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LEVELS OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS IN SOME ETHIOPIAN WINES

BY

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To

My brother Mesfin Minillu

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ABSTRACT

Levels of Essential and Non-Essential Elements in Some Ethiopian Wines

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The concentrations of thirteen metals (K, Na, Ca, Mg, Fe, Zn, Mn, Pb, Co, Ni, Cd, Cu and Cr) were determined in four brands of Ethiopian wines by flame atomic absorption spectrophotometry (FAAS) after digesting the wine samples with HNO_3-H_2O_2 mixture. Among the major metals potassium (694 – 767 mg/L) was found to be in the highest level, followed by magnesium (79.2 – 58.1 mg/L), calcium (37.1 – 28.4 mg/L) and sodium (24 – 24.4 mg/L). From the minor metals Iron, zinc and manganese were in the ranges of 1.42-3.16, 1.82- 2.7, and 1.04-1.88 mg/L, respectively, followed by copper (0.5-1.5 mg/L), nickel (0.18-0.2 mg/L), cobalt (< 0.065 - 0.091 mg/L), lead (0.14 – 0.31 mg/L) ,chromium (< 0.075 – 0.192 mg/L), and the toxic metal cadmium was below detection limit of the analytical procedure used in this study (< 0.014 mg/L). These metal concentrations of the Ethiopian wines were compared with published data on wines of different origin. The levels of metals in the Ethiopian wines are found to be comparable with wines of some other countries.

Key words: Essential and non-Essential elements, Toxic metals, Flame Atomic absorption spectroscopy (FAAS), acid digestion.

1. INTRODUCTION

1.1. Definition

The term wine describes an alcoholic beverage that contains products of the fermentation of the juice from the grapes, the fruit of the genus *Vitis* [1]. The word "wine" is derived from the Proto-Germanic *winam*, an early borrowing from the Latin *vinum* [2]. Yeasts digest the sugars found in the grapes by the processes of fermentation and produce alcohol (largely ethanol) and carbondioxide [1]. Grapes are not the only fruits used to make wine, though they are the most commonly used; wine is also made from the fermented juice of pears, apples, berries, and even flowers such as dandelions [1]. Wine naturally contains about 85 % to 89 % water, 10 % to 14 % alcohol, less than 1 % fruit acids, and hundreds of aroma and flavor components in very small amounts. Wine character its taste and smell is derived from many factors including the grapes it is made from, where they were grown, and the production techniques applied by the wine maker, or enologist [3].

1.2. Classification of wines

Wines are categorized using a number of different methods. Sometimes they are grouped into different categories by grape variety, region of origin, by color, by the name of the wine maker or viticulturalist, or by production technique. Three basic groups of wines are most easily distinguishable for the consumer; these are table wines, sparkling wines, and fortified wines [1].

Table wines also called still or natural wines are produced in many different styles and make up the majority of wines on the market [1]. They are consumed mostly with food. They may have an alcohol content that is no higher than 14% alcohol by volume. As such, unless a wine has more than 14% alcohol, or it has bubbles, it is a table wine or a light wine. Table wines are further classified by color, as red, white, or rose (pink); and by character, as sweet or dry. The pH range in dry table wine is generally around 3.1 - 3.6. Red wines are made from the skins of dark (red) grapes. White wines may be made from "white" (that is, green) grapes or from dark grapes, but with white wine the grape skins and pressed juice do not come into contact unlike 'red' wine. True rose wines are the products of dark grapes; their skins remain in contact with the juice only until it has turned into a pale pink [4]. Most table wines are fermented until they are 'dry' that is, all the grape sugar has been turned to alcohol by the yeast. Slightly sweet or off-dry wines are made by stopping the fermentation before all the sugar is gone or by adding grape juice back to the wine afterwards [1].

Sparkling wine is made from table wine that has undergone a second fermentation. The wine maker adds a measured amount of sugar and fresh yeast to the dry wine. This can happen in a closed tank, or directly in the bottle [1,4], which is the way the most famous sparkling wine, French champagne, is produced [1]. The yeast ferments the added sugar, but this time the carbon dioxide gas remains in the sealed bottle, creating carbonation. When the sparkling wine is poured into a glass or shaken in the open bottle the gas bubbles to the surface together with some wine forming champagne spray.

Fortified wines are often sweeter, and generally more alcoholic wines that have had their fermentation process stopped by the addition of a spirit, such as brandy, or have had additional spirit added after fermentation [1]. These types of wines contain additional alcohol (5 – 10 % higher than table wines) and are usually consumed in small amounts as aperitifs before meals or dessert wines after a meal.

1.3. Origin and distribution of wine

Wine has a long history dating back about 8,000 years and is thought to have originated in present day Georgia or Iran [5]. Wine is thought to have appeared in Europe about 6,500 years ago in present-day Bulgaria and Greece, and was very common in classical

Greece and Rome. And there are indications that viticulture was carried on in Mesopotamia (the region that is now Iraq and eastern Syria) about 5,000 years ago. Writings from the same time from China and Egypt also mention wine and vineyards. The highest-quality wines were reserved for the Egyptian kings and pharaohs, and wine was accepted as payment for taxes [1].

The Ancient Greeks introduced grapes such as *Vitis vinifera* and made wine in their numerous colonies in Italy, Sicily, southern France, and Spain. Dionysus was the Greek god of wine and revelry, and wine was frequently referred to in the works of Homer and Aesop [2].

Wine has also played an important role in religion since ancient times [6]. Since Roman times, wine (potentially mixed with herbs and minerals) was assumed to serve medicinal purposes as well. During Roman times it was common to dissolve pearls in wine for better health. Many of the major wine producing regions of Western Europe today were established by the Romans. Wine making technology improved considerably during the time of the Roman Empire. Many grape varieties and cultivation techniques were known. Barrels were developed for storing and shipping wine [4, 6].

Wine was equally important to the ancient Romans who looked to Bacchus, the Roman god of wine, to oversee all wine matters. Wine-making monastic orders produced wines not only for sacramental use, but also for marketing to produce revenue for the orders. Monks developed the predecessors of some modern grape varieties, and in abbey wine cellars they experimented with the forerunners of brandies and other wine-based liqueurs [1].

In the revival of commerce that preceded the Renaissance (14th century to 17th century), wine trading became a main attraction at lively fairs held in many French towns. The English were eager buyers of Bordeaux wines. For three centuries the English appetite for Bordeaux wines made the region prosper [1, 2].

The wine industry flourished in almost every new country settled by Europeans, as the colonizers took with them their knowledge of viticulture and often their own grape varieties. In North America, serious production of wine began in the mid-1800s [1].

Politics, war, and even fashions in drinking have often modified the trading and drinking of wine. Rivalry between England and France in the 15th century interrupted the profitable Bordeaux trade. In the 17th century cheap Portuguese wine entered the English market. A popular style for gin seized wine-drinking in England in the early 18th century. Then German Rhine wine became popular, followed by Madeira, produced in the Portuguese Madeira Islands, and then Spanish sherry. By about 1875 French wines dominated world trade, and since that time European wine industries have led the world in international wine trade [1]. Table 1.1 shows the top ten wine producer countries in 2004.

Table 1.1 World Top Ten Wine Producer Countries in 2004 [7].

Country of production	million liters	% share from the world total
France	5.738	19.2
Italy	5.300	17.8
Spain	4.298	14.4
USA	2.010	6.7
Argentina	1.546	5.2
Australia	1.381	4.6
China	1.170	3.9
Germany	1.004	3.4
South Africa	0.927	3.1
Portugal	0.748	2.5

1.4. Winery in Ethiopia

Vines were grown and wines made in Ethiopia for use in the Holy Communion Service of the Ethiopian Orthodox Church since the Middle Ages. Winemaking developed further with the arrival of Portuguese Catholic missionaries, who introduced grape varieties that were being cultivated in the Mediterranean regions of southern France at that time. As a result, grape wine came to replace the traditional wine made from honey as a common beverage. Extensive commercial wine production, however, did not get underway until about 1900 when Italian and Greek industrialists stepped in [8].

The best vineyards are about 8,300 feet (2,500 m) above sea level on the central plateau at Gouder, Dukem and Hollots. There are also vineyards along the Awash River valley. The main varieties grown are known locally as Tikur Weyn, Dibulbul Atter, Nech Dibulbul and Key Dubbe, all of which probably originated in Mediterranean countries and the Middle East [8].

Awash Winery is the first commercial winery in Ethiopia, established in 1943. It employs experienced oenologists trained in Italy. In collaboration with the Ethiopian Institute for Agricultural Research and the Debere-Zeit Agricultural Research Center of Alemaya University. Awash is conducting extensive research on more than 140 grape varieties to test their suitability for Ethiopian soil and climate. So far, about 20 are proving successful, including Grenache, Nebbiolo, Petit Syrah, Canno Nano, Dodoma Altico, Sylvaner, Ugni Blanc, Chenin Blanc, Zeirfandeler, Awash Negest, Shitto Woyne and Moskoko Gialo. The winery produces a range of well-made wines, including Gouder Red, Dukem Red, Awash Crystal White, Axumite Red, Kemila White as well as a Sparkling wine [8].

1.5. Major steps/processes during wine making

Winemaking, **or vinification**, is the process of wine production, from the selection of grapes to the bottling of finished wine. The science of wine and winemaking is known as *oenology*. Wine production can be generally classified into two categories: still wine production (without carbonation) and sparkling wine production (with carbonation). After the harvest, the grapes are crushed and allowed to ferment. Red wine is made from the must (pulp) of red or black grapes that undergo fermentation together with the grape skins, while white wine is usually made by fermenting juice pressed from white grapes, but can also be made from must extracted from red grapes with minimal contact with the grapes' skins. Rosé wines are made from red grapes where the juice is allowed to stay in contact with the dark skins long enough to pick up a pinkish color, but little of the tannins contained in the skins.

The Steps: the major steps in wine making are the following.

a. Harvest: The grapes are picked when they are ripe, usually as determined by taste and sugar readings.

b. Stemmer Crusher: This removes the stems from the grape bunches, and crushes the grapes (does not press them for red wines so that they are exposed to the yeast for fermenting and so the skins can better impart color to the wine).

c. Fermentation: Yeast turns the sugar in the wine primarily into carbon dioxide, heat and alcohol.

c. Maceration: This is how long the must (juice and grape solids) is allowed to sit, picking up flavor, color and tannin. Too long and the wine is bitter, to short and it is thin.

d. Pumping Over: Skin and other solids float to the top, and need to be pushed back down to stay in contact with the must. This "cap" can be punched down with a tool, or the must can be pumped form the bottom over the cap and submerge it that way.

End of Maceration: The winemaker must decide if the must has sat long enough.

e. Remove Free Run: The best quality wine is made just from the juice portion [4, 6].

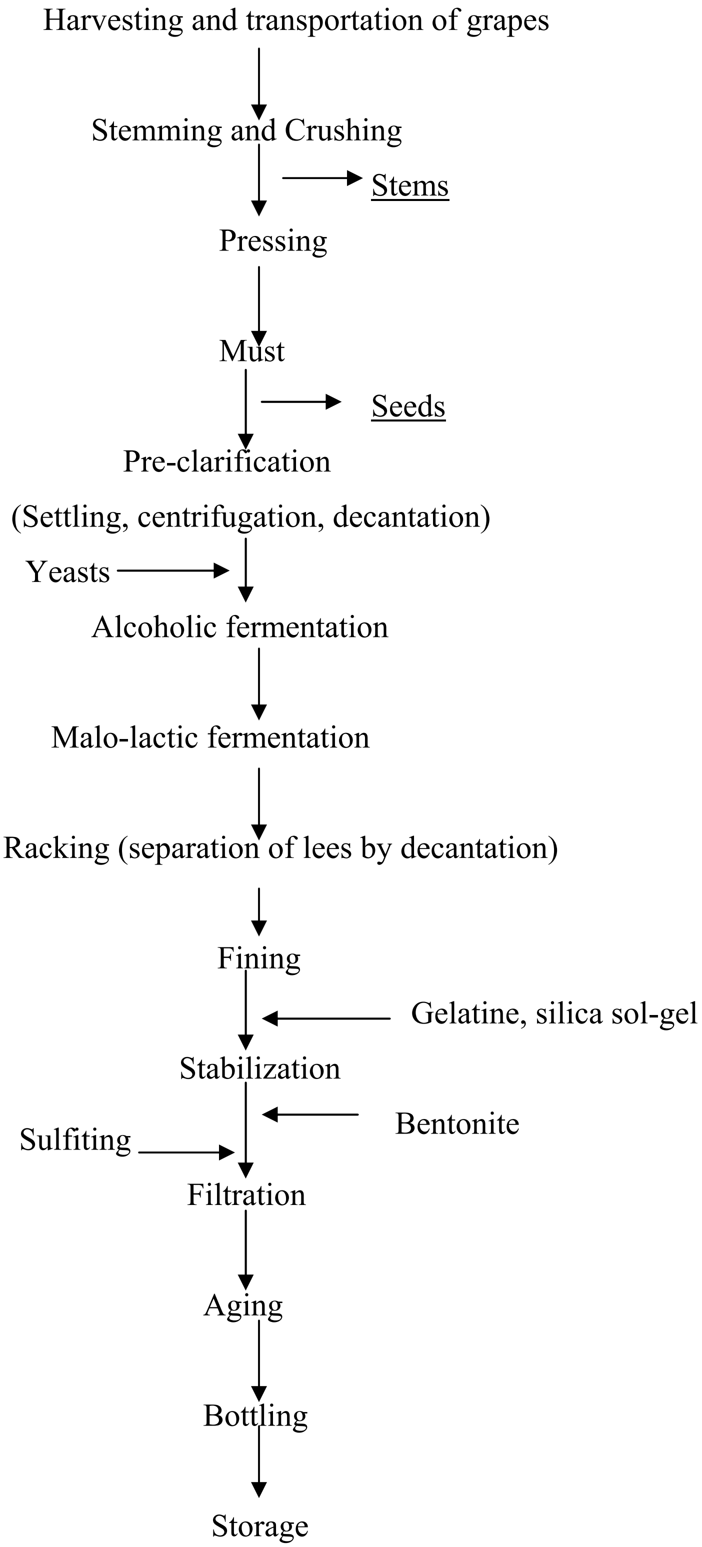

Figure 1.1. Flow scheme of white-wine vinification. For red and rose wines, pressing and separation are performed after fermentation [10].

1.6. Sources of metals in wines

The content of metals in wine can be attributed to the natural sources, the atmospheric deposition of airborne particulate matter on grapes and transfer of metals from the soil via the roots to the grapes and finally to wine, and to the contamination during the winemaking process [11].

Metals of primary, natural origin come from soil on which vines are grown and reach wine through grapes. The concentration of primary metals is characteristic and comprises the largest part of the total metal content in wine. It is connected with the maturity of the grapes, their variety, the type of soil in the vineyard, and the climatic conditions during their growth. The contribution of metals of a secondary origin is associated with external impurities that reach wine during growth of grapes or at different stages in winemaking (from harvesting to bottling and cellaring). During the growth of grapes in a vineyard, contaminations can be classified as geogenic (originating in the soil), from protection and growing practices, or from environmental pollution [9].

Accordingly, wines from vineyards in the vicinity of sea or ocean result in a higher Na content compared to wines from other regions [5–8]. Wind from sea or ocean affects the coastal region and vineyards located therein by a marine spray. Differences in K, Ca and Cu content can be due to fertilizers used for cultivation [9]. Application of pesticides, fungicides and fertilizers containing Cd, Cu, Mn, Pb and Zn compounds during the growing season of vines leads to increases in the amounts of these metals in wine [9]. Wines from vineyards located close to road traffic or situated in industrial areas contain higher levels of Cd and Pb because of vehicle-exhaust fumes or other emissions to air, water and soil [9].

Finally, there is an enological (winemaking) source of metals, as contamination may occur at different steps of wine production (Figure 1.1). The reason for this is the long contact of wine with materials (aluminum, brass, glass, stainless steel, and wood) from which wine-making machinery and pipes, casks and barrels used for handling and storing wine are made. This is the usual source of Al, Cd, Cr, Cu, Fe and Zn [9, 12].

Contamination with Na, Ca or Al can be associated with fining and clarifying substances (flocculants, such as bentonites) added to wine to remove suspended solids after fermentation and to reduce turbidity [7, 9, 12]. Ca concentration can also be affected by adding $CaCO_3$ or $CaSO_4$ for de-acidification of must and wine [13, 14] or enhancement of acidity of grape juices, respectively, [9, 12, 15].

A possible source of metals can also be attributed to residues of agrochemical products used as insecticides and fungicides as well as fertilizers, which contain salts of metals. A significant amount of metals is due to contact with the apparatus used in the wine production and packaging processes. Long maceration at excessively high temperatures probably causes a through extraction of metals from must. Technological features (pressing), chemical factors (alcohol content, must acidity) and physical parameters such as temperature could also affect metal extraction [11].

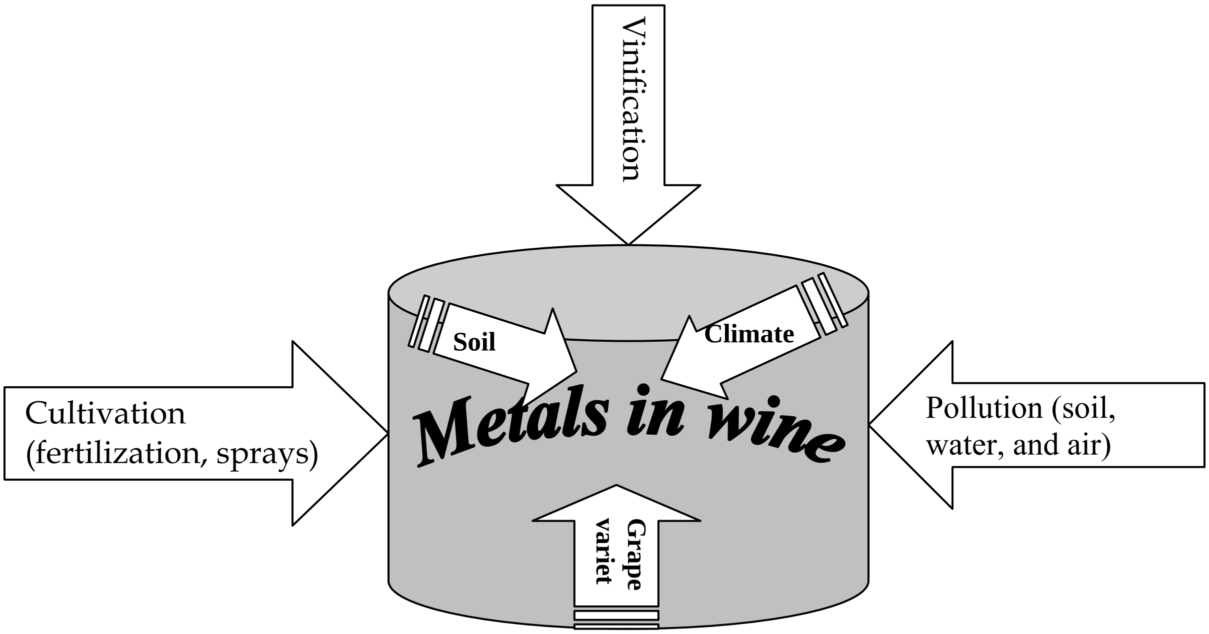

Figure 1.2. Endogenous (arrows inside) and exogenous (arrows outside) sources of metals in wine [9].

1.7. Chemical composition of wines

Wine contains a large number of inorganic as well as organic compounds. The principal dissolved species are inorganic ions (such as potassium, sodium and calcium), organic acids, polyphenols, proteins, amino acids and polysaccharides [9]. White wines are less abundant in dissolved substances than red wines, particularly with respect to polyphenols. Monomeric polyphenols, such as catechin, are generally considered to be more bitter, while polymeric tannins are highly astringent. Phenolic compounds not only contribute to the sensory characteristics of wine, such as colour, flavours and astringency, but may also act as antioxidants both free-radical scavenging and metal chelation. It has been proved that a moderate consumption of wine is related to a decrease in the risk of cardiovascular disease [11].

The composition of wine is summarized in Table 1.2. As any natural biological material, the composition of all components varies over a significant range. The widest and perhaps most important variation is in the composition and content of secondary plant metabolites, collectively termed polyphenols. The majority of grape polyphenols are present in the skins and seeds, and, as a result, the processing of grapes into wine has a greater effect on the total polyphenols content than does grape variety, although grape variety and growing conditions influence the spectrum of polyphenols present in the grapes. Aging increases various chemical processes that continue to alter composition. Each phenolic compound present in wine may have individual biological effects that are distinct from a contribution to a general antioxidant capacity as defined by gallic acid equivalents, and this simple fact of wine biology makes many of the fundamental interpretations about the health effects of wine complex [3].

Table 1.2. Composition of wine excluding polyphenols [3].

Component	Concentration(g/100 mL)
Water	80–90
Carbohydrates	
Glucose	0.05–0.1
Fructose	0.05–0.1
Pentoses	0.08–0.2
Arabinose, rhamnose, xylose	
Pectin	Trace
Inositol	0.03–0.05
Fucose	0.0005
Alcohols	
Ethyl	8.0–15.0
Other	
Methyl, higher, 2,3-butylene glycol, acetoin	0.3–0.19
Glycerol	0.30–1.40
Aldehyde	0.001–0.050
Organic acid	0.3–1.10
Tartaric, lactic, succinic, acetic, *p*-hydroxy-glutaric, galacturonic, amino, malic, citric, fumaric, oxalic, α ketoglutaric, aconic, citra-malic, malonic, pyrorocemic, pantothenic	
Nitrogenous compounds	0.01–0.09
Amino, ammonia, amide, protein humin	
Mineral compounds	0.15–0.40
Potassium, magnesium, carbon dioxide, phosphate sulfate, calcium, chloride, silicic acid, fluoride, aluminum, manganese, sodium, iron, boron, iodine, copper, rubidium, oxygen	

The metallic composition of wine depends on many factors, some of which are related to the specific production area: grape varieties, soil and climate, culture and wine making practices, yeasts and such. The kind of soil has a fundamental influence, as does the climatology and the geographical situation of the producing area. The variety of grape is also a very important factor in the content of metallic elements in wine. Other factors that contribute to the metal content are the availability of these elements in soil, fertilizing practices and processing conditions. Due to the influence of all these factors a great variability in the metal content in wines from different areas, regions and countries is observed. Determination of typical levels of metal in wines is a very useful tool to differentiate wines from different geographic origins and different Certified Brands of Origin (CBO) as well as to detect adulterations and falsifications of wines [13].

1.8. Minerals classification

Minerals are the building blocks of our bodies. They are required for body structure, fluid balance, protein structures and to produce hormones. They are a key for the health of every body system and function. They act as co-factors, catalysts or inhibitors of all enzymes in the body. Copper and iron, for example, along with other minerals are required for the electron transport system, and thus needed for all cellular energy production.

Minerals are classified into four groups: the macro-minerals, or those needed in large quantity, include calcium, magnesium, sodium, potassium, phosphorus, sulfur, iron, copper and zinc. Required trace minerals include manganese, chromium, selenium, boron, bromine, silicon, iodine, vanadium, lithium, molybdenum, cobalt, germanium and others. Possibly required trace minerals include fluorine, arsenic, rubidium, tin, niobium, strontium, gold, silver and nickel. Toxic metals include beryllium, mercury, lead, cadmium, aluminum, antimony, bismuth, barium, uranium and others.

These categories overlap slightly because assessing minerals that are required by humans is problematic. Some may be needed in minuscule amounts. Clinical studies to prove this by depriving people of vital minerals would be cruel and possibly disastrous.

Also, note that minerals needed in lesser quantities are usually toxic in greater amounts. Examples are copper, iron, manganese, selenium and vanadium. Even calcium and sodium are quite toxic in excess [14].

1.9. Essential and non-essential metals

We ingest metals everyday in our food, in the water that we drink, and in the air that we inhale. Some metals, the essential minerals, such as sodium (Na), potassium (K), calcium (Ca), nickel (Ni), cobalt (Co), chromium (Cr), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn) and magnesium (Mg), we need to ingest since they are required for normal growth and survival, while other metals such as cadmium (Cd), lead (Pb) and mercury (Hg) have no known biological function and some of these are capable of disrupting essential physiological processes. And they are only harmful to living systems. Other metals in the wrong form can be toxic. For example, chromium as the Cr^{+3} ion is an essential trace element important for maintaining correct blood sugar levels, but as the Cr^{+6} ion is a known human lung carcinogen [15]. A number of metabolic interactions between nutritionally essential and nonessential toxic metals may reduce the health hazard of the toxic metal.

1.10. Changes in metal content during wine production

As previously stated, various factors determine metal content in wines. First of all, there is the original amount absorbed from the soil and found in the grapes; then there are several decreasing factors connected with vinification processes that affect the final metal concentration in wine. Among the decreasing factors that apply to all metals, the most important is alcoholic fermentation, as can be seen from Table 1.3 from must to wine; most metals have their concentrations decreased to 5-10 % of the original amount, due to decreased solubility and precipitation as sulfides. Another decreasing factor is the so-called 'blue-fining' practice, in which potassium ferrocyanide is used to precipitate copper, iron and other metals that cause troublesome hazes and catalyze oxidation. While decrease of metal content is not a problem, increase of metal ions should be carefully checked because of two aspects: sanitary (toxic metals) and enological (alterations in organoleptic features). The main increasing factors are fining treatments and storage. In earlier times, most wine making equipments were made of iron or brass [16]. Wine acids are strong enough to dissolve the small amounts of Fe and Cu, and in the past, hazes were common problems throughout the wine industry. In recent years, the prevalent use of stainless steel and plastic materials has virtually eliminated metal haze problems, though steel might release small amounts of Fe, Ni, and Cr. Table 1.3 shows some metals' change in concentration during wine making.

Table 1.3. Lowering of metal content in wine after fermentation and blue fining.

Element	Fermentation (%)	Blue-fining (%)
Aluminum	20-30	0
Cadmium	29-41	- 80
Cobalt	17-24	77-100
Chromium	24-30	0
Copper	88-90	~ 100
Iron	0-19	63-80
Manganese	0-6	46-73
Lead	32-40	0-12
Vanadium	0-20	50-80
Zinc	0-31	78-8

1.11. The essential and non-essential elements and their roles in wine

The study of the mineral content of wines has been of great interest due to its influence on their stability, the possibility of toxicological risks, and because some of them were regulated by law. However, their major interest is to use the mineral content to characterize the wines by their geographical origin taking into account the relationship between the metallic content in samples and soil composition. In this sense, the knowledge of the mineral content can be useful to prevent fraud and to guarantee origin [17]. The following sections discuss the essential and non essential metals that will be analysed in this study and their roles (effects) in wines.

1.11.1. Sodium

Sodium is one of major metal in wine together with K, Mg, and Ca [10]. It regulates the cellular metabolism and maintains the cytoplasmic pH and the ionic balance of the yeasts. This balance is very important and related to proton transfer and enzyme activity in the plasma membrane [18]. The concentration of sodium in wines is correspondent to those of Ca and Mg or even it can be lower [10]. It is a major electrolyte of intracellular fluid. It is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure.

1.11.2. Potassium

Potassium is the other major metal [10] which is the natural component of the grape and its concentration in wine is a reflection of the grapevine status in the final stages of berry ripening [11]. The concentration of potassium is the highest in wines [10]. High potassium level could result in the precipitation of potassium hydrogen tartrate [11]. Precipitation of K and Ca tartrates changes pH, which enhances Cu and Fe oxidation in addition to forming Al, Cu and Fe clouding. Both oxidation and clouding affect wine conservation [10, 18].

1.11.3. Calcium

Calcium is a natural component of grape. Although the concentration of calcium in wine can be affected by the traditional practices of de-acidification ($CaCO_3$ addition) or plastering ($CaSO_4$ addition) elevated level of this metal can lead also to the onset of calcium tartrate precipitation [12].

Calcium also as one of the macro-elements regulates the cellular metabolism and maintains the cytoplasmic pH and the ionic balance of the yeasts [18]. It regulates the cellular metabolism and maintains the cytoplasmic pH and the ionic balance of the yeasts like that of Na. This balance is very important and related to proton transfer and enzyme activity in the plasma membrane [12]. Elevation of calcium levels tends to result in suppression of fermentation, presumably by interfering with the cellular uptake of magnesium [19].

1.11.4. Magnesium

Magnesium together with Ca, K, and Na take part in regulating the cellular metabolism of yeasts by maintaining adequate pH and ionic balance [10]. Elevating levels of external magnesium by supplementing growth media, or increasing intracellular concentrations of magnesium in yeast by cellular "pre-conditioning", resulted in a stimulation of yeast growth, sugar consumption rates and ethanol productivity [19].

1.11.5. Iron

The metallic content of a white wine appears to play an important role in its browning. Iron, copper, and manganese are among the metals most actively participating in the browning of white wines. The quantities in which these metals are present in white wines depend on diverse factors, such as absorption from the soil, contamination by atmospheric emissions, herbicidal treatments, the wine-making process, and the addition of fining agents [20].

The content of Cu(II) and Fe(III) ions (above 1 mg/L and 7 mg/L, respectively) can give unpleasant, astringent cupric and ferric tastes and be responsible for producing cupric and ferric cloudiness, especially when the content of tannic substances or pH is high [21]. However, this effect does not occur when wine contains a majority of Fe(II) ions and when the level of Cu, which catalyses Fe(II) oxidation, is low [10].

The must clarification process reduces cation content, especially iron and copper, whose presence in wine seems to be related to the concentration of solids suspended in the must

[18]. Iron catalyses oxidation of polyphenolic substances while Mn encourages formation of acetaldehyde, which reacts with polyphenolics in Fe mediated reactions to produce precipitate [21]. Resultant changes in the composition of wine lower quality and stability [10].

1.11.6. Cadmium

Cadmium is a ubiquitous environmental pollutant. It is nutritionally nonessential and toxic, and it interacts with the metabolism of three essential metals: calcium, zinc, and iron. Cadmium's pathway to man is from food, particularly leafy vegetables, grains, and cereals. Cadmium accumulates in the liver and kidneys and has a long biological half-life, from 17–30 years in man. Toxicity involves two organ systems, the renal and skeletal systems, and is largely the consequence of the interactions between cadmium and essential metals, particularly calcium [22]. It also affects hypertension, hepatic injury, reproductive toxicity, lung damage after inhalation exposure and bone effects.

The Cd levels in wines can be of technological origin due to residues of agrochemical products such as insecticides and fungicides, which can contain Cd. Generally, the Cd content in alcoholic beverage has been found to be quite low; however, when the consumption of large amounts of certain beverages causes the dietary intake of Cd to reach the maximum allowable levels [23]. Widely consumed wine could contribute an important fraction of the dietary intake of Cd. The maximum allowable concentration of Cd in drinking water is 5 mg/L, whereas for wine this concentration limit is 0.01 mg/L [24] by the International Organisation for Grapes and Wine.

1.11.7. Chromium

Chromium is an essential element which is involved in the metabolism of carbohydrates. As well as being associated with prevention of diabetes and cardiovascular disease, chromium seems to be influential in metabolism of cholesterol and various proteins. The toxicity of chromium depends on its chemical form, with hexavalent (chromium(VI)) compounds having a toxic, mutagenic and even carcinogenic nature, and the trivalent form (chromium(III)), which prevails in foods, having a low toxicity [25].

Some studies reported that the significant differences in chromium levels of wines are influenced by the type of fermentation, length of ageing, and quality of packaging. Chromium levels were found to increase from 2 to 10 fold in finished wines in relation to chromium concentrations in musts. Several elements, including chromium, contribute to haze formation and sometimes affect colour, aroma or taste. The chromium content of wines may also increase during the ageing process by means of prolonged contact with stainless steel or glass utensils and bottles [11]. In fact, the initial concentration of chromium in grapes can be increased or decreased by the technology involved in wine production [25]. The estimated safe and required dietary intake of Cr(III) is 0.05–0.20 mg/day [26].

1.11.8. Zinc

Contamination of vineyard soils with zinc results from the use of phytopharmaceutical substances containing zinc and from the use of mineral fertilizers. In mineral fertilizers we can find in average up to 500 mg of Zn/kg (ammonium sulfate, superphosphate, limestone, complex fertilizers) and in raw phosphates up to 1000 mg Zn/kg. Mobility of zinc in soil is closely connected to soil reaction. Zinc is hard to wash away therefore the contents of zinc are the highest in the surface layer [27]. Heightened content of zinc may influence negatively at the course and products of the yeast metabolism during the fermentation of alcohol (formation of acetaldehyde) [27].

Plants absorb zinc from the ground in small quantities. The zinc content in wines increases when zinc containers are used during the processing and ageing stages and also when zinc containing pesticides are used [12]. Zinc deficiency of vine occurs in the situation where in soil rich with phosphate ions surround the roots causing the change of zinc ions to insoluble zinc phosphate when contacting the roots. The surplus of phosphate ions deactivates zinc ions into insoluble zinc phosphate as well [17].

1.11.9. Copper

Copper can be carried over into grape juice from the use of copper-based vineyard sprays, although the concentration in freshly fermented wine is generally low owing to the ability of dead yeast cells to take up this metal. The main source of copper in finished product is a consequence of the practice of $CuSO_4$ addition for the removal of hydrogen sulfide [11].

High residual copper can contribute to an enhanced rate of oxidative spoilage, which ultimately results in the browning of the wine, particularly white ones. This phenomenon constitutes one of the principal enological problems for wine producers and some metal ions (Cu, Fe, Mn) are the activators of this process. In organoleptic terms, this phenomenon translates into a process of continuous oxidation, a loss of aromatic freshness and in the final stages, in the appearance of precipitates of condensed phenolic material in the bottled wine. Studies showed that application of ion exchange resins is extremely effective in lowering the content of copper, iron and manganese in white wines. The treated wines exhibited a reduced susceptibility to undergo browning, however, presented also lower polyphenolic and aromatic profiles [11].

Copper is desirable in wines up to content of 0.5 mg/L, because of its regulating effect on the numerous reactions during the ripping of wines [27]. During fermentation the content of copper decreases this is because of its consumption by yeast that needs it for development and growth. Another factor which would have contributed to decrease copper content during fermentation is its precipitation as a sulfide [28]. Cu, Fe and Mn form stable complexes with amino acids, polyphenols and melanoids. These occur during wine maturation and storage and determine ageing characteristics, final aroma, taste and even the color of the wine [10].

1.11.10. Lead

Lead and cadmium monitoring in wine is probably the most important, considering their cumulative toxic effect and relatively large consumption of wines [27, 28]. The presence of Pb in wine could be attributed to the soil or atmospheric deposition of airborne particulate matter on grapes and the winemaking process, wine processing equipment (valves, pipes, pumps, bronze tanks), and bottles (cork capsules used for sealed bottles) [29]. A positive correlation was reported between wine age and Pb concentration. It was concluded that the main contamination source was brass, and brass pipes and taps were always found in wineries known for elevated Pb levels [29].

Many studies have shown that wine can contain higher amounts of lead (Pb) than many other beverages. It has been observed that low levels of Pb exposure are correlated with irreversible fatal brain damage, hypertension, cardiovascular disease, kidney-dysfunction, impaired bone synthesis, impaired sperm production and osteoporosis. Several studies have shown that consumption of alcoholic beverages, especially wines, can influence the concentration of Pb in blood [23].

1.11.11. Cobalt

Cobalt is a relatively rare element with properties similar to iron and nickel. Cobalt is an essential element necessary for the formation of vitamin B_{12} (hydroxocobalamin), which catalyzes reactions, such as the synthesis of methionine, the metabolism of purines and folates, and the formation of methylmalonic acid and succinic acid. However, excessive administration of this trace element produces goiter and reduced thyroid activity [30]. There are few available data in the literature on the level of cobalt in wines, probably due to very low concentrations of the metal.

1.11.12. Manganese

Mn together with Cu and Fe are responsible for changes in stability of old wine and modification of the sensory quality of wine after bottling [10]. This phenomenon is called ‘‘browning’’, which involves a cascade of oxidation reactions of organic components of wine, leading to loss of freshness and aroma and appearance of condensed precipitates of tannins [20, 22]. The mechanism is complex; apparently, Cu^{2+}, Fe^{3+} and Mn^{2+} activate molecular oxygen and further oxidation reactions of organic compounds to aldehydes and ketones by forming reactive oxygen species (hydroxyl radicals) [10, 12, 21].

1.11.13. Nickel

There are few reliable data in the literature concerning nickel content in wines. Nickel is present in wines owing to the use of Ni-containing stainless-steel containers for wine fermentation and storage in modern cellar technology [2]. This metal is present in wines in very low concentrations. They showed that Ni content was generally constant during industrial processing and storage of wine [33]. Nickel, contributes to haze formation and sometimes colour aroma or taste effects. Lara *et al.* and Galani-Nikolakaki *et al.* [12, 31] showed that the nickel concentrations of the wines were independent of the type of the wine container and they did not change as a function of the wine storage time inside the container.

1.12. The health benefits of wine

In contrast to most foods and beverages that spoil quickly or that can spread disease, wine does not spoil if stored properly. The alcohol in wine, the ethanol, is present in sufficient concentrations to kill disease-causing microorganisms, and throughout history, wine was often safer to drink than water or milk. This property was so significant that before the connection between microorganisms, poor sanitation, and disease was understood, ancient civilizations regarded wine as a gift from the gods because it protected against disease [1].

Epidemiologic studies from different populations reveal that individuals with the habit of daily moderate wine consumption enjoy significant reductions in all-cause and particularly cardiovascular mortality when compared with individuals who abstain or who drink alcohol to excess. Moderate ethanol intake from any type of beverage improves lipoprotein metabolism and lowers cardiovascular mortality risk. The question now is whether wine, particularly red wine with its abundant content of phenolic acids and polyphenols, confers additional health benefits [3]. It was determined that a moderate intake of wine was unlikely to increase the risk of upper-digestive-tract cancer, whereas a moderate intake of beer or spirits did increase this risk.

1.13. The need to determine the essential and non-essential elements in wine

Modern societies appear to consume large amounts of wine, beer and other alcoholic products, sometimes increasing the daily intake of toxic metals above the maximum permissible levels [32]. The increasing global pollution of the environment requires a systematic monitoring of all kinds of food including wine.

Daily consumption of wine in moderate quantities contributes significantly to the requirements of the human for essential elements such as K, Ca, Mg, Cr, Co, Fe, Cu, Mn, Mo, Ni, Zn. On the other hand, metals such as Pb and Cd are known to be potentially toxic. At the same time, the analysis for certain elements in wines is of special interest due to the effect they seem to have on the organoleptic properties of wine. A typical example, copper is both an essential and a potentially toxic element for humans when in excess. On the other hand, the excessive presence of the elements Al, Fe, Zn and Cu has a definite negative effect on the organoleptic properties of the wine. Finally, the content of some metals can be used for the identification of the area where the wine comes from [32].

Several elements, including copper, iron, aluminum, zinc and nickel, contribute to haze formation and sometimes colour aroma or taste effects. Maximum acceptable levels of some metals in wine have been established (Table 3.9) by the Office International de la Vigne et du Vin (O.I.V.) for lead, cadmium, arsenic, zinc and copper [32].

1.14. Methods of determination of metals in wines

Metals affect the organoleptic characteristics of wine, including flavor, freshness, aroma, color and taste, mainly due to precipitates being formed (yeast, fining and filtration sediments) or clouding during wine fermentation, maturation and storage [10, 31].

Flame AAS (FAAS) [10, 12, 18, 32-39] is applied for measurements of alkalis and alkaline earth metals and Cu, Fe, Mn and Zn. In addition to atomic absorption, emission spectrometry (AES) is also common for metal determinations in wine [40-43]. With the same instrumentation as for FAAS but measuring emitted radiation or applying flame photometers, concentrations of easily ionized alkali metals (K, Li, Na and Rb) are determined [18, 39, 42]. Graphite furnace AAS (GF-AAS) has found application to determination of trace and sub-trace metals [10, 12, 32, 40]. Inductively coupled plasma AES (ICP-AES) facilitates multi-elemental analysis [12, 32, 41, 42].

The determination of trace metals in wines usually has been done by means of flame atomic absorption spectrometry (AAS). A direct determination is seldom used because of the significant interferences of the wine matrix [33, 40]. The interferences of sugar and alcohol can be partially compensated by the standard addition method or by the standard solutions prepared in a matrix similar to the wine matrix [21]. However, most workers recommended some pretreatment of the wine samples: evaporation of alcohol, extraction of metals by complexing agents into organic solvents or decomposition of organic matter [10, 31, 35, 43].

From an analytical point of view, wine is a fairly complex matrix owing to the content of its organic compounds, above all ethanol, which strongly influences transport properties of the sample toward atomization devices due to changes in density and surface tension with respect to aqueous standard solutions [44]. Various types of interferences are to be expected in metal determination. In fact, atomic spectrometry techniques are generally little prone to interference caused by organic compounds due to the high temperatures involved in the atomization steps. Moreover, being fast and sensitive, they are without doubt the most suitable techniques in metal determination in wines [42].

Flame-AAS is largely employed in wine analysis mainly due to the low cost of instrumentation that makes the technique easily accessible to most oenological laboratories. Considering the compromise between the cost and sensitivity needed, flame-AAS can be considered the technique of choice for alkaline and alkali-earth metal determination in wine. It is also well suited for Cu, Fe, Mn and Zn determination, with respect to concentration ranges of these metals in wines. It is not suitable for toxic or undesirable elements like As, Cd, Cr, Hg and Pb, except for highly contaminated samples or in an application with pre-concentration procedures.

The official methods for the determination of heavy metals in wine are based mainly on atomic absorption spectroscopy although several chromatographic studies have discussed ion complexations in wine samples [10, 45, 46]. However, there are a limited number of studies concerning the speciation of metals in wine and particularly in must, although wine quality depends on must composition and vinification technology. In this respect, advanced voltammetry predominantly in the differential pulse mode has become one of the most significant and suitable methods for investigations of the level, fate and transfer of heavy metals [11]. No spectral interferences are reported in literature concerning metal determination in wine with flame-AAS. From these indications, it appears clear that external calibration can be performed for quantitative determinations [42, 47].

As an alternative technique, capillary electrophoresis can be readily applied to the analysis of both anions and cations. Capillary electrophoresis has important advantages with respect to other commonly used techniques: rapid analysis, versatility, determination of several analytes in a single analysis and good detection limits. Because of these advantages, several research reports have described the determination of cationic mineral elements potassium, sodium, calcium and magnesium in a variety of food matrices [13].

1.14.1. Digestion of wine

As a biological sample, wine is complex matrix comprising water, alcohol, sugars and other inorganic and organic compounds, so, for metal determinations without any initial pre-treatment, usage of ethanol-containing standards or the standard-addition method is recommended to minimize physical and chemical interferences [20].

Ca, K, Mg and Na are usually measured only after dilution of original samples with water [12, 21]. Al, Cd, Cu, Fe, Ni, Pb and Zn can be determined without dilution [23, 24], after dilution with HNO_3 and HCl solutions and/or water [33], or following acidification with concentrated HNO_3 [23]. Addition of HNO_3 (to pH 1.5) is especially encouraged in direct analysis, since it prevents bacterial proliferation and sample spoilage [42]. Metals bound to organic ligands are also readily released under these conditions. Normally, it is necessary to decompose the wine because of possible matrix interferences [10, 31]. This can be done by wet digestion on a hot plate or in a microwave oven using concentrated HNO_3 [42], a mixture of concentrated HNO_3 and 30 % H_2O_2 [33, 34, 41], 30 % H_2O_2 [39] or 30 % H_2O_2 followed by adding concentrated H_2SO_4 [48, 49] or HNO_3 [42, - 51]. To catalyze oxidation, V_2O_5 can be added to reaction mixtures.

1.15. Scope and benefits of the study

So far, there has not been a systematic and extensive study for the elemental content of Ethiopian wines. A study was made for the polyphenolic content of Ethiopian Red wines by Geto [52]. The present study involves the analysis of industrially produced and bottled Ethiopian export standards Red and White wines. Analyses were performed for the elements sodium, potassium, calcium, magnesium, iron, manganese, cobalt, nickel, chromium, copper, and zinc, which are essential and cadmium and lead, which are considered to be non essential or even toxic.

This research is expected to give the following benefits

- Indicating the levels of the essential metals, that are needed by our body and by the wine for best quality, in the Ethiopian wines so that consumers will have the information when drinking the wines in preferences,
- Indicating the level of toxic metals in the wines, to make the wine consumers be aware of the levels and to take great care for their drinking habits,
- Giving information for the wine makers and cultivators about the quality of the wines in terms of the elemental composition, and so improve their production methods if there are any deficits or excess level in the metals studied.

1.16. Objectives of the study

The objectives of this study are:

- To develop optimum digestion procedure for the wine samples.
- To determine the levels of essential and non-essential elements (K, Na, Ca, Mg, Fe, Zn, Mn, Pb, Co, Ni, Cd, Cu and Cr) in Axumite Red Wine, Gouder Red Wine, Awash Crystal White Wine, and Kemila White Wine.
- To compare the levels of minerals among the four brands of wines.
- To compare the levels of minerals in the Ethiopian wines with data obtained from different parts of the world.

2. Experimental

2.1. Instrumentation

A refrigerator (Samsung Hitachi, Japan) was used to keep the wine samples cool till the analysis. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 g precision was used to weigh chemicals and reagents. 250 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus (Kjeldahl, Gehardt Gmbh & Co KG Type KB 40 S, Bonn, Germany) hot plate to digest the wine samples. BUCK SCIENTIFIC MODEL 210 VGP (East Norwalk, USA) flame atomic absorption spectrophotometer equipped with deuterium ark background corrector was used for analysis of the analyte metals (Na, Mg, Mn, Ni, Pb, K, Ca, Fe, Zn, Cu, Co, Cr, Cd) using air-acetylene flame. A micropipette (100 μL-1000 μL) was used for the volume measurement of reagents and standards. Acetylene gas for the FAAS was obtained from Chora Gas and Chemicals Company, (Addis Ababa, Ethiopia).

2.2. Reagents and Chemicals

Reagents used in the analysis were all analytical grade. HNO_3 (69-72 %) (Spectrosol, BDH, England) and H_2O_2 (30 %) (BDH Chemicals limited, Poole, England) were used for the digestion of wine samples. Lanthanum nitrate hydrate (99 %) (Riedel-de-Haën, Seelze,Germany) was used as molecular suppressor (to release calcium and magnesium from their common phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Na, Mg, Mn, Ni, Pb, K, Ca, Zn, Cu, Co, Cr, Cd (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for preparation of calibration standards and in the spiking experiments. Working standard solutions were obtained by suitable dilution from stock solution. Iron(III) chloride anhydrous (99 %, AVOCADO Research Chemicals ltd., Heysham, Lancs, England) was used for preparation of callibration standards and spiking of iron. Deionized water was used throughout the experiment for sample preparation and dilution, and rinsing of apparatus prior to analysis.

2.3. Procedures

2.3.1. Cleaning apparatus

As this research is interested in the analysis of metals in the ppm level, the cleanness of any apparatus used is of great interest. This is because the contaminations from the apparatuses may contaminate the sample and so lead to false results and hence incorrect conclusions about the level of that specific metal in the sample under analysis. Therefore, cleanness of apparatuses was given due emphasis in this study.

Apparatuses such as volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionised water, soaked in concentrated nitric acid for 24 hours, then rinsed with deionised water, dried in oven and kept in clean place until needed for use.

2.3.2. Sampling techniques employed

Sampling is a very crucial step (procedure) in analytical laboratories. This is because the sample needs to be as much as possible, representative of the material to be analysed or studied. Sampling is the process of collecting a small mass of a material whose composition accurately represents the bulk of the material being sampled [53].

For this project from the most popular bottled Ethiopian wines (Axumite Red Wine, Gouder Red Wine, Awash Crystal White Wine, and Kemila White Wine) five bottles for each brand were collected randomly from five supermarkets (groceries) at different sites of Addis Ababa. By taking 100 mL from each bottle of the same brand four bulk samples (500 mL each) were prepared. The bulk samples were shacked slightly to ensure mixing.

2.3.3. Digestion of the wine samples

From the bulk samples prepared in section 2.3.2., 10 mL wine samples were wet ashed (digested) to decompose the organic substances and make clear solution in triplicate according to the HNO_3/H_2O_2 procedure described by Lazos and Alexakis [33, 54]. The digestion procedure was optimized by taking the following points in to consideration.

- The amount of H_2O_2 and HNO_3 added to the sample
- The digestion time
- The maximum temperature required
- The color and clearness of the digest

A 10 mL aliquot of the bulk sample was taken and quantitatively transferred to a 250 mL digestion flask. To this flask a freshly prepared 7 mL HNO_3/H_2O_2 mixture in the ratio of 2:5 was added. The flask was shacked slightly in order to mix the acids sample mixture. The flask with the acid-sample mixture was then placed on the hot plate of the Kjeldahl apparatus and fixed with the condenser. By setting the temperature first to 60 ^oC and slightly increasing up to 180 ^oC, the heating continued until the solution becomes clear and colorless. After the digestion is completed the flask with the digest was removed from the hot plate and was let to cool. The cooled digest was transferred quantitatively to 50 mL volumetric flask and diluted to the volume by deionized water. The digest was kept in the refrigerator until the analysis by AAS.

2.3.4. Atomic absorption spectrophotometeric (AAS) determination of the metals

Na, K, Ca, Mg, Fe, Cu, Zn, Co, Ni, Pb, Cd, Cr and Mn were determined by AAS using an air/acetylene flame. Major elements (Na, K, Ca and Mg) were analysed by further diluting the digested wine samples. Dilution was required to bring the concentration within the linear range of the calibration. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Na, K, Ca, Mg, Mn, Fe, Cu, Zn, Ni, Co, Pb, Cd, and Cr were used for the preparation of calibration standards and in the spiking experiments. All analyses were carried out using flame atomic absorption spectrophotometer (BUCK SCIENTIFIC MODEL 210 VGB, USA) equipped with deuterium ark background correctors, for analysis of the analyte of metals in the mode and at the wavelengths specific for each metal. Sodium and potassium were analyzed in the emission mode of the instrument. The instrumental operating conditions for determination of metals wine samples using flame atomic absorption spectrophotometer are given in Table 2.1.

2.3.5. Instrument calibrations

Calibration is a set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, and the corresponding values realized by standards. The usual way to perform calibration is to subject known amounts of the quantity (e.g. using a measurement standard or reference material) to the measurement process and monitor the measurement response [55, 56, 57]. Instruments such as chromatographs and spectrometers, which require calibration as part of their normal operation, should be calibrated using reference materials of known composition (probably solutions of pure chemicals).

For the preparation of calibration curve, a series of four working standards were prepared for each metal. The working standards were prepared daily from the intermediate standards, that were earlier prepared from the stock solutions (1000 mg/L). The concentrations of the working standards and corresponding intermediate are given in Table 2.1.

Table 2.1**.** Instrumental operating conditions for determination of metals in wine samples using flame atomic absorption spectrophotometer.

Element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Lamp current (mA)
K	766.5	0.010	0.7	2.0
Na	279.5	0.002	0.2	3.0
Ca	422.7	0.010	0.7	2.0
Mg	285.2	0.001	0.7	1.0
Cu	324.7	0.020	0.7	1.5
Zn	213.9	0.005	0.7	2.0
Mn	279.5	0.001	0.7	3.0
Ni	232.0	0.04	0.2	7.0
Fe	248.3	0.030	0.2	7.0
Co	240.7	0.050	0.2	4.5
Cr	357.9	0.050	0.7	2.0
Cd	228.9	0.005	0.7	2.0
Pb	283.2	0.100	0.7	2.0

2.4. Method validation study

Method validation makes use of a set of tests that both test any assumptions on which the analytical method is based and establish and document the performance characteristics of a method, thereby demonstrating whether the method is fit for a particular analytical purpose. Typical performance characteristics of analytical methods are: applicability, selectivity, calibration, trueness, precision, recovery, linear range, limit of quantification, limit of detection, sensitivity, and ruggedness. To these can be added measurement uncertainty and fitness-for-purpose. Strictly speaking, validation should refer to an "analytical system" rather than an "analytical method", the analytical system comprising a defined method protocol, a defined concentration range for the analyte, and a specified type of test material [56, 57].

2.4.1. Precision

The closeness of agreement between independent test results obtained by applying the experimental procedure under stipulated conditions. The smaller is the random part of the experimental errors which affect the results, the more precise is the procedure. A measure of precision (or imprecision) is the standard deviation.

In this paper, it is the repeatability that was determined in terms of precision (% relative standard deviation (% RSD)).

2.4.2. Recovery test

If a reference material is not available and there are no other methods appropriate for comparison, then accuracy of an analytical procedure is investigated by spiking, into a sample. By spiking a suitable known amount of the analyte, or a chemical containing the analyte, into a test portion of a sample having a known concentration of the analyte, the recovery of the analyte can be determined. This is achieved by analysing the spiked test portion along with the original sample [57].

For this research, in order to demonstrate the validity of whole analytical procedure, the recovery was done as follows: 200 mg/L of K, 25 mg/L Mg, 10 mg/L Ca, 8 mg/L Na from the 1000 mg/L were spiked at once in to 10 mL wine sample and the remaining metals (1.5 mg/L of Fe, 1.05 mg/L Zn, 0.5 mg/L of Cu and Mn and Zn; 0.075 mg/L of Ni and Cr; and 0.01 mg/L of Cd were spiked at once in to another round bottomed flask containing 10 mL wine. Then same digestion procedure was followed for non- spiked and spiked wine samples side by side. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer. The recovery test for all samples was performed in triplicates.

2.4.3. Method detection limit (MDL)

MDL is the lowest concentration of analyte that can be detected and reliably distinguished from zero (or the noise level of the system), but not necessarily quantified; the concentration at which a measured value is larger than the uncertainty associated with it. MDL can be expressed in response units and is taken typically as three times the noise level for techniques or three times the standard deviation of the sample blank [55].

The procedure proposed by Galani-Nikolakaki *et al.* [31] was used. Six blank samples were prepared. Absolute ethanol was added to deionised water in order to achieve a final alcohol concentration similar to that of the wine sample (12 % v/v). The six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Na, K, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu) by atomic absorption spectrophotometer. The standard deviation for each element was calculated from the six blank measurements to determine method detection limit. Then the method detection limit for each metal was calculated by multiplying the pooled standard deviation of eighteen measurements times three.

2.4.5. Analysis for significance of differences between means (ANOVA)

T-tests and analysis of variance (ANOVA) are widely used statistical methods to compare group means. Mean values obtained for the metals studied in the four brands of wine were compared by One-Way ANOVA at 95 % level using SPSS 13 for windows (SPSS Inc. 1989 - 2004) assuming that there were significant differences among them when the statistical comparison gives $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Instrument calibration

The qualities of results obtained for metal analysis using AAS are seriously affected by the calibration and standard solution preparation procedures. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared daily by diluting the intermediated standard solutions. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 3.1. The calibration curve of each of metals of interest is shown in Figure 3.1.

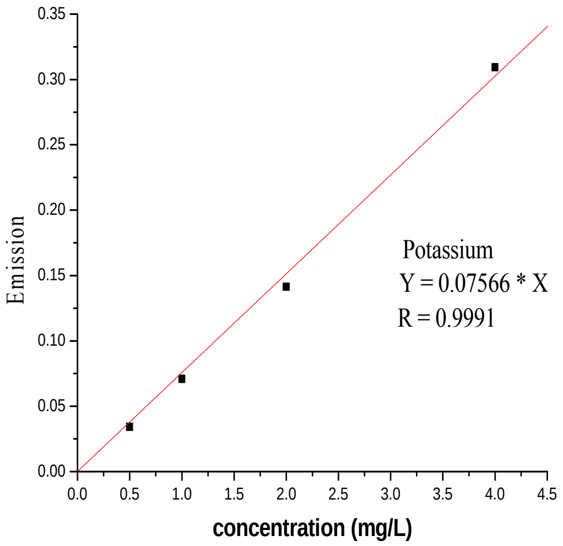

Figure 3.1a. Calibration curve of K standard solutions.

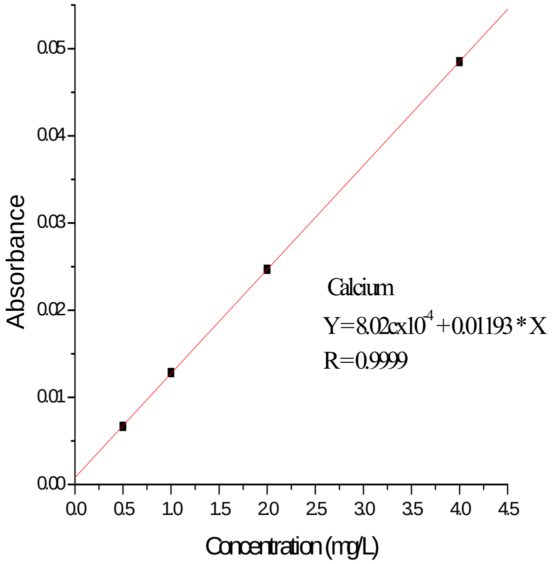

Figure 3.1b. Calibration curve of Ca standard solutions.

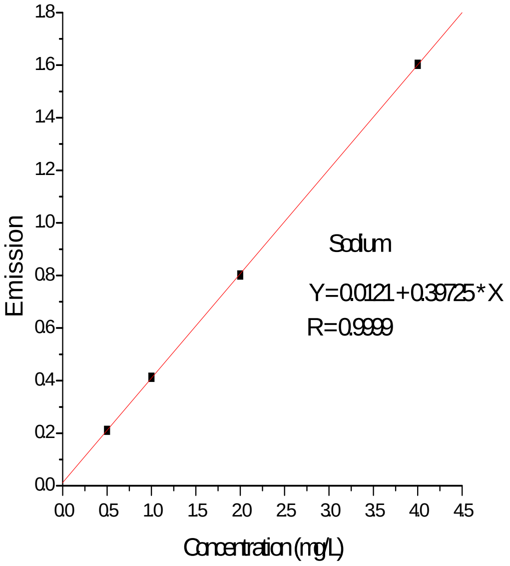

Figure 3.1c. Calibration curve of Na standard solutions.

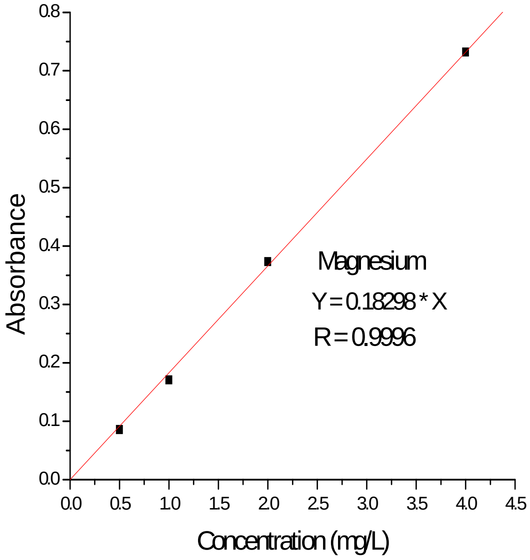

Figure 3.1d. Calibration curve of Mg standard solutions.

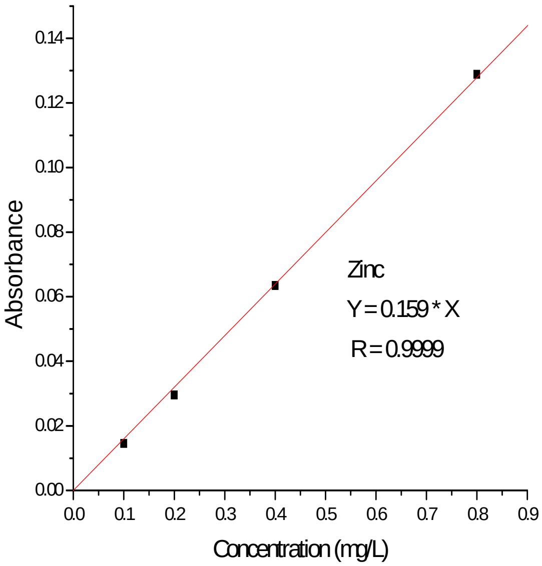

Figure 3.1e. Calibration curve of Zn standard solutions.

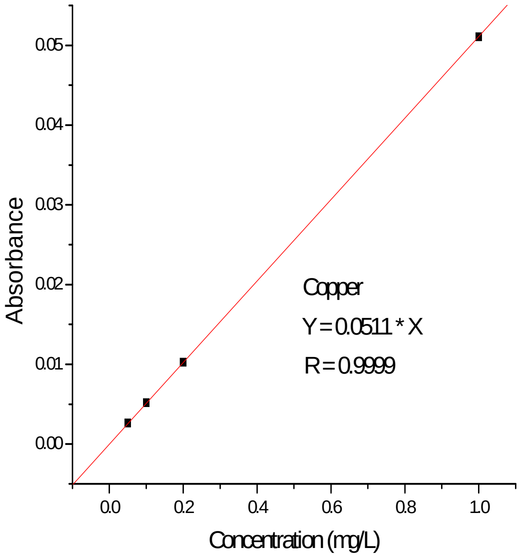

Figure 3.1f. Calibration curve of Cu standard solutions.

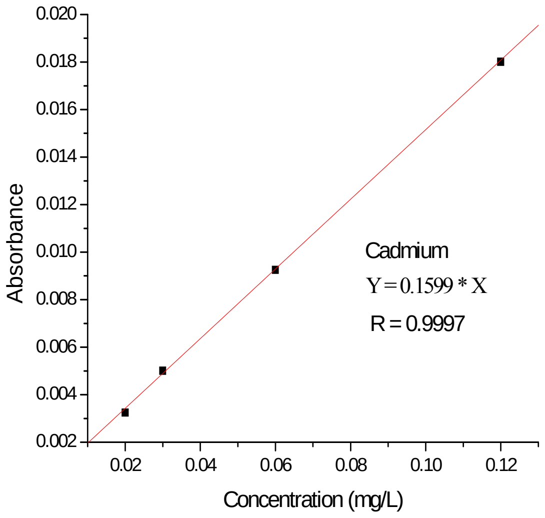

Figure 3.1g. Calibration curve of Cd standard solutions.

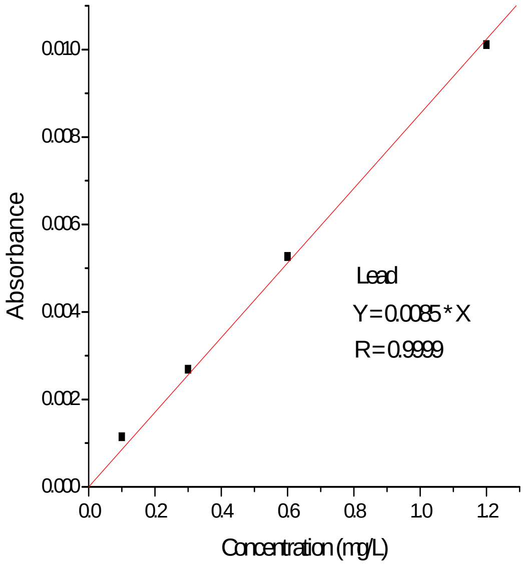

Figure 3.1h. Calibration curve of Pb standard solutions.

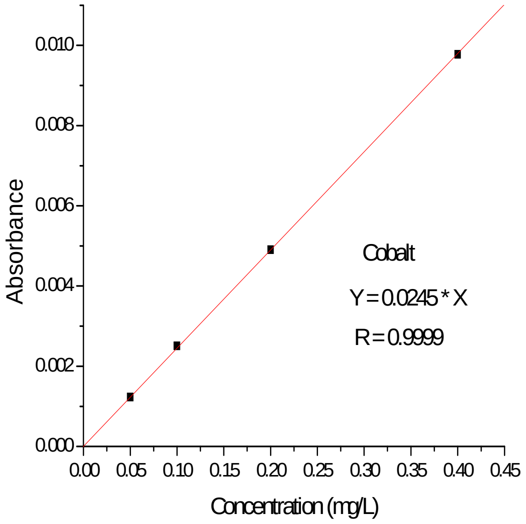

Figure 3.1i. Calibration curve of Co standard solutions.

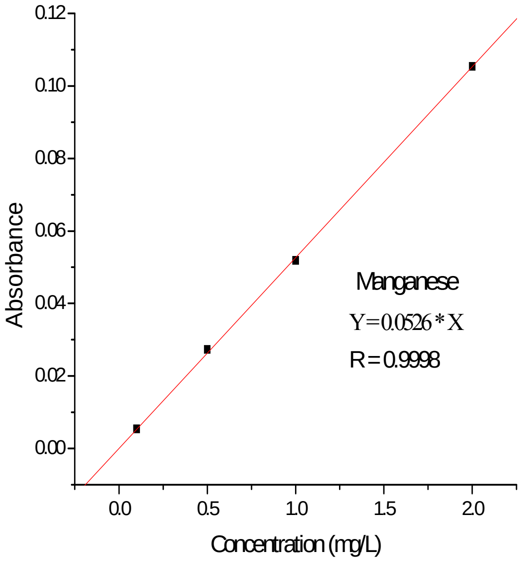

Figure 3.1j. Calibration curve of Mn standard solutions.

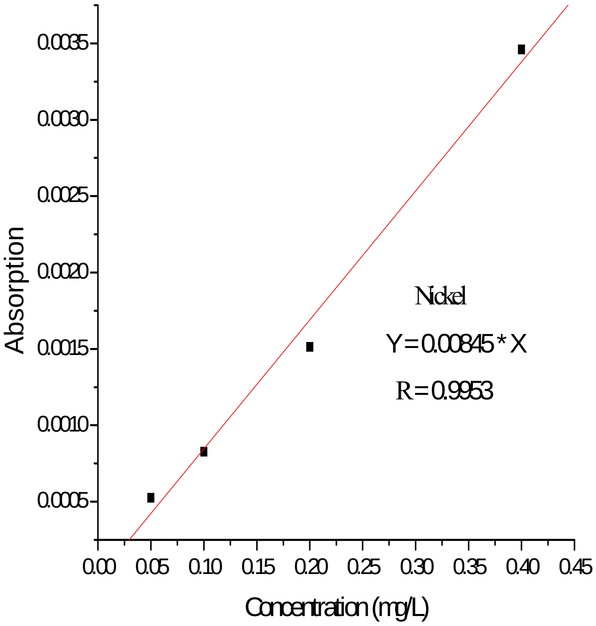

Figure 3.1k. Calibration curve of Ni standard solutions.

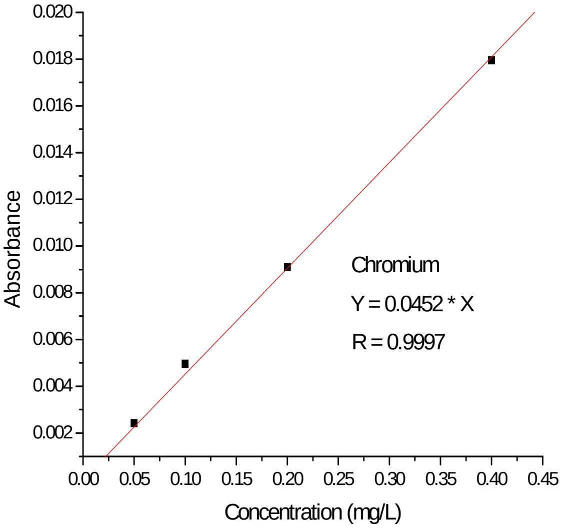

Figure 3.1m. Calibration curve of Cr standard. solution

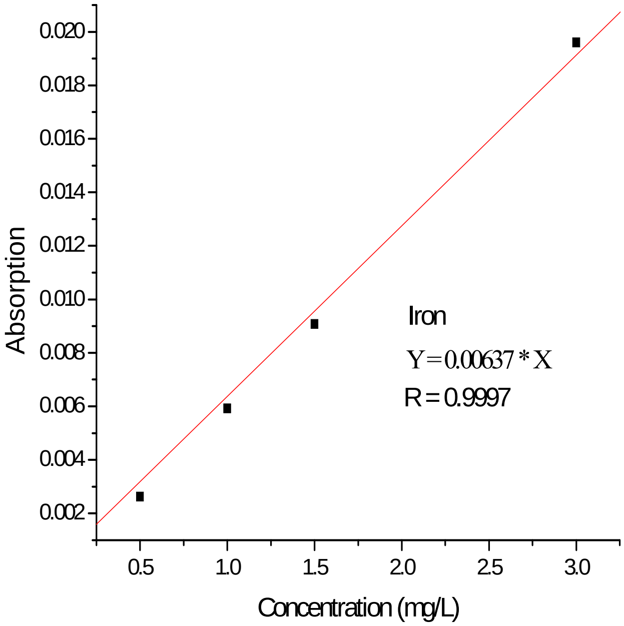

Figure 3.1l. Calibration curve of Fe standard solution

Figure 3.1 (a-m). Calibration graphs of the standard solutions of the metals.

Table 3.1. Working standards and correlation coefficients of the calibration curves for determinations of metals using flame atomic absorption spectrophotometer.

№	Metal	Concentration of intermediate standards (mg/L)	Concentration of working standards (mg/L)	Correlation coefficient of calibration curves
1	K	100	0.5, 1, 2, 4	0.9991
2	Na	100	0.5, 1, 2, 4	0.9999
3	Ca	100	0.5, 1, 2, 4	0.9998
4	Mg	100	0.5, 1, 2, 4	0.9996
5	Cu	10	0.025, 0.05, 0.1, 0.2	0.9998
6	Zn	10	0.1, 0.2, 0.4, 0.8	0.9997
7	Mn	10	0.05, 0.1, 0.2, 0.4	0.9998
8	Ni	10	0.05, 0.1, 0.2, 0.4	0.9953
9	Fe	10	0.5, 1, 1.5, 3	0.9997
10	Co	10	0.05, 0.1, 0.2, 0.4	0.9998
11	Cr	10	0.05, 0.1, 0.2, 0.4	0.9997
12	Cd	10	0.05, 0.1, 0.2, 0.4	0.9996
13	Pb	10	0.05, 0.1, 0.2, 0.4	0.9995

The correlation coefficients of all are > 0.999 except for Ni which is 0.9953, and these correlation coefficients show that there is very good correlation (relationship) between concentration and absorbance.

3.2. Optimization of digestion procedure of the wine samples

As wine consists of different matrix components that could affect the release and stability of the metals in it during AAS determination, there should be a means to destroy these matrix effects of the different organic components. The next question is how to do it. That means a procedure that is economical in terms of reagent consumption, time required, temperature, and the quality of the resulting solution. For the digestion of wine samples different researchers used different methods according to the wine sample origin. For this project a mixture HNO_3 and H_2O_2 were used and different combinations of the reagents, temperature, and time were looked for and the resulting solution was checked if it is clear and colorless. The combination or the method that needs minimum reagent consumption, minimum temperature and time of digestion, and gives clear and colorless solution was taken to be optimum condition. The trials during optimization and the result are given in the table 3.2.

Table 3.2. Optimization of digestion procedure.

Trial	Amount of Sample (mL)	Amount of reagents in the ratio of 2:5 HNO_3 : H_2O_2 (mL)	Maximum temperature needed (°C)	Total time taken (h)	Status of resulting solution
1	10	14	210	1:30	Clear & colorless
2	10	10	180	1:00	Clear & colorless
3	10	7	180	1:30	Clear & colorless
4	*10*	*7*	*180*	*1:00*	***Clear & colorless optimum***
5	10	5	210	1:00	Clear but yellowish
6	10	5	180	1:30	Clear but yellowish
7	10	14	210	1:00	Clear but yellowish
8	10	7	150	1:00	Clear but yellowish

10 mL of the wine sample was measured by the micropipette and quantitatively transferred to the 250 mL round bottom digestion flask. To this flask a freshly prepared 7 mL HNO_3:H_2O_2 mixture in the ratio of 2:5 was added. The flask was swirled gently in order to homogenize the sample-acid mixture. The flask was then placed on the hot plate of the Kjeldahl apparatus and fixed with the condenser. By setting the temperature first to 60 °C and then slightly increasing up to 180 °C, until the solution becomes clear and colorless. After the digestion is complete the flask with the digest was removed from the hot plate and let to cool. Finally the digest was transferred quantitatively to 50 mL volumetric flask and diluted to the volume by deionized

water. The digest was taken to AAS analysis as soon as digestion is complete.

3.3. Analytical figures of merit

Method validation is one of a number of concerted measures that analytical chemists can take to ensure that the data produced in the laboratory are fit for their intended purpose. In practice, fitness for purpose is determined by a comparison of the accuracy achieved in a laboratory at a given time with a required level of accuracy. Method validation therefore comprises the routine practical procedures that enable the analytical chemist to accept a result or group of results as fit for purpose, or reject the results and repeat the analysis [56]. There is a need to prove the quality of chemical analysis and so to demonstrate the reliability of measurements. Before reporting, the following operations were involved on the values obtained:
(a) removal of values lower than the method detection limit of (MDL),
(b) removal of statistically remote values (values which are out of the action limit, i.e. mean ± 3SD).

3.3.1. Precision

Precision is closeness of agreement between independent test results obtained under prescribed condition [56]. Precision can indicate two ideas; these are repeatability and reproducibility. The first term indicates within-run precision [57] whereas the latter term indicates the between-run precision. **Repeatability** is a term used when independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. And the measure of the precision can be expressed in terms of variance, standard deviation, and relative standard deviation. For this study the precision of the analytical method was evaluated in terms of pooled standard deviations at 95 % confidence limit and relative standard deviation (% RSD) of the eighteen measured values of one bulk sample.

3.3.2. Method detection limit

The method detection limit can be described as the concentration of the analyte of concern that gives a signal significantly different from the signal of the blank or background signal. The general accepted definition of method detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this study the detection limits were calculated as the concentrations that give signals equal to three times the pooled standard deviations of the six blanks, and are given in Table 3.3.

Table 3.3. Method detection limit of the metals in this analytical procedure.

Metal	K	Na	Ca	Mg	Cu	Zn	Mn	Ni	Fe	Co	Cr	Cd	Pb
MDL (mg/L)	0.09	0.13	0.26	0.15	0.026	0.048	0.02	0.121	0.18	0.065	0.075	0.014	0.12

From the table it can be seen that the method detection limits for all the metals are in the μg/L levels and allow the detection of trace levels of metals in the wine samples.

3.3.3. Recovery study

The validation (accuracy) of the optimized procedure and the whole analysis method should be checked for its validity. That is if the whole procedure is really working for the purpose it is intended for. Method validation studies can be done in different ways like analyzing certified reference materials under the same procedure done for the sample. Then comparing the concentration found with that of given or certified. The other way of checking the method validation is by the added and found or “spiking” method. "Spiking" is a way of creating a control material in which a value is assigned by a combination of formulation and analysis. This method is feasible when a test material essentially free of the analyte is available. After exhaustive analytical checks to ensure the background level is adequately low, the material is spiked with a known amount of metal [58, 59]. The reference sample prepared in this way is thus of the same matrix as the test materials to be analysed and of known analyte level - the uncertainty in the assigned concentration is limited only by the possible error in the unspiked determination. However, it may be difficult to ensure that the speciation, binding and physical form of the added analyte is the same as that of the native analyte and that the mixing is adequate [56, 60].

For this research as the certified reference material for wine samples were not available, the accuracy of the method was checked by recovery test. So as indicated in Table 3.4 the % recovery obtained was between 94 and 104, which shows the procedure had good accuracy to state that the method is valid for the purpose it is intended for, i. e for the determination of the mentioned metals in the wines.

Table 3.4. Recovery test for the metals.

Metal	*Determined value (mg/L)	Spiked amount (mg/L)	*Found in spiked (mg/L)	§ % Recovery
K	767 ± 15	200	961 ± 14	97± 2.7
Mg	79.2 ± 5.5	25	104 ± 5.3	99.2 ± 2.6
Ca	37.1 ± 2.8	10	46.8 ± 2.0	97 ± 1.8
Na	24.4 ± 2.1	8	32.0 ± 2.5	95 ± 1.2
Fe	3.16 ± 0.25	1.5	4.6 ± 0.22	96 ± 2.9
Zn	2.14 ± 0.15	1.05	3.15 ± 0.14	96.2 ± 0.35
Cu	1.46 ± 0.034	0.5	1.97 ± 0.042	102 ± 0.1
Mn	1.5 ± 0.078	0.5	1.97 ± 0.036	94 ± 0.85
Pb	0.25 ± 0.019	0.1	0.35 ± 0.022	100 ± 0.012
Cr	0.19 ± 0.015	0.075	0.264 ± 0.018	98.7 ± 0.2
Ni	0.19 ± 0.0101	0.075	0.267 ± 0.011	102.7 ± 0.11
Cd	ND	-	-	-
Co	ND	-	-	-

* Mean ± ts/√N, at 95 % confidence limit, t = 2.31, and 8 degrees of freedom

§ mean ± SD of the means

3.4. Levels of the Essential and Non-essential Metals in the Wine Samples

Official methods for the determination of heavy metals in wine produced by the Office International de la Vigne et du Vin and the American Society of Enologists are essentially based on atomic absorption spectrometry [52]. The levels of essential and non-essential elements (K, Ca, Na, Mg, Fe, Mn, Ni, Pb, Zn, Cu, Co, Cr, and Cd) were determined by air-acetylene flame atomic absorption spectrophotometer in the wine samples that were digested and diluted in deionized water.

The values are given in Table 3.5. As can be seen in the tables, from the analyzed metals Cr and Cd in the samples except for Axumite Red Wine were found to be below the limit of detection of the method. Co in Awash Crystal and Axumite was also below the method detection limit. From the major metals potassium was found to be the highest level in all brands of wine, followed by magnesium, calcium, and sodium. From the minor and trace heavy metals, iron and zinc are prominent. manganese and copper take the following order. Cr and Cd are found in very small amounts compared from the other metals in only one brand of wine that is Axumite Red Wine.

Table 3.5. Level of the metals in the Ethiopian Red and White wines.

Metal	Axumite red wine		Awash Crystal White Wine		Kemila White Wine		Gouder Red Wine	
	*Concentration (mg/L)	% RSD	*Concentration (mg/L)	% RSD	*Concentration (mg/L)	% RSD	*Concentration (mg/L)	% RSD
K	767 ± 15	2.3	694 ± 15	2.7	764 ± 19	3.0	73 ± 13	2.2
Mg	79.2 ± 5.54	8.5	66.0 ± 5.0	9.3	77.6 ± 5.6	8.8	58.1 ± 4.3	9.1
Ca	37.1 ± 2.8	9.2	28.4 ± 2.2	9.6	31.6 ± 2.3	9.1	28.4 ± 2.5	10.8
Na	24.4 ± 2.1	10.3	24.3 ±1.7	8.4	24.4 ± 1.6	8.3	24 ± 2.0	10.3
Fe	3.16 ± 0.25	9.7	1.49 ± 0.06	4.93	1.42 ± 0.075	6.5	2.33 ± 0.15	7.9
Zn	2.14 ± 0.15	8.6	2.7 ± 0.15	6.8	2.4 ± 0.184	9.4	1.82 ± 0.125	8.4
Mn	1.46 ± 0.034	2.8	1.56 ± 0.12	9.4	1.88 ± 0.15	9.8	1.04 ± 0.019	2.2
Cu	1.5 ± 0.078	6.4	0.55 ± 0.042	9.35	0.61 ± 0.036	7.3	0.5 ± 0.026	6.4
Pb	0.25 ± 0.0189	9.25	0.16 ± 0.012	9.18	0.31 ± 0.021	8.3	0.14 ± 0.005	4.4
Cr	0.192 ± 0.015	9.53	ND	---	ND	---	ND	---
Ni	0.19 ± 0.010	6.5	0.19 ± 0.011	7.21	0.18 ± 0.013	8.8	0.2 ± 0.004	2.5
Cd	ND	---	ND	---	ND	---	ND	---
Co	ND	---	ND	---	0.091 ± 0.007	9.4	ND	---

*Analytical values of all metals expressed as mean ± $ts/\sqrt{N}$ calculated for nine measurements, taking $t = 2.31$ at 95 % confidence limit ($p<0.05$), for eight degrees of freedom.

ND- Concentration of the metal was found to be below the method detection limit (Cr = 0.075, Cd = 0.014, Co = 0.065 mg/L)

3.4.1. The level of major metals in the wine samples

As the presence of the major metals (K, Na, Mg, and Ca) affects the stability of the finally bottled wine, their concentrations determination is one of the most common analyses in wine laboratories [58], to make their level in the suitable range. This range for the major metals is not as restrict as for the toxic metals from the health point of view, but for the enology of the wine, the interest for them is as great as the toxic metals. And there are many studies concerned with the study of those metals in wine.

In all the four brands of the analysed Ethiopian wines the major metals; K, Ca, Mg, and Na are found in appreciable amounts as expected to be found. The average contents of the major elements in the studied wines decreased in the order K (7340) > Mg (70.22) > Ca (31.4) > Na (24.24), all in units of mg/L. From the major metals, the level of potassium is the highest of all the metals followed by magnesium. This is the because of the rich concentration of K in grapes, which is derived from the soil and also from application of potash fertilizers. From the four brands of the Ethiopian wines Axumite Red wine has the highest potassium contents, followed by Kemila White wine, Gouder Red and Awash Crystal white wines.

Potassium is one of the elemental constituents of wines found most abundantly, and its level is dependant on the grapes variety, soil and climatic conditions, time of harvest and other variables such as the temperature of fermentation and storage, the pH. So its levels are different according to the type of wine and their geographical origin [11, 12]. When the concentration of Potassium is higher (thousands), Potassium hydrogen tartrate precipitate could result [11].

In this study, from all the metals the level of K is found to be the highest in all wine brands, as consulted in the literature. This result confirms that potassium is the natural component of grapes and its concentration is highest than any metal in wines. The range of the level of potassium in the wine samples is from 693 mg/L to 766 mg/L. Even though there is difference in the value, between the studied wine types, potassium was found to be the highest level in all the wine brands. This indicates that those studied Ethiopian wines could be good sources of potassium. And these high values are not in the ranges of thousands to affect the color of the white wines by forming precipitates. The level of the major element potassium is also affected by the fermentation process; potassium is released and consumed by yeasts during fermentation [18].

Magnesium plays a role in wines by taking part in regulating the cellular metabolism of yeasts by maintaining adequate pH and ionic balance [10]. The determination of magnesium in wines has more importance than just its role in the wine, because its high level also affects the health for those with kidney dysfunction, even though this is at very high level than found in wines [10].

In the Ethiopian wine types studied now, magnesium is the second in its concentration independent of the wine Brand. Magnesium is found in range of 58 mg/L - 79.2 mg/L. None of the concentrations of the metal magnesium is in the risk full range.

Sodium is found in the wine samples nearly equally (~24 mg/L). And this value is below the maximum permissible limit placed by the International Organization for Grapes and Wine, 60 mg/L. Bentonite and other additives, employed for the purification of wines from tarnishing components, can constitute an important source of base cations and/or rare-earth elements [59]. This lower level of sodium in the Ethiopian wines was as expected because the farms are very far from seas and oceans, so free from sodium salt addition from sea water.

Calcium concentration in wines can be affected by the traditional practices of de-acidification ($CaCO_3$ addition) or plastering ($CaSO_4$ addition). Elevated level of this metal can lead also to the start of calcium tartrate precipitation [12]. Elevation of calcium levels tends to result in suppression of fermentation, presumably by interfering with the cellular uptake of magnesium [19]. Calcium level in wines is also affected by the fermentation process like potassium does [18].

Calcium is the third metal in its abundance among the analysed wine samples, which is found in the range of 28 – 37 mg/L, this concentration is not as high as to interfere with the uptake of Mg. And these values may show that there are no as such exogenic sources of the metal during fermentation or viticulture as a whole. The comparison of the four major metals among the four brands of wines is shown in Figure 3.2.

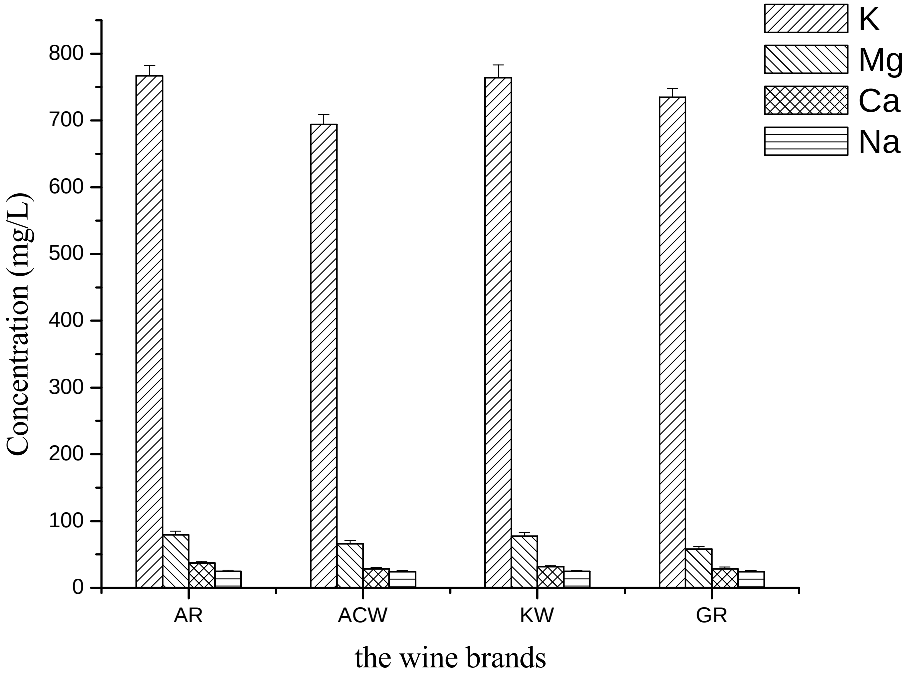

Figure 3.2. The average level of the major essential metals in the Ethiopian White and Red wine brands. Where AR= Axumite Red, ACW=Awash Crystal White, KW=Kemila White, and GR=Gouder Red

3.4.2. The levels of essential minor metals (Fe, Cu, Zn, Mn) in the wines analysed

Iron, Copper, Zinc and Manganese are micronutrients needed in low quantities by yeast during fermentation, but are also important since they take part in maintenance and function of biological processes. Some of them make up the prosthetic group of certain enzymes called metallo-enzymes. Copper, zinc, and iron contents in wine are monitored due to their effects on the organoleptic properties of the wines [59]. Grapes accumulate small amounts of the heavy metals by translocation from the roots or by direct contact from vineyard sprays, unless they are exposed to significant air pollution. The heavy metals are normally absorbed into the yeast cell membrane during fermentation. Increased concentrations of these metals in wines are results of contamination during post fermentation processing. The average contents of trace elements in the studied Ethiopian wines decreased in the order Zn (2.26) > Fe (2.1) > Mn (1.5) > Cu (0.79) all in units of mg/L.

Copper, up to content of 0.5 mg/L, is desirable in wines. But above this value cupric cloudiness (wine browning) is produced in white wines [21]. Copper is present in wine in trace amounts and acts as a catalyser in metabolic processes taking place in wine; more specifically it catalyses the synthesis of the auxines. A copper deficit in chlorophyll can induce its decomposition and decrease the yield of the vineyard [62]. To minimize the incidence of wine browning because of copper, it is generally recommended to maintain copper concentration below 0.3 – 0.5 mg/L [11].

In this study it is found that copper concentration in the Ethiopian wines is from 0.5 -1.5 mg/L. which is slightly higher than recommended for copper desirability in wine. Copper is found to be the least from the four metals (i.e. Fe, Cu, Zn, Mn); in the three wines except in Axumite (in which Cu ranked third above Mn).

Iron is one of those metals that actively participate in the browning of white wines. The level in which this metal is present in white wines depends on many factors, such as absorption from the soil, contamination by atmospheric emissions, herbicidal treatments, the wine-making process, and the addition of fining agents [20]. Evaluation of iron content in wines is of major importance either due to the changes in stability it may cause or to its effects on the oxidation and wine aging [12, 61].

The content of Fe(III) ion (above 7 mg/L) can give unpleasant, astringent ferric taste and be responsible for producing ferric cloudiness, especially when the content of tannic substances or pH is high [21]. However, this effect does not occur when wine contains a majority of Fe(II) ions [10]. At concentrations above 10 mg/L iron creates insoluble suspensions which are known as hazes [11].

The concentrations of iron in the studied Ethiopian wines are from 1.42 mg/L to 3.16 mg/L; the level of iron is not so greater as to create test problems and cloudiness in the white wines. Iron is found to be high in the Red wines and from the four metals compared (i.e. Fe, Cu, Zn, Mn), but the third in the white wines studied.

Zinc level in wines becomes high when zinc containers are used during the processing and ageing of wines [27], and also when zinc containing pesticides are used [12, 47]. Heightened content of zinc may influence negatively at the course and products of the yeast metabolism during the fermentation of alcohol (formation of acetaldehyde) [27].

The level of Zinc in the analyzed Ethiopian wines is in the range of 1.82 - 2.7 mg/L. This makes the first most abundant trace metal in the analysed Ethiopian wines followed by iron (1.42 mg/L to 3.16 mg/L). From the four metals (i.e. Fe, Cu, Zn, Mn); Zn is the second next to iron in the Axumite and Gouder Red wines, but it is the highest in the Awash Crystal and Kemila white wines.

Manganese is responsible for changes in stability of old wines and modification of the sensory quality of wines after bottling [10]. The Ethiopian wines are found to contain manganese content of values between 1.04 mg/L and 1.88 mg/L. Manganese is found to be the second highest metal in the white wines and the third in the Red wines studied, from the four metals compared. The graph for comparison of the four metals in the wines analysed is shown in Figure.3.3 to 3.6.

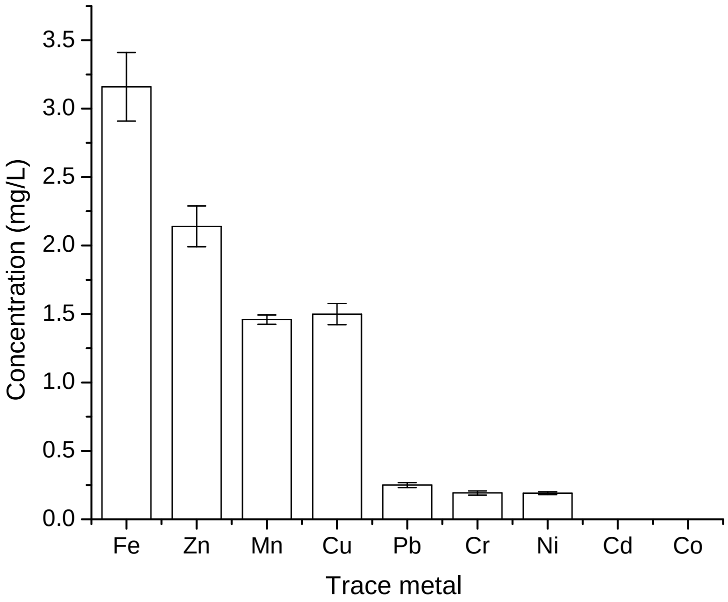

Figure 3.3. The average level of minor and trace metals in the Axumite Red wine brand.

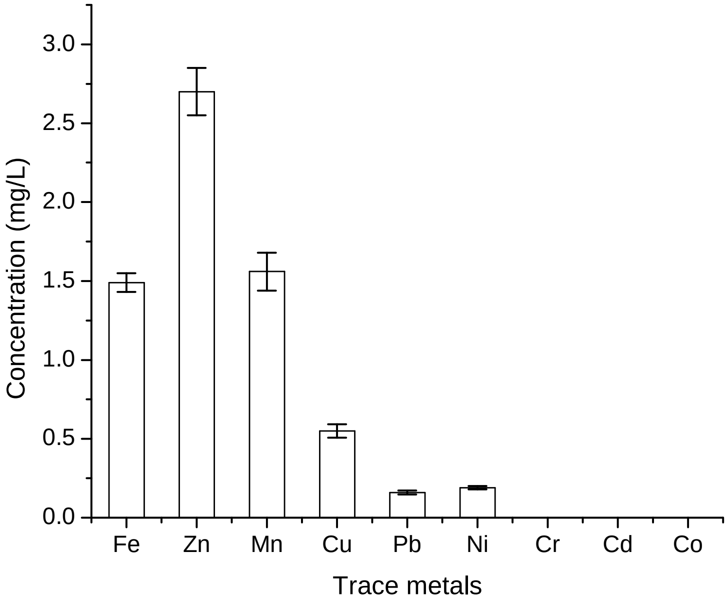

Figure 3.4. The average level of minor and trace metals in the Awash Crystal white wine brand.

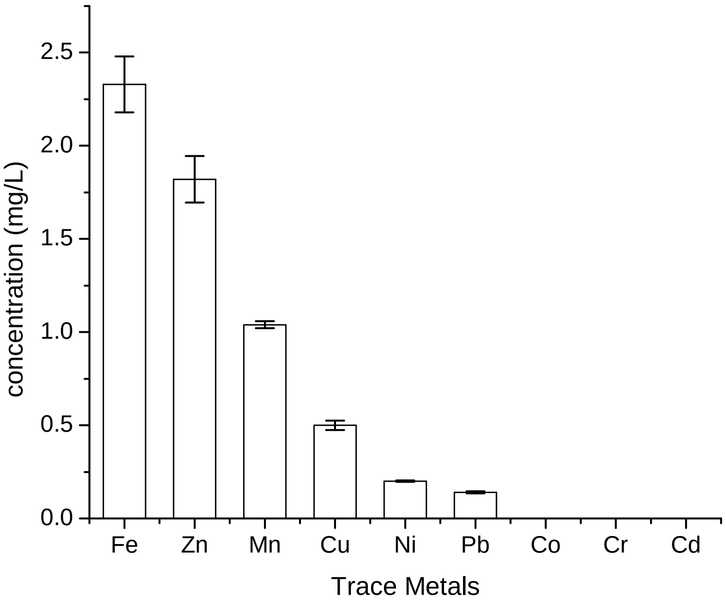

Figure 3.5. The average level of minor and trace metals in the Gouder Red wine brand.

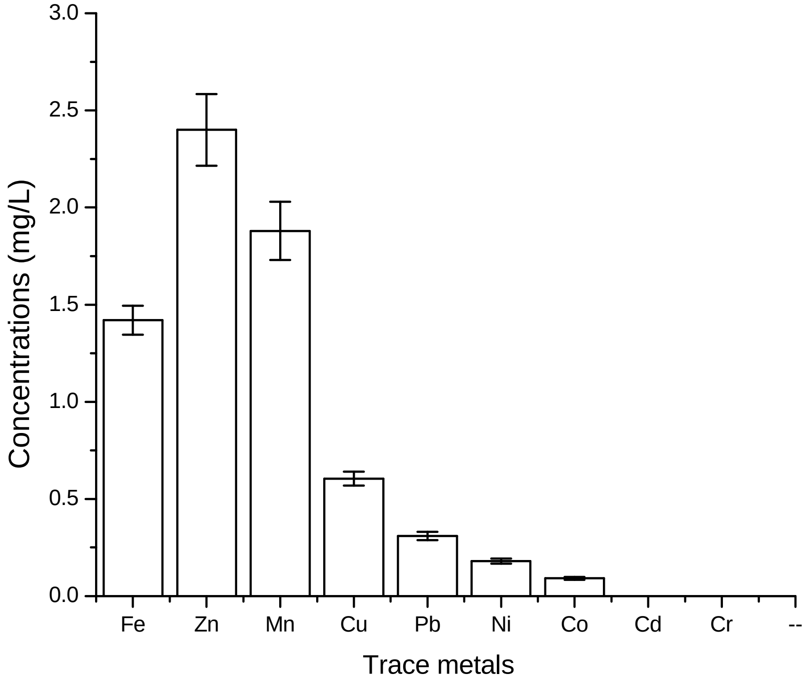

Figure 3.6. The average level of minor and trace metals in the Kemila white wine brand.

3.4.3. The levels of the essential trace metals (Ni, Co, Cr) and the toxic trace metals (Pb and Cd)

Nickel, in wines of modern cellar technology is due to the use of Ni-containing stainless-steel containers for wine fermentation and storage [52]. This metal is present in wines in very low concentrations. Studies showed that Ni content was generally constant during industrial processing and storage of wine [33, 62]. Nickel, contributes to haze formation and sometimes colour, aroma or taste effects.

In this study it is found that the level of nickel is in the range of 0.18 – 0.2 mg/L. From the other five trace metals (Ni, Co, Cr, Pb, and Cd), nickel takes the second rank in average, next to lead.

Cobalt in the analysed Ethiopian wines was found to be below the method detection limit (< 0.065 mg/L), except for one wine brand that is Kemila white wine, in which the level of cobalt was 0.091 mg/L.

Chromium levels were found to increase from 2 to 10 fold in finished wines in relation to chromium concentrations in musts. Chromium, contribute to haze formation and sometimes affect colour, aroma or taste. In fact, the initial concentration of chromium in grapes can be affected by the technology involved in wine production [25].

In the studied Ethiopian Red and White wines chromium was not detected except in one brand (Axumite Red wine) which is equal to 0.192 mg/L. To say this value is high or low the literature search didn't give any limit, it was not possible to comment on its level. Further study should be conducted in order to identify which species (i.e. Cr(III) or Cr(VI)) is found at what level.

The **Cadmium** level in wines can be of technological origin due to residues of agrochemical products such as insecticides and fungicides, which can contain Cd. Phosphatic fertilizers, are known to have higher concentrations of Cd [59]. The maximum allowable concentration of Cd in wine is 0.01 mg/L [24]. Cadmium was below the method detection limit in the Ethiopian wines under analysis.

Lead, a positive correlation was reported between wine age and Pb concentration. It was concluded that the main contamination source was brass. Brass pipes and taps were always found in wineries known for elevated Pb levels [49]. Many studies have shown that wine can contain higher amounts of lead (Pb) than many other beverages [23].

Lead concentrations in the Ethiopian wines are found in the range of 0.14 - 0.31 mg/L, some values are some how larger than the limit set by the O.I.V (International Organization of Grapes and Wine), as 0.2 mg/L. This may be caused by the pipes used in vinification and the water used having larger concentrations of lead. From Figures 3.3 – 3.6, for comparison of the trace metals Pb, Ni, Co, Cr, Cd; it is clear that lead is the highest in the Axumite Red and Kemila White wines and second in the Awash Crystal white and Gouder red (in which lead ranked next to Nickel).

3.5. Comparisons of the levels of the metals between the four brands of Ethiopian Wines

The level (concentration) of metals in wines is highly affected by the soil type on which the grapes are cultivated, which means on the geographical location of the grapes from which the wines are produced. Others like usage of fertilizers, pesticides, and herbicides also affect the metallic content of the wines. The third cause for the increase or decrease of metal contents in wines could be the location where the grape farm is found, with respect to nearness to oceans that could contaminate the vineyard by sodium. During vinification, the wines' metal content could be affected by the type of water, the components of the pipes, barrels and other materials involved in the process. So in the explanation for the differences in the metal content of the wines from the four brands, the above mentioned factors for the metal levels of wines could be taken in to consideration.

3.5.1. Comparison of the major metals (K, Ca, Mg, and Na) among the four brands

Concentration of the major metals in wines is dependent on the natural components of the grapes used to make the wine; the viticulture has little effect except the addition of some additives in fining and haze prevention. So the difference in the major metals concentrations is mainly dependant on the grape variety and the geographical location of the grapes cultivation.

From the four brands of wines, Axumite Red wine contains the highest level of K (766.76 ± 1.67 mg/L), followed by Kemila White (764.33 ± 2.44 mg/L), the third higher level is that of Gouder Red (735.14 ± 4.42 mg/L). And the least level of potassium out of the four brands is that of Crystal White (693.64 ± 2.48 mg/L). So the order is Axumite > Kemila > Gouder > Crystal, at the specified level of confidence. Figure 3.7 shows the level of the major metals in the four studied wines (i.e. Axumite Red (AR), Gouder Red (GR), Awash Crystal White (ACW), and Kemila White (KW)). The values are given at 95 % confidence level.

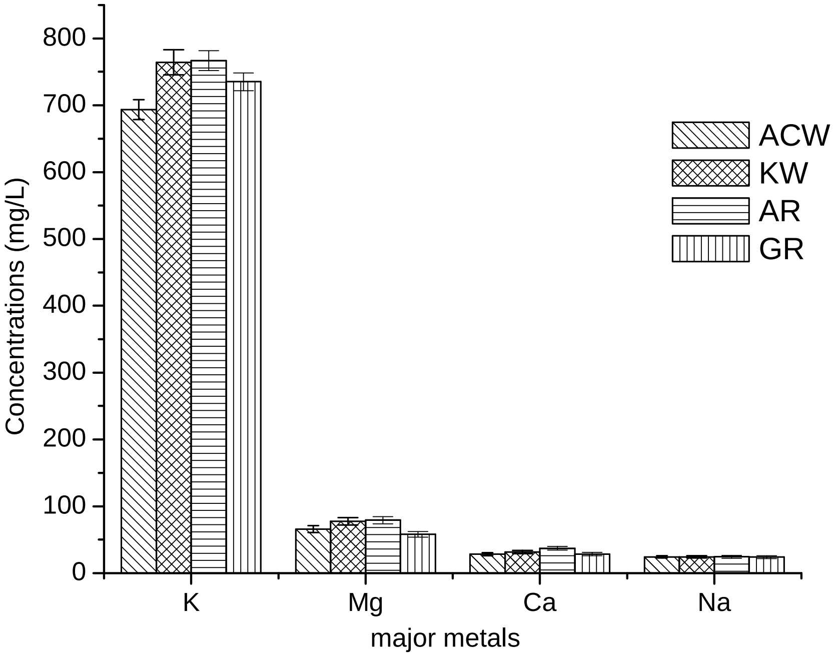

Figure 3.7. Graph comparing the level of the major metals among the four Ethiopian wines.

The mean values for magnesium in the four wines studied were in a range of 58.21-79.21 mg/L. The range (21) for the level of magnesium among the four brands of the Ethiopian wines is smaller when compared with the range (73) of potassium. Magnesium, generally does not cause a stability problem in wines, magnesium may be toxic to persons with kidney dysfunction, but at a level above that is normally found in wine [33]. The order in magnesium content of the wine brands is Axumite (79.2) > Kemila (77.6) > Crystal (66.0) > Gouder (58.1), all in units of mg/L.

Contribution to the calcium content in wines may be from calcium bentonite, like sodium bentonite, that is added to remove wine haze. Calcium ions can combine with oxalates and tartrates, which may precipitate after bottling [33].

In their calcium content the four wine brands can be arranged in the following order of Axumite (37.1) > Kemila (31.6) > Gouder = Crystal (28.4) all in mg/L. The calcium contents of the four wine brands varied in smaller range (8.67) in comparison with the Mg and K ranges.

The sources of sodium in wines, apart from natural concentration, are the addition of sodium salts such as sulfite, sorbate and sulfide [13]. The sodium levels found in wines from the four brands varied inconsiderably, with an average value of 24 mg/L. And the order was Kemila (24.4) > Crystal = Axumite (24.3) > Gouder (24.0) all in mg/L.

One should bear in mind that, the composition of metals in wine during fermentation, maturation and storage is not stable. In fermented wines, metal concentrations are lower than those in respective grape juices and musts, due to precipitation of K and Ca tartrates or other insoluble precipitates of Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn [10].

3.5.2. Comparison of the metals Fe, Cu, Zn, and Mn among the wine types analysed

The micronutrients Fe, Zn, Mn and Cu are needed in less quantity by yeast, but are also important since they take part in maintenance and function of biological processes. Some of them make up the prosthetic group of certain enzymes called metalo-enzymes. The presence of metals in wine notably influences its organoleptic quality [15]. A significant part of Cu, Fe, Mn and Zn is removed by adding $K_4Fe(CN)_6$ and precipitating the respective ferrocyanides to prevent wine from being susceptible to browning and formation of metal cloudiness [10, 63].

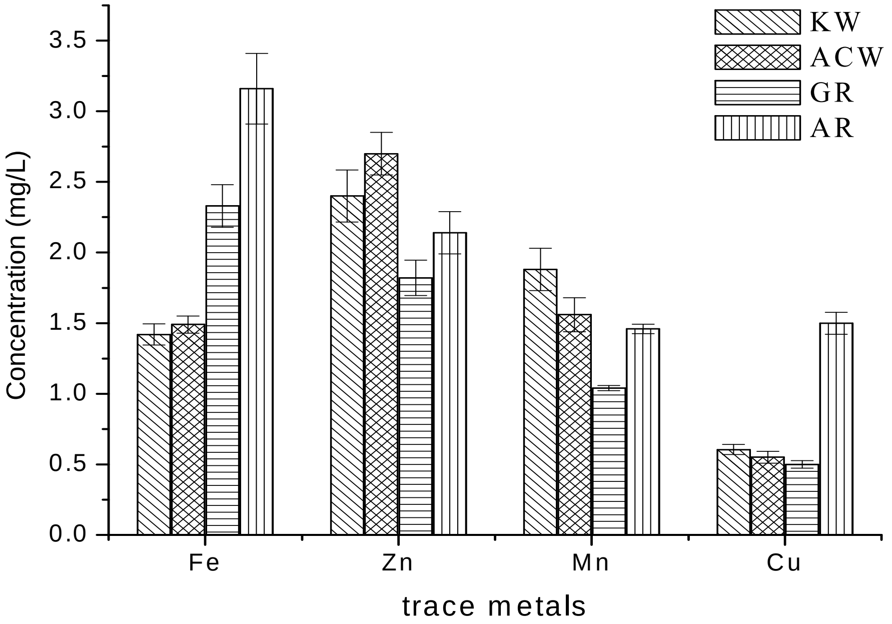

Figure 3.8. Graph comparing the level of essential trace metals among the four Ethiopian wines.

All the wines studied were determined to contain iron below 7 mg/L (mean value was 2.1 mg/L), considered to be the very low level to form ferric cases. The order in the iron content of the wines analysed was as follows; Axumite contains the highest (3.16 mg/L), Gouder takes the next rank (2.33 mg/L), and the last two are by the white wines, Crystal (1.49 mg/L) and Kemila (1.42 mg/L). So as can be seen from the figure 3.8, the white wines which are affected by high level of iron, are free from this because the level of iron is lower than the value responsible for white wines cloudiness.

The order in the level of copper in the four wines is Axumite (1.5) > Kemila (0.605) > Crystal (0.55) > Gouder (0.5) in the units of mg/L. All the wine samples except for Axumite have Cu contents below the 1 mg/L recommended by OIV to prevent formation of cupric cases. But all the levels of copper in the wines in this study are in the region of possible formation of cloudiness in white wines. New wines normally contain very small amounts of copper, 0.1-0.3 mg/L, copper in higher concentrations is due to accidental contamination of the wine, or prolonged contact with copper surfaces, although the content decreases considerably (80-95%) during fermentation, copper can also form precipitates; cloudiness occurs in wines containing > 0.2-0.4 mg/L [33].

In the case of zinc, all the samples presented a content of below 2.7 mg/L, much lower than 5 mg/L, the amount recommended by the OIV. Presence of zinc in wines may be originated from zinc-containing pesticides in addition to transport from the soil. In this study the level of zinc was in the range 1.82 - 2.7 mg/L. The order in the level of zinc in the wines studied was as follows; Crystal (2.7 mg/L) > Kemila (2.4 mg/L) > Axumite (2.14 mg/L) > Gouder (1.82 mg/L). Application of pesticides, fungicides and fertilizers containing Zn compounds during the growing season of vines leads to increases in the amounts of this metal in wine [10].

Manganese in small amount is a natural constituent of grape and wine. Manganese is among the metals most actively participating in the browning of white wines. The quantities in which this metal is present in white wines depends on diverse factors, such as absorption from the soil, contamination by atmospheric emissions, herbicidal treatments, the wine-making process, and the addition of fining agents [64].

The determined values for manganese in the Ethiopian wines are between 1.04 mg/L and 1.88 mg/L. Kemila was found to contain the highest level of manganese (1.88 mg/L), Crystal takes the next rank by having Mn content of 1.56 mg/L, followed by Axumite and Gouder having Mn level of 1.46 mg/L and 1.04 mg/L, respectively.

3.5.3. Comparisons of the metals Pb, Ni, Co, Cr, and Cd among the wine types analysed

The determination of cadmium, lead, copper and zinc in wines is of great interest from both enological and toxicological points of view; moreover, correct knowledge of these parameters is required by law. The amount of lead in wine has received extensive research due to its significance to public health and its exogenous origin. Grapes contain only traces of lead naturally, but they can be contaminated from fungicides, dust, car exhaust fumes and industrial pollution. Some lead is lost during alcoholic fermentation and much is removed during blue-fining treatment with ferrocyanide [33, 65].

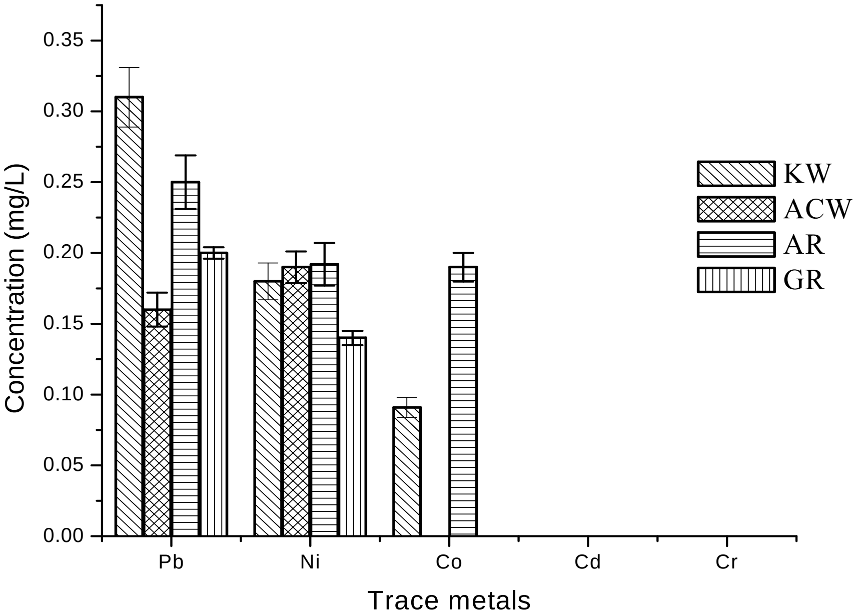

Figure 3.9. Graph comparing the level of essential trace and toxic metals among the four Ethiopian wines.

The level of lead is the highest in Kemila (0.31), followed by Axumite (0.25), Crystal (0.16), and Gouder (0.14) in mg/L. Ni is found in highest level in the wine brand Gouder (0.2 mg/L), and then in Axumite and Crystal (0.19 mg/L), least concentration of nickel is determined in

Kemila (0.18 mg/L).

Chromium in the studied Ethiopian wines was below the method detection limit for the three brands (i.e. Gouder, Crystal, and Kemila). But it was determined in small amounts (0.192 mg/L) in Axumite. Ingestion through food and beverages likely represents the principal route of chromium intake by humans [66]. Cadmium was below the method detection limit (0.014 mg/L) in all the four Ethiopian wines studied. The comparison of the levels of trace metals in the four Ethiopian wines is shown in Figure 3.9.

3.6. Comparison of the level of the metals in Ethiopian wines with data on the level of metals in wines from the rest of the world

In the analysed Ethiopian wines, the average value for potassium was found to be 740 mg/L, with a range of 693-766 mg/L. These wines had a slightly larger interval to that reported for French wines where the range is 265–426 mg/L [10]. A study [16] of three varieties of white Spanish wines, reported to have an average potassium content of 748 mg/L with a range 338–2032 mg L^{-1}. It can be seen that the potassium values for these Spanish wines have the widest range of those in the current discussion. The comparison of the major metals in Ethiopian wines and published data on wines of different origins is given in Table 3.6.

The lower content (24 mg/L) of Na in Ethiopian wines in comparison with literature data can be attributed to the considerably lower influence of sea salts in Ethiopian vineyards. Spanish wines have been reported to have an average sodium value of 3.5 - 300 mg/L but this depended on the region; grapes obtained from coastal vineyards were found to contain larger sodium levels than grapes from inland vineyards. The sources of sodium in wines, apart from natural concentration, are the addition of sodium salts such a sulfite, sorbate and (in some countries) sulfide [13]. A study [61] also showed high Na levels of Australian wines up to 256 mg/L.

The magnesium level in the Ethiopian wines was in the range of 58.1 - 79.21 mg/L with average value of 70.22 mg/L. The Mg values are found to be higher than reported for Austrian, Italian and Portuguese wines which are 68, 53-60, and 58-59 mg/L, respectively. But the Ethiopian wines contain lower amount of Mg than reported for Hungarian, Macedonian, and Spanish wines that are in the ranges of 72-100, 64-718 and 50-236 mg/L in that order. German wines also showed a wider range of 56–105 mg/L than the Ethiopian wines [10].

Table 3.6. Comparison of the concentrations of major metals in wines of different origin.

Wine	Concentration, mg/L				Ref.
	K	Na	Ca	Mg	
Australian	383 – 1482	ND – 276	7 – 150	78 – 718	[10, 61]
Austrian			118 – 140	47 – 362	[10, 61, 68]
Argentinean			10 – 15		[12]
Czech	493 – 3056	2.0 – 110	40 –210	7.8 – 138	[10, 21, 68]
French	265 – 426	7.7 – 14.6	65 – 161	55 – 96	[10]
German	480 – 1860	6 – 25	58 – 200	56 – 105	[36, 61]
Greek	955 - 2089	5.5 – 150	14.00- 47.5	82.50-122.5	[33]
Hungarian	489 – 1512	18.63 – 81.09	51 – 164	72 – 174	[10, 68]
Italian	750 – 1500	3.4 – 200	30 – 151	53 – 115	[10, 53, 61]
Macedonian	265 – 1100	5 – 310	30 – 120	64 – 718	[7]
Portuguese			108 – 114	58 – 59	[10]
Slovakian			62 – 236	57 – 145	[10]
Spanish	101 – 2032	3.5 – 300	12 – 241	50 – 236	[10, 46, 61]
U.S.A.	462 – 1147	7 – 106	17 – 94	100 – 245	[73]
Ethiopian	693 – 766	24	28 – 37	58 – 79	Present study

For calcium Australian wines generally vary from 7 to 139 mg/L [10] and in this study, the range is within these limits, being 28.4 to 37.1 mg/L with a mean of 31.4 mg/L. A mean of 60.7 mg/L has been presented for the calcium content of Spanish Penedes wines with a range of 23.4-37.0 mg/L. Similarly, German wines have been quoted as having a calcium range of

58–200 mg/L which is again higher than the values found in this work [10]. In the Argentinean wines there was a range of 10–15 mg/L, which is lower than the range in the Ethiopian wines.

The concentration of iron and copper in wine, due mainly to contact with equipment and soil contamination is usually low [67]. In the Ethiopian wines studied, the average copper and iron contents were 0.78 and 2.1 mg/L, respectively. From microelements, iron is a general component in all grape and wine varieties. Iron concentration depends on several factors, mostly on the soil, however iron levels may increase in wines due to the usage of steel devices during production. Table 3.7 shows levels of Zn, Cu, Fe, and Mn in wines of different origin.

Table 3.7. Comparison of the concentrations of Zn, Cu, Fe and Mn in wines of different origin.

Wine	Concentration, mg/L				Ref.
	Zn	Cu	Fe	Mn	
Argentinean	0.02 – 0.13	0.023 – 0.028	0.48 – 0.79		[12]
Australian		0.10 –1.8	0.06 – 11.49		[10, 61]
Czech		0.012 –6.827	0.9 – 5.2	0.28 – 3.26	[10, 21]
French	0.44 – 0.74	ND – 0.48	0.81 – 2.51	0.63 – 0.96	[10]
German	0.3 – 1.5	0.02–0.71	0.4–4.2	0.5–1.3	[10]
Greek	0.05 – 8.9	0.2 – 1.65	0.7 – 7.3	ND – 2.3	[31, 33]
Hungarian	0.6 – 1.9	0.15 – 2.57	2.03 – 23.7	0.12 – 2.9	[47, 68]
Italian	0.135 – 4.8	0.001 – 1.34	1.35 – 27.8	0.67 – 2.5	[43, 61]
Jordanian	0.11 – 3.03	0.03 – 2.60			[28]
Macedonian		ND – 1.8	0.1 – 4.0		[24, 26]
Portuguese		0.05 – 0.22			[67]
Serbian		0.10 – 0.63	2.7 – 12.2	0.62 – 4.08	[14, 27]
Spanish	ND – 4.63	ND – 3.1	0.4 – 17.4	0.1 – 5.5	[10, 39, 46]
U.S.A.	0.75 – 3.6	0.05 – 0.58	1.2 – 6.6	0.81 – 4.08	[73]
Ethiopian	1.82 – 2.7	0.5 – 1.5	1.42 – 3.16	1.04 – 1.88	present study

These values of copper and iron are within the limits quoted for Australian wines: trace to 2 mg/L for copper, and trace to 15 mg/L for iron [65]. Italian white wines found to contain <1 mg/L copper, which is lower than the values in this study. The iron values found in this study are among the lowest reported in the literature (0.48 – 0.79 mg/L Argentinean wines, 0.1– 4.0 for Macedonian wines). From the highest values, up to 7.3 – 23.7 mg/L have been reported for Hungarian wines, 2.7 – 12.2 mg/L for Serbian, 0.4 – 17.4 mg/L for Spanish wines were reported [10] and 1.07 - 7.52 mg/L for Nigerian [38] palm wines were some of the reported levels in other countries. The copper content in some Ethiopian wines were slightly larger than wines from Argentina which ranged between 0.023-0.028 mg/L [12]. But they are lower than those reported for Hungarian [68] and Czech [10, 21] wines whose range were 0.15 – 2.57 mg/L and 0.012 – 6.827 mg/L respectively.

At low concentrations, iron plays an important role in metabolism and fermentation processes, as an enzyme activator. Above trace levels, iron has other roles: altering redox systems of the wine in favor of oxidation, affecting sensory characteristics. At concentrations >10 mg/L, Fe(III) creates insoluble suspensions with tannin and phosphates which are known as hazes or "casses" [63], Iron is of importance to the wine maker because when it is present at > 7-10 mg/L, it may cause cloudiness or colour change; the content depends upon iron levels in soil and dust, and contamination during harvesting, transportation and processing [33]. Copper content of wines may be mainly originated from residues of copper-based pesticides in addition to transport from the soil [68].

As the amount of lead in wine has received extensive research due to its significance to public health and its exogenous origin, its determination in Ethiopian wines was crucial, and its level in the studied wines was 0.14 - 0.31 mg/L. These levels are higher than reported for Argentinean wines (0.05 – 0.09 mg/L) [12], and Spanish wines (0.001 – 0.096 mg/L) [16]. But some studies also showed higher lead levels in comparison with the Ethiopian wines studied now, for example Czech [10, 21] and Australian [10] wines have lead contents in the ranges of 0.1 – 1.253 and 0.009 – 1.1 in mg/L, respectively. Pb content of Portuguese wines was in the range 0.0079-0.071 mg/L [67].

Chromium(III) is one of the essential trace elements in the human body that is involved in glucose and lipid metabolism. However in excessive intake, particularly those of Cr(VI) it is considered to be highly toxic. Food and beverages are the most important source for Cr(III) intake by humans. Wine could contribute an important fraction of the dietary intake of Cr [26].

The chromium level in the analysed Ethiopian wines was found to be in the ranges of < 0.075 – 0.091 mg/L. the search for Cr levels of other countries gave lower contents of ND – 0.007 mg/L for Argentinean wines [12], ND – 0.2 mg/L wines of Greek [10], and 0.025 – 0.029 mg/L for Spanish wines [10, 39]. Studies on French wines were founds in the same range as Ethiopian wines with range of 0.006 – 0.09 mg/L [66].

The comparison made for zinc levels of the Ethiopian wines and wines of different origins showed that the Ethiopian wines are among the intermediate levels. Sixty-eight samples of red wines were analysed, for fifty-six elements by Marengo and Aceto [53] and they found Zn content in the ranges 0.135–4.80 mg/L. Argentinean wines are reported to have zinc level in the range 0.3–8.9, for Greek wines, the range was 0.3–1.5 for German wines, 0.6–1.9 in Hungarian wines and 0.81-8.88 mg/L for Nigerian wines [10, 38, 69 -71].

The determined values for manganese in the Ethiopian wines were between 1.04 mg/L and 1.88 mg/L. These levels are higher than reported for German 0.5–1.3 mg/L, 0.63–0.96 from French, but lower than from Spanish 0.5-5.5 mg/L in Spanish wines [10, 13].

From the literature there are reports indicating the level of zinc up to the range 0.075–3.67 mg/L for wines from U.S.A. [73]. All samples presented a copper and zinc content below the maximum amount recommended by the Office International de la Vigne et du Vin (OIV) for these elements. Increases in Cr, Cu, Fe and Zn contents have exogenous and/or endogenous origins. For red wines, it is a result of long maceration. Technological treatment and long contact of wine with vineyard equipment also increase metal concentrations, especially during maturation and (both producing disagreeable metal), contribute to an increase of Cu and Fe

Table 3.8. Comparison of the concentrations of Cd, Co, Cr, and Pb in wines of different origin.

Wine	Concentration, mg/L					Ref.
	Ni	Pb	Cd	Co	Cr	
Argentinean	ND	0.050 – 0.090	0.0010 – 0.0047		ND – 0.007	[12]
Brazilian		0.009 – 0.050	ND–0.0002			[10]
Czech	0.019 – 0.034	0.010–1.253	0.000055 – 0.0033	ND – 0.018	0.032 – 0.037	[10, 21]
French	ND – 0.052	0.006 – 0.023	ND–0.0002	0.004 – 0.011	0.006 – 0.09	[17, 66]
German				0.004–0.005	0.01 – 0.41	[10, 36]
Greek	ND – 0.5	ND–0.62	ND – 0.03	ND – 0.04	ND – 0.41	[10, 33]
Hungarian			0.00014 – 0.054	0.003–0.009	0.032 – 0.062	[10, 47, 68]
Italian	0.015 – 0.21	0.01 – 0.35	0.0012 – 0.0016	0.003 – 0.006	0.020–0.050	[10, 43, 61]
Macedonian			0.0001–0.0009		0.0089 – 0.0378	[24, 26]
Portuguese		0.0079 – 0.071		0.003–0.006	0.027–0.041	[10, 67]
Slovakian				ND – 0.017	0.006–0.055	[10]
Spanish	0.005 – 0.079	0.001 – 0.096	ND – 0.019	ND–0.040	0.025–0.029	[10, 39, 46]
Ethiopian	0.18 – 0.25	0.14 – 0.31	ND	ND – 0.091	ND – 0.091	Present study

content [10]. Cadmium was not detected (< 0.014 mg/L) in all of the analysed Ethiopian wines. $CuSO_4$ or $FeSO_4$ added to wine after fermentation to remove H_2S, which reacts with organic components to form mercaptans and then, after their oxidation, respective disulfides studies have shown higher contents of Cd in wine compared with the present data. Lazos and Alexakis

[33] reported Cd levels that ranged from not detectable to 0.03 mg/L in Greek white and red wine and from not detectable to 0.02 mg/L in Greek rose wine.

A wide variability of these data might result from different factors, both natural and exogenous. Natural factors include various factors such as soil composition and grape variety. Exogenous factors can be derived from the fermentation process, the wine-making system or from different kinds of contamination. The high concentration of Cd found in some wine samples could be due to the use of pesticides or fertilizers which contained salts of this metal [23]. Cork stoppers can also be sources of metals (Cd, Cu, Fe and Pb) in wines [72].

3.7. Analysis for significance of differences between means (ANOVA)

T-tests and analysis of variance (ANOVA) are widely used statistical methods to compare group means. While the independent sample t-test is limited to comparing the means of two groups, the one-way ANOVA (Analysis of Variance) can compare more than two groups. ANOVA use F statistic to test if all groups have the same mean. Therefore, the t-test is considered a special case of the one-way ANOVA. When comparing means of two groups (one degree of freedom), the t statistic is the square root of the F statistic of ANOVA ($F = t^2$) [74].

Comparing the means of all the four brands for their potassium content, at the 95 % confident level, the means are significantly different ($p < 0.05$). But the comparison made between Axumite and Kemila present no significant difference ($p > 0.05$). And any other pair comparisons indicate no significant difference between their means ($p < 0.05$).That means, K content of Axumite is significantly higher than any of the other wines K level. Also Awash crystal has significantly lowest level of potassium.

In the magnesium values as for potassium, all the four wine brands have significantly different means ($p < 0.05$). But Axumite and Kemila showed no significant difference ($p > 0.1$) in their Mg content. And the Mg content of Gouder is significantly lowest; Axumite and Kemila contain significantly higher Mg in them

The one way ANOVA also indicated that there exist statistically significant differences (significance level $p < 0.05$) among the means in Ca content of the wine groups. Axumite showed significantly highest ($p < 0.05$) level of calcium. And Awash crystal gave significantly the lowest Ca content from all the studied Ethiopian wines.

The statistical analysis for sodium content, presented the absence of significant differences among all the four wine brands and between any pair of the four wine brands studied ($p > 0.05$). That means all the four analysed Ethiopian wines contain significantly equal concentrations of the metal sodium.

The one-way ANOVA for iron showed that the four means are statistically different ($p < 0.05$), but no significant difference between the means of iron in Awash crystal and Kemila ($p > 0.05$) was found, where as iron in Axumite is statistically higher than it is in Kemila ($p < 0.05$).and also Axumite contains significantly higher iron than Kemila.

Mean of Zn values statistical analysis for their significance differences; turn out in presence of significant difference between the four wine brands ($p < 0.05$). And it was determined that the zinc level in Awash crystal to be the highest, Gouder contained significantly the lowest amount of zinc from the other wines analysed in this study. In mean of Mn no significant difference between Axumite and Awash Crystal was observed. Axumite has mean Cu content of significantly highest level from the others.

The analysis for the difference in means of Ni presented no significant differences among the four brands of the Ethiopia wanes ($p > 0.05$). Pb analysis showed that there was statistically significant difference between the wines ($p < 0.05$). ANOVA was not done for the elements Co, Cr, & Cd, this is because their level is below the method detection limit.

In summary, when the mean contents of the different metals of the Ethiopian Red and White wines were compared, it was seen that Axumite had significantly higher mean contents in potassium, calcium, magnesium, and zinc ($p < 0.05$), and Kemila contain significantly higher level of Pb ($p < 0.05$). The trace metal Cr was detected only in Axumite and the other trace metal Co was detected in Kemila wine only.

3.8. Comparison of the metal concentrations with limits set by international organization of wine

Many countries have maximum permissible level of some metals in wine. Considering both the enological and toxicological effects the metals in wines would have after some limit. The tolerable limits for some metals in wines are also set by the International Organization for Grapes and Wine (OIV). The maximum permissible levels in Australia, Germany, Italy and by OIV are given in Table 3.9 [32, 67].

The Ethiopian wines have nearly equal sodium levels as 24 mg/L, this amount is very much lower than the limit set for sodium by OIV.

Table 3.9 Permissible levels of some metals (mg/L) in some countries and by OIV*

Country	Na	Cu	Zn	Pb	Cd
Australia		5	5	0.2	0.05
Germany		5	5	0.3	0.01
Italy		10	5	0.3	
OIV *	60	1	5	0.2	0.01
Ethiopian wine**	24	0.5 -1.5	1.82 – 2.7	0.14 – 0.31	< 0.014

* Office International de la Vigea et du Vin (International Organisation for grapes and Wine)

** These are the mean values (mg/L) of the metals in the studied wines and not the permissible levels of the metals in Ethiopian wine.

From the major metals, sodium for example has maximum level of 60 mg/L in wines by OIV. In the Ethiopian wines analysed in this study it was found that the level was nearly equal as 24 mg/L, which is very much lower than the limit set.

Some of the analyzed wine samples contained lead to levels higher than the upper limit established by the Organization Internationale de la Vigne et du Vin (OIV). OIV has set a maximum limit of 0.2 mg/L for Pb in wine [17]. In the present study, lead concentrations of 0.25 mg/L and 0.31 mg/L were determined for Axumite and Kemila respectively. This shows the need to consider their grapes cultivation and viticulture. The content of iron did not present risks of "cases". All the samples presented an iron content lower than 3.5 mg/L, the maximum concentration being 3.16 mg/L for Axumite wine, therefore posing no risk of ferric cases, which is formed after iron concentration of greater than the range 10 mg/L [63].

In comparison with copper, no value greater than 1 mg/L was observed for the wine brands, except for Axumite (1.5 mg/L). And the three of the samples were found to have content below 0.65 mg/L, thus involving no risk of cupric cases. To minimize the incidence of these problems, it is generally recommended to maintain copper concentration below 0.3 – 0.5 mg/L. In the case of zinc, all the samples presented content below 2.75 mg/L, much lower than 5 mg/L, the amount recommended by the OIV [17].

4. CONCLUSIONS

Information about the total metal content of bottled wines is essential for not only winemakers and grape cultivators but also for customers. This is because metal concentrations have to be controlled in accordance with health-protection regulations. Accepted tolerable limits for metals in winemaking are critical for flavor, taste, color and long-term stability of final products, so they have a commercial impact. In this way, total metal contents are crucial parameters contributing to wine quality, while metal analysis is a part of quality assurance and quality control. And also, knowledge about metals is useful in testing wine authenticity and in investigating frauds or adulterations by blending wines and mixing hazardous substances.

In this study some Ethiopian export standard wines were analysed for their total contents of thirteen metals. The analysis was done by first optimizing the digestion procedure that worked well for the wines to be analysed. Then the wines were analysed by flame atomic absorption spectrophotometer (FAAS). During the analysis the necessary quality control procedures had been followed. The whole analytical procedure was checked for its validity by recovery tests, and the % recovery obtained was in the range of 100 ± 5 for all the metals analysed. This recovery range shows that the analytical procedure followed was valid for the purpose it was intended for (i. e. determination of metals in the Ethiopian wines).

It was determined that the selected most popular Ethiopian wines were found to contain potassium in the highest level followed by magnesium and calcium, which shows that the wines could be good sources of the essential elements mentioned. It was also found that the level of sodium is below the maximum limit set by the International Organization for Wine and Grapes (OIV). The toxic metal cadmium was not detectable in the wines. But lead was detected in the range 0.14 – 0.31 mg/L and found to be slightly higher than the limit set by OIV (0.2 mg/L) for the two Ethiopian wines, Axumite red and Awash Crystal White. In general, moderate wine consumption contributes for the daily nutritional requirements of many essential metals, including K, Mg, Na, Ca, Fe, Zn, Co, Cr, Cu, Mn, and Ni.

Further studies on Ethiopian wines should also address the speciation of metals in the wines that means analyzing the form of the metal in the wines. This is because the effect of the metal is dependant on the chemical form it exists in the wines. For example from the total Cr level what fraction is toxic and what fraction is essential. The understanding of the physicochemical forms under which a metal is present in wines (i.e. its speciation) deserves interest because these forms will dictate if undesirable phenomena occur, such as haze formation and oxidation enhancement rate due to Cu species [70]. A number of minor and trace elements concentrations in the Ethiopian wines have to be checked for their toxic levels.

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Declaration

I the undersigned confirm that the results reported in this work were obtained by research carried by me under the supervision of my advisor in the Faculty of Science, Department of Chemistry, Addis Ababa University in the academic year 2007/2008.

Name: Daniel Minillu W.Mariam Signature ______________________

This project has been submitted for examination with my approval as a university advisor.

Advisor: ______________________

Signature______________________

Place and date of submission: School of Graduate Studies
Addis Ababa University
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