Concentration of Elements based on Estimated Marginal Means

Arsenic varied strongly by both region and wine type, with the highest means in Mělník—especially for red—and comparatively low values for KH red and white. Using the OIV maximum acceptable concentration for wine (0.20 mg/L), most of the mean in this study exceed the limit except KH red and white and Prague white, pointing to local geogenic inputs and legacy agrochemical use as plausible drivers (e.g., historical arsenical pesticides and lithology-controlled soil As) (OIV, 2024a; Jiménez-Ballesta *et al.*, 2023; OIV, 2020). The clear Region \times Wine-type signal suggests that vineyard site and vinification choices (skin contact, blending) may modulate extraction and carry-over of As into finished wine (Huzum & Sirburadasanu, 2021; Jiménez-Ballesta *et al.*, 2023). Given arsenic's chronic toxicity, these exceedances merit targeted source investigation (soil/must mapping) and process controls (selective fining, blending) (OIV, 2020). The localized exceedances of As are periodically

reported near As-rich parent materials or where legacy agrochemicals were used (Jiménez-Ballesta et al., 2023).

Lead means were lowest and tightly clustered in Mělník and Mikulov but higher in Prague, with statistically significant effects of region, wine type, and their interaction. All reported means are below the current OIV maximum of 0.10 mg/. Regional differences are consistent with environmental deposition patterns (e.g., traffic and industrial sources) documented for Central European vineyards, where Pb can enter the soil-grape-wine chain via dust and surface contamination (Prokeš et al., 2025). Winemaking technology is a source of heavy metals in the final wine and contact with aluminium, brass, stainless steel, and wood winemaking equipment (winemaking machinery, pipes, casks, and barrels), filtration materials (diatomous earth, bentonite), or some additives can lead to Pb contamination (Shimizu *et al.*, 2020). The concentration of Cadmium was relatively similar across all the regions and wine type except for slightly higher concentration in white wine from KH (Kutná Hora), Mikulov and Prague. The concentrations were below the OIV limits and they do not pose any significant health threat. This pattern—low absolute values and weak spatial structure—is typical for Cd in modern European wines and indicates low agronomic input and minimal process contributions.

Zinc differed by both region and wine type, with the highest white wine means in Mikulov and the lowest in Prague, yet all values were comfortably below the OIV maximum (5 mg/L) (OIV, 2024a). Zn in wine is often influenced by vineyard nutrition regimes and contact with galvanized equipment; your Region × Wine-type effect aligns with those process-linked explanations (OIV, 2024a; OIV, 2020).

Copper varied significantly, with a standout high mean in Mikulov red that exceeded the OIV limit of 1.0 mg/L. Elevated Cu in a single region/wine-type combination may be consistent with use of copper-based fungicides (e.g., Bordeaux mixture), which remain authorized—and common—in European viticulture, including organic systems (EFSA/EC updates on Cu compounds; recent agronomic and ecotoxicological work) (OIV, 2024a; Garinie *et al.*, 2024; AMS, 2022). A large number of symptoms/ailments, comprising anemia, depressed growth, dermatitis, dwarfism, electrolyte imbalance, gastro-intestinal and neurological disorders, lethargy and nausea, have been associated with Cu and Zn deficiency in humans, as well as with toxicity due to excessive intake (Kostic et al., 2010)

Iron was highest in Mikulov (particularly white and red) and lowest in Mělník white, yet remained well beneath commonly cited guidance values (~10 mg/L) used in oenological

practice for quality/instability control (protein haze/oxidation), rather than a strict toxicological ceiling (OIV methods and secondary summaries) (OIV, 2019; Durguti et al., 2020). Iron is essential for life in relatively high amounts but which at higher than certain threshold concentrations may become toxic (Durguti et al., 2020). The application of fining agents, such as bentonite, copper sulfate and other environmental pollution may contribute also to wine contamination with Iron (Dumitriu et al., 2019)

5.5 Comparison between Region and Wine Type

Arsenic levels were significantly lower in KH compared to Mělník and Mikulov, yet higher than in Prague. Meanwhile, Mělník and Mikulov wines held markedly more arsenic than Prague wines. In terms of wine types, red wines exhibited significantly lower arsenic than both white and rosé. These outcomes may be as a result of arsenic content heavily influenced by region-specific soil lithology and historical agrochemical use, especially arsenical pesticides.

Mikulov wines displayed the highest zinc concentrations, significantly exceeding those from KH, Mělník, and Prague—while KH also surpassed Prague. These regional differences likely reflect varying viticultural practices or equipment contact that inadvertently introduce zinc into wines.

Prague wines showed significantly higher lead levels than those from KH, Mělník, and Mikulov, though overarching concentrations remained far below the OIV limit of 0.10 mg/L (OIV, 2024a). The elevated Pb in Prague may reflect proximity to urban pollutants or legacy environmental contamination; however, the compliance with regulatory caps suggests minimal health risk currently.

Cadmium levels presented no statistically significant differences by region or wine type, and values remained well below the 0.01 mg/L OIV limit (OIV, 2024a). This consistency indicates minimal regional or processing influence on cadmium levels—a reassuring finding for consumer safety.

Mikulov wines, especially rosé, exhibited the highest copper concentrations, significantly exceeding levels in KH, Mělník, and Prague. Notably, rosé wines contained more copper than red wines. This is aligned with known precautions in viticulture: copper-based fungicides (such as Bordeaux mixture) can accumulate in soil and be transferred to grapes and wines, particularly when skin contact is prolonged or spray frequency is high (Komárek et al., 2008; OIV, 2024a).

Iron levels in Mikulov wines exceeded those in KH and Mělník. Though under the oenological guideline of ~10 mg/L, elevated iron may impact wine stability and oxidative properties (OIV, 2019). Mikulov producers might benefit from monitoring strategies to manage iron-related treatment effects and ensure quality preservation.

5.6 Comparison between the mean of Bulková's study (2024) and this study

The mean concentration of Arsenic in Bulková's study (0.26212 mg/L) was significantly higher than in the present study (0.18177 mg/L), with a mean difference of 0.08034 mg/L ($p < 0.001$). Higher arsenic levels in some wines are often associated with environmental factors such as soil contamination or anthropogenic pollution affecting the vineyards, as supported by multiple studies analyzing trace elements in wine from different origins (Komárek et al., 2018). Variability in arsenic levels may also reflect differences in analytical methods or grape variety (Abdulla et al., 2025).

Cadmium (Cd) levels were markedly higher in Bulková's findings (0.00102 mg/L) compared to this study (0.00006 mg/L), with a mean difference of 0.00096 mg/L ($p < 0.001$).

No significant difference in copper levels was found between Bulková's study ($M = 0.12812$) and this ($M = 0.14200$) study ($p = 0.755$). This suggests similar copper content across both studies. Copper in wine largely originates from vineyard fungicides and equipment contact during winemaking (e.g., copper sulfate), but levels tend to be controlled within narrow ranges due to regulatory standards and winemaking practices, explaining similar concentrations across studies (Alkış et al., 2014).

Iron content did not differ significantly between Bulková's study ($M = 0.91318$) and this ($M = 0.97717$) study ($p = 0.369$), indicating broadly comparable levels.

Lead concentrations were slightly higher in Bulková ($M = 0.01375$) than in this study ($M = 0.00925$), but the difference was not statistically significant ($p = 0.169$). Lead content in wines usually reflects environmental pollution and contamination during winemaking; similar values suggest comparable exposure levels or good contamination control (Gajek et al., 2021).

Zinc levels were higher in this study ($M = 0.63951$) than in Bulková ($M = 0.56892$), but this difference was also not significant ($p = 0.257$). Zinc concentrations in wines have shown

considerable variability linked with soil composition and winemaking equipment, but usually stay within safe consumption limits (Płotka-Wasyłka et al., 2018)

The elements whose concentration differed significantly between the studies are Arsenic and Cadmium while differences in copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) were not statistically significant.

5.7 Concentrations of toxic elements in wine digested with nitric acid and nitric acid + peroxide

For arsenic, the mean concentration was higher using nitric acid alone (0.14813 mg/L) compared to the combination with peroxide (0.06413 mg/L), though this difference was not statistically significant ($p = 0.062$). This aligns with studies showing that nitric acid digestion efficiently recovers arsenic due to its ability to degrade organic arsenic species into inorganic forms, with limited advantage from peroxide addition in some matrices (Sommer And Ünlü, 2006).

Lead showed similar concentrations between the two methods (0.0104 mg/L with nitric acid alone vs. 0.0114 mg/L with peroxide), with no significant difference ($p = 0.731$). Nitric acid alone is effective for lead extraction, and the addition of peroxide may not enhance recovery significantly, consistent with studies reporting effective lead recovery using nitric acid digestion and microwave methods, sometimes optimized with peroxide for enhanced efficiency (Adebowale, 2021; Gende and Schmeling, 2022).

Cadmium concentrations were very low, with no significant difference between methods ($p = 0.170$). Nitric acid digestion is generally sufficient for cadmium extraction, as supported by findings from digestion recovery studies showing adequate cadmium recovery with nitric acid alone (Abbruzzini et al., 2014).

Zinc showed a significant difference ($p = 0.027$) between methods (0.9813 mg/L with nitric acid alone vs. 1.1694 mg/L with peroxide). Studies have indicated that peroxide enhances organic matrix breakdown, improving zinc recovery in complex samples (Abbruzzini et al., 2014; Ishak et al., 2015 & Gende and Schmeling, 2022). Ishak et al., (2015) reported that a high recovery was obtained for Zn in the digestion using HNO₃ than the combination with peroxide.

For copper and iron, the combination method with peroxide resulted in significantly higher measured concentrations (copper: 0.0846 mg/L vs. 0.0493 mg/L, $p = 0.014$; iron: 1.3688 mg/L vs. 1.0825 mg/L, $p = 0.007$). This reflects the oxidative power of hydrogen peroxide combined with nitric acid, which facilitates more complete digestion of organic matter, improving recovery of these metals, as confirmed by research on digestion of tissue and environmental samples (Rajahalme et al., 2021; Gende and Schmeling, 2022). Gende and Schmeling, (2022) reported that it was found that a 4:1 ratio between nitric acid and hydrogen peroxide yielded the best percent recovery with higher than 95%.

6.0 CONCLUSION

During this study, a comprehensive analysis of potentially toxic elements in wines from anthropogenically polluted regions was conducted through acid digestion and subsequent Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The study revealed elevated concentrations arsenic in red and white wines produced from Mělník and Mikulov, which were above safety limits established by International Organisation of Vine and Wine (OIV). This finding highlighted the utmost danger of wine consumption from specific areas, which posed a public health issue because of long-term exposure to such poisonous substances. Arsenic concentrations were high in Kutná Hora, with some values exceeding the limit and others approaching it. These findings highlight the importance of regular testing, as Kutná Hora was initially presumed to be the only polluted region, yet our results revealed two additional regions with problematic arsenic levels. Consequently, our hypothesis (H1) and Aim 2 were not supported, since higher concentrations were found in regions originally expected to be non-polluted.

The comparison of toxic element concentrations in wines digested with only nitric acid versus a nitric acid–hydrogen peroxide mixture revealed significant differences in zinc, copper, and iron levels. This directly fulfills Aim 5, which was to evaluate and compare the two digestion methods. Although most elements did not show significant differences between digestion methods, the main focus of this study—arsenic—did, with concentrations differing statistically, likely due to its potential volatility. This supports hypothesis 2, which proposed that direct analysis and digestion methods yield different concentrations of potentially toxic elements (PTEs).

With regards to the measured trace element concentrations, this study generally falls in line with what Bulková (2024) reported. Concentrations of Ba, Ni and Zn were notably higher in this study, while Al, B, Mn and Fe were greater than or equal to Bulková's ranges. Similar pattern or partial detection elements were found in Be, Co, Cr, Cu, Sb, V, and Pb. This shows constant trace element profile of both datasets which confirms the handling process of sample.

The first aim was achieved by selecting the most appropriate type of acid digestion method from the literature review and the equipment in the laboratory. It may not have been the most appropriate method, given the explosions and potential volatility of As, which likely resulted in obtaining lower arsenic concentrations than Martina. Aim 2 was met by comparing Kutná Hora wines with other regions with higher PTE levels. Unexpectedly, the concentrations at the other locations—especially for arsenic—were higher than those observed in Kutná Hora. The result revealed significant difference in the concentration of Kutná Hora and other regions in Arsenic, Lead, Zinc, Copper and Iron. Although Aim 3 was partially fulfilled as differences between red, white, and rosé wine types were found, no consistent trend was seen. The result of comparing the concentrations of PTE revealed significant difference in the concentrations of Arsenic between Red wine against white and also red against Rose. Comparative analysis with Bulková's data was fully addressed Aim 4, establishing the acid digestion technique as both effective and consistent. The result of the comparison revealed significant difference in the concentration of Arsenic and Cadmium from the two studies.

The findings of this research contributed to new information on the occurrence of toxic contaminants in wine, enhancing awareness and transparency in wine labelling norms. Further research may deal with the efficacy of different treatments during the wine-making process and the effect of geographical variation on elemental content. Last but not least, this research set the stage for the development of customized interventions for safeguarding consumer health and enhancing wine production quality standards in affected regions. Further potential research worth considering - finding out what caused the Mikulov samples to have such high arsenic contents.

The comparison of the results indicated that there was no specific trend on the level of contamination and the maximum concentration of toxic element were not specific to a particular location. These results stress the need for overall stringent monitoring in the viticulture sector regardless of the location to safeguard consumers. This not only identifies health risks of toxic element intake through wine but also necessitates the requirement for regulatory agencies to implement stricter testing regimens.

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