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Quantitative determination of macro- and micro-nutrients and trace elements in the system soil-grapevine in two vineyards of Veneto

Supervisor
Prof. Rossano Piazza

Assistant supervisors
Dr. Fabiana Corami

Dr. Sarah Pizzini

Graduand
Luca Perissinotto
Matriculation Number 844433

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Ai miei genitori Maria e Luciano

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1 Introduction

1.1 Abstract

The grapevine belongs to the family of *Vitaceae* and is a widely cultivated plant throughout the world where approximately 80% of its grapes are used for wine production and a 13% are consumed as table grapes becoming one of the most popular fruits [1].

The quality characteristics of wines are influenced by environmental and anthropogenic factors and can be identified by variation in contents of macro- and micro-elements and trace elements forming their chemical composition.

The aim of this thesis is to better understand the fluxes in the system soil-plant of the *Vitis vinifera* (Linnaeus, 1753) such as its capacity to extract macro- and micro-elements and trace elements (*e.g.* Al, Cr, Fe, Ni, Cu, Zn, As, Cd, Sn, and Pb) from the growth substrate, the transport of these elements in the rhizosphere and their mobility through the xylem towards the aerial parts of the plant.

The system soil-plant has been studied by analysing soil and different parts of vines (*i.e.*, leaves, flowers, stems and grapes) of three different *cultivars* (Glera, Garganega e Cabernet-sauvignon) in two vineyards of Veneto located in Visnà di Vazzola (TV) and Cologna Veneta (VR).

The quantification of these elements could be helpful to identify the distribution of the analytes in soil and in plant with its differences among the *cultivars* and to better understand this natural resource, emblem of the Mediterranean culture.

1.2 The annual cycle of the *Vitis vinifera*

Vitis vinifera is a deciduous, woody perennial plant and its annual cycle can be divided according to the phenological Eichorn-Lorenz stages which establish a description and a categorisation of all grapevine physical changes associated to its growth processes. However, some differences may occur when it is cultivated for commercial production.

The first stage is *the dormancy* which starts from leaf fall of the previous year until the beginning of spring; during this period grapevines are dormant, consist entirely of woody tissue and focus all stored energy in the process of root growing which is highly sensible to temperature fluctuation.

The hardiness of the grapevines to cold temperature, which negatively influences the roots growth, varies considerably across *cultivars* which have been adapted to different climates through hybridisation.

In spring temperature *the bud break* starts, stored starch is converted to sugar, sap begins to move in the vine, buds begin to swell and later burst.

The newly emerged shoots grow very rapidly for several weeks in the absence of stress and cluster inflorescences become visible, usually opposite the third and fourth leaves on a shoot [1a].

The phase of *bloom and fruit set* starts with the flowering period which strongly depends on weather and can be very short under warm and dry conditions or longer under very cold and wet conditions. The carbohydrates, accumulated in the previous year, are the principal source of energy for the pollination which, for most commercial *cultivars*, occurs in the same plant without requiring a separate polliniser *cultivar*. After pollination, the flowers are separated by abscission and form clusters, leaves on each shoot became fully expanded and now are the principal source of energy enabling the newly-formed berries to develop rapidly for approximately five to seven weeks, due to cell division, until the *veraison*. During this period weather influences the next year growing season and leaves which have been well exposed to sunlight will result in more fruitful buds.

The *veraison* ends with harvest and in the maturing process berries expand further becoming soften, and accumulating sugar, pigments, and other flavour compounds while organic acids decrease and change forms [1a]. The visual indicators of maturity on red *cultivars* are clearly shown by a change of colour while on the white ones are subtler.

The bark of green shoots turns brown from the base under the process of lignification becoming woody. After harvest, grapevine leaves continue to photosynthesize until frost, if temperatures are warm enough; during this very important period the vines accumulate carbohydrates for future growth

and sugars, converted to starch, are mostly stored in perennial structures such as roots and trunks for the winter period [1a].

1.3 Berry growth

The most important process among those described in the Eichorn-Lorenz stages, is the growth of the berry which involves a series of chemistry changes inside the plant from the formation of the flower to the grape cluster. The grape cluster is an inflorescence and consists of a peduncle, cap stems (also called pedicels), a stem, and berries that are made up of seeds, flesh (or pulp), skin, and the bloom, or waxy layer on the outside of the skin, that helps to prevent water loss and gives a lighter appearance to a dark-coloured *cultivar*.

All necessary nutrients are provided by the vascular system, namely the phloem and xylem. The xylem plays an important role in the early stages of berry growth until the *veraison*; it transports water, mineral nutrients, growth regulators, sugars, and other nutrients from the root system. The phloem is responsible for carbohydrate transport from the leaf canopy to the vine, and subsequently, to the berry for which becomes the primary source of nutrition after the start of the *veraison*. Berry size increases with an increase in sugar content differently by *cultivar* [2a]. This process is summarised below in three stages [2].

The first stage lasts approximately 60 days and starts after the pollination, with the bloom and the formation of berries followed by a rapid cell division which occurs inside them. The berry expands in volume reaching at least half of their final size. During the initial stages of berry growth, it accumulates solutes principally in the skin, such as tartaric acid, malic acid in the flesh and little amount of sugar. Other important acids in the berry are the hydroxycinnamic acids which are present in the flesh and skin, are involved in browning reactions and are precursors to volatile phenols like tannins. Tannins, accumulated in the skin and seeds during the first growth phase of the berry, are responsible for bitterness and astringency; this accumulation has a strong influence on the quality and on the characteristics of red wine, which includes colour, stability, and mouth-feel.

The second stage, called the *lag phase*, is distinguished by a pause in berry growth, during which seed embryos start to grow rapidly for 5 to 10 day, cells expand reaching their final size and accumulate acids and tannins, which reach their maximum levels at the *veraison*.

The third stage starts with the *veraison*; during this period the berry doubles in size and several changes occur like the softening, the colouring and the accumulation of sugars in opposition to a decrease of acids. In particular, the reduction of malic acid is strongly correlated to the climate: grapes from warm region typically have less malic acid, whereas cooler regions produce grapes with higher levels of malic acid. Acids are not the only substances which decline; during the second growth phase, indeed, seed tannins were oxidised and fixed to the seed coat in relation to the exposure to sunlight of the berries.

The most significant changes which occur after the *veraison* are an increase of the concentrations of sugars like glucose and fructose derived from sucrose as a consequence of different factors such as hang time, crop load, canopy size, disease, and water. Anthocyanins, that are secondary metabolites in red grapes, and volatile flavour compounds like terpenoids in white grapes, are responsible for the wine quality [2a].

1.4 *Vitis vinifera* domestication

About 60 species of *Vitis* are mainly found in the temperate zones of the Northern Hemisphere and almost equally distributed between America and Asia [1]. The wild grapevine is a heliophilous liana that grow generally along river banks, and in alluvial and colluvial deciduous and semi-deciduous forest from western Europe to the Trans-Caucasian zone and around the Mediterranean Basin [3]. The cultivation and domestication of the grapevine took place between the seventh and the fourth millennia BC in a geographical area between the Black Sea and Iran. Subsequently, thanks to the human activity, cultivated forms of grapevine would have been expanded in the Near East, Middle East and Central Europe where a secondary domestication occurred. The most ancient testimonies of grapevine cultivation in Italy date back to the ninth century BC.

The domestication process forced by humans selected specific phenotypic traits such as size, shape and colour of berries ignoring other characteristics like seed shape, even though some differences occur when domestication syndrome caused a change from dioecious wild individuals to hermaphroditic cultivated plants influencing also their germination ability [3].

During this long period different *cultivars* have appeared over the centuries of cultivation, according to the tastes of grape growers and wine makers that selected different species. *Vitis vinifera* and its *cultivars* are widespread all over the world, but in USA species such as *Vitis labrusca* (Linnaeus),

Vitis riparia (Michaux), *Vitis aestivalis* (Michaux), *Vitis rupestris* (Scheele) and *Vitis rotundifolia* (Michaux) are also used in wine-making [1].

The recent distribution of the wild grapevine is strongly fragmented probably because of the anthropogenic pressure and other factors like allochthonous pathogens as occurred in European vineyards with the 'Phylloxera crisis'. This phytophagous insect attacks the roots, with a considerable impact on both cultivated varieties and wild grapes, causing such an endangered condition to reduce the wild grapevines on the verge of extinction [3].

The *cultivar* name is largely used for wine differentiation and product labelling; some examples are the *cultivars* Cabernet-sauvignon, Shiraz (Syrah), Chardonnay, Sauvignon blanc and Riesling which have been established genotypes of *Vitis vinifera* and have a reputation for producing premium quality wine [4].

The production of wines is subjected at denomination of origin, a pillar of the European wine industry and a synonym of quality and economic importance (EU Reg. 479/2008).

In Italy the labels DOC and DOCG (controlled – and guaranteed – designation of origin, from the Italian *Denominazione di Origine Controllata e Garantita*) are controlled by several public bodies and by the Italian law (Legislative Decree 61/2010) [5].

The quality, aroma characteristics and health safety of wine consumption are influenced by environmental factors like geography, climate (temperature, precipitations, humidity, wind, etc.), soil composition and grape variety as well as by anthropogenic factors; these last factors may impact with the pollution of vineyards and viticulture management practices such as use of seed preservatives, chemical sprays, fertilisers, grape-growing approaches, and winemaking technology and storage [6].

1.5 Nutrients, micro-nutrients and toxic elements in plants

Knowledge of the accumulation of mineral elements in plants and their distribution in the different parts of fruit has been considered essential for biochemical and physiological studies which, supported by recent improvements in analytical techniques, led to better understand elements functions or their physiological role [7].

Macro-nutrients are nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulphur (S) and are generally found in plants at concentrations greater than 0.1% of dry tissue weight; micro-nutrients (*e.g.* Cu, Fe, Zn, Mo or Ni) are generally found at concentrations less than 0.01% of dry tissue weight [8].

Elements such as Co, Se or V have been shown to be beneficial, while other elements (*e.g.* As, Cd, Cr, Hg and Pb) may enter in plants following the same path of an essential nutrient, however, not only they have not any known benefit function, but they can even be toxic [8]. The trace elements, which concentration is highly variables among plant species, depending on soil composition and geographical zone, are those elements present in very low concentrations in the organism, below 0.01%, while the concentrations of ultra-trace elements are even lower, below 0,0001% [7].

The presence of mineral elements in soil and plants, in particular trace and ultra-trace elements, is considered to be closely linked to the geological composition of the underlying mother rock, the physico-chemical properties of the soil and the specific take up ability of the plant [7].

The definition of nutrient's essentiality for a plant has been set with three criteria [9]. To be essential an element must be necessary for a given plant to complete the vegetative and/or reproductive stages of its life cycle, cannot be replaced by another element to achieve a specific function, and have to be directly involved in the plant metabolic or structural processes [8].

According to the criteria explained above, essential elements may have multiple functions in a plant, both structural and biochemical, and they can be classified in one or more of the following six types [10]:

1) Type I – nutrients bounded to the structure of carbon compounds;
2) Type II – nutrients required for energy storage and transport;
3) Type III – nutrients linked with the structure of cell wall;
4) Type IV – nutrients integrated as constituents of molecules required for metabolism;
5) Type V – nutrients responsible of the activity of the enzymes;
6) Type VI – nutrients involved in the saline balance.

The principal macro- and micro-nutrients are shown in Table 1.

Table 1: Average concentration, in dry matter of plant, of the main nutrients and micro-nutrients and their principal function; the bolded elements have been indagated in this thesis work.

Mineral concentrations of typical whole plants				
		Concentration in dry matter		
Element	Chemical symbol	ppm or %	μmol g^{-1}	Function and main roles in plants
Macro-nutrients				
Nitrogen	N	1.5%	1000	Chlorophyll, nucleic acids and proteins (I, VI)
Potassium	K	1%	250	Enzyme activator, osmotic balance (V, VI)
Phosphorus	P	0.2%	60	Energy supply (*e.g.* ATP), nucleic acids (I, II)
Sulphur	S	0.1%	30	Nucleic acids and proteins (I)
Calcium	Ca	0.5%	125	Cell walls, enzyme activator, signalling (III,V)
Magnesium	Mg	0.2%	80	Chlorophyll (IV,V)
Silicon	Si	0.1%	30	Cell walls (III)
Micro-nutrients				
Nickel	**Ni**	**0.05 ppm**	**0.001**	**Enzyme component (IV)**
Molybdenum	Mo	0.1 ppm	0.001	Enzyme component (*e.g.* nitrogenise) (IV)
Cobalt	Co	0.1 ppm	0.002	Nitrogen fixation in legumes (IV)
Copper	**Cu**	**6 ppm**	**0.1**	**Respiration and oxido-reduction (IV, V)**
Zinc	**Zn**	**20 ppm**	**0.3**	**Enzyme activation (IV,V)**
Sodium	Na	10 ppm	0.4	C4 photosynthesis in some plants (IV)
Manganese	Mn	50 ppm	1.0	Chlorophyll synthesis, energy transfer (IV, V)
Boron	B	20 ppm	2.0	Cell wall stability (III)
Iron	**Fe**	**100 ppm**	**2.0**	**Chlorophylll synthesis, energy transfer (IV, V)**
Chlorine	Cl	100 ppm	3.0	Photosynthesis, osmotic balance (V, VI)

Bioavailability is the fraction of the total content of a chemical substance in a specific environment which, in a certain period, is available for the uptake by a living organism [11]; the knowledge of bioavailability provides information regarding element transfer and their possible toxic effects [12]. The physico-chemical characteristics of the soil and the membrane transporters are the main factors which determine the availability of nutrients to uptake by plant roots that are mainly found in top layers [10]; the nutrients dissolved in soil water are those that are generally available for the uptake and the plant can directly influence nutrient availability in the area around the root surface, called rhizosphere. In fact, roots, thanks to the excretion of organic acids, mediate localised changes in pH encouraging the growth of particular types of bacteria and fungi that can directly influence the water solubility of many nutrients, making them available for uptake by plant roots according to their requirements. Furthermore, some plant roots excrete specific enzymes and chelating molecules to improve soil nutrient availability, for example, phosphatases and siderophores for Fe [10]. The key steps in the uptake of nutrients, each mediated and regulated by specific transporter proteins, are: the

transport across the plasma membrane from the soil into root cells, the storage in the vacuole and the loading of the long-distance transport systems in plants (phloem and xylem) and the unloading into the growing tissues such as leaves or seeds [10].

Plants absorb essential and non-essential elements from soil in response to concentration gradients induced by selective uptake of ions by roots or by diffusion of elements in the soil; being the foundation of the food chain some of these elements that are toxic, even in low concentrations, may be transported to higher levels of the food chain [13].

Plants could be classified into three categories relating to the level of accumulation of elements which differs among and within species [13]:

1) Excluders: those that grow in metal-contaminated soil and maintain the concentration in shoot at low level even when in soil there is a critical value which results in relatively unrestricted root-to-shoot transport;
2) Accumulators: those that concentrate metals in the aerial part;
3) Indicators: where uptake and transport of metals to the shoot are regulated so that internal concentration of these elements reflects the external levels, at least until toxicity occurs.

The principal effects in plants stressed by heavy metal/metalloid are various biochemical reactions produced by the displacement of protein cationic centres, and consequently a metabolic disturbance, or the increase of reactive oxygen species [13].

Metals and metalloids can affect physiological and biochemical processes differently, since they may be nutrients and/or may exert toxic effects in plants, in relation to their speciation. Roots of plants are the initial contact site for trace elements which can also be absorbed from plant leaves due to the deposition on their surfaces [14].

Micro-nutrients can be redistributed via xylem and phloem to supply aerial plant parts and this process, in polluted soil, may cause an accumulation of undesirable large quantities of metal/metalloids which can damage or influence negatively the plant and their products surpassing the defence strategies of the plants like the retention in roots due to an induced insolubilisation [15].

However, the total concentration of metals/metalloids, macro- and micro-nutrients in soil does not provide any specific information about their mobility and availability, because its related to their chemical form in soil and to how plants take up these elements [16].

Metals in soil can be classify into five major geochemical forms in relation to their availability [16]:

1) Exchangeable;
2) Bound to the carbonate phase;
3) Bound to Fe and Mn oxides;

4) Bound to organic matter;
5) Residual metal.

However, these elements in soil can change in their chemical behaviour, probably because of their capacity to react to form organic compounds and with enzymes secreted by microorganisms [16].
Metals, after the interaction with roots, are transported into the cells where some of them are transferred to the apoplast, the space outside the plasma membrane within which substances can diffuse, and later migrating thought the plasma membrane into the cytoplasm, affecting some nutrients status, while others may bound to cell wall substances [16].
When a metal/metalloid is released into the root xylem flows upwards following the transpiration stream from the roots to shoot parts and only if there would be no sequent redistribution given by the phloem, which varies in a wide range within the plant species, these elements would accumulate primarily in photosynthetically active leaves [15].
In the following paragraph, uptaking processes and effects in vines of the elements studied (Al, Cr, Fe, Ni, Cu, Zn, As, Cd, Sn, and Pb) will be illustrated.

1.5.1 Aluminum (Al)

Al occurs naturally mostly in the form of Al silicates and is required by plant, in trace amount, to carry out some biological processes. Al exists in the oxidative states Al (II) and Al (III), have a high anion affinity and chemical one to fluoride (F^-) and hydroxyl (OH^-), and easily form complex with carboxyl, carbonyl, and phosphate [19]. Al (III) is water-soluble and, thus, is toxic to plants even at micro-molar concentrations; to tolerate Al toxicity some plants form organic acid complexes with Al within leaves and outside roots [19].
In acidic soil condition, Al is the mineral mainly responsible for the inhibition of plant cell growth, division of root cells, and affecting the signal transduction pathway and Ca homeostasis thought the inhibition of enzyme phospholipase C. Others damaging effect caused by Al are a distortion of ATPase activity, a production of ROS in cytosol and in mitochondria and, in the worst scenario, an induction of nuclear apoptosis that results in programmed cell death [19]. Al also interferes, in general negatively, with the metabolisms and the uptake of different nutrients, including Cu, Zn, Ca, Mg, K, P, and Fe [19].
Al polymerisation in presence of various chelating agents such as phosphate raises the pH of soil, with a subsequent increase in Al concentration causing the loss of monomeric Al [19].

Plant uptake and absorption of Al occurs, through endocytosis which involve specific carriers, in soil with near-neutral pH values, where Al-organic matter complexes can be solubilised. This process can also be exalted in relation to the physico-chemical characteristic of the group to which Al is complexed. For example, Al chloride exposure causes a concentration of Al in xylem sap 15 times greater than Al oxalate exposure [19].

The initial uptake site of Al is the root cap and mucilaginous layer covering epidermal cells; its accumulation is strongly in the root apex and, in the elongation zone, forms super-oxides and peroxides.

1.5.2 Chromium (Cr)

Cr is one of the most harmful elements for the environment; its natural sources are ultramafic and serpentine rocks aschromite ($FeCr_2O_4$) and complexes with other metals/metalloids in minerals like crocoite ($PbCrO_4$), bentorite $Ca_6(Cr, Al)_2(SO_4)_3$ and tarapacaite (K_2CrO_4); the antropogenic sources instead are mostly related to several industrial processes [13].

The most stable and prevalent oxidation state of Cr is Cr (III), relatively insoluble in water, which tends to form hydroxide precipitates with Fe at typical ground water pH values.

Cr (VI), formed in abundance of oxygen or Mn oxides which oxidize Cr (III), is the most toxic species because of its high oxidizing potential, solubility, and mobility across the membranes, producing genetic mutations in living organisms and through the environment.

Cr in plants is differently taken up among and within species and is not an essential element even if in some cases, at low concentrations (0.05-1 mg L^{-1}), seems to promote growth and yield of plants. Concentrations higher than 1-5 mg L^{-1} of the available fraction in soil produce alterations in some plant metabolic processes (*e.g.* growth inhibition, decrease in chlorophyll synthesis, and chlorosis).

The principal pathway of Cr to the plant is its reduction and/or complexation with root exudates, such as organic acids, which increase the solubility and mobility of Cr through the root xylem [13]. Both Cr (VI) and Cr (III) enter in the root cells by the symplast pathway where Cr (VI) is reduced and accumulated in the cortex also because is poorly translocated to aerial parts where it is mobilised and accumulated inside tissues depending on its chemical form.

Cr enter into the plants as Cr (III) by a passive mechanism, while Cr (VI) uptake may occour through sulphate or phosphate transport system, or through active mechanism. Cr (VI) is retained in the vacuoles by the cell wall, while the uptake of Cr is inhibited by the presence of SO_4^{2-} and Ca^{2+} ions. At neutral pH, Cr (VI) compounds are tetrahedral and are transported across cell membranes through

similar tetrahedral ion channels, while Cr (III) is octahedral and transported through diffusion along the membranes. Cr (VI) may damage roots and reduce uptake of macro-elements such as P, Fe or Ca [13].

1.5.3 Iron (Fe)

Fe is an essential nutrient for plants and is required for life-sustaining processes from respiration to photosynthesis. Due to its chemical proprieties, Fe participates in electron transfer and is responsible reversible of redox reactions between Fe^{2+} and Fe^{3+}.

Although Fe is the fourth most abundant element in the earth's crust, it is not readily available to plants which to maintain its concentration of 10^{-9}–10^{-4} M to succeed in an optimal growth developed different uptaking process due to the low solubility of Fe in soil solution.
In well-aerated soils at physiological pH, the concentrations of free Fe^{3+} and Fe^{2+} are less than 10^{-15} M; this causes agricultural problems also because one third of the world's cultivated soils are calcareous and considered Fe deficient [23].

The two most important process that plants have developed to uptake insoluble Fe (III) in soils are:

- Strategy I plants (dicotyledons and nongraminaceous monocotyledons): mobilize Fe through acidification of the rhizosphere, causing the dissolution of Fe/Al oxides, and the upregulation of Fe reductases, which reduce Fe (III) to Fe (II);
- Strategy II plants (graminaceous monocotyledons): can complex Fe (III) through the release of high-affinity Fe (III) root exudates, phytosiderophores [22].

Once Fe has entered in the symplast in roots, Fe is bound to various chelators, facilitating it remaining in solution, preventing the formation of participates in the formation of hydroxyl radicals and allowing its short- and/or long-distance transport. Organic acids, such as citrate, are known to bind Fe^{3+} while nicotianamine (NA) forms stable complexes with both Fe^{2+} and Fe^{3+} [23].

1.5.4 Nickel (Ni)

Ni occurs abundantly, as a free-metal or as a complex with Fe, in igneous rocks that are the main natural sources of Ni in soil, and in surface-waters. In these two matrices, the average concentration of Ni is lower than 100 and 0.005 ppm respectively. Three important examples of anthropogenic sources di Ni are smelting, burning of fossil fuel and industrial wastes.

Its prevalent form in soil is Ni^{2+} thanks to its stability over a wide range of pH and redox conditions. Ni toxicity in plants occur when its concentration is more than 10 mg kg^{-1} (dry mass) with different impact relating to the of plant species, growth stage, cultivation conditions, concentration and exposure time. The principal effects of its toxicity are the inhibition of mitotic activities, reduction in plant growth, plant water relation and photosynthesis, inhibition of enzymatic activities as well as nitrogen metabolism, interference with the uptake of other essential metal ions and even induction of oxidative stress [25].

The uptake of Ni in plants is mainly carried out through the root system via passive diffusion or active transport based on its form and concentration in the soil or nutrient solution.

Like other metal ions Ni uptake generally declines at higher pH values of the soil solution due to the formation of less soluble complexes, and the limit its bioavailability and is affected by Ca ion. The inhibitory effect of various metal ions on absorption and translocation of Ni^{2+} from roots to shoots varied as $Fe^{3+} > Co^{2+} > Ca^{2+} > Mg^{2+} > NH_4^+ > K^+ > Na^+$, however Ni can also enter in the plants via leaves.

The path of Ni transport in plants, often helped by organic acids and amino acids potential metal chelators, is from root to shoot where makes an exit transpiration stream via xylem [25].

1.5.5 Copper (Cu)

Cu is a transition heavy metal with three valence states: Cu°, Cu^{+1}, and Cu^{+2}. Is an abundant and essential micro-nutrient in various rocks and minerals and is required in plants for a variety of metabolic processes. Furthermore, Cu plays a key role in several physiological processes such as photosynthesis, respiration, carbohydrate distribution, and protein metabolism but, like others micro-nutrients, its excess can disturb the normal plant growth affecting biochemical reactions and physiological processes [20].

The main symptoms of a Cu excess are a reduction in the growth of the roots, resulting in less exploration of the soil by the roots and in a consequently damage to their cell membranes, and a significant decrease in the uptake of nutrients and water. Other effects of a Cu excess are related to the oxidative stress caused by the increased concentration of reactive oxygen species (ROS) like superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), which can damage all biomolecules [20]. Damage to the cell membranes results in lower selectivity and may causes membrane breakage and leakage of the cell contents [21].

The mechanisms of Cu mobilisation and its uptake by roots from soil remain still unclear. However, Cu in soils is strongly associated with organic matter, as well as Fe and Al oxides and, although no studies have confirmed the mobilisation and uptake mechanism of Cu, it seems that there is a strong overlap between Fe and Cu uptake, since they are competitors for the same uptake path [22].

1.5.6 Zinc (Zn)

Zn is an essential micro-nutrient for plants and its assimilation is due to a characteristic complex physiological which is mainly governed by Zn transporters and metal chelators of plant system [24]. Zn is the only metal that is part of all six enzyme classes (oxidoreductase, transferase, hydrolases, lyases, isomerases and ligases) and regulates their activities.

Zn is also responsible of the membrane integrity, can provide an alleviation of oxidative and heat stress, is an intracellular second messenger and is involved in several other plant physiological processes such as hormone regulation and maintenance of CO_2 concentration in mesophyll [24].

Zn in soil have as primary source the physico-chemical weathering of underlaying mother rocks and most of agricultural soils contain Zn in the range of 10-300 µg g^{-1}. However, all the Zn presents in soil is not available to plants because its availability depends on many factors like physico-chemical properties of soil, activity of plant roots and micro-flora in rhizosphere.

In soil Zn exists as insoluble complexes non available to plants (> 90%) or in adsorbed and exchangeable form which concentrations ranges from 0.1 to 2 µg g^{-1}.

Plant can also actively absorb Zn thought roots thanks to ion exchange via and release of organic acids to obtain available form for the uptake [24].

The pH value, the redox potential and the presence of organic matter in soil are the most important factor which affect Zn distribution and solubility. Indeed, the principal form of Zn taken up by roots is Zn^{2+} and this form is enhanced by low pH value and low concentration of soluble organic matter;

even the organic ligand-Zn complexes can be absorbed by plant roots through more complex path [24]. There are two physiological strategies involved in the uptake of divalent cations like Zn^{2+}:

- Strategy I: involve efflux of reductants, organic acids (*e.g.* citric acid, malic acid, oxalic acid or tartaric acid) and H^+ ions, which enhance solubility of Zn complexes and release Zn^{2+} ions for absorption by root epidermal cells;
- Strategy II: involves efflux of phytosiderophores (phytometallophores) which form stable complexes with Zn and their subsequent influx into root epidermal cells [24].

Inside the root surface Zn^{2+} must, using the symplastic route or the apoplastic one, pass through different tissues (epidermis, cortex, endodermis, and pericycle) before reaching the xylem to be transported to shoot where a regulation system, performed by Casparian strip and the xylem loading process, prevent an excessive accumulation of this metal inside the plant. This regulation system is also involved in the pathway of others metal cations and nutrients. In phloem Zn mobility is higher and further translocation to various plant organs or sinks are mediated by short and long-distance pathways [24].

Generally, Zn levels are higher in roots than the aerial parts (leaves and shoots). Due to the system described above, indeed, it is an indirect way to protect the aerial photosynthetic shoots from toxicity defects, even if some intraspecific variations in pattern of Zn accumulation may occur [24].

1.5.7 Arsenic (As)

As is a metalloid of great environmental concern due to its toxicity and abundance in ground water and surface soil. As disperses into soil and water through natural process like the disintegration of rocks and minerals and lixiviation (the process of removing soluble constituents of matter by liquid permeation) or anthropogenic influence through smelting and mining processes, agricultural practices, fabrication and consumption of wood preservatives, and food additives.

The mobility and availability of As in the environment depends on its chemical form and speciation. Inorganic As in the form of arsenite – As (III) – is less mobile but has been considered more toxic than inorganic arsenate – As (V). However, both species have been considered harmful to living organisms due to their capability to alter metabolic pathways. As (III) binds to sulphur groups from enzymes and proteins and to thiol groups from phytochelatins, whereas As (V) binds to amino-, or reduced nitrogen groups. As (V) is the most common and stable form of As, found in aerobic soils and is therefore more available for plant uptake [13].

The transport and availability of As in soil is strongly dependent on the soil pH. At low pH values (pH 4) As is found complexed with Fe, whereas at high pH values (pH 6-8) it is mostly bound to Ca. Moreover, the presence of Fe and Mn oxides also increases As mobility and its availability in soil [13].

Generally, plants uptake and mobilize As (V) through the phosphate transport channels and because of their chemical similarity, As (V) competes with phosphate for root uptake and interferes with metabolic processes like ATP synthesis and oxidative phosphorylation.

As (III) can be coordinated to sulphur ligands and transported as As (III)-tris-glutathione complex. As accumulation and resistance vary among plant species due to genetic differences and diversity in detoxification processes which involve As mobilisation from roots to aerial parts of the plant (translocation) which is principally controlled by the external As concentration [13].

Inside plant tissues, As (V) is reduced to As (III) and/or bio-transformed to less toxic organic compounds such as dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), or as inorganic As (III) complexed with thiol groups. The majority of plants are able to synthesize enough arsenate reductase, which reduces most of the As (V) to As (III); when plants were exposed to As (III), a percentage of the As was oxidised to As (V) in the soil and was then absorbed by the roots [13].

1.5.8 Cadmium (Cd)

Naturally occurring Cd levels are extremely low; naturally Cd concentrations in non-contaminated soil vary from 0.01 to 5 mg kg^{-1} of soil. The main sources of spread Cd pollution are fertilisers produced from phosphate ores and the inappropriate disposal of wastes that containing Cd, increased in populated areas around the world because of its industrial applications [13].

As regard plants, the electrochemical potential gradient of the plasma membrane in the root cells drives Cd and other cations inside them. External factors such as Fe concentration can reduce the uptake of Cd as confirmed by the studies developed in barley (*Hordeum vulgare*; Linnaeus 1753), and maize (*Zea mays*; Linnaeus 1753) plants.

As regards Cd transport, the low-affinity cation transporter (LCT1) responsible for Ca transport in wheat may be also responsible for Cd transport in many plants. However, it seems that in other plants as the maize, the entrance of Cd into the root symplast is unregulated, but its translocation towards the shoots is controlled and restricted to some extent by unknown factors [13].

1.5.9 Tin (Sn)

Sn is a heavy metal which is naturally present as a component of many soils due to the natural weathering of bedrock and present a high variability of concentration in it, with an average concentration of 2 mg kg^{-1} approximately.

The principal sources of Sn in nature, beside the weathering of bedrock, are volcanic eruptions, and anthropogenic activities like mining, industrial processes and agriculture [17].

Sn has a very low soil solubility, which increases with decreasing pH values, while decrease with increase of organic matter due to its affinity whit it. Furthermore, Sn^{2+} translocation and its uptake by plants is minimal. Nevertheless, some recent studies shown that at high concentrations Sn may be incorporate to the plant tissue, especially in the roots [18]. This suggests that Sn is retained in the apoplastic regions of the roots, resulting in poor transport to the aerial parts of the plants. The inorganic Sn forms are less toxic, nevertheless the effects of their presence in soil and plant are still little known [18].

1.5.10 Lead (Pb)

Pb seems to not have any functions in biological systems and is considered toxic. The major source is metal smelting and other human activities like agriculture and industry.

Pb has low solubility and availability for plant uptake because it precipitates as phosphates and sulphates, chemicals commonly found in the rhizosphere of plants, or it is immobilised in soil where it forms complexes with the organic matter [13].

Pb is not an essential element so plants do not have channels for Pb uptake and how this element penetrate into the root tissue is still unclear. Some plant species can tolerate Pb through its complexation and inactivation, while other species undergo toxic effects caused by an impediment of some metabolic pathways, process that inhibits seed germination, plant growth and chlorophyll synthesis [13].

The absorbed Pb remains in roots making them the first barrier for the Pb translocation to the above ground plant parts; however, once inside the roots, most of the Pb is bound to ion exchangeable sites in the cell walls and to extracellular precipitation as phosphate and carbonate. However, unbound Pb may move through Ca channels and accumulate near the endodermis [13].

Low concentration of Pb can be stopped by the Casparian strip of the endodermis becoming a partial barrier for is movement into the central cylinder tissue.
However, Pb may also concentrates in the phloem tissues suggesting the presence of Pb movement thought the xylem towards leaves and returning movements through the phloem to the plant body. In the same way as other toxic elements, Pb can be found complexed by the cysteine-rich low molecular weight polypeptides widely known as phytochelatins [13].

2 Materials and Methods

2.1 Description of the sites

The first vineyard, located in Visnà di Vazzola (TV), is a private winery with a cultivated area of 2,58 hectares. The vines in this site are *Vitis vinifera*, *cultivar* Glera (white grapes) subdivided in three group of different ages: ten, two and one year. According to the soil chart of Veneto 1:50.000 ARPAV (Veneto regional environmental prevention and protection agency, 2015) the area was signed as RAM1 (Ramera soil), categorised with the W.R.B. like Endogleyic Fluvic Cambisols (Calcaric, Orthosiltic) [3a]. This soil is part of an undifferentiated alluvial plain and mainly formed by clay and silts with the following characteristics: deep-medium texture, absence of stones, from strongly calcareous at surface to extremely calcareous deeply, alkaline with a mediocre drainage [3a].
The second vineyard located in Sant'Andrea di Cologna Veneta (VR) in a private garden that has a cultivated area of 0.05 hectares in which the owner produce wine for personal usage. The vines in this site are *Vitis vinifera*, *cultivar* Garganega (white grapes) and *Vitis vinifera*, *cultivar* Cabernet-sauvignon (red grapes) subdivided in three group of different ages: forty, twenty and ten years. According to the soil chart of Veneto 1:250.000 ARPAV (2015) the area was signed as BR4 ("*Bassa pianura recente*"), categorised with the W.R.B like Fluvic Cambisol [3a]. This soil has the same characteristic described for the Visnà di Vazzola soil with a lower presence of carbonates [3a].

2.2 Sample treatment

2.2.1 Sampling shedule

Soil, roots, flowers, leaves, stems and grapes were sampled in different moments following the natural vegetative cycle of the vineyard. Specifically, soil was sampled in April 2017, flowers and roots in May 2017, stems, leaves and grapes in September 2017.
Leaves, stems and grapes of Visnà di Vazzola were not sampled due to an early harvest, as summarised in Table 2.

Table 2: Matrices selected and their relative amount.

Matrices	**Total amount**	**Samples from Visnà di Vazzola (TV)**	**Samples from Sant'Andrea di Cologna Veneta (VR)**
Soil	**11**	**5**	**6**
Roots	**11**	**5**	**6**
Flowers	**11**	**5**	**6**
Leaves	**6**	**0**	**6**
Stems	**6**	**0**	**6**
Grapes	**6**	**0**	**6**

2.2.2 Sampling procedures

One of the first aspects that have been considered in sampling soil is the spatial variability, although this is often low in soil intended for viticulture, due to a relative homogenisation caused by the anthropogenic activity of cultivation. Another key aspect of the sampling is to obtain a sample that is representative of all the sampling area characteristics. For this reason, soil samples were taken along the row of vines, in the corners and in the centre of the two vineyards, as shown in Figures 1-2.
Sampling procedures include the following steps:

- Registration of GPS coordinates of the sampling point;
- Removal with a shovel of the surface 2-3 cm ground layer;
- Coring with a hand pedologic auger and sampling every 10 cm until it reaches 40 cm of depth.

All samples were signed and then carried to the laboratory in polyethylene bags in order to minimize any source of contamination. Once in the laboratory, soil samples were sieved (2mm mesh) and have been stored at -18°C until the analysis.
According to their age, plants of *V. vinifera* have two maximum root absorptions: 20-30 cm and 60-80 cm; only soil sampled at the first maximum root absorption was analysed.

Roots, flowers, leaves, stems and grapes have been sampled in a similar way minimizing the distance from the relative previous soil sample point (Tables 3-4).
Suitable materials were employed to collect samples from the plant (*e.g.* scissors, ceramic knives and polyethylene bags). Once in laboratory samples, except grapes, were cut with a ceramic knife clean with ultrapure water (Elga PURELAB Ultra), then put in sterilised polypropylene vials and stored frozen before freeze-drying. The grapes did not require a freeze-drying treatment, so they were stored directly in polyethylene bags after a hand-removal of the stems and a clean with ultrapure water (Elga PURELAB Ultra).

Table 3: Characteristics of the Visnà di Vazzola (TV) sampling points.

VISNÀ DI VAZZOLA (TV)	
SAMPLE NAME	**CHARATERISTICS OF THE VINE**
1	Two years old, *cultivar*: Glera
2	Two years old, *cultivar*: Glera
3	Ten years old, *cultivar*: Glera
4	Ten years old, *cultivar*: Glera
5	Ten years old, *cultivar*: Glera

Table 4: Characteristics of the Sant'Andrea di Cologna Veneta (VR) sampling points.

SANT'ANDREA DI COLOGNA VENETA (VR)	
SAMPLE NAME	**CHARATERISTICS OF THE VINE**
A	Ten years old, *cultivar*: Cabernet-sauvignon
B	Forty years old, *cultivar*: Garganega
C	Ten years old, *cultivar*: Cabernet-sauvignon
D	Forty years old, *cultivar*: Cabernet-sauvignon
E	Twenty years old, *cultivar*: Garganega
F	Forty years old, *cultivar*: Garganega

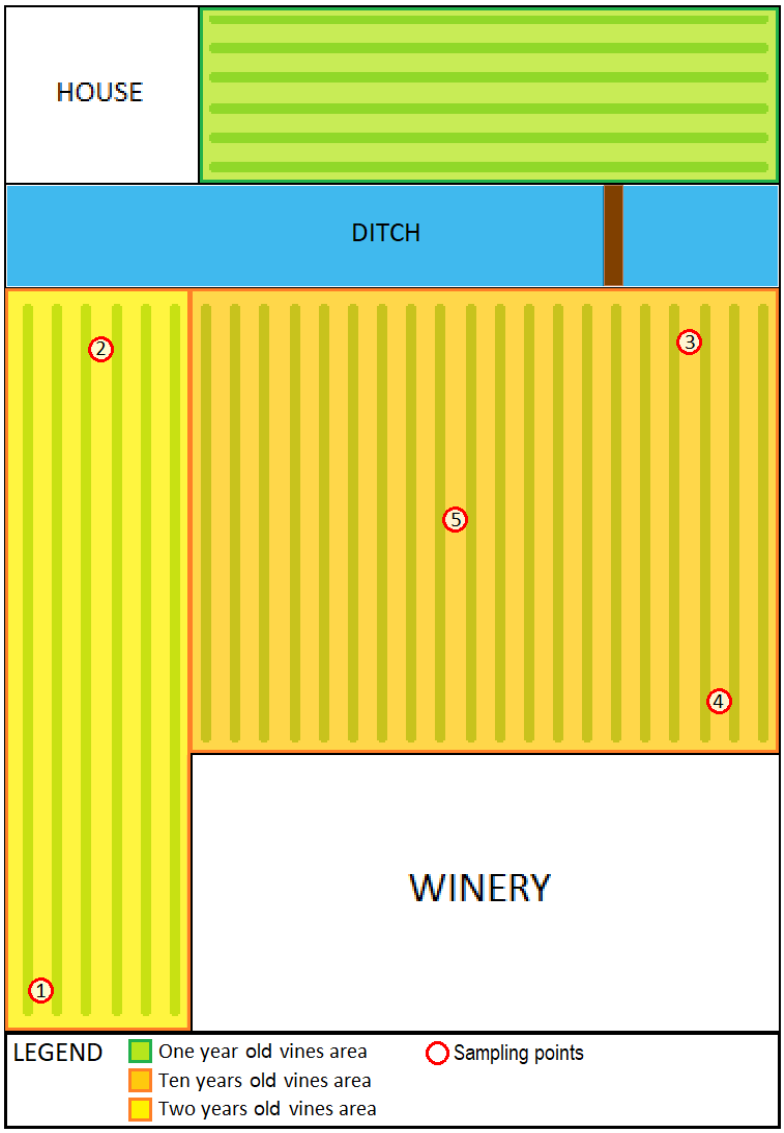


Figure1: Visnà di Vazzola (TV) sampling scheme.

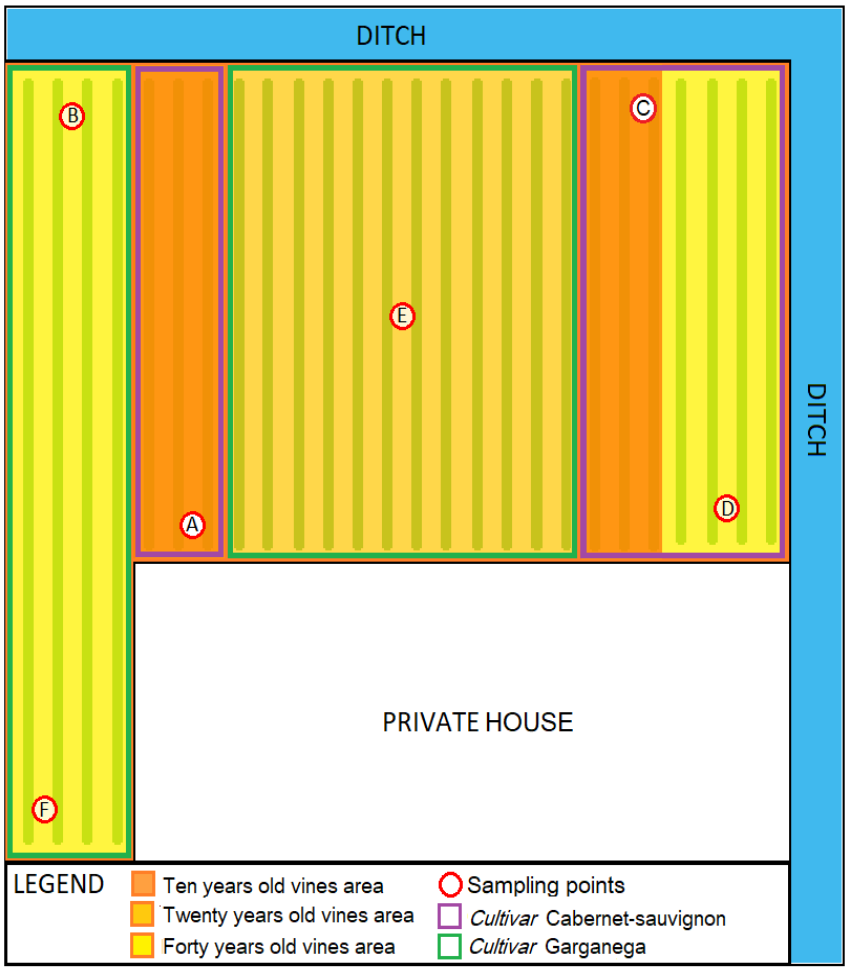


Figure 2: Sant'Andrea di Cologna Veneta (VR) sampling scheme.

2.2.3 Soils characterisation

The pH has been determined with the method III.1 [26]: in a suspension between soil and distilled water with a ratio of 1:2.5 pH value is measured via pH-meter.

Total carbonates have been analysed with the method V.1 [26]: a gas-volumetric determination of the CO_2 produced by treating the sample with hydrochloric acid was carried out using a calci-meter "Dietrich-Frühling".

The cation exchange capacity has been analysed with the method XIII.2 [26]: after mono-saturating the soil with barium chloride, a solution of Mg sulphate is added and insoluble barium sulphate is formed by complete exchange between Ba/Mg. Excess of Mg in solution is determined by complexometric titration with EDTA.

2.2.4 Sample freeze-drying

Samples were freeze-dried with a Freeze Dryer Edwards Ltd. Modulyo® at the operative condition of temperature: -50˚C, pressure: 0.5 mbar for a week.
Freeze-drying is a sublimation process where water is directly vaporised from its solid phase, usually at pressure lower than 4 mbar, avoiding chemical, physical and enzymatic changes and obtaining a highly porous mass having the same size and shape as the originally frozen material. The final result of the freeze-drying is a completely dried sample, ready for the next process.

2.2.5 Sample grinding

Samples were ground with a Mixer Mill MM 400.
The mixer mill is structured by three essential parts: the mixer mill, a couple of grinding jars and the grinding balls. This instrument performs radial oscillations in a horizontal position and the inertia of the grinding ball causes them to impact with high energy on the sample material at the rounded ends of the grinding jars where the sample is grinded. At the same time the movement of jars is perfect to mix and homogenise the sample. To obtain the expected result it is possible to program the frequency of the oscillation and the time of action. Jars with grinding balls in Teflon® have been used.
One small fraction of the lyophilised sample was inserted in the Teflon® jar and threated for 15 minutes at a frequency of 28 oscillations per second. After every single process of grinding and prior the next grinding, jars and spheres were cleaned carefully with ultrapure water and an aqueous 2% solution (v/v) of HCl ROMIL UpA and, then, left under laminar flow hood overnight.

2.2.6 Acid mineralisation

The acid mineralisation procedure is essential to convert a solid homogenised and grounded sample into a liquid solution to be further analysed in ICP-MS (inductively coupled plasma mass spectrometry). The mineralisation process is an acid digestion in closed vessel of suitable material,

in order to minimize any possible contamination and losses of volatile elements. Ethos 1 Microwave digestion system (Milestone) has been used for all the digestions; structured with ten Teflon vessels, transparent to microwaves, it is specifically designed for closed acid digestion.
Replicate of samples were weighed individually and directly inside a vessel. In every digestion procedure, samples were put in nine of the ten vessels and reagent blank was put in the remaining vessel. For plant samples, hydrogen peroxide UpA ROMIL-UpA™ and Nitric Acid UpA ROMIL-UpA™ (1:8) were employed, while for soil samples Nitric Acid UpA ROMIL-UpA™, Hydrofluoric acid UpA ROMIL-UpA™ and hydrogen peroxide UpA ROMIL-UpA™ (3:1:1) were employed. H_2O_2 is an enhancer of the oxidation process, while HF increases the solubilisation of silicates. Cleaning sessions were operated prior any digestion. To avoid any contamination, especially for the plant samples, vessels were thoroughly cleaned by heating them in a muffle-furnace at 600°C and then cleaning them before the digestion at least 6 times. Once digested, samples were recovered and diluted 1:4.5 and stored frozen until the analysis. Before the analysis, samples were thawed and diluted 1:10 (in order to achieve an acid concentration of 2% approximatively, according with the requirement of the ICP-MS).

2.3 Sample Analysis

The elements of interest (Al, Fe, Ni, Zn, Cu, Cr, As, Cd, Sn, and Pb) were analysed using an inductively coupled plasma mass spectrometer ICP-MS 7500 Series Agilent Technologies.
The instrument has these five essential components [27]:

1) *Sample introduction system*: a peristaltic pump continuously pumps the sample solution into a nebuliser that disperses it using a stream of Ar gas. Formed a sample mist, the sample passes through a double-pass spray chamber where the larger sample droplets are removed by collision with the spray chamber wall; excess of sample solution is constantly removed by the same peristaltic pump used for the introduction of the sample. The fine sample aerosol that exits the spray chamber passes directly into the injector tube of the horizontally mounted ICP torch.

2) *ICP (inductively coupled plasma) torch:* the ICP torch is comprised of three concentric quartz tubes through which streams of Ar pass. This structure forms three different streams that are

named, starting from the inner section, carrier or nebuliser gas (responsible of deliver the sample aerosol in the plasma torch), auxiliary gas and plasma gas. At the end the torch is situated inside the 4-turn work coil, which generate an oscillating radio frequency (RF) current at 27.12 MHz; seeding the plasma with electrons and forcing collisions of Ar atoms, this intense RF field generates and sustains the Ar plasma. High temperatures at the centre of plasma ranges from 8,000 to 10,000 K, allowing an instantaneous de-solvation and ionisation of the sample. Ar plasma is a good source of singly charged cations allowing most of the elements of interest to be ionised with efficiency and controlling the mass flow of the Ar gas and optional gasses contributes to very good signal stability.

3) *Interface*: it connects the ICP torch system at ambient pressure to the mass spectrometer at $1 \cdot 10^{-5}$ bar thanks to a series vacuum chambers. The analytes ionised by the Ar plasma are extracted into the first vacuum stage, an expansion region evacuated by a rotary pump, through a hole (1 mm) in the front plate (the sampling cone). The ions then pass through a second orifice (0.4 mm) called the skimmer cone, which acts as a differential aperture between the interface and intermediate vacuum stage which contains the ion optic system and is evacuated by a turbomolecular pump. Ions are extracted from the interface stage and collimated by two conical extraction lenses prior to focusing by the ion optics. To achieve high signal sensitivity, the ion beam must be focused before entering the quadrupole mass analyser. The ion lenses perform the dual role of focusing the ion beam (alternately repelling and attracting the ions) and preventing photons and neutral species from reaching the detector resulting in excellent detection limits.

4) *The quadrupole mass analyser*: this component consists of four long metal rods with a hyperbolic cross section which are arranged parallel to each other and have RF and direct current (DC) voltages applied to them. By varying these voltages, the rods act as a mass filter allowing only ions of a specific mass-to-charge ratio to pass through the centre of the quadrupole at any given combination of applied voltages; the others collide with the rods. After passing through the quadrupole, ion signals are measured by the electron multiplier detector.

5) *Detector*: the electron multiplier (EM) has many dynodes and when an ion enters in the EM, the ion hits the first dynode and a shower of electrons is generated. The electrons then hit the next dynode generating more electrons. The generated current intensity is directly proportional to the number of ions who reach the detector.

The quantitative analysis with the ICP-MS require a sequence of calibration standards of pre-defined increasing concentration to create a calibration curve for each selected element; these concentrations range to cover entirely the expected concentration in the samples.
The principle is to measure the instrument signal intensity related to the unknown concentration of a specific element of a solution (sample) and compare it with the signal intensity of the same element in a solution which its concentration is known (standard). This is possible only because the signal is directly proportional to concentration in solution.
Another important requirement is the internal standard (Rh at a concentration of 100 ppb), one single solution of an element that is not considered in the analysis, that has an atomic mass and ionisation potential similar to analytes and is employed to compensate the drift of the instrument, instability and matrix effects; to keep the instrument at the best operating conditions an aqueous solution of HNO_3 2% (v/v) was flushed after each analysis.
Measurement error expressed as the percentage of the relative standard deviation (RSD%) is < 10% and expressed as the standard error percentage (SE%) is < 5% for all the elements analysed.

2.4 Evaluation of traceability

The elements concentrations will be studied in all matrices considered and the traceability will be assessed by calculating the transfer factor.
The transfer factor for an element from soil in plant is generally calculated using the formula [28]:

$$TF = \frac{Mp}{Ms}$$

where:

TF = Transfer Factor;

M_p = metal content in plant (mg kg^{-1});

M_s = acid soluble concentration of an element in soil (mg kg^{-1}).

In this study the total concentration of the elements of interest was quantified only in soil samples. However, the total concentration of the elements of interest in roots is the concentration which plants

take up from soil, hence it can represent the bio-accessible and mobile concentration of trace elements. Thus, the formula aforementioned can be used to evaluate the transfer factor from roots to any other part of the vine [29].

If the transfer factor is above 1, there is a high transport or the "ending" have a concentration higher than the "starting". If the factor is near 1, there is the possibility of a total transport, and if the factor has a value below 1, there is a lower transport as the value is near the zero.

3 Results and discussion

In this paragraph will be used acronym for the name of the sample in relation to the belonging vineyards (CV for Sant'Andrea di Cologna Veneta and VdV for Visnà di Vazzola) and the chemical elements focus of this study will be mentioned whit its chemical symbol omitting the isotope: Al (27), Cr (52), Fe (56), Ni (60), Cu (63), Zn (66), As (75), Cd (111), Sn (118) and Pb (208).
The results will be discussed in this order: soil, roots, flowers, leaves, stems and grapes. As aforementioned results for grapes, stems and leaves of the Visnà di Vazzola vineyard (TV) are not available because of an early harvest due to warm temperature.

3.1 Soil characterisation

Table 5: Physico-chemical characterisation of soil. CEC = Cation-exchange capacity in cmol kg^{-1}; Tot CO_3^{2-} = total carbonates.

SAMPLE	**VDV1**	**VDV2**	**VDV3**	**VDV4**	**VDV5**	**CVA**	**CVB**	**CVC**	**CVD**	**CVE**	**CVF**
pH	7.80	7.46	7.56	7.04	7.24	6.34	7.04	6.92	6.66	6.80	6.48
CSC	34.470	42.441	47.823	30.788	20.188	44.991	52.218	32.564	38.495	24.935	41.433
Tot CO_3^{2-}	16.23	7.27	10.93	23.41	15.37	6.83	7.58	2.76	4.06	2.76	5.23

In the Table 5 are expressed the data related to the physico-chemical characterisation of soil at the depth of 20-30 cm (maximum uptake for vines).
The pH values in VdV samples range from 7.04 in VDV4 to 7.80 in VDV1, while in CV samples the values are lower and range from 6.34 in CVA to 7.04 in CVB.

The Cation-exchange capacity (CEC) ranges in VdV soil from 20.188 in VDV5 to 47.823 cmol kg^{-1} in VDV3, whereas in CV soil the values are higher and ranges from 24.935 in CVE to 52.218 cmol kg^{-1} in CVB.

The total carbonate is expressed as a percentage value of carbonate in soil; in VdV ranges from a 7.27% in VDV2 to 23.41% in VDV4, while in CV values are lower and ranges from a 2.76% in CVC to 6.83% in CVA.

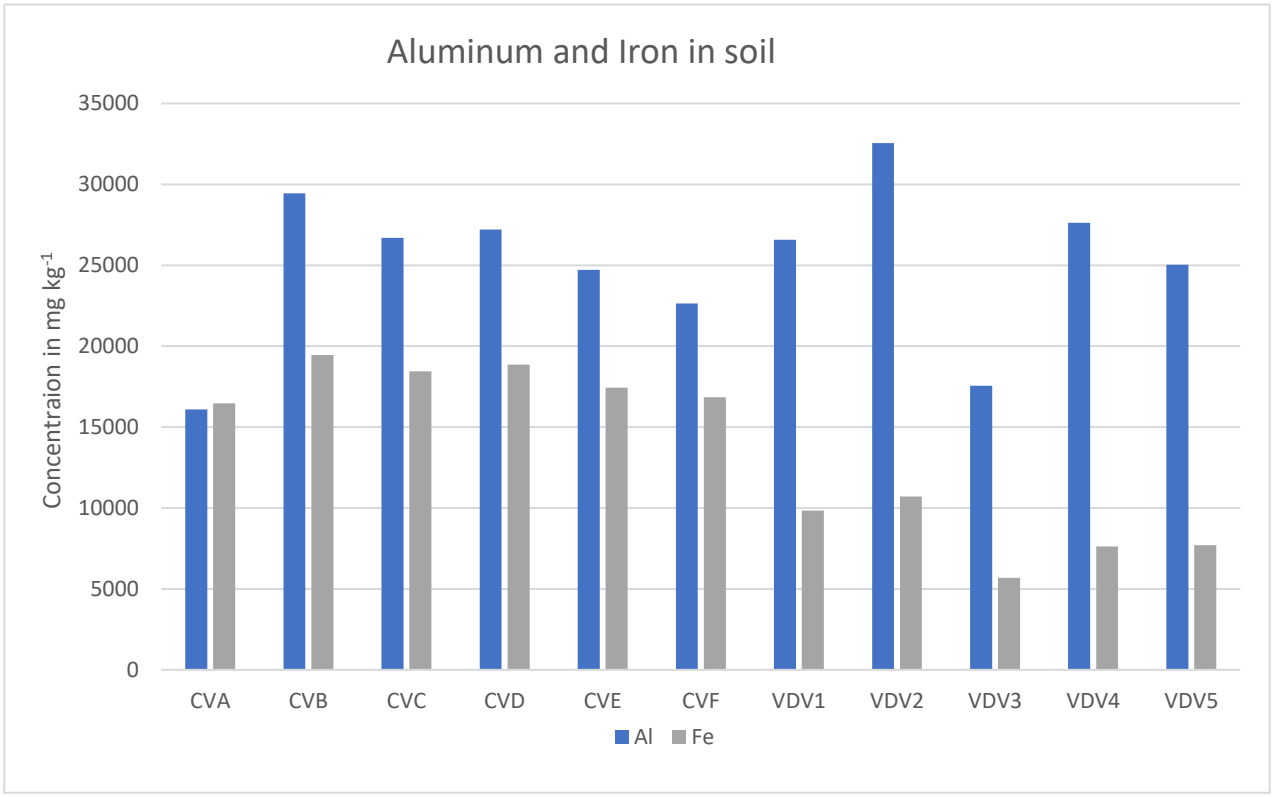
3.2 Elements concentration results

3.2.1 Elements concentration in soil

Figures 3-5 show the concentration of all elements in soil of both vineyards. In CV soil the elements with a very high concentration in were Al and Fe, which range from 16093 to 29445 mg kg^{-1} and from 16466 to 19452 mg kg^{-1} respectively. The same elements vary in soil from VdV vineyard from 17559 to 32544 mg kg^{-1} and from 5681 to 10715 mg kg^{-1} respectively (Figure 3). The elements with a very low concentration in all samples are Sn and Cd that range in CV soil from 3.50 to 4.08 mg kg^{-1} and from 0.227 to 0.316 mg kg^{-1} respectively, whereas in VdV soil range from 0.712 to 2.32 mg kg^{-1} and from 0.111 to 0.409 mg kg^{-1} respectively (Figure 5). Delineating a decreasing order for the concentration in both vineyards it is possible to say that in soil of CV CVB, CVC, CVD, and CVF present the same sequence (Al > Fe > Cu > Zn > Cr > Pb > Ni > As > Cd > Sn) instead, in CVA Fe is higher than Al, and in CVE Zn is higher than Cu.

In soil from VdV, VDV1, VDV2, and VDV5 present the same sequence (Al > Fe > Cu > Zn > Cr > Ni > Pb > As >Sn > Cd), while in VDV3 Zn showed a higher concentration than Cu and in VDV4 Pb showed a higher concentration than Ni (Figure 4). This strongly similarity among the samples, for each site, with some little exceptions, is probably due to the treatments applied to the soil to keep it at the best condition for the cultivation of the vines and to the low spatial variability of the sites itself.

Figures 3-5: Concentration in mg kg^{-1} of all element analysed in soil for all samples.



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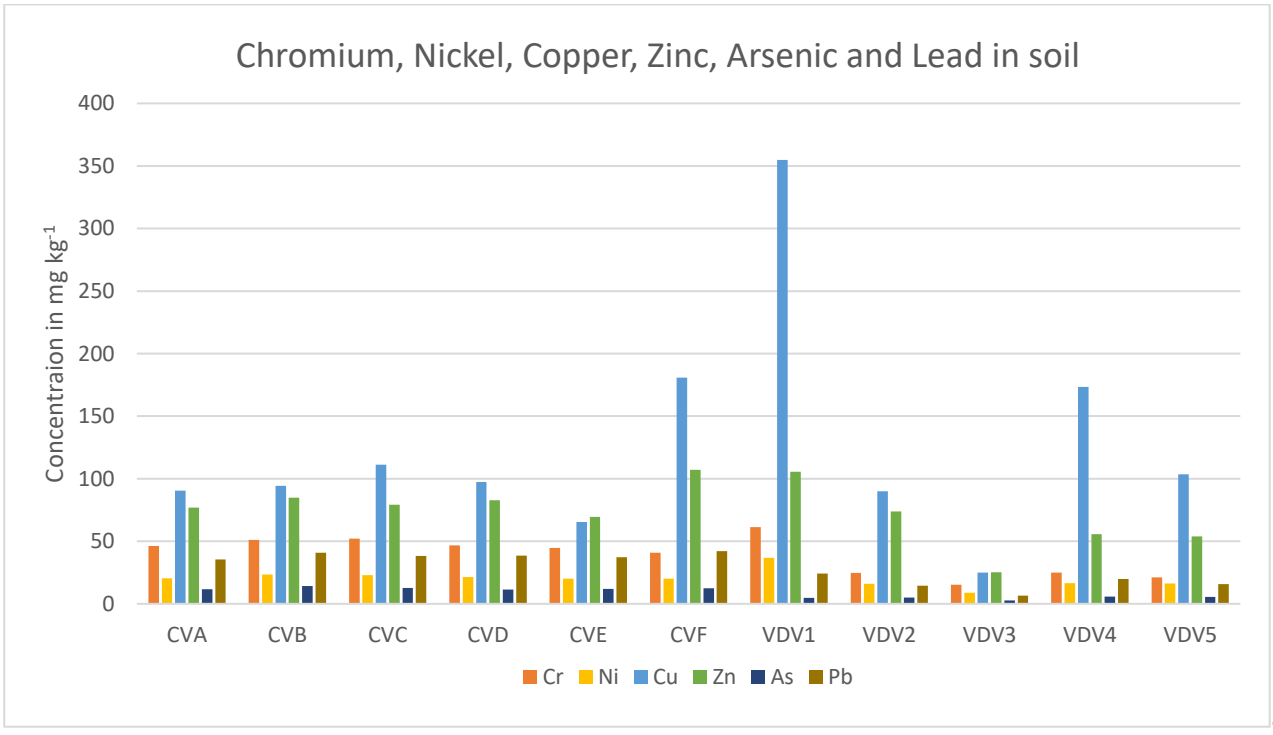

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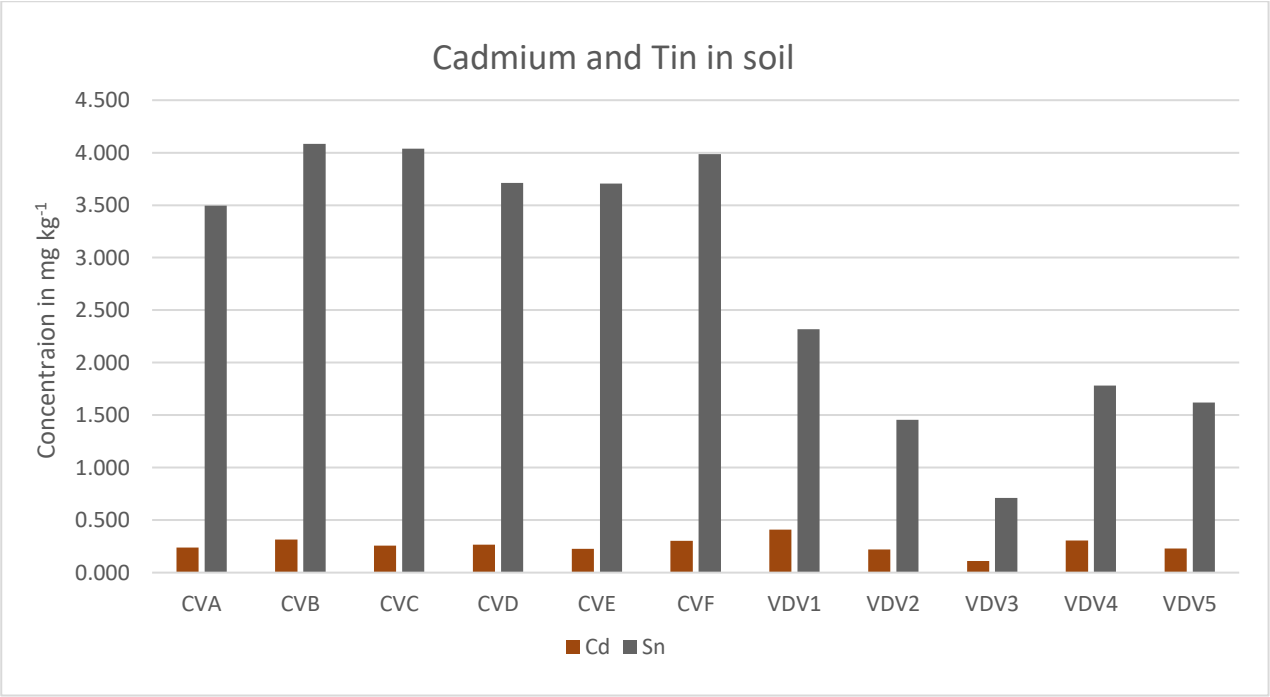

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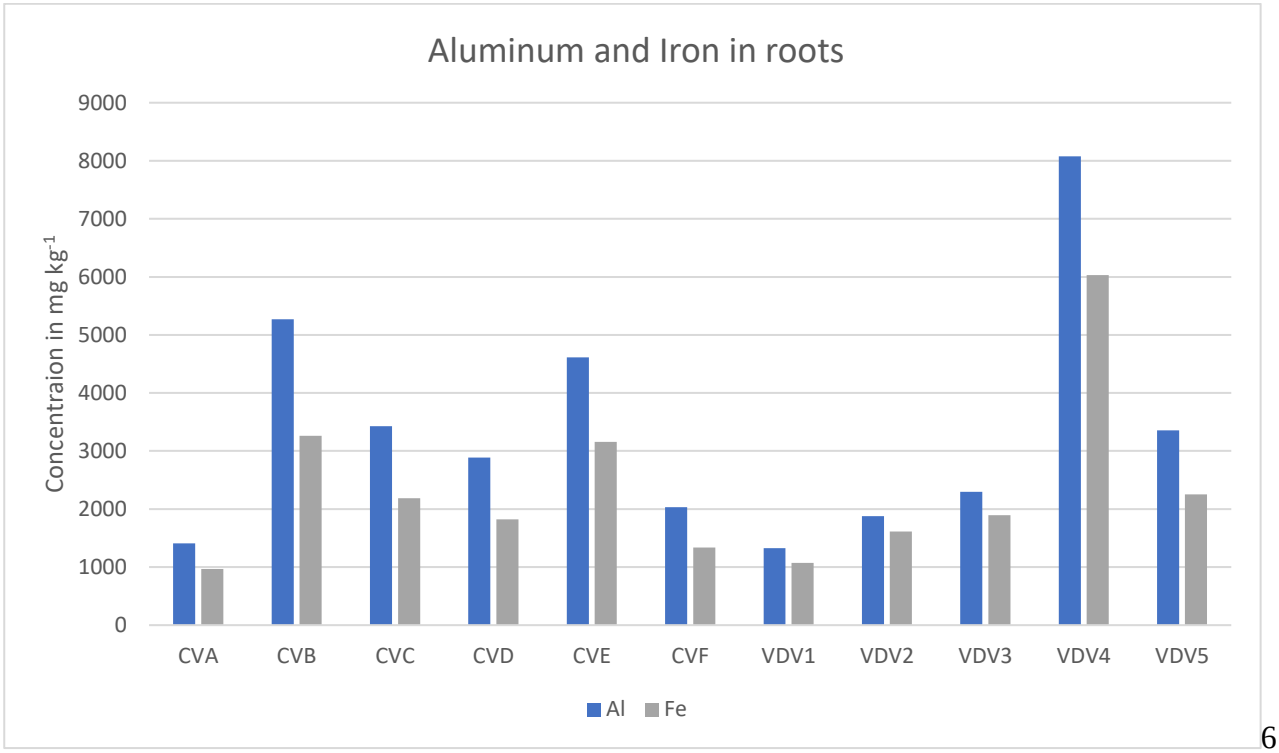
3.2.2 Elements concentration in roots

The Figures 6-9 show the concentration of all elements in roots of both vineyards. Al and Fe showed very high concentrations, which range from 1409 to 5270 mg kg^{-1}, and from 966 to 3263 mg kg^{-1} respectively in roots of CV. In VdV roots Al ranges from 1876 mg kg^{-1} to 8074 mg kg^{-1} and Fe ranges from 1071 mg kg^{-1} to 6030 mg kg^{-1} (Figure 6). As and Cd showed the lowest concentration among all the elements studied; in CV samples the range of concentration observed was from 0.811 to 2.139 mg kg^{-1} for As and from 0.048 to 0.208 mg kg^{-1} for Cd. The same elements vary in root samples of VdV from 0.514 to 2.338 mg kg^{-1} and from 0.124 to 0.218 mg kg^{-1} respectively (Figure 9). In CV roots, CVB, CVD, and CVE showed the same sequence of element concentration in decreasing order (Al > Fe > Cu > Zn > Cr > Ni > Pb > As > Cd > Sn) instead, in CVC Zn was higher than Cu and Ni higher than Cr. In CVF Ni concentration was higher than Cr, while CVA has a completely different sequence (Al > Fe > Zn > Cu > Pb > Ni > Cr > As > Cd > Sn). In VdV roots, VDV1, VDV2, VDV3, and VDV5 showed the same sequence order (Al > Fe > Cu > Zn > Ni > Cr > Pb > As > Cd > Sn) instead, in VDV4 the concentration of Cr was higher than Ni (Figures 6-9).

Sn present concentration under the limit of detection in CVA, CVE, CVF, VDV1, and VDV2. VDV4 has higher concentration of all elements in relation to the other samples, suggesting a probably different uptake by this specific vine.

Figures 6-9: Concentration in mg kg^{-1} of all element analysed in roots for all samples.



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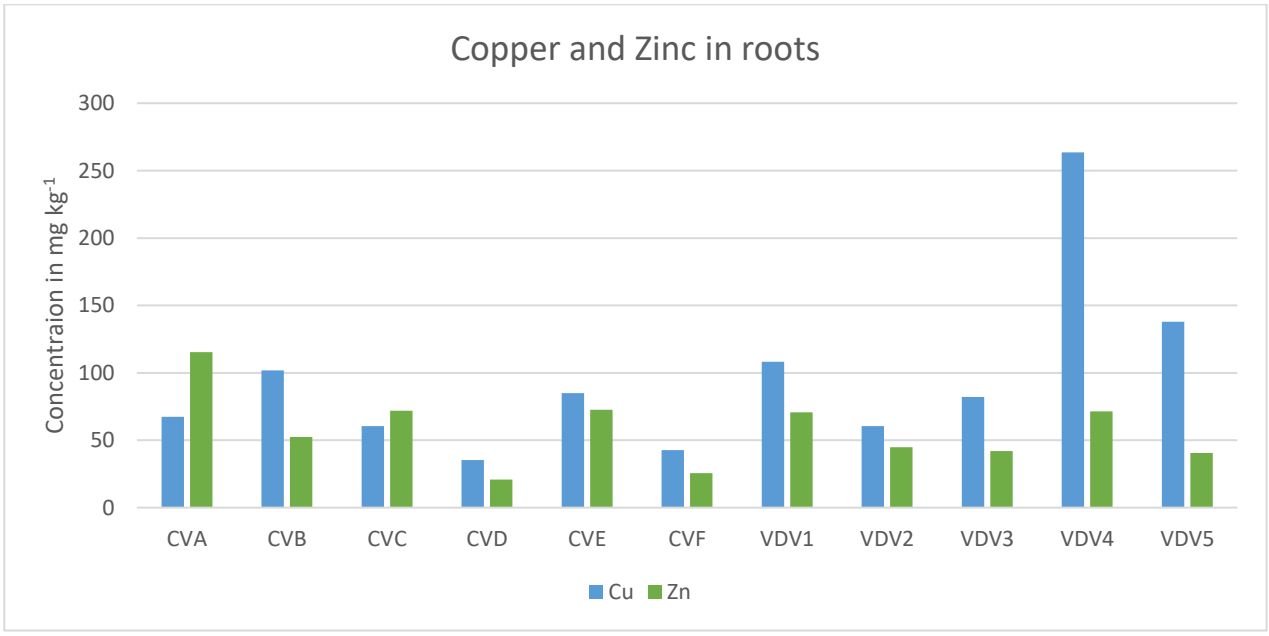

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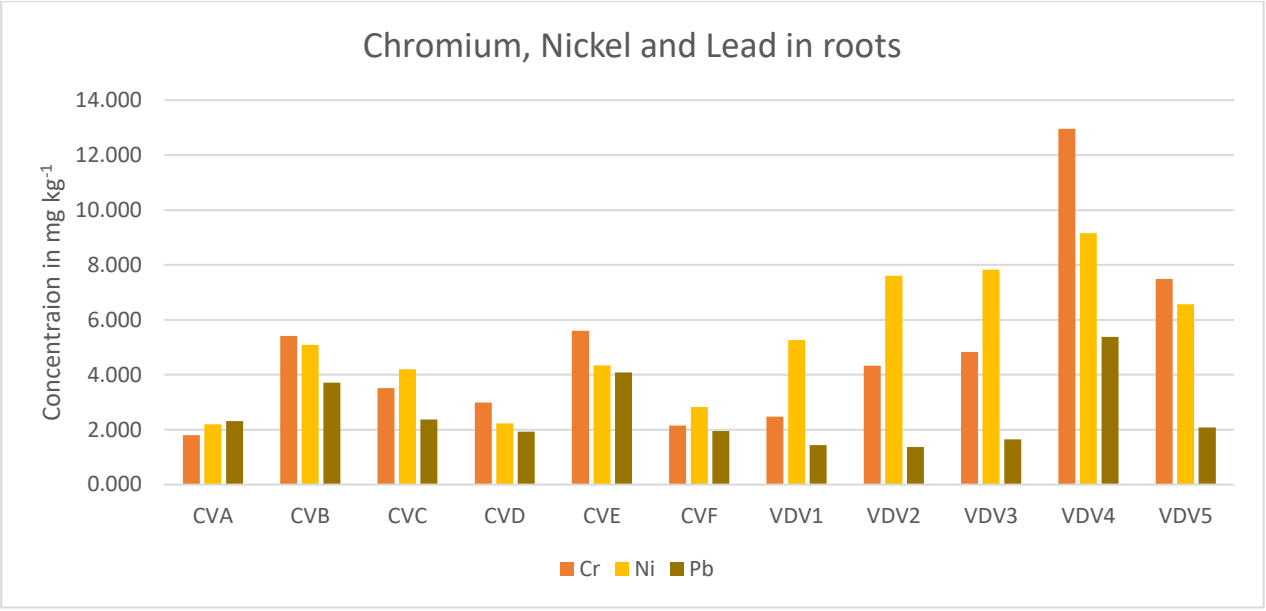

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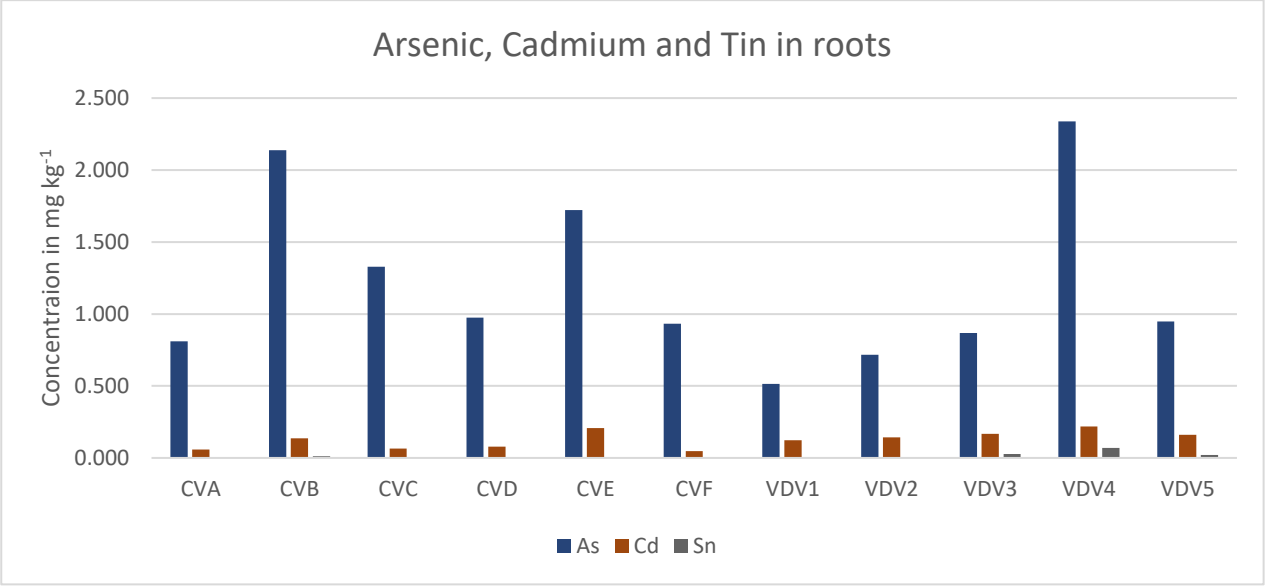

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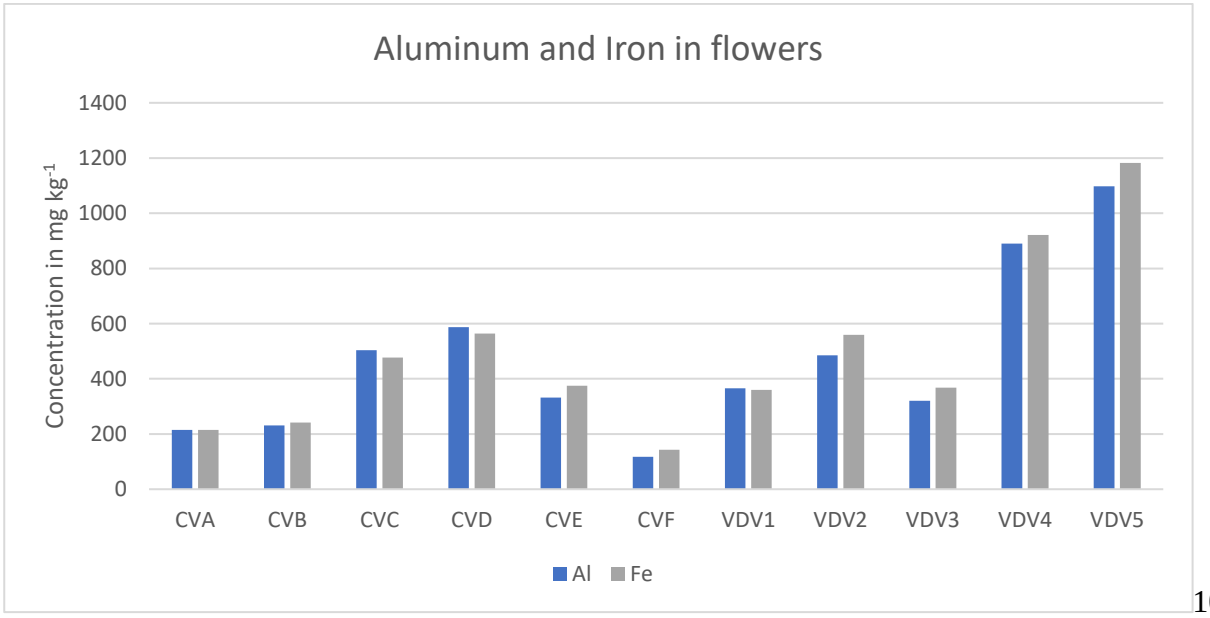
3.2.3 Elements concentration in flowers

The Figures 10-13 show the concentration of all elements in flowers of both vineyards.

As for the other matrices, Fe and Al showed a very high concentration, which range in CV samples from 143 to 564 mg kg^{-1} and from 117 to 587 mg kg^{-1} respectively, while in flower samples of VdV Fe ranges from 360 to 1.183 mg kg^{-1} and Al from 321 to 1097 mg kg^{-1} (Figure 10). In CV flowers, Sn and Cd showed very low concentration which were from 0.022 to 0.135 mg kg^{-1} and from 0.002 to 0.007 mg kg^{-1} respectively. In the flowers from VdV a similar situation occurs with concentration

of Sn that ranges from 0.009 to 0.028 mg kg^{-1} and values of Cd that range from 0.012 to 0.024 mg kg^{-1} (Figure 13). CVA, CVC, and CVD showed the same sequence of element concentration in decreasing order (Al > Fe > Cu > Zn > Cr > Ni > Pb > As > Sn > Cd), while in CVB, CVE, and CVF Fe concentration was higher than Al and Ni concentration was higher than Cr. In VdV flowers, VDV3 and VDV4 showed the same sequence of element concentration in decreasing order (Fe >Al > Zn > Cu > Ni > Cr > Pb > As > Cd > Sn) (Figures 10-12). Sn showed a higher concentration than that of Cd in VDV2 and VDV5, while in VDV1 Al concentration was higher than Fe concentration.

Figures 10-13: Concentration in mg kg^{-1} of all element analysed in flowers for all samples.



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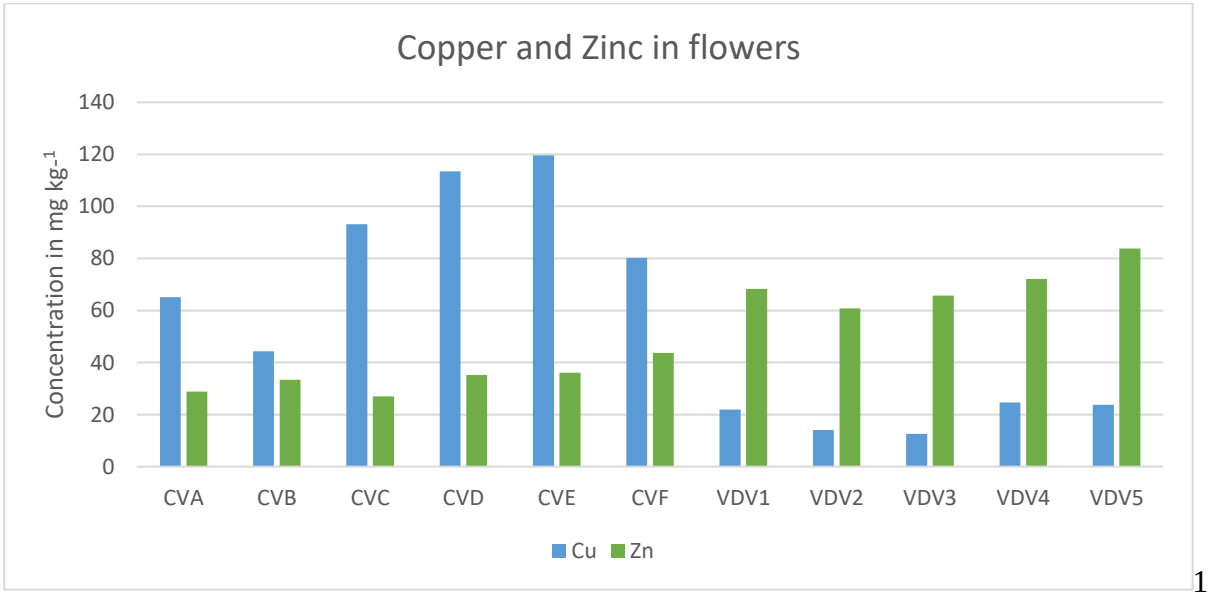

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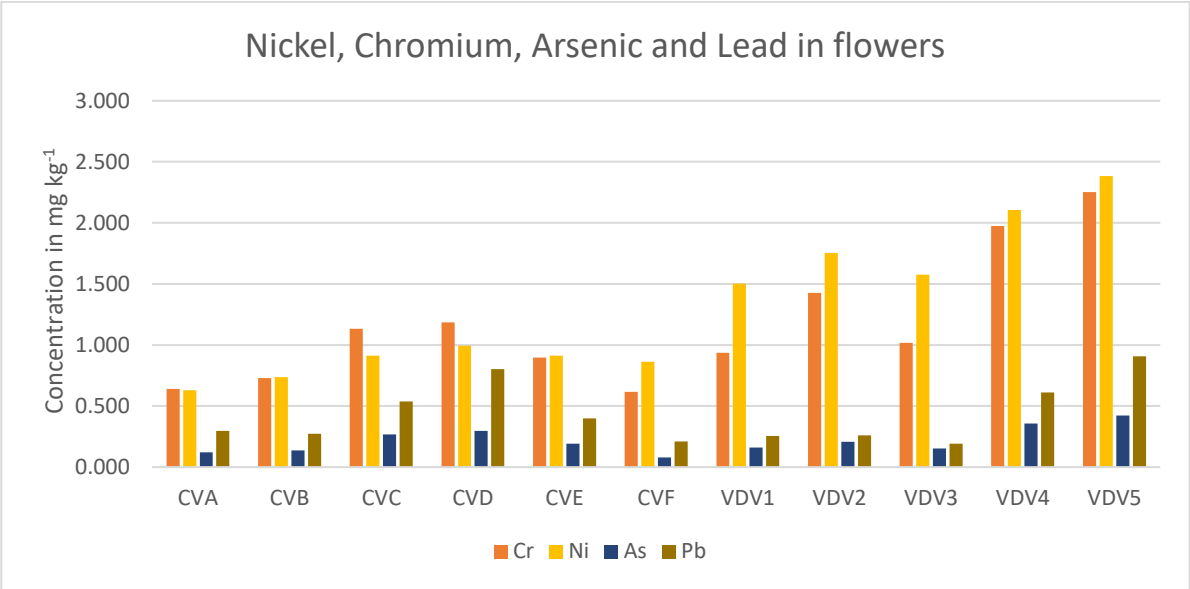

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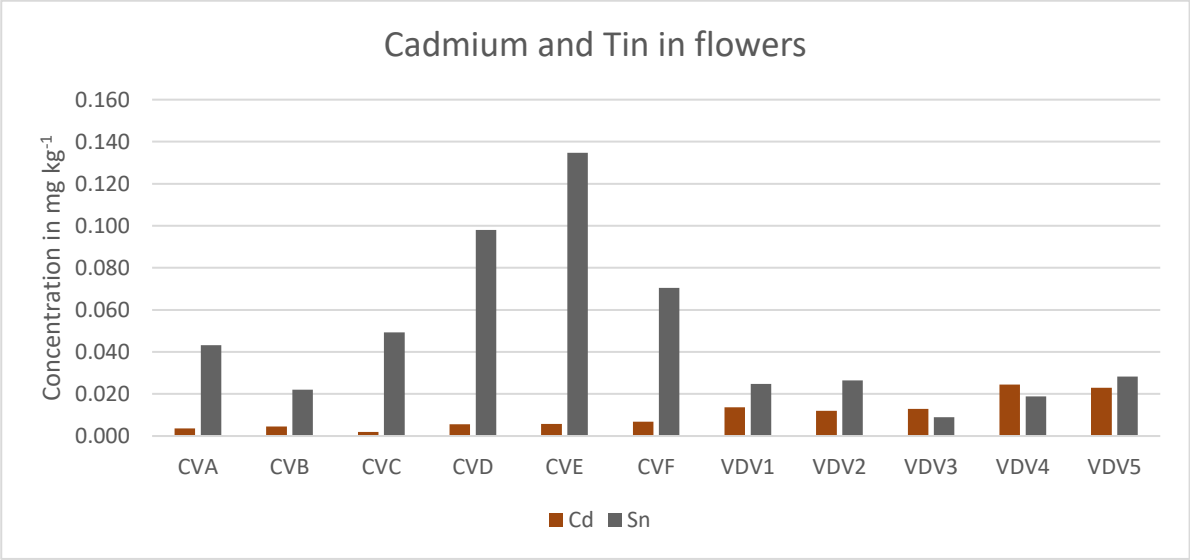

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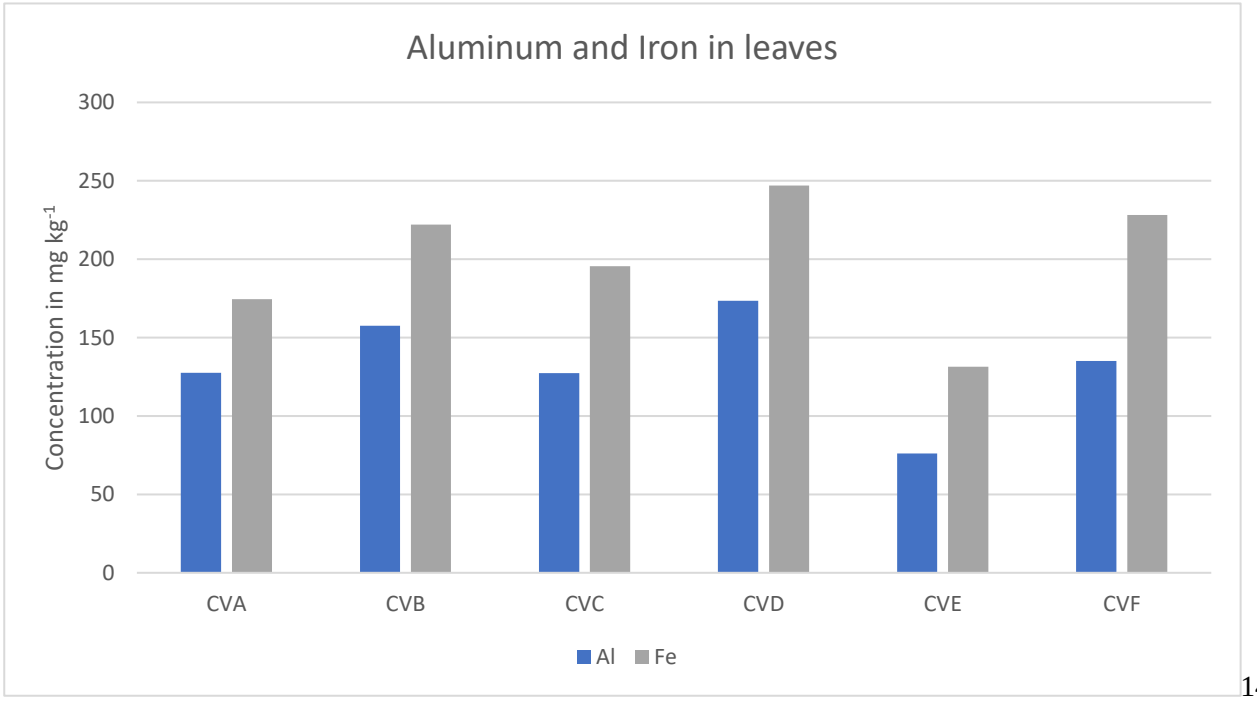
3.2.4 Elements concentration in leaves

The Figures 14-17 show the concentration of all elements in leaves from CV vineyard. As observed for roots and flowers, Fe and Al showed very high concentrations that range from 131.4 to 247.0 mg kg^{-1} and from 76.2 to 173.4 mg kg^{-1} respectively (Figure 14). Sn and Cd showed very low concentrations, which range from 0.029 to 0.167 mg kg^{-1} and from 0.005 to 0.011 mg kg^{-1} respectively (Figure 17). CVA and CVF showed the same sequence of elements in decreasing order (Fe > Al >

Zn > Cu > Ni > Cr > Pb > As > Sn > Cd). In CVD and CVE, the concentration of Cr was higher than that of Ni, while in CVB and CVC the concentration of Cu was higher than that of Zn (Figures 15-16). Cr, Ni and Zn showed their lowest concentrations in CVA, while Al, Fe, Cu, As, Cd, Sn and Pb showed their lowest concentrations in CVE. Cu, Zn, As, Cd, Sn and Pb showed the highest concentrations in CVB, while the highest concentrations of Al, Cr, Fe were observed in CVD.

Figures 14-17: Concentration in mg kg^{-1} of all element analysed in leaves for all samples.



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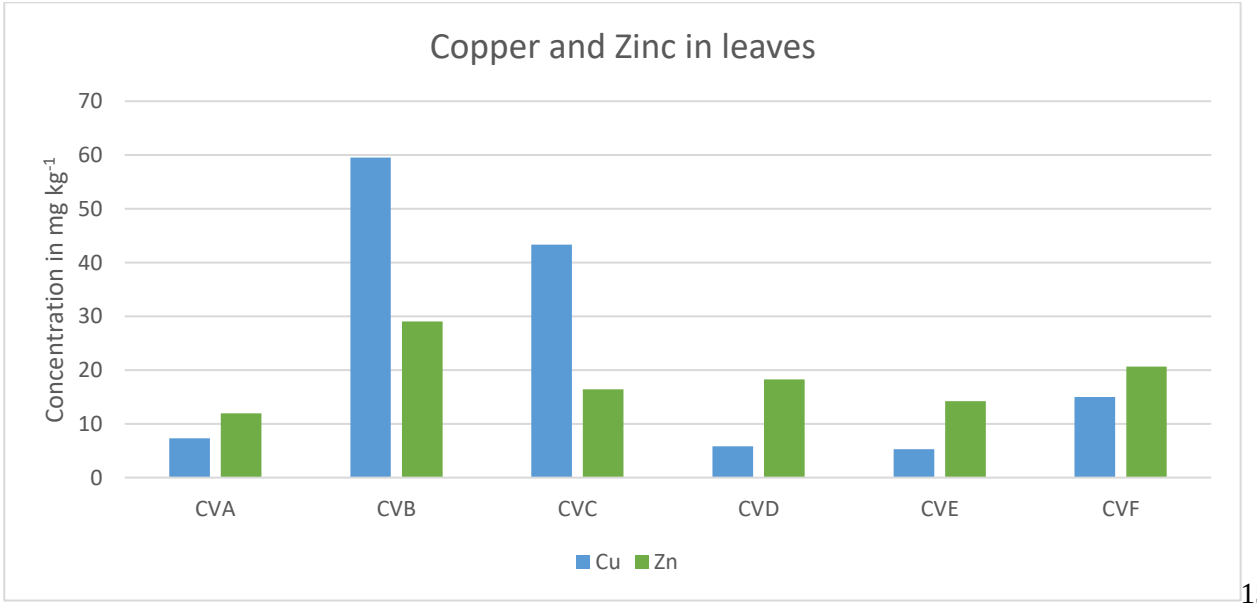

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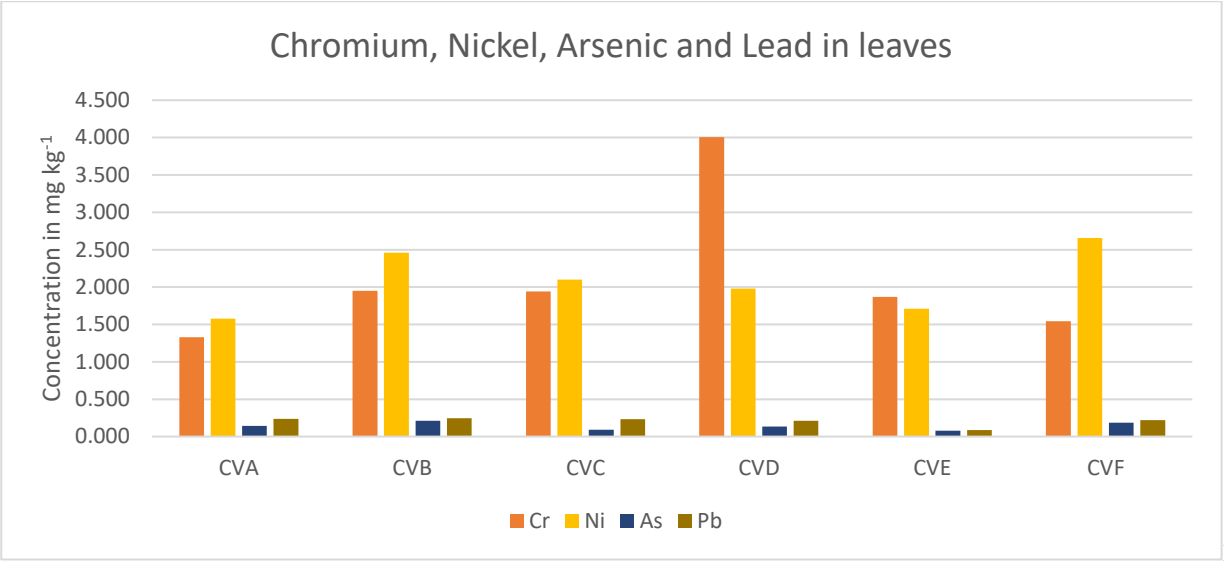

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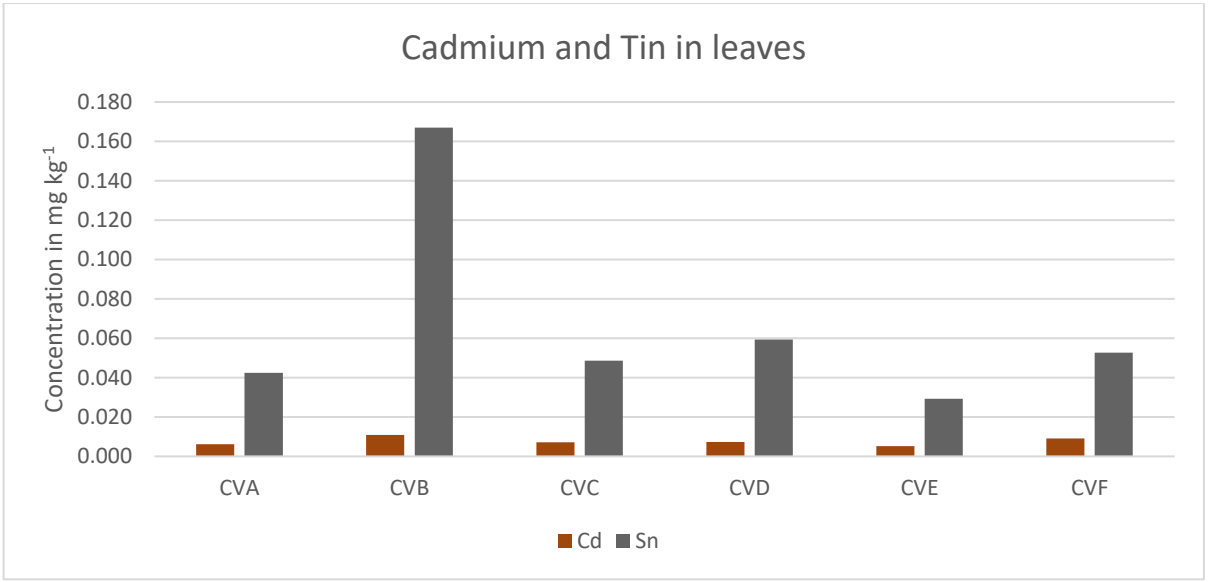

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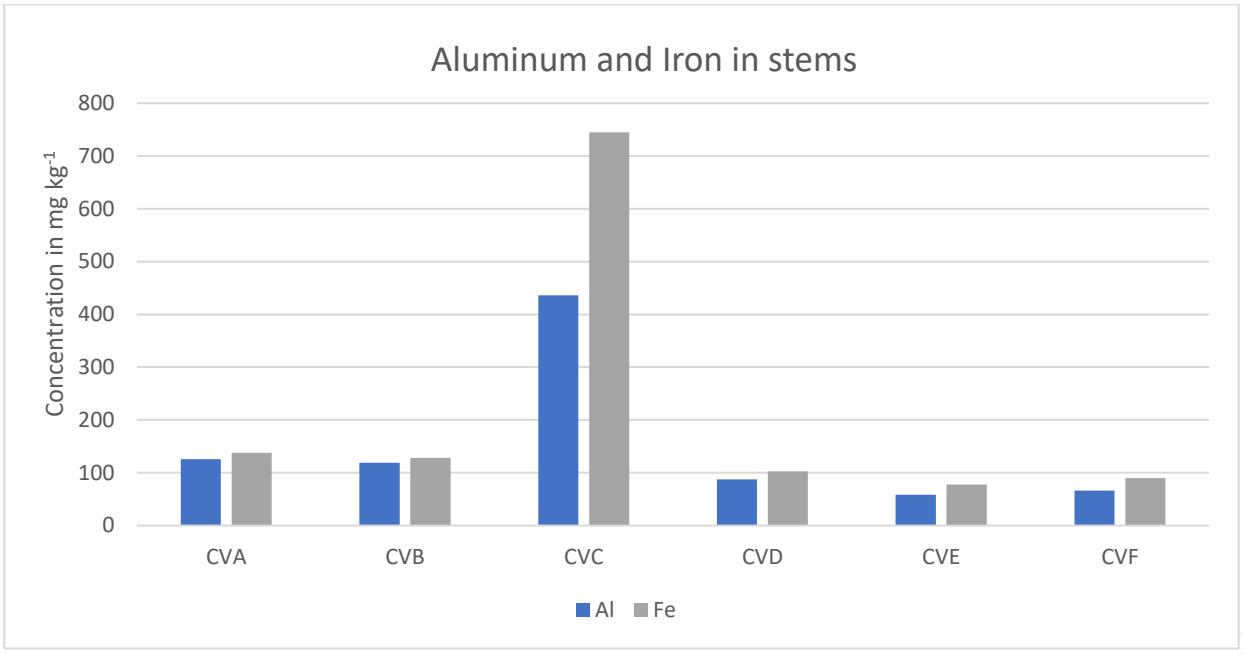
3.2.5 Elements concentration in stems

The Figures 18-21 show the concentrations of all elements in stems from CV vineyards. As observed in other parts of the plants, Fe and Al showed very high concentrations that range from 77.9 to 744.9 mg kg^{-1} and from 58.3 to 436 mg kg^{-1} respectively (Figure 18). Every sample has a singular sequence of element concentration in decreasing order. Cu is higher than Zn in CVA, CVC, and CVF stems samples, instead for the others (CVB, CVD, and CVE) is the opposite (Figure 19). Cr is higher than Ni in all samples except for CVB stems where is the opposite. The sequence (Pb > As > Cd) is present in CVA, CVE, and CVF but in CVA stems Sn is higher than these elements, whereas in CVD the

sequence of the elements at low concentration is (Pb > As > Sn > Cd) (Figures 20-21). In CVC, the highest concentration of all elements was observed, while the lowest concentration of Al, Cr, Fe, Ni, Cu, As, Cd and Pb was observed in CVE. CVC showed higher values than the other sample suggesting a probable different accumulation pattern for this vine.

Figures 18-21: Concentration in mg kg^{-1} of all element analysed in stems for all samples.



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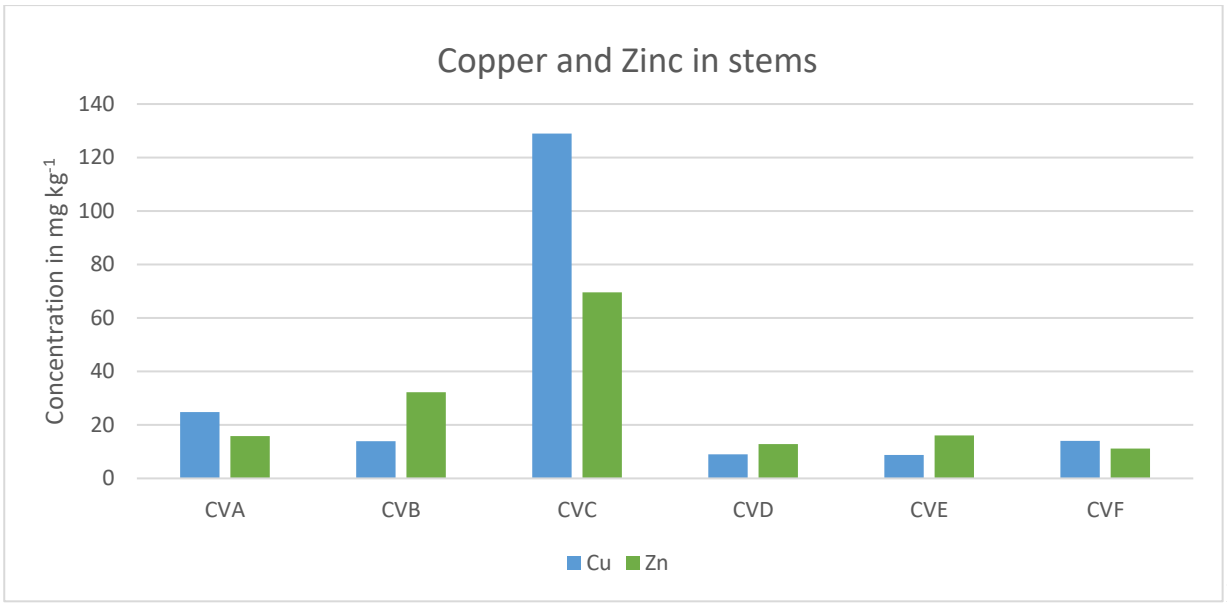

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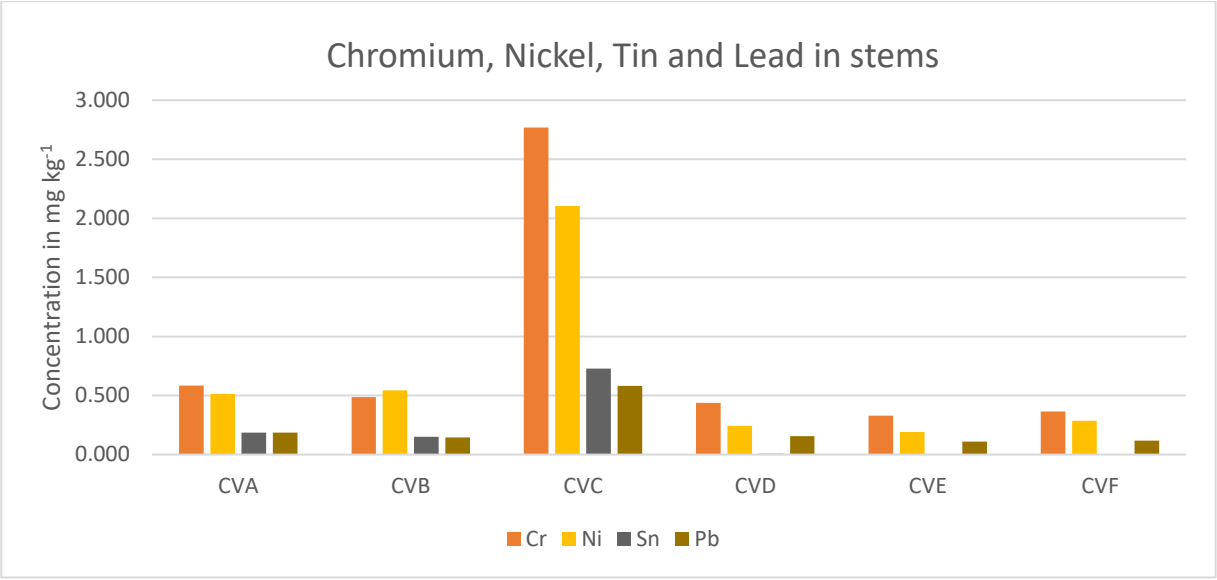

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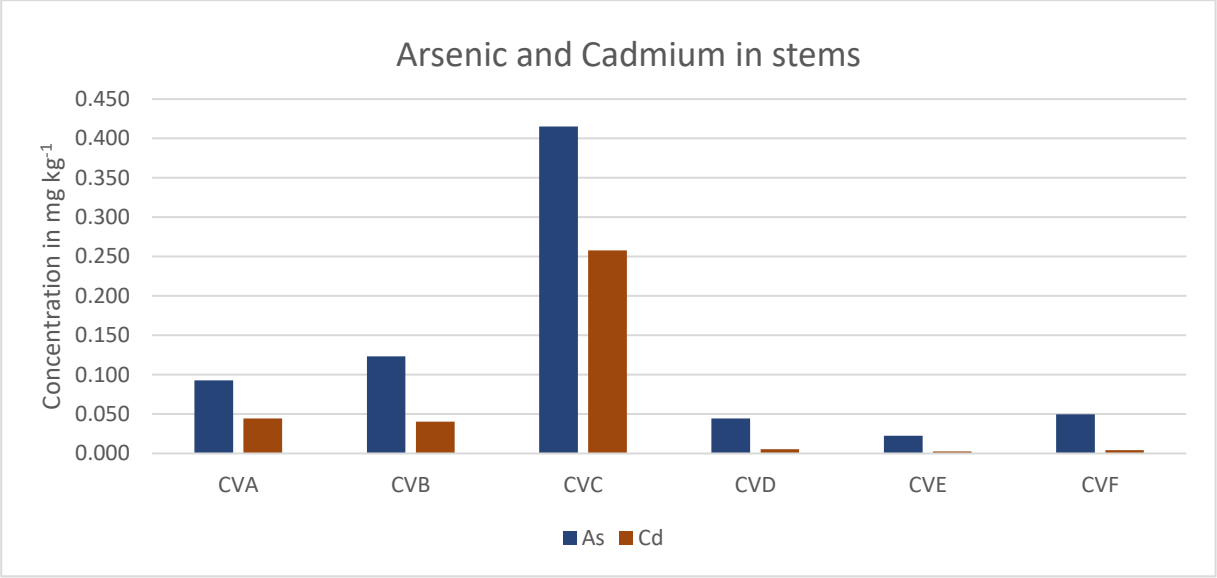

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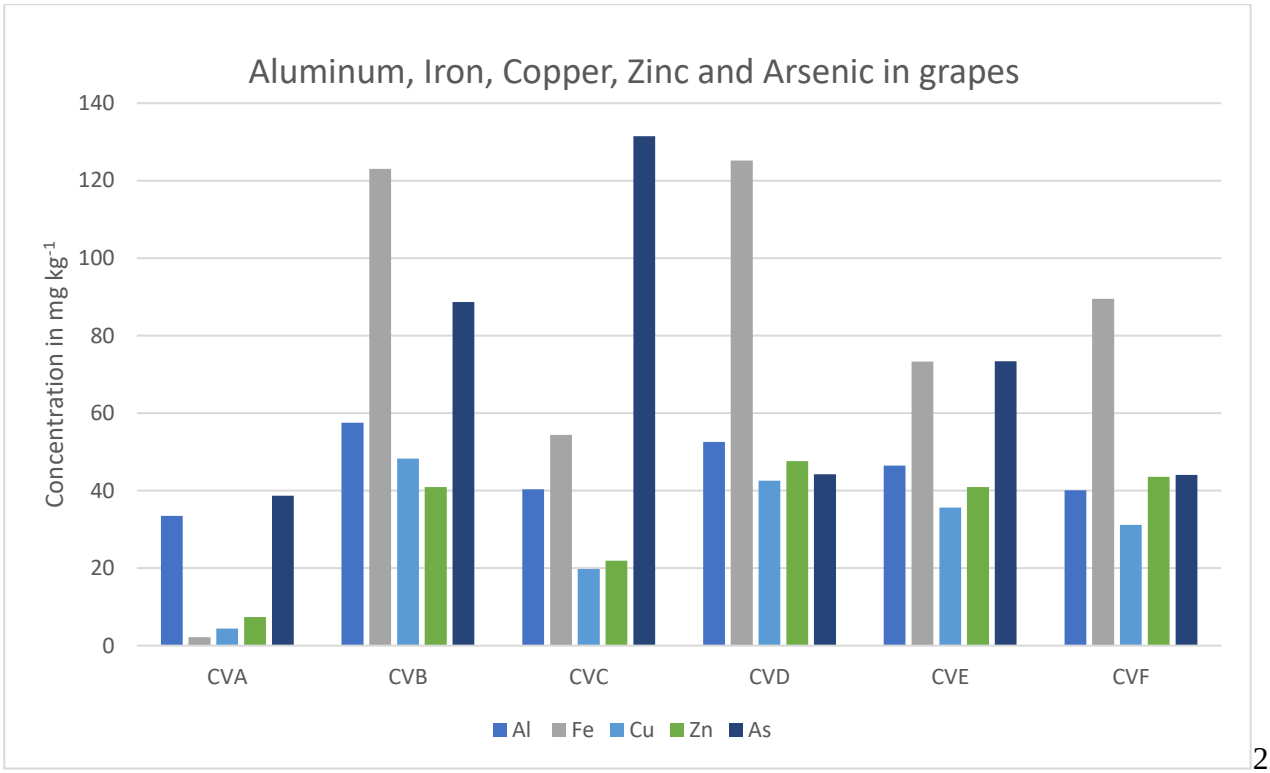
3.2.6 Elements concentration in grapes

The Figures 22-23 show the concentrations of all elements in grapes from CV vineyard. In every sample a singular sequence of element concentration in decreasing order was observed.

As differently from the other matrices, present very high concentrations, ranging from 38.68 to 131.46 mg kg^{-1} in all the samples, especially in CVA, CVC and CVE grapes where is the element with the highest concentration. As for the other grape samples, Fe is the elements that shows the highest values (Figure 22). The other elements that present a relevant concentration are Zn and Cu, in this decreasing order, for CVC, CVD, CVE, and CVF while in CVB grapes it is the opposite and in CVA both elements present low concentrations (Figure 22). Sn is lower than the detection limit for all the

samples. In CVA grape sample, the lowest concentration of Al, Cr, Fe, Cu, Zn, As, and Pb were shown, while the highest concentration of Al, Cu and Cd were shown in CVB (Figures 22-23). In CVC the highest concentration of As was shown, while the highest concentration of Fe and Zn were observed in CVD. CVE grapes shows the highest concentration of Cr, Ni and Pb.

Figures 22-23: Concentration in mg kg^{-1} of all element analysed in grapes for all the samples.



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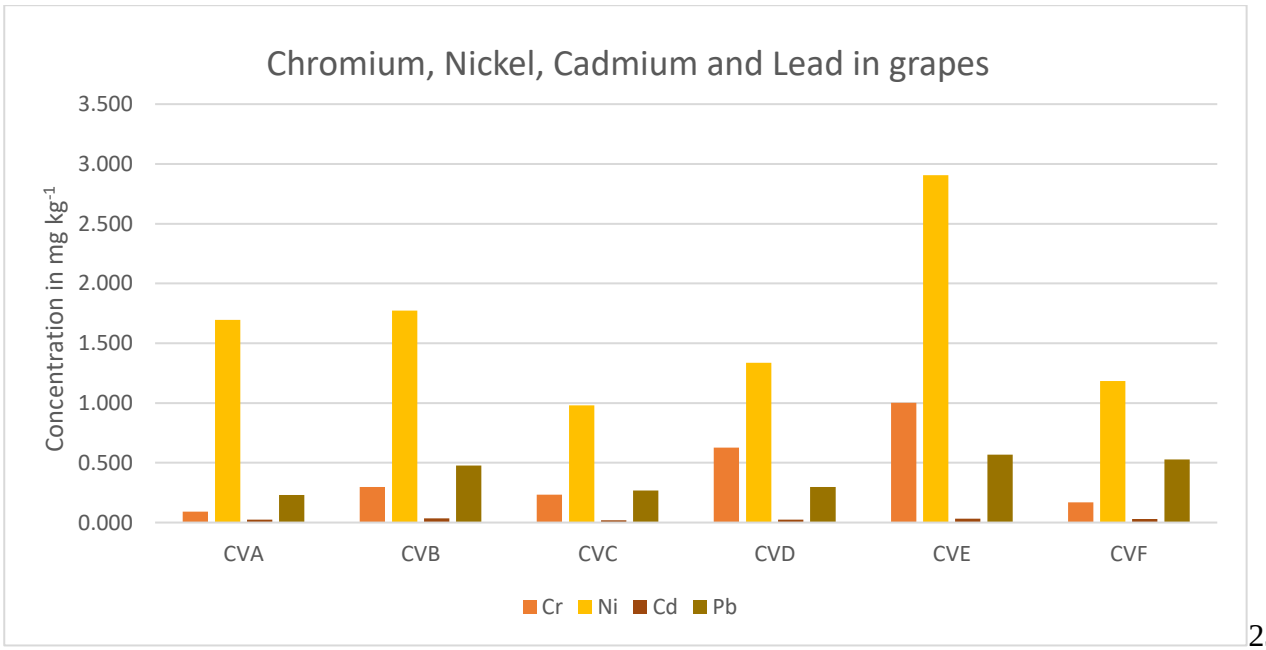

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3.3 Evaluation of the traceability of all elements with the transfer factor

3.3.1 Transfer factor for metal/metalloid roots to flowers

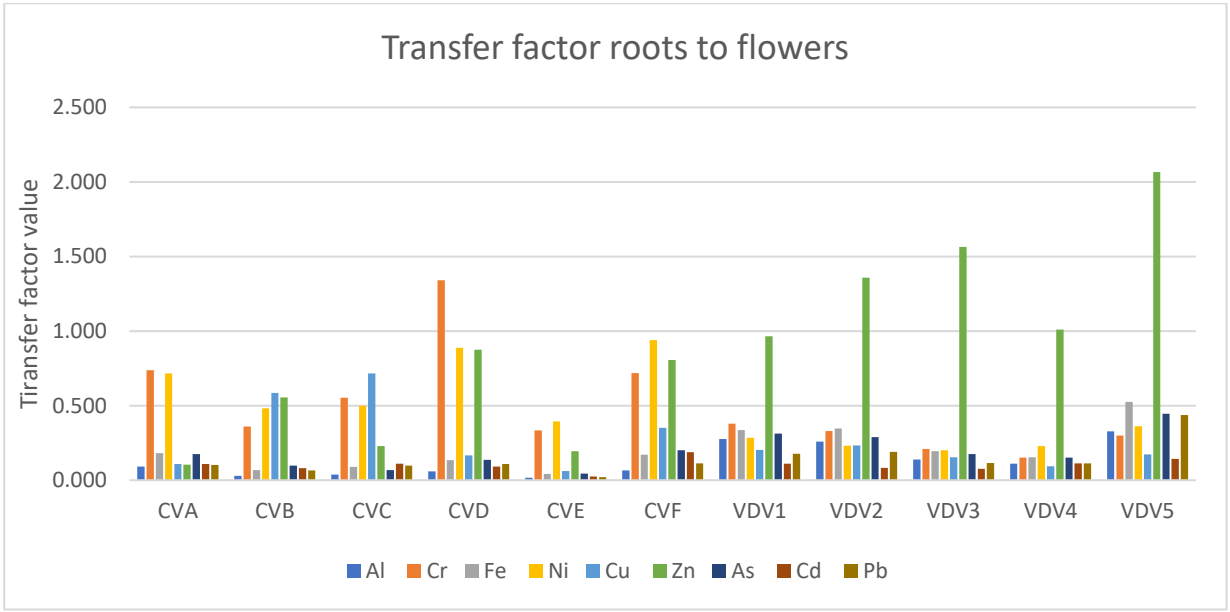
In Figure 24 TFs from roots to flowers for both vineyards are shown.

Sn has high TF values in CVC and CVD caused by its concentration in roots near to the limit of detection or the zero value (denominator of the formula) and because of that will be not considered as appropriate and omitted in the Figure 25.

In CV vineyard, TFs of Cr and Ni are relevant in all samples and only in CVB and CVC the TF of Cu was high as well. In this vineyard the lowest TFs of Al, Cr, Fe, Ni, Cu, As and Pb were observed in CVB, while the highest TFs of Cr, Fe, Ni, Cu, Zn, As, Sn and Pb were observed in CVD.

In VdV vineyard, Zn presents the highest TF among the elements in all the samples, ranging from 0.965 to 2.067; the TFs of Fe, Al, As, Cd and Pb were higher than those observed in CV. As regards VdV sampling sites, VDV1 has the lowest TF of Zn, VDV2 shows the lowest TF of Cd while the lowest TFs of Al, Cr, Fe, Cu, As, Sn and Pb were observed in VDV4. On the contrary, the highest TFs of Fe, Ni, Zn, As, Cd, Sn and Pb were observed in VDV5.

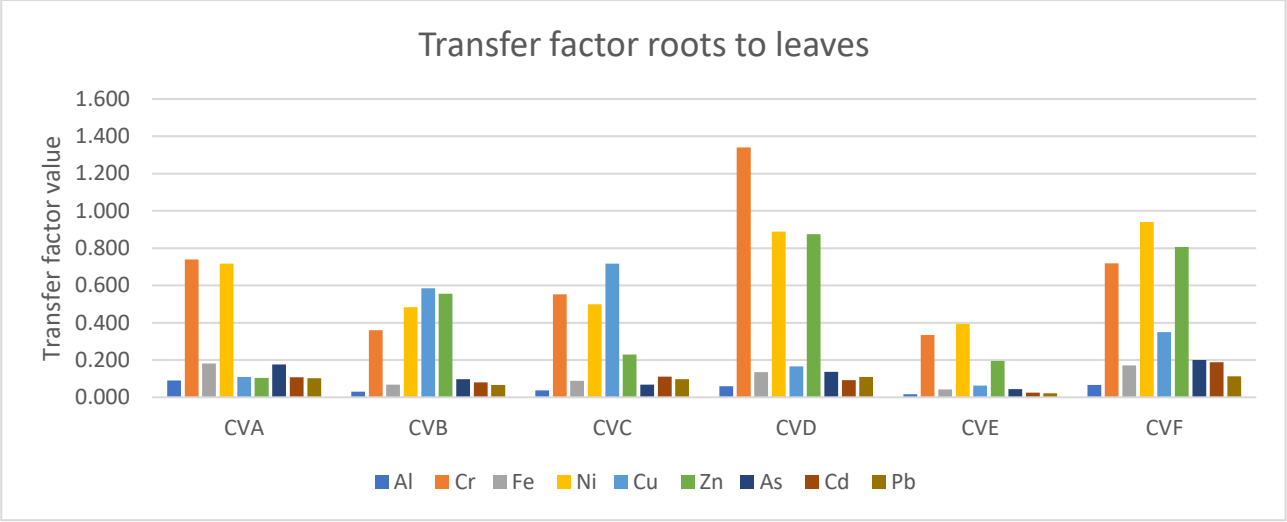
Figure 24: Transfer factor roots to flowers in all samples.



3.3.2 Transfer factor for metal/metalloid roots to leaves

In Figure 25 TFs from roots to leaves in CV vineyard are shown. The TF values change among the samples: in CVA, CVD, and CVE the highest TF values belong to Cr and Ni, in CVF it belongs to Ni and Zn, in CVB belong to Cu and Zn and in CVC to Cu and Cr. In CVA TFs of Al and Fe were highest, while TF of Zn was the lowest. The lowest TFs of Al, Cr, Fe, Ni, Cu, As, Cd and Pb were observed in CVE, while the highest TFs of Ni, As, Cd and Pb were observed in CVF. The highest TF of Cu and the lowest of Sn were observed in CVC. In CVA TFs of Al and Fe were highest, while TF of Zn was the lowest. The elements that present very low value in all the samples considered are Cd, Pb and Al. For the reason previously explained the TF value of Sn have been.

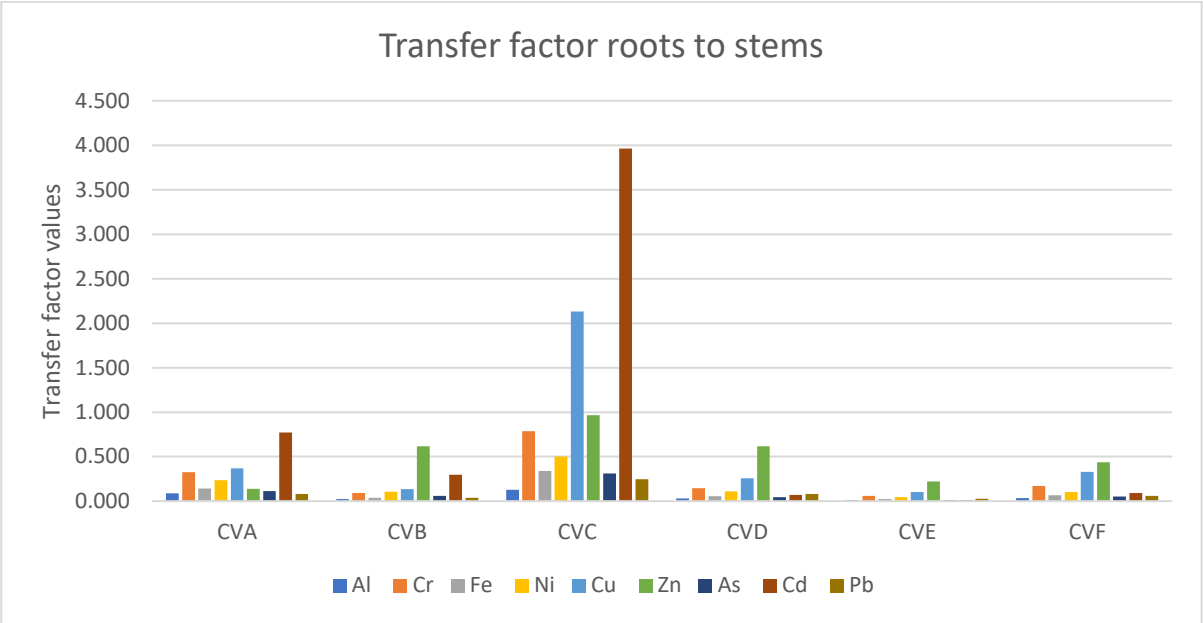
Figure 25: Transfer factor roots to leaves in all samples.



3.3.3 Transfer factor for metal/metalloid roots to stems

In Figure 26 TFs from roots to stems in CV vineyard are shown. As observed for samples of leaves, TF values change a lot among the sampling sites. The highest TF of Cd was observed in CVA and CVC, while the highest TF of Zn was observed in CVB, CVD, CVE, and CVF. Other TFs which may be relevant for all samples were those of Cu and Cr. CVC has the highest TF of all elements analysed among the samples in agreement with the concentration observed. The lowest TFs of As, Zn, Al and Pb were observed in CVA, while in CVE all the other elements showed their lowest TFs values. For the reason previously explained the TF value of Sn have been.

Figure 26: Transfer factor roots to stems in all samples.

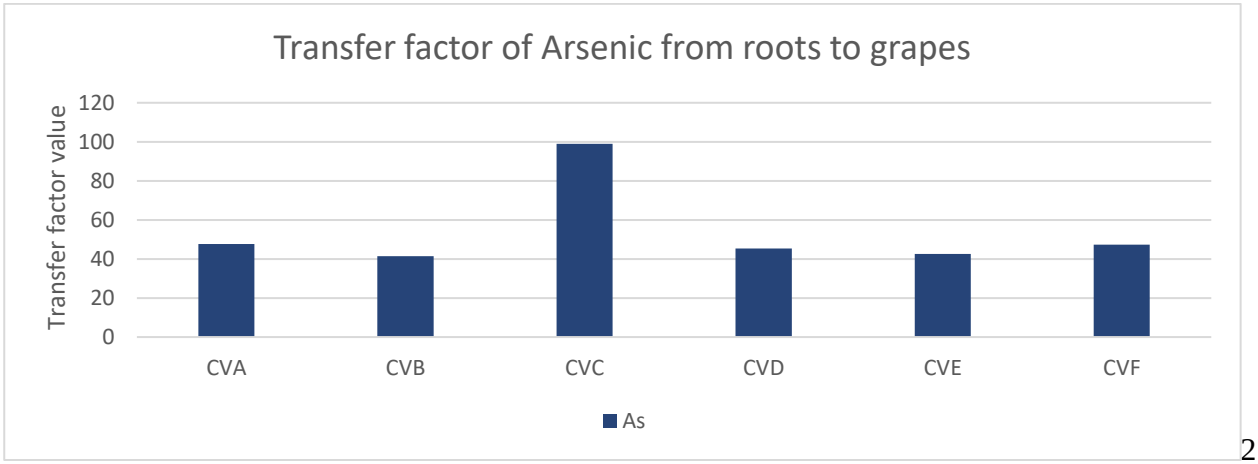


3.3.4 Transfer factor for metal/metalloid roots to grapes

In Figures 27-29 TFs from roots to grapes in CV vineyard are shown. The TF value of As was the highest, presenting values from 30 to 100 times higher in comparison with the other elements (Figure 27). Although some differences, other relevant TFs were those of Zn, Cu and Ni in samples CVB, CVC, CVD, CVE, and CVF. The lowest TF values among the elements were observed for Fe and Al in all the samples (Figure 29).

The lowest TF value for Cd was observed in CVE, while its highest TF was observed in CVF. The TF of Pb was highest in CVF, while it was lowest in CVA, where TFs of Cr, Fe, Cu and Zn were lowest, as well (Figure 29). As aforementioned, the TF value of Sn has been omitted.

Figures 27-29: Transfer factor roots to grapes in all samples



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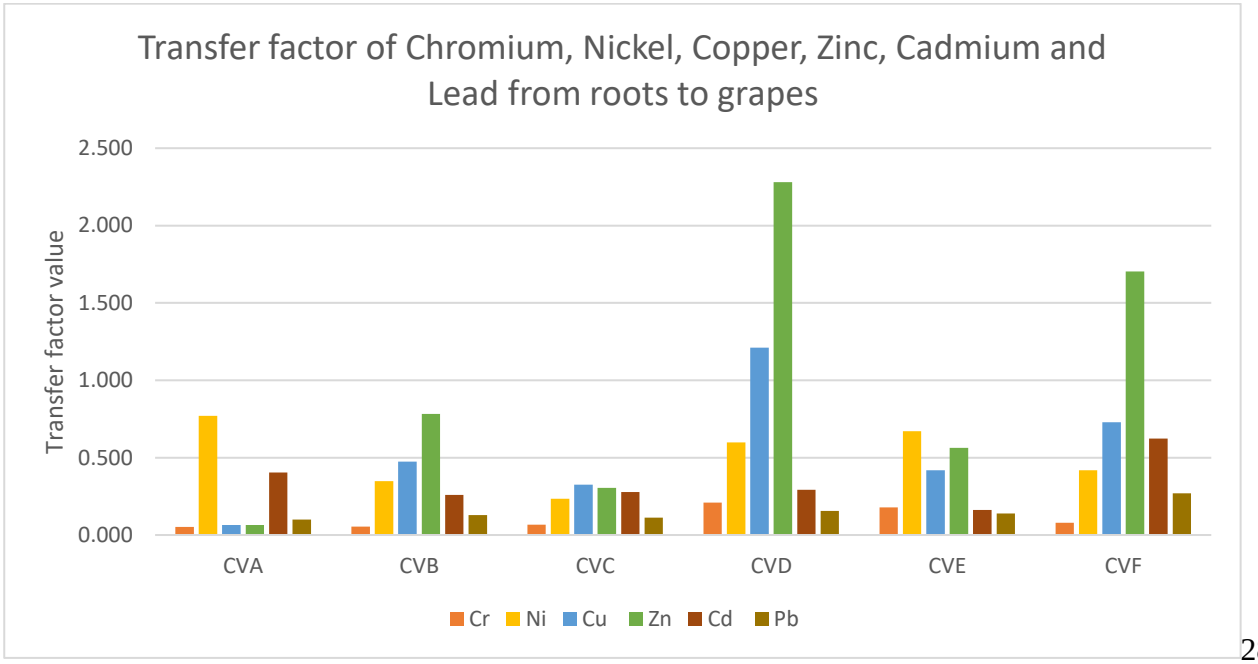

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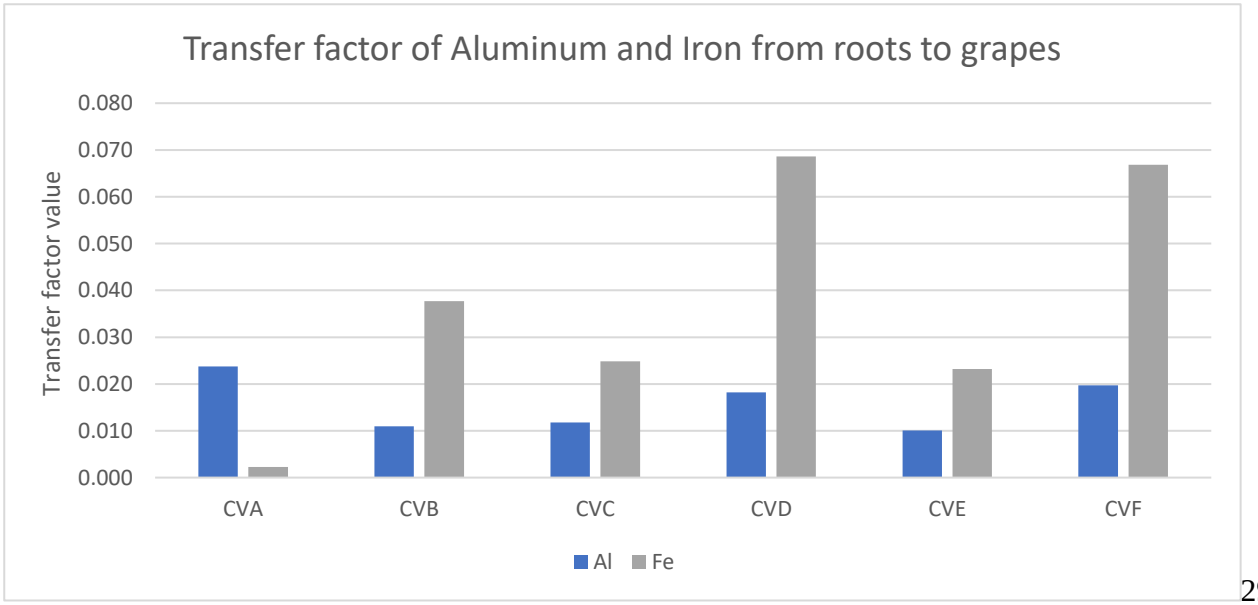

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3.4 Discussion

3.4.1 Soil characterisation discussion

Table 6: Physico-chemical analysis of soil.

Soil analysis	**Visnà di Vazzola (TV)**	**Sant'Andrea di Cologna Veneta (VR)**
pH	7.04 - 7.80	6.34 - 7.04
Cation-exchange capacity	20.188 - 47.823 cmol kg^{-1}	24.935 - 52.218 cmol kg^{-1}
Total carbonates	7.27 % - 23.41%	2.76 % - 6.83%
Soil taxonomy classification	Fluvic Cambisols (Calcaric)	Fluvic Cambisol

In Table 6 are synthetically shown all the data related to the physico-chemical analysis of the soils of both vineyards; more details are available in Table 5.

According with recent interpretative indication of the ARPAV [4a], it is possible to distribute the samples in three categories: VDV1, VDV2, and VDV3 belong to the sub-alkaline range (pH values varying from 7.3 to 8.0), samples VDV4, VDV5, CVB, CVC, CVD, and CVE belong to the neutral range (pH values comprised in the range 6.7-7.3), while CVA and CVF belong to the sub-acid range (pH values ranging from 6.0 to 6.6).

The soil of VdV vineyard may be classified between the neutral range, in which are present the best condition for the growth of many crops, promoted by a strong microbiological activity and by the solubilisation of mineral elements, and the sub-alkaline range in which, the presence of carbonates influences negatively the solubilisation of some macro-nutrients like K and Mg.

The soil of CV vineyard instead, may be classified between the neutral and the sub-acid range where the solubilisation of nutrients is enhanced so much that the concentration in plant of Fe, Al, Cu and Ni may reach toxic levels, and the absorption by plant of Ca, Mg, K and P is limited by the leaching.

The Cation-exchange capacity (CEC) seems to confirm the consideration done for the pH. VDV4, VDV5, and CVE could be included into the high activity range (18-32 cmol kg^{-1}) whereas the other samples, that present a CEC value above 32 cmol kg^{-1} could be included in the very high activity range.

A high activity means a good presence of clay, probably illite or chlorite, or organic matter and a good water holding capacity, in agreement with the categorisation of the soil expressed in the section

2.1. Together with pH values, the CEC values suggest a low solubilisation of Al^{3+} for the plant in both vineyards.

The total carbonate results implying a division of the sample in three categories: poorly calcareous (1-5% of total carbonate) for CVC, CVE, and CVD; moderately calcareous (5-10%) for CVA, CVB, CVF, and VDV2, and highly calcareous (10-25%) for VDV1, VDV3, VDV4, and VDV5. According to the soil categorisation it is possible to define the soil of CV vineyard as "from calcareous to poorly calcareous" and the soil of VdV vineyard as "highly calcareous"; this agrees with the descriptions for both soils presents in the soil map of ARPAV 2015 [3a].

3.4.2 Soil analysis discussion

The concentration of elements present in the soil of the two vineyards under investigation is shown in Tables 7 - 8; these values has been compared with the limits set by the Italian Legislative Decree 152/2006, shown in Table 9.

Table 7: Element total concentration, expressed in mg kg^{-1} in soil of Sant'Andrea di Cologna Veneta (VR) vineyard; concentration in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
CVA	**16093**	46.1	**16466**	20.4	90.4	77	11.6	0.240	**3.50**	**35.4**
CVB	**29445**	51.1	**19452**	**23.4**	94.3	84.8	**14.1**	**0.316**	**4.08**	41
CVC	26696	**52.2**	18438	22.8	111.2	79.3	12.8	0.256	4.04	38.3
CVD	27210	46.7	18855	21.3	97.3	82.7	**11.5**	0.265	3.71	38.6
CVE	24721	44.6	17428	20.2	**65.5**	**69.5**	11.9	**0.227**	3.70	37.3
CVF	22640	**40.8**	16843	**20**	**180.9**	**107.1**	12.3	0.304	3.99	**42.0**

Table 8: Element total concentration, expressed in mg kg-1, in soil of Visnà di Vazzola (TV) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
VDV1	26583	**61.3**	9850	**36.8**	**354.9**	**105**	4.84	**0.409**	**2.32**	**24.1**
VDV2	**32544**	24.7	**10715**	16.1	90	73.8	5.01	0.221	1.46	14.5
VDV3	**17559**	**15.2**	**5681**	**9**	**25**	**25.4**	**2.73**	**0.111**	**0.71**	**6.6**
VDV4	27618	24.8	7622	16.6	173	55.6	**5.71**	0.307	1.78	20
VDV5	25038	21.2	7698	16.3	104	53.9	5.51	0.230	1.62	15.7

Table 9 Limits set by Italian law for metals in soils, expressed as mg kg^{-1} (Legislative Decree 152/2006)

Metal/Metalloid	Limit (mg kg^{-1})
Chromium	150
Nickel	120
Copper	120
Zinc	150
Arsenic	20
Cadmium	2
Lead	100

As regards Sn concentration values, a regionally derogation can be granted, from the limits set by the national law, due to the high values present in the Italian soils. The only element which shows values above the limit is Cu for VDV1 (354.9 mg kg^{-1}), VDV4 (173.4 mg kg^{-1}) and CVF (180.8 mg kg^{-1}). The other elements studied in both vineyards show values in agreement with the limits reported in column A of the Italian Legislative Decree 152/2006.

The high presence of Cu is probably due to the treatment applied to the vines (*i.e.*, Bordeaux mixture). The concentration of the elements in soil strongly change among Italian regions; however, the concentration of Cu in both sites is comparable with the concentration reported in other wine growing regions in Italy (from 2 to 375 mg kg^{-1}) [30] and in other countries like France (from 22 to 398 mg kg^{-1}) [31]. The concentration of Cd (from 0.227 to 0.316 mg kg^{-1} in soil from CV vineyard and from 0.111 to 0.409 mg kg^{-1} in VdV) agrees with the background levels of Cd (0.40 mg kg^{-1}) reported in literature for soils of other countries [32], such as in the Castelon region, Spain (0.358 mg kg^{-1}) [33]. Al and Fe concentrations are strongly higher than those of all the other elements in both vineyard soils with values that are all above 15000 mg kg^{-1} for Al and all above 5000 mg kg^{-1} for Fe. This information supports the hypothesis suggested in the evaluation of the characteristics of the soils where the CEC values proposed a significant presence of clay of the illite and chlorite group.

The possibility of relation between the soil chemical characteristics and the age of the vines (of a same *cultivar*), with which has been sub-dived the vineyard area for both sites, as it is shown in the Figures 1-2, seems not to be supported by the concentration data.

The sample VDV1 and VDV2, for example, has been sampled from the area in which there are two years old vines of the *cultivar* Glera, and for all the analysed element there is a strong difference in the concentration as it is shown in Table 8. The same situation occurs between the sample CVB and CVF (area in which there are forty years old vines of the *cultivar* Cabernet-sauvignon) and among the sample VDV3, VDV4 and VDV5 (area in which there are ten years old vines of the *cultivar* Glera).

However, in opposition at the examples cited above, the sample CVA and CVC and the sample VDV4 and VDV5 shown similar concentrations for each element, but these examples are not enough to support the first hypothesis even because similar concentration for each element can be found in samples with completely different characteristics like in CVC and CVD.

3.4.3 Roots analysis discussion

Table 10: Element total concentration, expressed in mg kg^{-1}, in roots of Sant'Andrea di Cologna Veneta (VR) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
CVA	1409	1.8	966	2.20	67.3	115.4	0.811	0.058	BDL	2.31
CVB	5270	5.41	3263	5.09	101.9	52.3	2.139	0.136	0.011	3.72
CVC	3427	3.52	2187	4.20	60.5	71.9	1.329	0.065	0.003	2.37
CVD	2886	2.99	1824	2.23	35.2	20.9	0.976	0.079	0.002	1.92
CVE	4612	5.60	3157	4.34	85	72.7	1.723	0.208	BDL	4.08
CVF	2031	2.15	1339	2.83	42.7	25.6	0.932	0.048	BDL	1.95

Table 11: Element total concentration, expressed in mg kg^{-1}, in roots of Visnà di Vazzola (TV) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
VDV1	1327	2.48	1071	5.26	108.2	70.7	0.514	0.124	BDL	1.43
VDV2	1877	4.33	1612	7.61	60.4	44.8	0.717	0.142	BDL	1.37
VDV3	2299	4.83	1897	7.83	82	42.0	0.868	0.168	0.027	1.65
VDV4	8074	13	6031	9.16	263.5	71.3	2.338	0.218	0.071	5.38
VDV5	3355	7.49	2252	6.56	137.8	40.5	0.949	0.160	0.020	2.07

The concentration of the elements present in roots of the two vineyards under investigation is shown in Tables 10-11; these values strongly varies among the samples and this may be due to the different ages of the vines and, consequently, to the structure that have developed in the ground layers. However, the concentrations of the elements in roots, like is shown for soil analysis, do not suggest any relation with the age of the vine because there are similarities among samples with different characteristics like VDV3 and VDV2 or CVB and CVE, and also relevant differences among samples with similar characteristics like VDV3 and VDV4 or CVA and CVC. Generally, in the two vineyards analysed, the sequence of element concentration found in roots, in decreasing order, reflects, with some exceptions, the sequence found in the soil samples (high concentration of Al, Fe, Cu, and Zn and low concentration of As, Cd, and Sn). Cu seems to be more taken up than Zn by the samples CVB, CVD, CVE and by all the samples analysed in VdV site. Comparing the concentration of all elements between soil and roots, a decrease is observed except for Cu, which has similar ranges in both sites for both matrices. It is also possible to divide the elements, basing on their concentration, in elements that highly taken up and stored by the roots (Al, Fe, Cu, Zn, and Cd) and elements poorly absorbed and stored by the roots (Cr, Ni, As, Sn, and Pb). For example, Cr in CV vineyard ranges from 40.8 to 52.2 mg kg^{-1} in soil, while in roots it ranges from 1.8 to 5.6 mg kg^{-1}. The concentration of Al is the highest, compared to the other elements, in each sample but, averagely only an 13% of the concentration present in soil is present in roots, in agreement with the consideration done in the characterisation of the soil were a low solubilisation of this specific element is proposed.

3.4.4 Flower analysis discussion

Table 12: Element total concentration, expressed in mg kg^{-1}, in flowers of Sant'Andrea di Cologna Veneta (VR) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
CVA	215	0.641	214	**0.629**	65.1	28.9	0.121	0.004	0.043	0.296
CVB	231	0.730	242	0.738	**44.3**	33.4	0.136	0.004	**0.022**	**0.273**
CVC	503	1.132	477	0.912	93.2	**27**	0.268	**0.002**	0.049	0.538
CVD	**587**	**1.185**	**564**	**0.995**	**113.5**	35.2	**0.297**	0.006	0.098	**0.802**
CVE	332	0.897	375	0.913	119.6	36.1	0.191	0.006	**0.135**	0.399
CVF	**117**	**0.617**	**143**	0.863	80.2	**43.7**	**0.080**	**0.007**	0.070	0.210

Table 13: Element total concentration, expressed in mg kg^{-1}, in flowers of Visnà di Vazzola (TV) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
VDV1	365	0.937	360	1.50	22	68.3	0.161	0.014	0.025	0.256
VDV2	485	1.428	559	1.75	14	60.9	0.208	0.012	0.027	0.261
VDV3	321	1.016	368	1.58	12.6	65.7	0.152	0.013	0.009	0.191
VDV4	891	1.974	921	2.11	24.7	72.1	0.356	0.024	0.019	0.610
VDV5	1097	2.253	1183	2.38	23.8	83.8	0.423	0.023	0.028	0.907

The concentration of the elements present in flowers of the two vineyards under investigation is shown in Tables 12-13.

In flowers Fe, compared to the other elements, showed the highest concentration in CVB, CVE, CVF and in all the samples of VdV apart from VDV1; in the other samples (CVA, CVC, CVD, and VDV1) the element with this characteristic is the Al. This group of samples, which show concentration of Al higher than that of Fe, have in common that belong all to the same *cultivar* (Cabernet-sauvignon) with the exception of VDV1. Although Fe and Al are the elements that have the highest concentrations in flowers, the TF values from roots to flowers reveal that the fluxes of Cu and Zn are the most relevant in CV vineyard, where their value ranges from 0.435 to 3.225 and from 0.250 to 1.687, while in VdV vineyard only the flux of Zn is relevant (from 0.965 to 2.067). This seems to be also confirmed by the concentration of these two elements observed in the studied sites. Cd seems not to be transported in flowers; it has a very low concentration in both sites (from 0.002 to 0.007 mg kg^{-1} in CV flowers and from 0.012 to 0.028 in VdV flowers). This hypothesis is also supported by the TF values that, for this element, are averagely the lowest in each sample respectively to the other elements. Comparing the two sites, As and Pb showed similar concentrations, while Cu had a higher concentration in VdV than that found in CV.

The sample CVD is related to a forty years old vine and shows the highest concentration for Al, Cr, Fe, Ni, Cu, As, and Pb, suggesting a probable connection between time and accumulation in this tissue. However, the same situation does not occur for the other vines of forty years old from the same site (CV vineyard) showing even lowest values, in comparison with the other sample, for the elements Al, Cr, Fe, and As.

3.4.5 Leaf analysis discussion

Table 14: Element total concentration, expressed in mg kg^{-1}, in leaves of Sant'Andrea di Cologna Veneta (VR) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
CVA	127.5	1.33	175	1.58	7.31	11.9	0.143	0.006	0.042	0.237
CVB	157.6	1.95	222	2.46	59.54	29.0	0.209	0.011	0.167	0.245
CVC	127.4	1.94	195	2.1	43.35	16.4	0.089	0.007	0.049	0.231
CVD	173.4	4	247	2	5.84	18.3	0.133	0.007	0.059	0.210
CVE	76.2	1.87	131	1.7	5.29	14.2	0.076	0.005	0.029	0.088
CVF	135.1	1.54	228	2.66	14.97	20.6	0.187	0.009	0.053	0.221

The concentration of the elements present in leaves of the CV vineyard is shown in the table 14.

As is shown in flower analysis discussion, if compared to the other elements, Fe and Al show the highest concentrations and Sn and Cd the lowest in all the samples. The relevant TF values are related to Cr (from 0.333 to 1.341), Ni (from 0.394 to 0.939), and Zn (from 0.104 to 0.875). This suggests that a small fraction of Fe and Al flows from roots to leaves, while a high amount of Cr, Ni and Zn is transported from roots to flowers. The relation between age of the vine of a same *cultivar* and concentration of the elements in this matrix seems to be supported by the similarities in the concentration of the elements, with the exception of Cu and Sn, between CVA and CVC and between CVB and CVF. This is also confirmed by the presence of higher concentration, for the majority of the elements, in the samples related to forty years old vines (CVB, CVD and CVF) than the concentration present in the twenty (CVE) and ten years old (CVA and CVC) vines. For the reasons, the concentration of element in leaves seems to be dependent to the age of the vine. Considering instead, vines of the same age but different *cultivar* (CVA, CVD and CVE), it is not possible to highlight any relevant differences in the concentration of the elements but, the TFs of the same samples suggest a higher transport of Cr and a lower one of Cu in Cabernet-sauvignon *cultivar* (CVD) compared to Garganega *cultivar* (CVA and CVE). However only three samples can not be considered enough to validate this hypothesis and in future studies should be considered a higher number of samples.

3.4.6 Stem analysis discussion

Table 15: Element total concentration, expressed in mg kg^{-1}, in stems of Sant'Andrea di Cologna Veneta (VR) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
CVA	125.4	0.585	138	0.515	24.8	15.8	0.093	0.044	0.187	0.186
CVB	119.3	0.487	128	0.543	13.9	32.2	0.123	0.040	0.150	0.145
CVC	**436**	**2.768**	**745**	**2.106**	**129**	**69.6**	**0.415**	**0.258**	**0.728**	**0.581**
CVD	87.6	0.439	103	0.244	9.01	12.9	0.044	0.005	**0.006**	0.155
CVE	**58.3**	**0.328**	**78**	**0.191**	**8.76**	16.1	**0.023**	**0.002**	BDL	**0.110**
CVF	66.2	0.364	90	0.287	14.1	**11.1**	0.050	0.004	BDL	0.118

The concentration of the elements present in stems of the CV vineyard is shown in Table 15.

Like presented for flowers and leaves, Fe and Al show the highest concentration among elements in stems sample, but Cd show the highest TF in CVA and CVC, as Zn for CVB, CVD, CVE and CVF, being the major elements transported from the roots.

Al and Fe in fact, together with As and Pb, present very low TF values, indicating that only a small fraction has been transported from roots. The difference observed in the concentration of Cu and Zn in roots is similar in stems (*e.g.* Cu is higher than Zn in CVA, CVC, and CVF), but it is not related to the TF values for these elements. Considering the concentrations of the elements and the calculated transfer factor roots to stems is not possible to highlight any relevant differences between vines of different *cultivars* and the same age or similarity between vines of the same *cultivar* and age.

3.4.7 Grape analysis discussion

Table 16: Element total concentration, expressed in mg kg^{-1}, in grapes of Sant'Andrea di Cologna Veneta (VR) vineyard; concentrations in blue are the lowest, the red ones are the highest for the same element.

	Al	Cr	Fe	Ni	Cu	Zn	As	Cd	Sn	Pb
CVA	33.5	0.092	2.18	1.7	4.39	7.42	38.7	0.023	BDL	0.231
CVB	57.5	0.298	123	1.8	48.26	40.94	88.7	0.035	BDL	0.478
CVC	40.3	0.234	54.4	0.981	19.74	21.95	131.5	0.018	BDL	0.267
CVD	52.6	0.628	125	1.33	42.61	47.61	44.3	0.023	BDL	0.299
CVE	46.4	1.001	73.3	2.91	35.62	40.89	73.4	0.033	BDL	0.567
CVF	40.1	0.170	89.5	1.18	31.18	43.57	44.1	0.030	BDL	0.528

The concentration of the elements present in grapes of the CV vineyard is shown in Table 16.

The most relevant elements present in grapes, considering only their concentrations, are As, Fe, Al, Cu and Zn. If compared to other elements, As present the highest concentration in the samples related to the younger vines of the site (CVA and CVC, that are ten years old and CVE that is 20 years old) instead, in the older vines of the site (CVB, CVD, and CVF, that are forty years old) the elements which present the same characteristics is Fe. However, TFs support the hypothesis of a transport of As, which shows the TF highest value, in all samples indicating that grapes might be a possible sink of this element. However, in another study the concentration of As in grapes in vineyards in Trentino is similar to the leaf concentration or lower [34]. It was suggested that the high presence of As is probably correlated to the treatment with As-containing pesticides [35]. The distribution of Cu and Zn in stems and roots is not observed in grapes where Zn is generally higher than Cu; these two elements show relevant TF values which support what observed for their concentrations. Comparing concentrations with TF values, differences between the two *cultivars* or among the age of the vines cannot be highlighted.

The Commission Regulation (CE) 1881/2006 established in section 3.1.13 and 3.2.15 the maximum levels of Cd and Pb in the grapes set, respectively, at 0.05 and 0.2 mg kg^{-1} (wet weight). Comparing these values to the concentrations present in our samples is possible to affirm that Cd concentrations in all samples are under the law limit. Conversely, the Pb concentrations are all above the limits set by the European Commission.

4 Conclusions

The element concentrations and the TF values found in this study shown different patterns in soil and in all the parts of plant considered (roots, flowers, leaves, stems, and grapes). In soil and roots elements show their highest concentration, with the exception of As, which shows its maximum in grape samples. It was hypnotised that As might be considered as a sink even if could be mainly present due to the treatment with As-containing pesticides.

In soil all the elements, with the exception of Cu, have concentrations under the limits set by the Italian law; the high concentration present in Visnà di Vazzola vineyard, especially in VDV1 and VDV4 samples, are probably due to the treatment applied at the vines by the winery owner. In all grape samples have been found Pb concentrations that exceed the maximum concentration set by the European law.

The high variability among a small number of samples of different *cultivars* and ages, and the absence of a part of the matrices considered in this case of study for the Visnà di Vazzola vineyards, do not allow to highlight a transfer trend in all matrices, although while Al is higher than Fe in soil and roots, the opposite was observed in the other parts of the plants, and leaves concentrations seems to be related to the age of the vine. For an in-depth understanding of the transport in the different part of the vines, more plants of the same *cultivar* and of the same age should be considered for further analysis.

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