UNIVERSITY OF NOVA GORICA
GRADUATE SCHOOL

DETERMINATION OF THE ACTUAL UPTAKE OF ESSENTIAL NUTRIENTS BY DIFFERENT PARTS OF *Vitis vinifera* L. cv. 'REBULA'

DISSERTATION

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ABBREVIATIONS AND SYMBOLS

AL-method	Ammonium lactate soil extraction
ANOVA	Analysis of variance
ATP	Adenosine-5'-triphosphate
BSN	Bunch stem necrosis
CEC	Cation exchange capacity
CRM	Certified reference material
CV	Coefficient of variation
D.W.	Dry weigh
DAD	Diode array detector
dd-H_2O	Doubly deionised and purified water (~18.0 MΩ cm^{-1})
DTPA	Diethylene triamine pentaacetic acid
EC	Electrical conductivity
EDTA	Ethylenediamine tetraacetic acid diethylene triamine pentaacetic acid
FAAS	Flame atomic absorption spectrometry
FAN	Free amino nitrogen content
FC	Folin-Ciocalteu method
FT-IR	Fourier transform infrared spectra
GAE	Gallic acid equivalent
GLM	General linear model
HCAs	Hydroxycinnamic acid esters, Hydroxicinamates
HPLC	High performance liquid chromatography
ICP	Inductively Coupled Plasma Analyser
IRMM	Institute for reference materials and measurements
LOD	Limit of detection
LOQ	Limit of quantification
NIST	National institute of standards and technology
NPK fertilization	Application of nitrogen, phosphorus and pottasium fertilizers
OM	Organic matter

ORP (E_h / pE)	Oxidation/reduction potencial (E_h - The milivolt difference in potential between a Pt electrode and the standard H electrode; pE - The negative log of the electron activity)
PCA	Principal component analysis
PCs	Principal components
PP	Polypropylene
PTFE	Polytetrafluoroethylene
SO4	Selecion Oppenheim Nr. 4 rootstock
SPE	Solid phase extraction
SRMs	Standard reference materials
SS	Soluble solids
TA	Total titratable acids, total acidity
TEA	Triethanolamine
trans–CoTA	*trans*–coutaric acid
trans–CTA	*trans*–caftaric acid
trans–FTA	*trans*–fertaric acid

1 INTRODUCTION

Nutrients are in addition to water basic requirements for plant growth and performance that are absorbed from the soil and air surrounding the plants. A well-balanced nutrient supply has known to be crucial for all the crops in order to avoid excessive growth or mineral deficiency, since mineral elements affect plant physiology and, thereafter, also plant development (Bergmann, 1992).

Soils vary in the amounts and composition of mineral nutrients (macro- and micro-elements) but also in the degree of the uptake availability by the roots. Nutrient storage capacity and accessibility are influenced by soil texture, rooting depth, and organic matter content, but the availability is modified by soil moisture and pH (Keller, 2005). Parameters mentioned above can affect several chemical and biochemical processes occurring in the soil: precipitation - dissolution, adsorption - desorption, complexation - dissociation, and oxidation - reduction, which in progress control the mobility and plant-availability of nutrients (He et al., 2005). Plant-available nutrients are taken up as ions, dissolved in the soil solution and their uptake depends also on the water flow through the soil-root-shoot pathway (Keller, 2005).

Viticulture is one of the most important and extended branches of agriculture in Slovenia. Statistical data from the year 2011 show that vineyards in Slovenia cover 16351 ha, representing 60.9% of the utilized agriculture area by land under permanent crops (Structure ... , 2012). Around 40% of all Slovenian vineyards (providing 50% of Slovenian grape production) are located on the western part of Slovenia, known as Primorska winegrowing region (divided into Goriška Brda, Vipava valley, Karst and Slovenian Istria region) (Vršič and Lešnik, 2005).

Goriška Brda are known for its sub-Mediterranean climate with lots of sun, high average annual precipitations (1200 mm), and especially for Eocene flysch (marls and sandstones) soil type, which provides the best basis for high quality wines (Rusjan et al., 2006; Pedološka karta … , 1998).

Vitis vinifera L. cv. ʻRebulaʼ is a very old, domestic, white grape cultivar typical for

Goriška Brda region, having reach history and long tradition (Rusjan, 2003). It is the major white grape cultivar in Primorska winegrowing region, representing 11.8% of all cultivars here, and the number one in vineyards of Goriška Brda demonstrating almost a quarter (23.5%) of all grape varieties (Škvarč and Brdnik, 2007).

Nutrient deficiencies and/or toxicities are widespread and have been documented for various soils all over the world. Nutrient deficiencies occur when the plant cannot acquire sufficient amounts of nutrients for its internal needs, whereas an excessive supply of elements, especially trace metals (*e.g.* Zn, Cu, Mn), results in toxicity to the plant (He et al., 2005). Specific deficiency symptoms appear on all plant parts, but discoloration (chlorosis) of leaves is most commonly observed. Deficiency symptoms of low mobile nutrients (like Fe and Zn) appear initially and primarily on upper leaves or leaf tips, while deficiency symptoms of mobile nutrients (like Mg) appear primarily on lower fully expanded leaves (Marschner, 1995).

Potassium (K) is the most abundant cation that can be found in grape berries (Hradzina et al., 1984) and it is highly important for four biochemical roles: enzyme activation, cellular membrane transport processes and translocation of assimilates, anion neutralization and osmotic potential regulation (Mpelasoka et al., 2003). During wine making, K affects the pH of must and wines and thereby their chemical and microbiological stability (Jackson and Lombard, 1993). Magnesium (Mg) accounts for the highest chemical activity between divalent cations in the cytoplasm, and contributes to neutralize sugar compounds, organic acids and amino acids. K and Mg are phloem-mobile elements, thus they can be transported to grape berries (Etchebarne et al., 2009). Interaction between these two elements is one of the widely known antagonisms in grapevine, and often leads to Mg deficiency (Capps and Wolf, 2000; Haefs et al., 2002).

Iron (Fe) and zinc (Zn) are also known to be important mineral elements for grapevine nutrition. Fe deficiency is recognised as one of the main abiotic stresses affecting fruit tree crops growing in calcareous soils in the Mediterranean area (Tagliavini and Rombolá, 2001). The limited availability of Fe in the soil for root absorption, leads to reduced yield and quality losses (Bertamini et al., 2002; Mengel,

1994). Also Zn deficiency may limit plant growth, since the element plays multiple important roles in various physiological and metabolic processes of the plant (Marschner, 1995).

Despite several studies (Conradie and Saayman, 1989; Hilbert et al., 2003; Jackson and Lombard, 1993; Keller, 2005; Topalović et al., 2011) where grape quality has been investigated from the perspective of impacts by soil type, climate, cultivar or season variation, there are only limited data available in the literature concerning correlations between plant mineral status (or fertilisation trials) and polyphenols these being rare in case of hydroxycinnamic acid esters in white grapes.

Nutrient availability can be improved by soil or foliar application of a needed element. Soil fertilization is the most ancient normal fertilization practice, but foliar fertilization, which has been developed in the last 60 years, may improve nutrient uptake when compared with soil application, particularly for nutrients that can be sorbed on the soil minerals (Kannan, 2010; Tejada and Gonzales, 2004). However, the uptake of one mineral does not depend only on the nutrient availability in the soil, since interactions among elements – synergism and/or antagonism – can influence the plant nutrient uptake (Bergmann, 1992; Farago, 1994).

On the other hand, repeated use of metal-enriched chemicals such as fungicides and pesticides, farm manures, and chemical fertilizers can contribute variable amounts of trace elements, especially heavy metals (*e.g.* Cu, Mn, Mo, Zn, Cd) (He et al., 2005). Viticulture represents an important agricultural practice in many countries (Slovenia included) and long-term use of metal-enriched chemicals in vineyards has resulted into increased concentrations/accumulation of these pollutants in soil and other environmental compartments (Komárek et al. 2010; Rusjan et al., 2006).

In the years 1992–2010, the consumption of mineral fertilizers and plant nutrients per hectare of agricultural land decreased by 30.6% in Slovenia. In addition, notable decrease of average consumption of plant nutrients (N, P_2O_5, K_2O) per hectare of utilised agricultural area has been observed in Slovenia (from 135 kg ha^{-1} in 2002 to 95 kg ha^{-1} in 2009), being still higher compared to other EU member countries (from

72 to 77 kg ha^{-1}). Documented decrease (for 29.6%) is mostly attributed to the requirements of the Nitrates Directive (91/676/EEC) and the principles of good farming practice in fertilization, to which all agricultural holdings have been committed in the last decade (Sušin, 2011).

Since elements affect vine growth and production, the nutrient status of grapevines becomes an important parameter also for sustainable production. Moreover, deficiency and toxicity symptoms may be confused also with drought, diseases, insect and other damage, so correct diagnosis may be difficult without experience and foliar analysis. In any case, the mineral composition of leaves could change as dependent on cultivar and rootstock (Christensen, 1984; Peuke, 2009).

1.1 THEORETICAL BACKGROUNDS

1.1.1 Essential elements and terminology

The term essential element (or nutrient) was proposed by Arnon and Stout (1939, op cit Marschner, 1995) who described three criteria to be met for the essentiality:

(1) the plant cannot complete its life cycle in the absence of the element,
(2) the function of the element must not be replaceable by another mineral element, and
(3) the element must be directly involved in plant metabolism (as a constituent of an essential metabolite, or required for the proper functioning of an essential enzyme system or be uniquely involved in maintaining the overall ionic composition of tissue).

Table 1 lists 22 elements generally accepted as essential for higher plants. Depending on the content of a given nutrient for the plant growth, the nutrient is referred as ‘macro-nutrient’ (macro-element, major nutrient, main element) or ‘micro-nutrient’ (micro-element, trace element) (Bergman, 1992; Marschner, 1995). Some of the micro-nutrients (usually defined as ‘beneficial’ (Marschner, 1995) or ‘functional’

(Bergman, 1992)) are enclosed in brackets because either their essential character is disputed or they have proved to be essential only in some plants or in certain metabolic processes.

Table 1: *Elements those are essential for all higher plants (Bergman, 1992: 12).*

Basic elements in organic matter (organic nutrients)	*Mineral elements*		
	Macro-elements	*Micro-elements*	
Carbon (C)	*Calcium (Ca)*	*Boron (B)*	*(Aluminium (Al))*
Hydrogen (H)	***Magnesium (Mg)*** [a]	*Chlorine (Cl)*	*(Cobalt (Co))*
Oxygen (O)	*Nitrogen (N)*	*Copper (Cu)*	*(Sodium (Na))*
	Phosphorus (P)	***Iron (Fe)***	*(Nickel (Ni))*
	Potassium (K)	*Manganese (Mn)*	*(Silicon (Si))*
	Sulphur (S)	*Molybdenum (Mo)*	*(Vanadium (V))*
		Zinc (Zn)	

[a] *Studied elements are bolded.*

Most micro-elements listed in Table 1 are predominantly constituents of enzymes (except Cl and B), where they can be directly or indirectly involved in the catalytic function of the enzyme, thus being essential only in small amounts. On contrary, macro-nutrients like N, S, and P are important constituents of organic compounds, such as proteins or nucleic acids but they can act as osmoregulators (like K) as well. K and Cl are the only mineral nutrients that are not constituents of organic structures. They function mainly in osmoregulation, in the maintenance of electrochemical equilibrium in cells and their compartments and in the regulation of enzyme activities (Marschner, 1995).

Differences in function of individual nutrients are reflected in plant shoots average concentrations that are sufficient for adequate growth (Table 2). These values can vary considerably depending on plant species, plant age, and concentration of other mineral elements (Marschner, 1995). Macro-nutrients are needed in the range (or more) of 1 g kg^{-1} (1000 ppm or 0.1%) of plant's dry weigh (D.W.), while

micronutrients are required in plant tissue at level of 100 mg kg^{-1} (100 ppm) D.W. or lower (Farago, 1994).

Table 2: *Average concentrations of mineral nutrients in plant shoot that are sufficient for adequate growth (Marschner, 1995: 5).*

Element symbol	*g kg^{-1} D.W.*	*Element symbol*	*mg kg^{-1} D.W.*
N	*15*	*Cl*	*100*
K [a]	*10*	***Fe***	*100*
Ca	*5*	*Mn*	*50*
Mg	*2*	*B*	*20*
P	*2*	***Zn***	*20*
S	*1*	*Cu*	*6*
		Mo	*0.1*

[a] *Studied elements are bolded.*

Besides nutrient classification described in the Table 1 also other classifications were proposed in the literature. One of them divides the elements on the basis of physicochemical properties into ‘metals’ (K, Ca, Mg, Fe, Mn, Zn, Cu, Mo) and ‘non-metals’ (N, S, P, B, Cl) (Marschner, 1995).

One has to mention that micro-elements like Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are known also under the names like ‘heavy metals’, ‘trace metals’ or even ‘toxic metals’. The qualifier ‘heavy’ is not rigorously defined but most authors use it to describe metallic elements with an atomic density greater than 6 g cm^{-3} (Alloway 1995; Farago, 1994).

1.1.2 Plant nutrient sources

According to Farago (1994) plants can reach nutrients from:

(1) natural sources in soil:

- released from soil reserves (parent material),

- as a product of biological residues decomposing (*e.g.* roots, straw, manures),
- biological nitrogen fixation (only for some plants, *e.g.* legumes),

(2) air as dry deposition,

(3) water (precipitation) as wet deposition, and

(4) fertilizers in the case of crop plants (anthropogenic input).

The main source of elements in soil is the parent materials (underlying geological material (bedrock) in which soil horizons are formed) from which they are derived by physical and chemical weathering. Unfortunately nowadays 'normal' (inherited from parent material) abundance of an element can be found only in remote or mountain areas, where impacts of human activity is relatively small (He et al., 2005). However, some soils have been found to have a high background of some micro-elements, due to extremely high concentrations of these elements in the metal-rich parent material (like marine black shales and metal mineral deposits) (Farago, 1994).

On the other hand, in urban areas or agricultural land with a long history of crop production, the concentrations of micro-elements in soil can be much higher, but mainly due to long-term agricultural practise with fertilizers. Other important anthropogenic processes in the agro-ecosystem include inputs of micro-elements through the use of pesticides, organic manures, industrial and municipal wastes, irrigation (use of domestic and industrial wastewaters), and wet or dry deposits (emissions from large industry sources like iron and steel industry, metal smelters and metal refineries) (He et al., 2005).

The concentrations of trace elements in soils can vary a lot. For example, the average concentration of Zn in worldwide soils is 10-300 mg kg^{-1} (ppm), but some metal-rich soils may contain more than 10 g kg^{-1} of Zn, because of particular parent materials or contamination (He et al., 2005).

1.1.3 Inputs and outputs of micro-elements from agro-ecosystems and their impact on the environment

As already mentioned, modern agriculture practice increases the pool of minerals in the soil the most. Fungicides, farm manures and chemical fertilizers are applied to increase crop production and lower the bad impacts of pests and insects. At the same time, these chemicals can contain impurities like Cd, Cu, Pb, Zn, Fe, Mn, and As (heavy metals) which may enter the soil together with other active substances. Many farmers correct the soil deficiencies of micro-nutrients like Cu, Zn, Mn, Fe, and B by application of agrochemicals containing those (Fageria et al., 2002).

Beside micro-element containing fertilizers, those containing macro-nutrients like N (*e.g.* ammonium sulphate, ammonium nitrate, urea, NPK compounds), P (*e.g.* superphosphate), and K (*e.g.* potassium sulphate) are extensively used for stimulating plant growth as well. These macro-element fertilizers are also important sources of micro-elements, which can be purposely added (*e.g.* Cu, Zn, B, Fe, and Mn) to meet the demand of plant growth for these elements, or they can be naturally present as in the case of phosphorus fertilizers made out of phosphate rocks, which may contain As, Cd, Cr, Cu, Pb and Zn (Senesi et al., 1999).

In addition to soil applications, foliar spraying of metal-containing chemicals is used for curing or preventing diseases of grape and fruits like apple, citrus, cherry, and peach. Accumulation of Cu due to the extensive use of copper-based fungicides (such as the Bordeaux mixture; copper sulphate and lime) was established in some viticulture soils in France, Italy (Brun et al., 1998) and in Slovenia (Rusjan et al., 2007).

Outputs of trace elements from agro-ecosystems include crop harvest, losses by leaching and surface runoff. Crop harvest accounts for a big proportion of the output of trace elements, although the precise amounts of metal removal vary greatly with the type of soil, crop variety, and climate conditions. For most fine texture soil, leaching of trace elements is limited because of the strong binding of these elements with soil colloids, whereas for sandy soils, especially under acidic conditions,

leaching can be an important output (He et al., 2005). Surface runoff losses of trace elements are often associated with transport to the environment of particulates that contain adsorbed trace elements and organic-metal complexes. Transport of the metals may result in an increased content of heavy metals in the groundwater or surface water (Alloway, 1995).

Pollutants from fertilisers or other anthropogenic sources are non-degradable and they accumulate in the upper layers of soils as chemical forms that are often more reactive than native ones (Gleyzes et al., 2002). When a soil is contaminated with heavy metals, microorganisms in the soil are the first living organisms subjected to their impacts. Heavy metals decrease microbial biomass by directly killing or biochemically disabling organisms in soil (Giller et al, 1998). A combination of Zn and Cu at high concentrations had an additive adverse effect on the amounts of soil microbial biomass present (Chander and Brookes, 1993). An increase in heavy metals concentrations by sewage sludge application decreased biomarkers of actinomycetes, arbuscular mycorrhizal fungi, and total fungi, but surprisingly increased relative amount of bacteria, that have been observed to be more resistant to high concentrations of heavy metals than other microbial populations (Kelly et al., 1999). Beside the impacts on the soil microorganisms, metal pollution accumulated in agricultural soil influences also higher organisms and water. Toxic metals (*e.g.* Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn) can be taken up directly by humans and animals through the inhalation of dusty soil or they may enter the food chain as a result of their uptake by edible plants and animals or leach down to groundwater and contaminate drinking water resources, and may cause, in both cases, hazards to the health of humans and animals (Senesi et al., 1999).

1.1.4 Nutrient uptake by plants

Plant uptake of various elements from the soil and through the root system depends on particular plant, botanical structure of specific tissue, soil type and element as well (Ražić et al., 2005).

Table 3: *Plant mobility of same essential nutrients, their absorption forms by plant roots and optimal soil pH values for the assimilation (Bavaresco et al. (2010); Fregoni, 1997: 518; Marschner, 1995).*

Nutrient	*Absorption form of nutrients*	*Optimal soil pH for the assimilation*	*Plant (phloem) mobility*
Nitrogen	NH^{4+}, NO^{3-}	*6.0 – 8.0*	*High*
Phosphorus	HPO_4^{2-}, $H_2PO_4^-$	*6.5 – 7.5*	*High*
Potassium [a]	$\mathbf{K^+}$	***6.0 – 8.0***	***High***
Calcium	Ca^{2+}	*7.0 – 9.0*	*Low*
Magnesium	$\mathbf{Mg^{2+}}$	***6.0 – 8.5***	***High***
Sulfur	SO_4^{2-}	*5.5 – 9.0*	*High*
Iron	$\mathbf{Fe^{2+}}$, $\mathbf{(Fe^{3+})}$	***3.0 – 6.5***	***Intermediate***
Zinc	$\mathbf{Zn^{2+}}$	***3.5 – 7.0***	***Intermediate***
Copper	Cu^{2+}	*5.0 – 7.5*	*Intermediate*
Manganase	Mn^{2+}	*3.0 – 6.5*	*Low*
Boron	$B(OH)_3$	*5.0 – 7.2*	*Intermediate*
Molybdenum	MoO^{4-}	*6.5 – 9.0*	*Intermediate*

[a] *Studied elements are bolded.*

Plant-available nutrient ions are dissolved in the soil solution (the interfaces between the soil matrix and water in the soil), so nutrient uptake depends on water flow through the soil-root-shoot pathway and on element concentration in the soil solution. Nutrients are often concentrated in the biologically active surface soil, but water and nutrient availability varies greatly in both space and time. Different nutrients are often available in different locations; *e.g.* nitrate (NO_3^-) leaches into the subsoil much more rapidly than potassium (K^+), which diffuses much faster than phosphate ($H_2PO_4^-$). As a consequence, superficial roots may take up soil-immobile nutrients (such as K and P), while deeper roots procure soil-mobile nutrients (such as NO_3^-) (Keller, 2005; Marschner, 1995). Soil and plant mobility of ionic forms of same essential nutrients are presented in Table 3.

Another important soil compartment, where chemical processes occur, is the plant-root interface known as rhizosphere. This is a complex and heterogeneous cylinder of

soil that surrounds the plant root at a distance of up to 2–5 mm. The conditions in the rhizosphere can be very different from those in the bulk soil. This is the local environment from which the root takes up nutrients, excretes inorganic and organic species capable of complexing micro-elements, and in which there is shedding and decomposition of parts of the root surface (Farago, 1994; Marschner, 1995).

The roots of many plant families are associated with particular symbiotic (mycorrhizal) fungi, which are very important for the mineral nutrition of plants. Since mycorrhizal fungi effectively increases the absorptive area of the root, they can assist in the uptake of nutrient (macro- and micro-elements) ions (Gosling et al, 2006), and on the other hand they can increase the tolerance of their host plants to heavy metals (*e.g.* Zn, Mn) when the metals are present at toxic levels (Christie et al., 2004).

Also grapevines are known to form mycorrhizas with arbuscular mycorrhizal fungi under normal field conditions (Karagiannidis et al., 1997). These associations are especially important for the uptake of P and several others nutrients like Zn, Cu, and Mn (Schreiner, 2003; Karagiannidis and Nikolaou, 2000).

Ions can be absorbed into the root by either passive (based on diffusion of ions in the soil solution into the root endodermis) or active process (against a concentration gradient with the use of metabolic energy - adenosine-5'-triphosphate (ATP)). Some ions (*e.g.* NO_3^- and K^+) are taken up passively across ion channels when their availability in the soil solution is high (generally in the mM range, for instance, after fertilizer application) and actively (and very selectively) across carriers, when availability is low (usually in the μM range) (Tester and Leigh, 2001). This ensures that grapevine roots absorb nutrient ions over a wide range of external concentrations. For ions, which are absorbed into the root by the same mechanisms are likely to compete with each other. For example, Zn absorption is inhibited by Cu^{2+} and H^+ (Alloway, 1995).

There is a close relationship between the metabolism of the shoot and the root. It is generally accepted that the xylem forms the main path for upward movement of

water and ions from the roots to the leaves, thanks to transpiration and phloem counter-flow (Sattelmacher, 2001). Most of the essential macro-elements are transported in the xylem as inorganic ions (Table 3). However, metals ions of Fe, Cu, and Zn will usually be transported with special organic acids salts and chelates (*e.g.* by citrate, malate, ethylenediamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA)) (Farago, 1994). Once the elements are in the xylem vessels, they can move throughout the whole plant. The rate and extent of movement within plants depends on the metal concerned, the plant organ and the age of the plant (Alloway, 1995).

Beside the root uptake from soil, plants can receive the elements also through the leaves, especially by the use of foliar fertilizers (spraying). Foliar uptake of elements is believed to consist of two phases: non-metabolic cuticular absorption (major route), and metabolic mechanisms which account for element accumulation against a concentration gradient (diffusion) (Farago, 1994). Metal interactions can occur in foliar absorption as well as in the root (Alloway, 1995).

1.1.4.1 Soil pH and redox potential

One of the most important soil parameter, influencing the mobility of elements and consequently its assimilation is the soil pH value. The recommended pH (determined in $CaCl_2$ solution) for grapevines is between 5.5 and 7.5. Vines can grow outside this range, but are more likely to suffer nutrient toxicity or deficiency. The data about optimal pH values for the assimilation (mobility) of some essential elements are presented in Table 3. The availability of micro-nutrients Fe, Cu, Zn, Mn decreases as the soil pH increases due to the hydrolysis reactions (through the splitting of water molecules in their hydration shells) (Sinskey, 2009).

In addition, some elements (*e.g.* C, N, O, S, Fe, Mn and others) are affected by variations in oxidation-reduction (redox) status in the soil. Redox reactions in soils are frequently slow but are catalised by soil microorganisms which are able to leave over the full range of pH and pE conditions normally found in soils (Alloway, 1995).

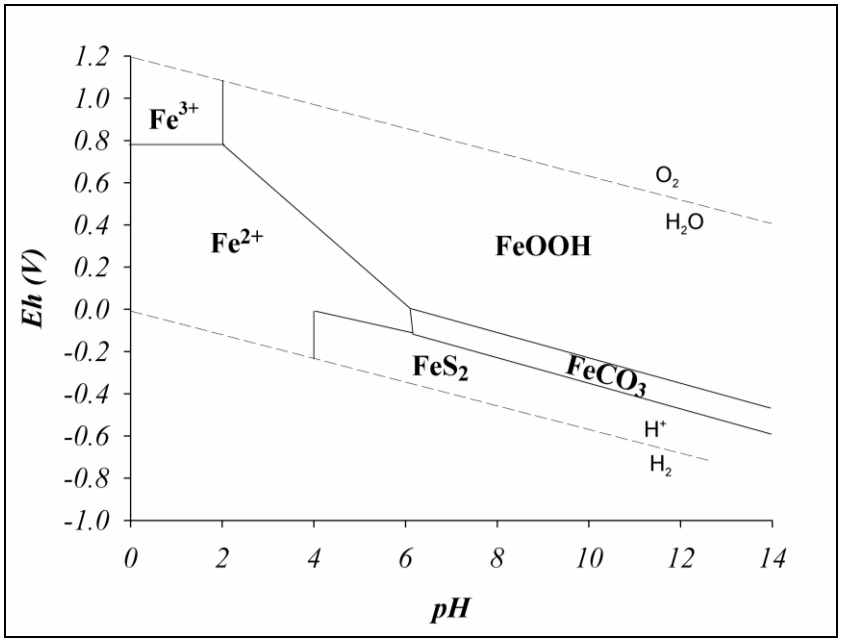

Figure 1: *The E_h-pH diagram of various iron ions and compaunds (Bohn et al., 2001: 124).*

Large positive values of E_h (the milivolt difference in potential between a Pt electrode and the standard H electrode) or pE (the negative log of the electron activity; E_h (mV) = 59.2 pE) favour the existence of oxides species (*e.g.* Fe^{3+}, NO_3^- Mn^{4+}), and low or negative values of E_h or pE are associated with reduced species (*e.g.* Fe^{2+}, NH_4^+, Mn^{2+}, S^{2-}). It is difficult to get accurate readings, however oxic soil conditions usually give values in the range +300 to +800 mV (pE 5.1-13.5). Moreover, red and brown colours of soils are a good indicator of oxic conditions. On the contrary, the grey colour is an indicator of reducing conditions (Alloway, 1995; Bohn et al., 2001).

The combined effect of E_h and pH conditions on the element form is illustrated by an E_h/pH diagram, as shown in Figure 1. Iron is present in the soil as oxide, hydroxyoxide, hydroxide, phosphate, carbonate, etc. The concentrations of Fe in ionic forms are extremely low (related to pH and redox potential from 10^{-20} to 10^{-6} mg L^{-1}). Iron chelates of Fe^{3+} and occasionally of Fe^{2+} ion forms are dominant sources of soluble iron in the soil (10^{-4} - 10^{-3} mg L^{-1} in soil with high organic matter; Bergmann, 1992). Figure 1 shows that goethite (FeOOH) dissolves to Fe^{2+} under

reduced and moderately acidic conditions. The Fe^{3+} ion predominates under strongly acidic and oxidizing conditions. Fe^{2+} and FeOOH are the predominant states in typical well-aerated soils (Bohn et al., 2001).

1.1.4.2 Interactions among elements

Beside soil parameters, the uptake of the elements depends also on individual elements' interactions. One element can reduce the uptake of the other, which is known as antagonism. On the other hand, the uptake can be increased by each other - this interaction is known as synergism (Farago, 1994).

***Table 4**: Negative (-) and positive (+) interactions among N, K, P, Mg, Fe and Zn.*

	Application of nutrient					
Interaction	*N*	*P*	*K*	*Mg*	*Fe*	*Zn*
N			–			
P	+/–					
K		–		–		
Mg	–	+	–			
Fe	+/–	–				–
Zn	+/–	–	–		–	

Antagonistic and synergistic interactions can occur between macro- and micro-elements themselves, some micro-elements may affect the absorption of macro-elements and vice versa. Moreover, those interactions may appear between the elements in the soil (particularly in the rhizosphere), or between the elements within the plant and those in the soil on the root surface (Alloway, 1995). Some known antagonistic (negative) and synergistic (positive) interactions between N, K, P, Mg, Fe and Zn are presented in Table 4 (Aciksoz et al., 2011; Alloway, 1995; Bergmann, 1992; Loneragan et al., 1979; Kutman et al., 2011; Marschner, 1995; Skinner and Matthews, 1989; Wolf et al., 1983).

1.1.5 Plant nutrients and their functions

Plants require an adequate supply of macro- and micro-elements for their normal physiological and biochemical function (Bergmann, 1992). Major physiological functions and deficiency symptoms of basic elements (N, P, K) and Mg, Fe, and Zn, are summarized in Table 5.

Each of essential elements has its unique physiological functions. The functions of N and P are so special, that they cannot be replaced by other elements in the plant metabolism. For instance, other macro-and micro-elements can be replaced by other cations, but only to the certain level, depending on the element and its function (Marschner, 1995).

As for other crops, for grapevine three basic mineral nutrients – N, P, and K – generally limit bunch production in addition to Mg, Zn, Mn, Fe, and B (Farago, 1994). Of all mineral nutrients, nitrogen is the one that grapevines need in highest quantity, that most often limits growth, and that is the most potent in terms of influencing fruit yield and quality (Keller, 2005). Therefore, it is not surprisingly that it is the most studied element (Jackson and Lombard, 1993; Marschner, 1995; Topalović et al., 2011).

Potassium is the most abundant cation present in the grape berry (Hradzina et al., 1984). Due to its high phloem mobility, it accumulates primarily in the berry skin tissues during ripening as a result of K remobilization from mature leaves. It neutralizes organic acids and it plays an important role in controlling the acidity and pH of the grape juice (Coombe, 1992; Poni et al., 2003). Several factors can induce K deficiency: high K fixation, high K buffer power of soils, competition with soil Mg and water shortage in summer (Tagliavini et al., 1996). Since the direction of K transport is often towards the developing organs, symptoms of K deficiency are usually first displayed by the basal, older leaves as a result of their continuous supply to the younger ones. In grapevine, the leaf browning induced by K deficiency is especially pronounced on heavily cropped vines after veraison (change of colour of the grape berries). In that time berries become a primary sink for K, an effect also

aggravated by water stress (Poni et al., 2003). The most obvious symptom of K deficiency is decreased grapevine yields, which happen because of vegetative growth reduction (Mullins et al., 1992).

Table 5: *Functions and deficiency symptoms of common macro- and micro-nutrients in grapevine (Bergmann, 1992; Mullins et al., 1992).*

Mineral nutrient	*Major functions*	*Deficiency symptoms*
N	*Structural component of amino and nucleic acids, proteins, nucleotides, chlorophyll and metabolic enzymes*	*Chlorosis of basal leaves; yield reduction*
P	*Used in high-energy bonds (ATP); structural component of nucleic acids, phospholipids, phosphoproteins*	*Chlorosis of leaves (reddening on leaves of red cultivars); reduced berry set and yield*
K	*Involved in enzyme activation (carbohydrate metabolism and transport); act in osmosis and ionic balance; control the acidity and pH of grape juice*	*Chlorosis of basal leaves; yield reduction*
Mg	*Cofactor and activator of many enzymes involved in protein synthesis, RNA formation, synthesis of chlorophyll and other leaf pigments, phosphorylation processes and others*	*Chlorosis of basal leaves*
Fe	*Enzyme activator (part of prosthetic groups, involved in redox reactions, bridging element between enzyme and substrate)*	*Chlorosis of apical leaves first*
Zn	*Functional, structural, and regulatory cofactor of enzymes (synthesis of RNA, indoleacetic acid, and others)*	*Stunted shoot growth, 'little leaves', chlorosis of apical leaves*

The major function of Mg is being a central atom of the chlorophyll molecule; therefore the most obvious visible symptom of Mg deficiency is chlorosis of older basal leaves, as a consequence of Mg being transferred from the older to young leaves, growing organs and especially to the seed and fruit, where it is most needed. Mg deficiency can frequently be observed on sandy soils in high rainfall regions, poorly drained sites, or alkaline soils due to the fact that Mg is leached from the soil (Marschner, 1995). Mg deficiency, however, can be induced not only by low Mg concentration in the soil, but also by high concentrations (antagonism) of other ions such as H^+ (low pH), K^+ (heavy application of fertilizers), NH_4^+ (ammonium sulphate as a fertilizer), Ca^{2+}, Mn^{2+}, and Al^{3+} ions (in acid soils with pH $\leq$ 5), even on soils that otherwise have good Mg status (Bergmann, 1992). One of the typical symptoms connected with Mg deficiency in vines is so-called 'bunch stem necrosis' (BSN) or 'stalk necrosis' or 'stem dieback'. BSN is one of the most serious physiological grapevine diseases leading to cluster injury in the ripening stage and causing yield and quality losses (Capps and Wolf, 2000). It is caused by an imbalance between K ions on the one hand and Ca and Mg ions on the other (Haefs et al., 2002).

Iron is one of the important plant micro-nutrient, being among the elements in the highest degree affected by soil properties (*e.g.* pH, redox potential, presence of $CaCO_3$). Green plants must absorb Fe continuously (Fe^{2+} (ferrous) is preferred form) during growth because it is not transferred from older to younger leaves. As described in section 1.2.4.1, the concentrations of Fe in ionic forms are extremely low. Plants are therefore assumed to improve iron availability through specific uptake mechanisms – Fe uptake strategy I and strategy II – or by other non-specific mechanisms (*e.g.* exudation of organic acids by the root, root-induced decrease in pH). Roots of plants with 'strategy I' (dicotyledons and monocotyledons except gramineous plants) develop more root hairs and they exude more protons (H^+ ions), phenolic compounds and organic acids, some of which have chelating properties. On the other hand, gramineous plants exude increasing amounts of iron chelating substances (non-proteinogenous amino acids) known as 'phytosiderophores', which form chelates with Fe^{3+} in the rhizosphere and on that way facilitate the iron uptake (strategy II) (Bergmann, 1992).

There are many factors like interactions of Fe with other elements, soil and environmental factors that can affect iron uptake and its utilization by plants (depend on the species and variety) and as a consequence induce leaf chlorosis (on the youngest leaves). Nevertheless, Fe deficiency is fairly widespread in vines, especially on soils that are rich in lime, with the pH value above 6.5 (Bergmann, 1992; Fregoni, 1997). Iron chlorosis can be one of the limiting factors for fruit crop production, since growers are usually not using Fe treatments to face major yield and quality losses and marked reductions in the vineyards longevity. However, *Vitis* species, as other plants, differ to their susceptibility to Fe chlorosis (Tagliavini and Rombolà, 2001).

Table 6: *K, Mg, Fe, and Zn concentrations in blades and petioles for assessing the nutrient status of grapevines.*

Element (unit)	*Deficient*	*Low to marginal*	*Adequate*	*High to excessive*
Blades [a]				
K (g kg^{-1})	*<10*	*10-12*	*12-14*	*>14*
Mg (g kg^{-1})	*<2.0*	*2.0-2.3*	*2.3-2.7*	*>2.7*
Fe (mg kg^{-1})	*<50*	*50-100*	*100-250*	*>250*
Zn (mg kg^{-1})	*<20*	*20-30*	*30-150*	*>150*
Petioles [b]				
K (g kg^{-1})	*<10*	*10-25*	*25-35*	*>35*
Mg (g kg^{-1})	*<3.0*	*3.0-5.0*	*5.0-10*	*>11*
Fe (mg kg^{-1})	*<25*	*25-30*	*31-100*	
Zn (mg kg^{-1})	*<15*	*15-25*	*30-60(120)* [c]	

[a] *Concentration ranges reported by Fregoni (1997: 528).*

[b] *Concentration ranges reported by Fregoni (1997: 533) and/or Sinskey (2009: 77-78).*

[c] *According to Sinskey (2009) adequate range is up to 120 mg kg^{-1}.*

Zinc plays an important part as a metal component or as functional, structural, or regulatory cofactor of a large number of enzymes. The mobility of Zn within the plants is poor, but better than that of Fe. Zn is relatively immobile (with minimal solubility at pH 7) in typical agriculture soil except in acid soils where H^+ promotes its uptake (Farago, 1994). Zn deficiency commonly occurs on soils with pH values of

6.5 to 8.0. Owning to its poor mobility and the low concentration in the soil solution, direct contact between root and the soil particles plays a crucial role in the uptake process. Plants absorb Zn mainly as Zn^{2+} ions. At the same time Zn concentration in plants is species specific because of different absorbtion and translocation ability, and is influenced by the age and vegetation state of the plant as well (Bergmann, 1992; Farago, 1994). The symptoms of Zn deficiency are not the same in all plants. If the grapevine lacks of Zn, typical the formation of small and/or mottled leaves (known as 'little leaves') can be noticed and sometimes shortened internodes. In addition, other deficiency symptoms like intervenial leaf chlorosis and clusters with few berries of different sizes may occur.

Deficit, optimal and toxic concentrations limits of four studied elements - K, Mg, Fe, and Zn in leaf blades and petioles of most grapevine cultivars (Fregoni, 1997; Sinskey, 2009), which are important parameters for evaluating the nutritional status of grapevines, are presented in Table 6.

1.1.6 Grape quality parameters influenced by mineral nutrition

It is well known that the grape ripening process depends on different factors, such as climatic conditions and cultural practices. The first cannot be controlled and change from one year to the next, resulting in differences of composition in matured grapes. In this way, final products (wines) show different characteristics between vintages, which greatly influence their quality (Pérez-Magariño et al., 2002).

The most important parameters determining the grape and consequently wine quality are soluble solids and organic acid contents, pH and phenolic potential. Soluble solids (SS) are expressed as ºBrix, ºBalling, ºBaumé, or ºOechsle. At, or close to maturity (18 ºBrix), soluble soilids levels are within 1% of actual sugar content (glucose and fructose). Sugar levels indicate potential alcohol yield after fermentation and the likelihood of residual sugars remaining (Jackson and Lombard, 1993). Moreover, the content and the composition of sugars are relatively stable in grape berries between years (Liu et al., 2006).

Organic acids consist mainly of tartaric, malic, and citric acids and can be measured by titration and expressed as total titratable acids (TA) *e.g.* as tartaric acid equivalents. Development of grape acids is again dependent on photosynthesis, lack of which seldom limits TA. The reduction of TA during maturation is related to the respiration rate of the berry and is a function of temperature. Malic acid is the chief acid influenced by respiration. Wine with too much acid (10 g L^{-1} TA equivalents and above) is tart to the taste and deacidification may be required. In warm/hot climates acid may be too low (below 6-7 g L^{-1}), producing a bland wine which can be adjusted with addition of tartaric or citric acid (Jackson and Lombard, 1993).

A pH level above 3.60 in the wine may cause problems. High pH levels in wines incerase the relative activity of micro-organisms such as bacetria, lower the colour intensy in red wines, bind more sulphur dioxide and reduce the free SO_2 content, and can shorten the ability of wine to age.

Most of the studies conducted on nutrients focused on the effects of nitrogen, which is known to be widely impact on yield and fruit quality (Jackson and Lombard, 1993; Keller, 2005; Topalović et al., 2011). Nitrogen promotes higher yields, enhances grape juice pH value (when combined with potassium) and reduces soluble solids and anthocyanin content (Jackson and Lombard, 1993). Potassium is thought to be involved in the translocation of solutes (*e.g.* sugars) into the berry (Mullins et al., 1992) and it appears to be linked with higher acidic contents (*i.e.* malate) (Conradie and Saayman, 1989), high pH (Morris et al., 1987) and poor colour in red wines (Jackson and Lombard, 1993). Nitrogen deficiency and also water stress causes plant growth to be reduced and an increased biosynthesis of grape polyphenols is stimulated (Castellarin et al., 2007; Hilbert et al., 2003). Among polyphenols, there are still missing data discerning the relationship between mineral fertilisation and the occurrence of hydroxycinnamic acid esters (HCAs) in wine grapes.

1.1.6.1 Polyphenol compounds

Phenolic compounds (known as phenols, phenolics, polyphenols, polyphenolic acids etc.) are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found in plants (Spanos and Wrolstad, 1992). They represent the third most abundant constituent in grapes and wines after carbohydrates and fruit acids (Nawaz et al., 2006).

In general, grape and wine phenols can be divided into two groups: (1) non-flavonoid (including hydroxybenzoic and hydroxycinnamic acids (HCAs), volatile phenols, stilbenes (*e.g.* resveratrol) and miscellaneous compounds like lignans and coumarins) and (2) flavonoid phenolic constituents (comprising anthocyanins, flavan-3-ols and flavonols) (Montealegre et al., 2006).

Phenols play an important role in the quality of grapes and wines because of: (1) the colour (anthocianin content, copigmantation); (2) the sensory properties, astringency, bitterness and structure of wines (Chamkha et al., 2003; Robichand and Noble, 1990). On the other hand, hydroxycinnamic derivatieves are known as oxidation substrates and browning precursors (Singleton et al., 1978). Browning of the must is one of the technical problems in the early stages of grape juice making (Sapis et al, 1983). The levels of HCAs derivatives seem to constitute an important factor in the browning of grapes, since polyphenol oxidase does not exhibit the same affinity to all substrates (Romeyer et al., 1983). In addition to properties they contribute to quality of grape and wine, phenolic compounds (especially anthocyanins) are known to have a wide range of physiological, biochemical and pharmalogical functions in human bodies (Nawaz et al., 2006).

The concentration of phenolic compounds in grapes depends on the cultivar (variety, species), season and is influenced by viticultural (crop load, processing practices, winemaking procedures), management and environmental factors such as soil conditions and climate (*e.g.* water uptake) (Jackson and Lombard, 1993; Romeyer et al., 1983; Yang et al., 2009). However, the phenolic profile of wine is not the same as that of fresh grapes (Nagel et al., 1979) because significant changes in phenolic

composition occur during the wine making process, both very early at the grape crushing step and during wine fermentation and ageing (Singleton et al., 1978). Moreover, there is big difference between phenolic profile of red wines in comparison to white wines, due to diverse technology and wine making processes. Phenolic compounds in red wine are derived from the grape`s skin, as well as from grape seeds, grape stems, or grape pulp, all of which are important sources of flavanols that are transferred to the wine during maintenance together with the grape juice at the first stage of wine fermentation. Flavan-3-ols (catechins and oligomeric proanthocyanidins) are mainly present in the skins and in the seeds, while anthocyanins mostly accumulate in the skin. On the contrary, white wines are usually made from the free running juice, without the grape mash (without maceration of the solid parts), having no contact with grape skins. This is thought to be the main reason for the relatively low phenol content of white wine (50 to 350 mg L^{-1} of gallic acid equivalent) in comparison to red wines (800 mg L^{-1} to 4 g L^{-1}) (Chamkha et al., 2003; Yang et al., 2009).

The major phenolics present in white wine are the hydroxycinnamic acids (Singleton et al., 1978). In fruits and vegetables, HCAs are mainly derived from *p*-coumaric, caffeic, and ferulic acids and predominantly occur in esterified form with quinic acid or glucose (hydroxycinnamoylquinic acids) (Winter and Herrmann, 1986). In vacuoles of grape skin and pulp cells of grape, the quinic acid esters are uniquely replaced by L-tartaric acid (hydroxycinnamoyl tartaric acid esters) and are named caftaric acid [caffeoyl-L-(+)-tartaric acid], coutaric acid [*p*-coumaroyl- L-(+) tartaric acid], and fertaric acid [feruloyl- L-(+)-tartaric acid] as first reported by Ribéreau-Gayon (1965, op cit Romeyer et al., 1983). Caftaric acid is by far the most predominant HCA in gapes and wine followed by coutaric acid and, albeit in smaller amounts, fertaric acid (Ong and Nagel, 1978). Natural form of both cautaric and caftaric acids is *trans* and the *cis*-isomer arises in grape and wine on exposure to ultraviolet light (Singleton et al, 1978).

Hydroxycinnamoyl tartaric acid esters are relatively unusual in nature, and they are characteristic of the genus *Vitis* with the exception of *Vitis rotundifolia*. However, in *Vitis* species the content of *trans*-caftaric acid of berry juice was found to vary

between 5 and 1337 mg L^{-1} (mean content of all species was 292 mg L^{-1}) in comparison to white and red varieties of *Vitis vinifera* where contents were between 16 and 295 mg L^{-1} (mean content was 127 mg L^{-1}), and between 48 and 430 mg L^{-1} (mean content was 163 mg L^{-1}), respectively (Singleton et al., 1986a).

Singleton et al. (1986b) reported that grapes tend to maintain a relatively constant content of hydroxicinamates. The concentration of *trans*- and *cis*-forms of caftaric and coutaric acids, their ratio to each other, and the relative proportion of the *cis* forms is largely varietally (genetically) set. It appears that the biosynthesis is maintaining the concentration of caftaric and related acids relatively constant as the berry ripens. It is also believed that vineyard location and vintage year have relatively little influence compared to genetic characteristics of the mother vine, but they do not appear to be without influence.

1.1.7 Physicochemical forms (species) in the soil

Soil is physically and chemically complex heterogeneous material. Metals may be present in several different physicochemical forms (species) (Lake et al., 1984):

(1) as free or complexed ions in soil solution,
(2) as exchangeable ions,
(3) as organically bound,
(4) adsorbed, co-precipitated or occluded with metal oxides, carbonates, or phosphates and other secondary minerals,
(5) as ions in crystal lattice of primary minerals.

The first three forms are believed to be in equilibrium with each other, the equilibrium being affected by pH value, redox potencial, and the concentration of metals and ligands. Metal cations present in soil in these three forms are considered to be the most ‘available’ to plants, while successive forms under the points (4) and (5) are representing decreasing degrees of availability. Metals occluded in primary minerals may also become available through weathering in long time, while metals occluded by highly stable secondary minerals may become plant-available over very

long period of time or after drastic pH or redox changes (Lake et al., 1984; Zhang et al., 2002).

The distribution of metals among soil components is important for assessing the soils potential to supply sufficient macro- and micronutrients for the plant growth and retaining of heavy metals toxic amounts as well (mobility of metals in the environment and possible contamination of ground waters) (Quevauviller et al., 1997). The degree of metal association with different geochemical phases strongly depends upon the physico-chemical conditions of the soil: the pH value, temperature, amounts and forms of oxides and carbonates, cation-exchange capacity (CEC), nutrient status (synergism and antagonism), organic matter content (OM), and soil texture (Alloway, 1995; Farago, 1994).

The most important chemical processes affecting the behaviour and bioavailability of metals in the soils are those concerned with the adsorption of metals from the liquid phase on to the solid phase. These processes control the concentrations of metal ions and complexes in the soil solution and thus exert a major influence on their uptake by plant roots. The major chemical processes that control mobility and availability of trace elements in the soil include precipitation-dissolution, adsorption-desorption, and chelation. The relative importance of each process is dependent on soil reactions and subjected to rhizospheric effects (Alloway, 1995; He et al., 2005).

Precipitation–dissolution is an important process that controls the solubility of elements in calcareous soils and soils with a pH above 7.0. Many trace metals (*e.g.* Zn, Fe, Cu, Mn) can form insoluble precipitates, which may control the solubility of metals in the soil solution (He et al., 2005). In addition, trace metals (Zn, Fe and many others) can also co-precipitate with secondary minerals including clay minerals, Fe and Mn oxides and calcite (Ca carbonates) (Alloway, 1995).

Metals can be adsorped onto surfaces of soil colloids through non-specific adsorption (by static electric force) and specific adsorption (formation of covalent bonds between the ion and the surface). Most metals exist mainly as cations in the soil solution, and their adsorption (CEC) therefore depends on the density of charges on

the surfaces of the soil colloids (Alloway, 1995). On the other hand, specific adsorption is strongly pH dependent and is related to the hydrolysis of the metal ions. The metals most able to form hydroxyl complexes are specifically adsorbed to the greatest extent (*e.g.* Al, Fe and Mn) (Alloway, 1995).

Another process involved in the adsorption of metals is chelation, during which trace elements (*e.g.* Cu, Fe, Mn, Zn and others) forms stable complexes with organic ligands (*e.g.* humic, fulvic, and organic acids) or other chelating reagents like EDTA or DTPA (He et al., 2005). Chelation has been reported to be responsible for increased availability of trace elements (especially for Fe) in the plant rhizosphere, especially for those plants that can excrete organic acids in response to low availability of the metals (Marschner, 1995).

1.1.8 Estimation of the 'phytoavailable' metals in the soil

A complex variety of abiotic and biotic processes affect metal phtoavaiability; these include adsorption onto and desorption from minerals, and interactions with soil, plants and microbs (Basta et al., 2005). The soluble fraction of metals is generally considered to be phytoavailable, which is not always 'true'. Ideally, a soil test used to assess the plant-accumulation of metals is one which extracts metals under conditions similar to those exerted by the plant in the rhizosphere (Menzies et al., 2007). One way to estimate bioavailability of nutrients in plants is by calculation of the relationship between extractable fractions of the metals from soils and the total metal concentrations in the plants. If the accumulation of an element by a plant correlates significantly with the extractable fraction in soils, it can be assumed that the extractable fraction is readily available to plants. The higher the correlation coefficients, the more suitable the recommended extraction method for establishing the relationship between the elemental speciation of heavy metals in soils and plant availability (Zhang et al., 2002).

A widely-used technique for understanding element distribution in soil (different species) is known as fractionation (Templeton, 2000). Both single (García et al.,

2005; Helmke and Sparks, 1996; Lindsay and Norvell, 1978; Pueyo et al., 2004; Quevauviller et al., 1997) and sequential extractions methods (Gleyzes et al., 2002; Sánchez et al., 2005; Száková et al., 1999) can be used to assess the amount of mobile or potentially mobile metal species, that in turn may correlate with plant-available contents under certain environmental or agricultural conditions (Rao et al., 2008). A schematic example diagram of these two approaches is shown in Figure 2.

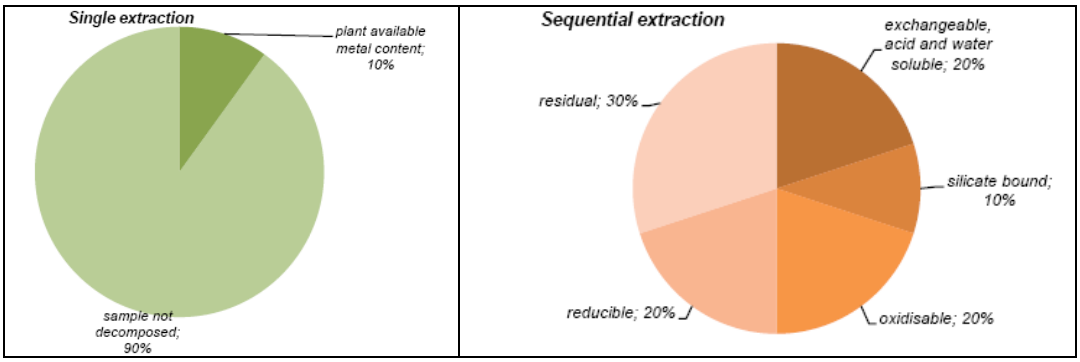

Figure 2: *Two different approaches (single and sequential extraction) applied in general to the determination of metals in soil (% values shown above are only tentative as they are metal and matrix dependent) (Rao et al., 2008: 292).*

A variety of single soil extractants are currently used for the evaluation of micro-and macro-nutrient availability in soils. The frequently used reagents can be grouped into four categories (Zhang et al., 2002): (1) chelating agents, (2) diluted acids (organic and inorganic), (3) un-buffered salt solutions, and (4) buffered salt solutions. Some of the most used extractants with corresponding extracted elements are summarised in Table 7.

The most widely used extractants for cationic micro-nutrient (*e.g.* Zn and Fe) are DTPA and EDTA (García et al., 2005; Haq and Miller, 1972; Lindsay and Norvell, 1978; Norvell, 1984; Quevauviller et al., 1997). Both methods are used also in agricultural laboratories in Slovenia (KIS - Agricultural Institute of Slovenia) and Italy (YARA Italia S.p.a.). DTPA and EDTA extractants use organic ligands capable of forming a strong complex with metal as the bases for the extraction process. This approach was developed as a chemical representation of the phytosiderophore release strategy used by metal deficient plant, and was intended to be used for testing metal

availability (particularly Zn, Cu, Fe, and Mn) in near neutral and calcareous soils (Lindsay and Norvell, 1978). However, nowadays it has been used for widely varying soils and to estimate also non-essential or toxic metal (Cd, Cr, Ni, Pb etc.) availability (see Menzies et al., 2007 and references therein). For Cd, Zn and Ni, Menzies et al. (2007) reported that DTPA remove approximately 11% of the total soil metal, a concentration greatly in excess of that which would be removed by plants over many years and decades.

Table 7: *The most used extractants to predict phytoavailability of metals.*

Extractant	*Elements*	*Reference*
Chelating agents		
0.05 mol L^{-1} EDTA	*Zn, Cd, Cr, Cu, Ni, Pb*	*Quevauviller et al. (1997)*
0.005 mol L^{-1} DTPA/TEA	*Zn, Fe, Mn, Cu*	*Lindsay and Norvell (1978)*
Mild acids		
0.43 mol L^{-1} CH_3COOH	*Zn, Cd, Cr, Cu, Ni, Pb*	*Quevauviller et al. (1997)*
Un-buffered salt solutions		
0.01 mol L^{-1} $CaCl_2$	*Zn, Cd, Cu, Pb, Fe, K, Ca, Mg, Mn, Na*	*Pueyo et al. (2004)*
1 mol L^{-1} NH_4NO_3	*Zn, Cd, Cu, Pb, Fe, K, Ca, Mg, Mn, Na*	*Pueyo et al. (2004)*
Buffered salt solutions		
1 mol L^{-1} CH_3COONH_4	*K, Mg*	*Helmke and Sparks (1996: 559)*

On the other hand, the use of neutral salt solutions as extractants is advocated on the assumption that the phytoavailable metals are mostly located on mineral surfaces and can be displaced by the other cations (exchangeable fraction). Unlike chelating extractants, neutral salts remove the metal from the soil solid phase by swamping the soil with the desorbing cation (McLaughlin et al., 2000). A variety of neutral salt extractants have been proposed for the measurement of trace metals in soils, including NH_4Cl, NH_4NO_3, CH_3COONH_4, $NaNO_3$, $CaCl_2$, $MgCl_2$ and others. In fact, each of the different extractants has been reported to provide various benefits when

compared to the others (Menzies et al., 2007). However, two methods using un-buffered salt solutions are standardised in Europe (0.01 mol L^{-1} $CaCl_2$ solution in The Netherlands and 1 mol L^{-1} NH_4NO_3 solution in Germany).

There are many methods proposed for estimating the bio-availability of major and trace elements in soil. The extractants developed are not universal reagents, but remain, to varying degrees, soil and crop specific (Rao et al., 2008). In Slovenian vineyards are K and Mg added on regular basis according to recommendations for yearly side-dressing based on the ammonium lactate-extractable K_2O ('AL-method'; Vršič and Lešnik, 2005) and $CaCl_2$-extractable Mg, respectively; while in other European countries (*e.g.* Italy) extraction using ammonnitrate, barium chloride or ammonium acetate is performed for determination of plant-available K and Mg. Therefore, harmonisation of extractable procedures will be advisable in order to obtain comparable data among laboratories.

1.2 RESEARCH GOALS

The main research goals of this thesis is devided to five point:

(1) Evaluation the uptake of K, Mg, Fe, and Zn into grapevine leaves and grape berries

Despite the obvious importance of soil fertilization in plant growth and production, the knowledge and understanding about nutrient availability, the actual uptake from different fertilizers and how they are affecting grapevine physiology and productivity is surprisingly poor (Keller, 2005). Moreover, there are not many data available in literature as regard the effects of applied elements on the concentration of other micro- and macro-elements within the plant. Thus, the ***first*** aim of this experiment was to study effects of basic N, P, K soil fertilisation in combination with foliar or soil application of fertilizers containing Mg, Fe, and Zn (individual or in combination) on the nutrient status of grapevines 'Rebula' (i.e. element concentration in the petioles). An experimental trial was carried out on a local white variety named 'Rebula' (*Vitis vinifera* L.), grafted on SO4 rootstock, during four

seasons (2008-2011). The uptake study was conducted in pots to minimize factors affecting the plant uptake (pH, moisture, soil, location, etc.) as all vines were grown in the same, sieved soil collected in the vineyard of Goriška Brda. Nutrient uptake was determined by measuring the concentrations of K, Mg, Fe and Zn in leaf petioles and whole grape berries using FAAS. All results have been statistically evaluated with appropriate tests (ANOVA, Student-Newman-Keuls´s test) to weigh up the effect of fertilizing (foliar spraying and fertirigation) on the concentrations of studied elements in the plant`s organs.

(2) Evaluation the interactions on element uptake

The uptake of one mineral does not depend only on its availability in the soil, since interactions among elements – synergism and/or antagonism – can influence the plant nutrient uptake. Interaction between K and Mg is one of the widely known antagonisms in grapevine, and often leads to Mg deficiency (Capps and Wolf, 2000). The ***second*** aim of this experiment was to study the relationships (positive and negative interactions) between K, Mg, Fe, and Zn in different plant organs (leaf petioles and grape berries). Simple correlation analysis of content data was used to evaluate the interactions on element uptake.

(3) Evaluation the impact of basic N, P, and K fertilization coupled with soil or foliar applications of fertilizers containing Mg, Fe, and Zn on the grape quality parameters (e.g. soluble solids, organic acids, HCAs)

Together with climate and soil parameters, nutrient supply becomes a key factor concerning yield and fruit quality (Etchebarne et al., 2009). Despite several studies where grape quality has been investigated from the perspective of impacts by soil type, climate, cultivar or season variation, there are only limited data available in the literature concerning correlations between plant mineral status (or fertilisation trials) and quality parameters, these being rare in case of micro-nutrients. Among polyphenols, there are still missing data discerning the relationship between mineral fertilisation and the occurrence of hydroxycinnamic acid esters in wine grapes. Thus, the ***third*** aim of this experiment was to evaluate the impact of basic N, P, and K fertilization coupled with soil or foliar applications of agrochemicals containing Mg, Fe, and Zn on the grape quality parameters – berry weight, soluble solids, organic

acid content, FAN, and HCAs in the local white grape cultivar ‘Rebula’. Grape quality parameters were determined by WineScan spectrometer. Hidroxycinnamic acids in grape juice were quantified by HPLC–DAD method. All results have been statistically evaluated with appropriate tests (ANOVA, Student-Newman-Keuls´s test, simple correlation analysis) to weigh up the effect of fertilizing (foliar spraying and fertirigation) on the grape quality.

(4) Finding out the correlations between estimated plant-available soil fractions and concentration of element in the plant leaves

There are many methods proposed for estimating the bio-availability of major and trace elements in soil. However, the extractants developed are not universal reagents, but remain, to varying degrees, soil and crop specific (Rao et al., 2008). Therefore, the ***fourth*** research goal was to tested extractants from different categories (EDTA and DTPA as chelating agents; CH_3COOH as diluted acid; $CaCl_2$ and NH_4NO_3 as un-buffered, and CH_3COONH_4 as buffered salt solution) using rhisospere pot soil in order to found the most suitable extracting solution for alakline soil in terms of (1) pH of extractant and soil extract, (2) extraction efficiency and (3) statistically significant correlations between soil extract and element content in the leaf petioles of ‘Rebula’ grapevines.

(5) Estimation the reasonableness of fertilization on alkaline soil consisting of carbonates (flysch soil)

The statistical processing of data obtained in the four-annual experiments will allow us to compare data on the soil/foliar nutrient application treatments and on ‘Rebula’ vine nutrient uptake. With all results gathered we plan to estimate reasonableness of fertilization on alkaline soil, typical for Goriška Brda wine-growing region.

2 EXPERIMENTAL

2.1 POT EXPERIMENT

2.1.1 Site and climate

A pot trial was performed during four consecutive growing seasons (2008–2011) in Podsabotin, within Western Slovenia winegrowing district (Goriška Brda), an area characterised by a typical sub-Mediterranean climate with frequent dry periods in summer and with an average annual rainfall of 1200 mm (Rusjan et al., 2006).

Meteorological data were recorded at two weather stations located close to the experimental trial. The one located in Capriva del Friuli (ARPA-OSMER FVG, Italy) was used in 2008 and weather station Biljana (WMR 200, Oregon Scientific, Oregon, U.S.) was used in the following years (Annex A). During the four-year experiment, the total annual rainfall was 1856, 1481, 2050 and 1297 mm in 2008, 2009, 2010 and 2011, respectively. From the beginning of April to the end of September, the amount of rainfall was 503 and 671 mm in 2009 and 2011 respectively, while in 2008 was 869 mm and nearly double (1204 mm) in 2010. In the last two years, the average temperatures were approximately 2 °C lower in comparison to the seasons 2009 and 2011 (21.1 °C on the average).

2.1.2 Plant material and growing conditions

The experiment was set up using ‘Rebula’ (*Vitis vinifera* L.) grapevines grafted on SO4 rootstock (Selection Oppenheim Nr. 4), that were planted in the year 2007 in 21 L plastic pots filled with soil, sieved to pass a 20 mm screen, collected from the surface layer (approximately 0–10 cm) of a typical vineyard of Goriška Brda. The pots were equipped with a plastic saucer, which prevented contamination with soil as well as growing of roots into the surrounding soil.

After planting during the vegetative period (from bloom to harvest), vines were sprayed periodically (every 2 weeks) to avoid diseases, therefore following a fungicide strategy plan based on integrated pest management rules adopted in the area. All the vines were protected against typical grapevine diseases *i.e.* downy (caused by *Plasmopara viticola)* and powdery mildew (caused by *Uncinula necator*) by spraying appropriate fungicides (0.3% (w/v) Mikal (Rhone Poulenc, Lyon, France) and 0.3–0.5% (w/v) Kumulus DF (BASF SE, Ludwigshafen, Germany)).

Pots were maintained outdoor and covered with nets in order to avoid hail damage. The vines obtained water from rainfall and from a drip irrigation system during the summer time (approximately 1.5 L every 2 days) in order to avoid water stress.

Vines in pots were placed in two parallel rows (one longer than other, due to the space availability) and spaced 0.5 m apart from each other. Each vine had its own 80 cm higher plastic cane for support and every 10 vines a larger woody stake was added in order to wire the plastic canes and also vine shoots (Figure 3).

Figure 3: *Pot experiment (Brataševec, April 2009).*

Vines were trained vertically and annual pruning retained one shoot per vine bearing 5–to–6 buds. On the average 4 canes had developed during the summer season and after first sampling (in June) only one cluster per cane was retained (4 clusters per vine as maximum crop load). At the same time (in 2010 and 2011) the length of three shoots per vine was measured to determine the vine growth.

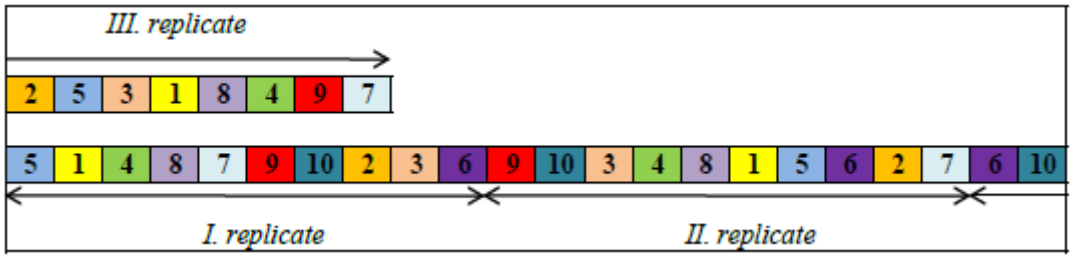

Figure 4: *The experiment setting up. Numbers (#1-10) are representing different treatments (for detailes see Table 8).*

2.1.3 Treatment layout

The same experimental design was maintained in every season from 2008 to 2011. Nine treatments (along with the Control–untreated vines) were applied in a randomized design with three replicates and 4 vines for replication (Figure 4). The rate of pre-plant mineral fertiliser (50 N, 90 P_2O_5, and 140 K_2O in kg ha^{-1}) was decided based on the soil analysis that was performed before grapevine planting by the Agricultural and Forestry Institute of Nova Gorica, Slovenia (Annex B), and according to recommendations for yearly side-dressing (Vršič and Lešnik, 2005).

Since the amounts of nutrient are usually given per hectare of vineyard, was the rate of each element calculated per pot, considering a field with 5000 vines ha^{-1}. Nitrogen as ammonium sulphate (20.6% N; Adriatica S.p.A, Rovigo, Italy) was applied each year in the spring, while phosphorus as phosphate fertilizer (26% P_2O_5; Timac Agro, Zwentendorf, Austria) and potassium as potassium sulphate (50% K_2O; Marchi Industriale S.p.A., Florence, Italy) were applied in 2008 only. In addition, agrochemicals containing Mg - 'Bittersalz' ($MgSO_4$ x 7 H_2O, 16% MgO; Compo GmbH & Co. KG, Münster, Germany), Fe - 'Foliacon Fe' (Fe complex with amino

acids, 5% (w/w) Fe; Green Has, Canale d'Alba, Italy) and Zn - 'Zinc 25' (water soluble Zn complexed by carboxylic acids, 25% (w/w) Zn; Green Has, Canale d'Alba, Italy) have been added each year before bloom (in May), individually or combined. Mg, Fe, and Zn applications were calculated according to producer recommendations (except #6 where concentration was triplicated) to simulate the actually fertilization treatment performed in the vineyard. In order to understand the effectiveness of Mg and Fe soil *vs*. leaf fertilization, a comparison between foliar spraying (l; leaves) and fertirigation (s; soil) was applied to the experimental vines.

Table 8: *Description of treatments applied in the pot experiment (2008-2011) with 'Rebula' grapevines.*

Exp	*Treatment*	*Concentration/quantity of fertilizer (typology of application)*
#1	*Control*	*Untreated (no added nutrients)*
#2	*NPK*	*10 g N, 18 g P_2O_5, and 28 g K_2O per pot (into the soil)* [a]
#3	*NPK Mg l*	*3%* [b] *'Bittersalz' (foliar spraying)* [c]
#4	*NPK Fe l*	*0.15% 'Foliacon Fe' (foliar spraying)* [c]
#5	*NPK Mg Fe l*	*3% 'Bittersalz' + 0.15% 'Foliacon Fe' (foliar spraying)* [c]
#6	*NPK Mg Fe hl*	*9% 'Bittersalz' + 0.45% 'Foliacon Fe' (foliar spraying)* [c]
#7	*NPK Mg s*	*3% 'Bittersalz' (fertirigation)* [c]
#8	*NPK Fe s*	*0.15% 'Foliacon Fe' (fertirigation)* [c]
#9	*NPK Mg Fe s*	*3% "Bittersalz" + 0.15% 'Foliacon Fe' (fertirigation)* [c]
#10	*NPK Zn l*	*2% 'Zinc 25' (foliar spraying)* [d]

[a] *N, P, K were added in pots #2-10. N-fertilizer (10 g N) was added once per year in 2008, 2009, 2010, and 2011. P- fertilizer (18 g P_2O_5) and K-fertilizer (28 g K_2O) were added in 2008, only.*

[b] *% (w/v)*

[c] *Mg an Fe-fertilizers were applied before bloom in 2008, 2009, 2010 and 2011.*

[d] *Zn-fertilizer was applied before bloom in 2009, 2010 and 2011.*

All fertilizers and fungicides used in the experiment were checked if besides labelled elements contain also other studied elements (K, Mg, Fe, or Zn). We dissolved them in double deionised water (n = 2) and analysed them by flame atomic absorption spectrophotometer (FAAS) (Annex C).

2.2 INSTRUMENTS AND OTHER EQUIPMENTS

A pH-meter HI 8417 (HANNA instruments, Woonsocket, U.S.) was used to measure the pH of grape juice, soils, soil extracts and extraction reagents. A pH/Redox/Temperature-tester HI 98121 was used to measure the oxidation/reduction potential (ORP) of the soil (directly in the field and in the laboratory).

For mineral concentration determination, an atomic absorption spectrometer (SpectrAA-10, Varian, Victoria, Australia) with air–acetylene flame (FAAS) and a single element hollow cathode lamp was used. Measurements were performed at 766.5 nm for K, 285.2 nm for Mg, 248.3 nm for Fe, and at 213.9 for Zn.

Grape quality parameters (*e.g.* soluble solids, pH, organic acid contents, and free amino nitrogen content) were assessed by the Agricultural and Forestry Institute of Nova Gorica (Slovenia) using a WineScan FT 120 spectrometer (Foss, Hillerød, Denmark) fitted with a Michelson interferometer generating Fourier transform infrared (FT–IR) spectra. Soluble solids (° Brix) of free run juice were determined using a digital hand refractometer (model WM-7, Atago, U.S.), too.

High performance liquid chromatography (HPLC), an Agilent 1100 series system (Agilent Technologies, Waldbronn, Germany) with an auto injector and a diode array UV–visible detector (DAD G1315A) recording at 280, 320 and 530 nm was used to detect the phenolic compounds (HCAs). For HCAs separation, the column, a PFP–2 100 A Luna (250 x 4.60 mm; Phenomenex, Torrance, USA), 5 μm particle size, was used. For total phenol determination with Folin-Ciocalteu (FC) method an Agilent 8453 UV-visible spectrophotometer was used. Readings were taken at 765 nm.

A 2-mm stainless sieve (Retch, Haan, Germany) was used to obtain a soil fraction suitable for further soil analysis.

For sample (soil, grape, leaves) grinding, a Mixer mill MM 400 (Retsch, Haan, Germany) with grinding cap and balls of zirconium oxide was applied.

The soil and grape skin polyphenol extractions were obtained by using a horizontal shaker (Kambič, Semič, Slovenia).

A sand-bath with thermostatic control between 50 and 300 °C (ST72; Harry Gestigkeit, Düsseldorf, Germany) was used for evaporation of acids in wet digestion procedures of plant and soil samples.

For the phenol solid phase extraction (SPE) procedure a Supelco Visiprep 12 SPE vacuum manifold (Sigma-Aldrich, Bratislava, Slovakia) was used.

A centrifuge (5804 Eppendorf, Hamburg, Germany) was used for centrifugation of grape juice samples and for the soil extractions.

A filter paper (25 μm Schleicher and Schuell, Dassel, Germany) and a memebrane filter (0.45 μm Chromafil Xtra PTFE filter 45/25, Macherey-Nagel, Düren, Germany) were used for filtration of grape juice before analysis. A filter paper (Assistent, Germany) was used for filtration of soil extractions.

Cartridges Strata-X 33 μm Polymeric Sorbent (patent pending) 200 mg/6ml (Phenomenex, Castel Maggiore, Italy) were used for phenol SPE procedure.

All dilutions were performed using adjustable micropipettes (Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany and Brand GmbH & Co. KG, Wertheim, Germany).

2.3 REAGENTS, STANDARDS AND REFERENCE MATERIALS

Almost all the reagents used for mineralization (wet digestion) of plant, grape and soil samples were of suprapure grade and purchased from Merck (Darmstadt, Germany): nitric acid (65% HNO_3), hydrochloric acid (30% HCl), hydrofluoric acid (40% HF), and hydrogen peroxide (30% H_2O_2). Perchloric acid was of analytical-grade (70% $HClO_4$, Fluka, Buchs, Switzerland).

The reagents used to prepare the extracting solutions were analytical-grade: ethylenediamine tetraacetic acid (0.5 mol L^{-1} EDTA solution, pH 8.0), diethylene triamine pentaacetic acid (DTPA), ammonium nitrate, triethanolamine (TEA) purchased from AppliChem (Darmstadt, Germany), acetic acid (≥ 99.8%; Sigma-Aldrich, Steinheim, Germany); sodium carbonate (≥ 99.0%, anhydrous; Fluka, Buchs, Switzerland); ammonium hydroxide (25%, J. T. Baker, Deventer, The Netherlands); potassium chloride (Sigma-Aldrich (Riedel-de Haën), Seelze, Germany), and strontium nitrate (98%, anhydrous, Alfa Aesar, Karlsruhe, Germany). Calcium chloride, provided by Acrōs Organics (New Jersey, U.S.) was of extra pure quality (96%, anhydrous).

Methanol used for HPLC analysis was of HPLC grade and purchased from Sigma-Aldrich (Steinheim, Germany) and other reagents used for the polyphenol determintation were analytical-grade: formic (~98%) and perchloric acid (Fluka, Buchs, Switzerland); Folin–Ciocalteu reagent (Merck, Darmstadt, Germany), L-glucose (Sigma-Aldrich, Steinheim, Germany). Potassium metabisulfite (Esseco, San Martino di Trecate, Italy) and ascorbic acid (Laffort Enologie, Bordeaux, France) were commercial grade.

All solutions and dilutions were prepared using doubly deionised and purified water (~18.0 MΩ cm^{-1}; dd-H_2O) obtained from a Bernstead ultrapure water system (Thermo Scientific, Braunschweig, Germany).

Standard stock solutions of K and Fe (100 mg L^{-1}) and of Mg and Zn (25 mg L^{-1}) were prepared from the single-element standard solutions (J. T. Baker, Deventer, The Netherlands) containing 1000 ± 2 mg L^{-1}metal ions. Standard working solutions were prepared from stock solutions on a month basis. All the solutions were acidified to 2% (v/v) with HNO_3.

For phenols quantification gallic acid (≥ 98.0%, Fluka, Buchs, Switzerland) and *trans*-caftaric acid (≥ 97.0%; Sigma-Aldrich, Steinheim, Germany) were used (calibration curves).

Plant and soil standard reference materials (SRMs) from National Institute of Standards and Technology – NIST (Gaithersburg MD, U.S.) and a certified reference material (CRM) from the Institute for Reference Materials and Measurements – IRMM (Geel, Belgien) were used for quality control purposes. For wet digestion procedure the following SRMs were used: 1) leaf samples: 1572–citrus, 1547–peach, 1573 and 1573a–tomato leaves; and 2) soil samples: 2709–San Joaquin, 2710–Montana I, and 2711–Montana II soil. A sewage sludge amended soil (CRM 483) was used for evaluation of EDTA and acetic acid-extractable Zn.

Lab glassware, plastic and polytetrafluoroethylene (PTFE) beakers (used for metal determination) were rinsed three times - first with once and then with double deionised water - after being soaked in a HNO_3 (10%, v/v) bath overnight. In addition, the glassware used for the preparation of extracting solutions was soaked for half an hour in 0.05 mol L^{-1} EDTA solution. The glassware used for the polyphenol determination was cleaned only with once and double deionised water (no using detergents). The polypropylene (PP) centrifuge tubes were used only once. Preparation of reagents and standards were performed in a clean laboratory used only for atomic absorption spectrometry.

2.4 ANALYTICAL PROCEDURES

2.4.1 Determination of K, Mg, Fe and Zn in leaf and grape samples

Sample preparation steps were done according to Hoenig (2001) with some modifications, following the proposed methods for environmental trace element analysis.

2.4.1.1 Sampling and homogenisation

Sampling and phenology dates in the years 2008-2011 are presented in Table 9. Leaves (2–3 leaves per vine of each plant) were sampled at berry set (end of June, leaf opposite to the cluster or mid-shoot leaves in 2008, since there were no grapes) and at veraison (beginning of August, mid-shoot leaves) (Haefs et al., 2002; Sinskey, 2009). All grape clusters (in 2009, 2010 and 2011) were collected at harvest (end of August–beginning of September). Each sample was put in its own clean plastic bag and transported to the laboratory for further analysis.

After sampling, the blades were separated from the petioles. For grape analysis approximately 20–25 undamaged berries (retained with pedicels) were randomly selected on all clusters from each replicate. Both, leaf and berry samples were first washed up with tap water and then with deionised water in order to remove dust and other residues from the surface. All samples were then oven-dried at 105 °C to a constant weight (berries firstly at 60 °C to avoid juice drainage from the berries). Pedicels were removed from berries after drying up.

Table 9: *Sampling and phenology dates during the experiment.*

		Leaf sampling		*Grape sampling*
Year	*Bloom*	*Berry set*	*Veraison*	*Harvest*
2008	*June 6–June 15*	*June 23*	*August 12*	*No grapes*
2009	*May 27–June 5*	*June 17*	*August 3*	*September 7*
2010	*June 6–June15*	*June 23*	*August 21*	*September 5*
2011	*May 28–June 6*	*June 20*	*August 10*	*August 31*

2.4.1.2 Mineralization (wet digestion) of plant material

After homogenisation and grinding were the samples digested with HNO_3 (left overnight to prevent strong foaming, covered with a lid) and H_2O_2 in the PTFE beakers on a sand-bath (sand temperature = 120–140 °C) according to Hoenig et al. (1998) without HF step. H_2O_2 was added with care (after sample heating,

approximately 30 min) in small amounts (4 times with 0.5 mL) to avoid possible strong foaming due to the sample composition. The samples were heated at the same temperature for the next 1-2 h to remove the acid by evaporation. The dry residues were re-dissolved in 0.5 ml of HNO_3 with 1–2 min heating, and filled to an appropriate final volume with dd-water in 30 mL PP centrifuge tubes. Preparation steps and mineralization procedure for all three samples are presented in Table 10. All analysis were performed on duplicate (and in triplicates in 2008 and 2009). The mineral contents were then analysed by FAAS, which will be described later on.

Table 10: *Leaf and grape sample preparation steps and mineralization procedure.*

Sample	*Quantity per replicate*	*Oven-drying (°C)*	*Grinding time (min)* [a]	*Sample weight (g)* [b]	*Volume (mL) of*		
					HNO_3 [b]	*H_2O_2*	*Final*
Blades	*8–12*	*105 (3 days)*	*2.5*	*0.5*	*5*	*2*	*25*
Petioles	*leaves*	*105 (3 days)*	*1*	*0.3*	*3*	*2*	*20*
Grapes	*20–25*	*60 (3 days)*	*4*	*0.5*	*5*	*2*	*25*
	berries	*105(1 week)*					

[a] *Grinding at 28 Hz s^{-1} using 2 balls.*

[b] *The ratio between the sample weight and volume of acid (w/v) was determined according to Hoenig et al. (1998). For petioles, a smaller sub-sample was used due to limited sample quantity.*

2.4.2 Determination of other major elements in leaf blades

In addition to K, Mg, Fe and Zn determination, two leaf blade samples were analysed for other major elements like nitrogen (N), phosphorus (P), sulphur (S), calcium (Ca), boron (B), manganese (Mn) and copper (Cu) by outsourcing lab YARA Italia S.p.a. To evaluate the differences in nutritional status between the control vine and NPK treated vines blades of treatment #1 and #2 were sampled at veraison 2011.

2.4.3 Grape quality analysis

At harvest time (Table 9) all clusters were collected and samples of 100 undamaged berries per replicate (from four vines, see paragraph 2.1.3) were prepared for further analysis in the laboratory. Samples were weighed in order to determine the average berry weight and then skins and pulp were crushed gently and the free running juice was collected in dark 125 mL flask and stored at 4 °C until analysed for quality parameters.

Before analysis grape samples were centrifuged and filtered through 25 μm filter paper. We used WineScan spectrometer for analysis of the following parameters: soluble solids (*i.e.* sugar content (°Brix) and specific weight), pH value, organic acid content (titratable (TA) expressed as tartaric acid equivalents in g L^{-1}, tartaric and malic acids in g L^{-1}); free amino nitrogen content (FAN in g L^{-1}). All analyses were performed with WineScan spectrophotometer only in 2010 and 2011. In 2009 we measured soluble solids (° Brix) and pH (using a refractometer and pH meter) as well.

2.4.4 Sample preparation and processing prior to polyphenol analysis

In 2009 and 2011, another sample of 50 berries was retained and added with 1 g of potassium metabisulfite to inhibit polyphenol oxidase after berry crushing. Vrhovšek (1998) reported that the addition of this substance in stated amounts had no apparent deleterious effect on chromatographic analyses of individual phenols

Each grape sample was pressed with fingers (berry by berry) and carefully squeezed without crushing. The juice obtained was then filtered through gauze and the volume was measured in 50 ml glassware measuring cylinder. The skins and solid parts residues on the gauze were then put into 250 ml conical flask and extracted three times (10 min at 150 rpm using the horizontal shaker) with 30 ml portions of 6% (w/v) perchloric acid in dd-water (Vrhovšek, 1998). Perchloric acid was added to precipitate proteins and inhibit polyphenol oxidase. The three skin extracts were

combined after filtered through a gauze and the total volume was measured (in 100 ml glassware measuring cylinder) to refer the data (milligrams per litre) to the volume of an un-oxidized juice. This sample was considered representative of the total content of phenols in skins grapes. Juice and skin extract have been separately stored into two 25 ml dark flask at –20 °C for further analysis: 1) FC spectrophotometrical total phenols determination; and 2) HPLC–DAD quantification of individual hydroxycinnamic acids (HCAs) derivatives.

For HPLC analysis, collected juice was additionally protected with 1 g of ascorbic acid in order to reduce any quinine formation until phenol determination with HPLC (Vrhovšek, 1998). Sampling, extraction, and analysis of grapes were performed once for each treatment replicate. Phenols analysis was performed as soon as possible, but no later than 2 months after preparation.

2.4.4.1 Total phenols determination

For total phenol determination with FC method we had to preclean the samples with solid phase extraction, using Strata-X cartridges according to Mozetič et al. (2006). According to that procedure columns were (pre)conditioned by sequentially passing drop wise 5 ml each of methanol and dd-water using a SPE vacuum manifold. Three millilitres (or 1.5 ml in the case of skin extract) of centrifugated (4000 rpm, 10 min) and membrane filtered (0.45 μm) grape juice or skin extract passed through the cartridge to adsorb phenolic compounds. Each cartridge was washed two-times with 1 ml of dd-water. The adsorbed fraction was than eluted with 5 ml of methanol and collected in a conical 15 ml centrifuge tubes. All samples were prepared in triplicate (three successive extractions of each sample on the same cartridge). Total phenols were determined only in 2009.

Methanolic extract was than quantitatively transferred (each centrifuge tube was washed three times with dd-H_2O) into 100 ml volumetric flask and total phenols were determined according to the colorimetric reaction method of Folin–Ciocalteau (Singleton and Rossi, 1965).

To each flask were added: approximately 60 ml of dd-H_2O and mixed; 5 ml of Folin–Ciocalteau reagent, mixed and after 30 s to 8 min also 15 ml of 20% sodium carbonate solution– an alkaline solution which allow to phenols to react rapidly with phenol-oxidizing reagent (Singleton and Rossi, 1965), and at the end completed with dd-H_2O to 100 ml. A blank sample was prepared at the same procedure with no sample passed through the cartridge for each series of measurements.

The solutions were allowed to sit at room temperature for 90 min to develop a blue colour. Before spectrophotometrically measurements, all the sample solutions have been membrane filtered (0.45 μm) directly into Quartz cuvette (Hellma) (three times with the same sample solution), since a white precipitate was formed in some samples. Readings were taken at 765 nm.

Total phenolics were expressed as gallic acid equivalent (GAE) in g L^{-1}. Five-point calibration curve (from 50 to 500 mg L^{-1}) was prepared in triplicate in two ways (in order to calculate the extraction efficiency): 1) using the same procedure as described above for cartridge extraction of the grape juice; and 2) directly (standard was added directly into the flask without the extraction process). In both cases, 1 mL of each standard solution was analysed.

2.4.4.2 HPLC–DAD quantification of grape phenolic compounds

Aliquots of juice samples and skin extract were membrane filtered (0.45 μm) and injected directly onto the HPLC to determine to determine *trans*-caftaric acid (*trans*-CTA) and total HCAs content (sum of *trans*- CTA, *trans*-coutaric (CoTA) and *trans*-fertaric (FTA) acids) (Mozetič et al., 2006). HPLC system with an auto injector (20 μl injection volume) and a DAD UV–Vis detector recording at 280, 320 and 530 nm, was used to detect the phenolic compounds. The column was kept at 25 °C. A constant flow rate of 1 ml min^{-1} was used with two solvents: solvent A, 2.2% (v/v) aqueous formic acid; solvent B, methanol. The following linear gradient was used: in 24 min from 12% to 33% B, hold for 5 min at 100% B to wash the column and then return to the initial conditions to re-equilibrate for 10 min. The method was prepared

in accordance to already published HPLC-DAD method of HCAs in grape juice and skin extracts (Mozetič et al., 2006). However some modifications were needed in solvent gradient due to change of the column used. The identification of compounds was achieved by comparing retention times and their UV–Vis spectra at 320 nm with our database (Mozetič et al., 2006) as well as by the addition of an external standard (*trans*-caftaric acid), which was used also for the quantification of total HCAs content in the years 2009 and 2011.

Calibration curves (were performed in duplicate in three different media in concentrations ranging between 4.8 mg $L^{-1}$and 363.6 mg L^{-1}: 1) water; 2) grape juice matrix (20% glucose solution, 1 g of potassium metabisulfite and 2 g of ascorbic acid per 50 ml of solution); and 3) natural grape juice to see possible impact of background on phenol determination. Two blank samples (*i.e.* a sample with no added caftaric acid) have also been prepared for each media and analysed with the proposed HPLC-DAD method. Limit of detection (LOD) and limit of quantification (LOQ) were determined (Skoog et al., 2004). All analyses were performed twice. One sample of natural grape juice has been run six–times to calculate the measurements repeatability of retention times and peak areas of monitored compounds.

2.4.5 Soil sampling and characterisation

Soil was sampled over the entire depth of the pot (0–25 cm) using the stainless steel auger (1–2 samples per pot; three replicates of the same treatment represented one sample); 1) before the experiment set up (in 2007) and 2) during the experiment (2009-2011) (Table 11). The soil samples were (dispersed on a sheet of paper) and air-dried (1 week), ground to pass through a 2–mm stainless sieve, homogenised, and stored at room temperature in polypropylene flasks until use.

Soil organic matter (OM) and cation exchange capacity (CEC), electrical conductivity (EC), total content of $CaCO_3$ and soil texture (sand, silt and clay content) were determined by the reference laboratory YARA Italia S.p.a. (Milan, Italy) in 2007 and in 2011. Soil pH was measured in deionised water, 0.01 mol L^{-1}

$CaCl_2$, and 1 mol L^{-1} KCl using a 1:5 (w/v) of soil: solution ratio (Benton Jones, 2000; Sinskey, 2009). Redox potential was measured *in situ* in 2011 (two pots per one treatment replicate) and in the laboratory in a homogenised soil paste prepared by mixing approximately 5 g of soil with deionised water (Patrick et al., 1996).

2.4.5.1 Total K, Mg, Fe and Zn contents in soil samples

A soil subsamples (Table 11) were oven-dried at 105 °C to constant weight (2–3 days) and grind (28 Hz s^{-1}, 4 min, using 2 balls) to receive a homogenised soil material. The soil samples (0.3 g) were left overnight in the PTFE beakers (covered with a lid) with 3 mL of HNO_3 and evaporated to small volume (0.5 mL) on a sand-bath (the first step was similar to that of leaf samples). In the second step 2.5 ml HNO_3, 2.5 ml $HClO_4$ and 5 ml HF were added and heated to perchlorate fumes. The samples were slowly heated for 3 hours on a sand-bath (T_{sand} 150 °C) to dryness. The residues were then re-dissolved in 3 ml of HCl solution (1:1, v/v), heated for 1 min on a sand-bath and diluted to the final volume of 50 ml with dd-water in PP centrifuge tubes. The procedure was already described by Ure (1995: 69) and the volumes of acids were adjusted to the quantity of soil sample used for our analysis. The mineral contents were then analysed by FAAS, which will be described in the following sections.

2.4.5.2 Extraction procedures – determination of plant available fraction

Soil extracts were obtained following the extracting conditions already described (see Table 12 for the references) with some modifications (*e.g.* soil:solution ratio, shaking time and shaker type). Soil samples were weighted in the 250 mL polypropylene bottles followed by extractant additions (w/v soil:solution ratios are given in Table 12). Extractions were accelerated by shaking soil mixtures in an horizontal shaker at 200 rpm for 2 hours (or for 16 hours when acetic acid was used) at 22 ± 2 °C. According to Quevauviller et al. (1997) the standard extraction protocol with *e.g.* EDTA and acetic acid stipulates a speed of 30 ± 10 rpm (for 1 and 16 hours,

respectively) using an end-over-end shaker. Since, this shaker was not available for our experiment we performed the extractions on a horizontal one, by elevating the extraction speed in all cases.

Table 11: *Date and characteristics of soil sampling and analysis performed during the pot experiment in our laboratory and by YARA Italia S.p.a. (Milan, Italy).*

Year	*Soil sampling*	*Analysis*
2007	*May 14 (0–25 cm)*	◙ *one united sample of all pots*
	before the experiment set up	● *one united sample of all pots*
2009	*May 18 (0–25 cm)*	● *s# 1, 2, 10*
	before application of Mg, Fe, or Zn	◘ *s# 1, 2, 7, 8, 9,10*
		■ *s# 1, 2, 10*
	June 17 (0–25 cm	◘ *s# 1, 2, 4, 7, 8, 9,10*
	at berry set	■ *s# 1, 2, 10*
2010	*June 19 (0–25 cm)*	◘ *s# 1, 2, 4, 7,10*
	in the middle of berry set and veraison	
	October 19 (0–25 cm)	● *s# 1, 2, 10*
	after harvest	◘ *s# 1, 2, 4, 7,10*
		■ *s# 1, 2, 10*
		◙ *s# 1 and 2*
2011	*November 5 (0–25 cm)*	● *s# 1, 2, 10*
	at the end of the experiment	◘ *s# 1-10*
		■ *s# 1, 2, 10*

Legend:

◙ Complete soil analysis performed by YARA Italia S.p.a.: OM, CEC, EC, pH_{H2O}, $CaCO_3$ total, N total; extractable P, K, S, Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn; soil texture (sand, silt and clay content).

● pH_{H2O}, pH_{KCl}, and pH_{CaCl2} and redox potential (*Eh*); ◘ Extractable K, Mg, Fe, and Zn; ■ Total K, Mg, Fe, and Zn content

Table 12: *Extractants used in our experiment with corresponding references in the literature to predict the phytoavailability of K, Mg, Fe, and Zn in 'Rebula' grapevines.*

Extractant	*Ratio (w/v), time* [a]	*Reference*	*Method modification*
Chelating agents			
0.05 mol L^{-1} EDTA [b]	*5:50, 2 h*	*Quevauviller et al. (1997)*	*Speed, time (20-40 rpm, 1h)*
0.005 mol L^{-1} DTPA/TEA [c]	*10:20, 2 h*	*Lindsay and Norvell (1978)*	*Speed (20-40 rpm)*
Mild acids			
0.43 mol L^{-1} CH_3COOH [d]	*1:40, 16 h*	*Quevauviller et al. (1997)*	*Speed (20-40 rpm)*
Un-buffered salt solutions			
0.01 mol L^{-1} $CaCl_2$ [d]	*5:50, 2 h*	*Pueyo et al. (2004)*	*Speed (20-40 rpm)*
1 mol L^{-1} NH_4NO_3 [d]	*10:25, 2h 5:25* [e]*, 2h*	*Pueyo et al. (2004)*	*Speed and ratio (50-60 rpm, 10:25)*
Buffered salt solutions			
1 mol L^{-1} CH_3COONH_4 [f]	*5:50, 2h*	*Helmke and Sparks (1996: 559)*	*Time and mode (25 ml of extractant shaking for 30 min in a centrifuge tube, repeated two times)*
Other			
dd-H2O	*5:50, 2h*	*Benton Jones (1999: 109)*	*Ratio and time (5:25, 30 min)*

[a] *Soil:solution ratio (w/v); duration at 200 rpm.*

[b] *EDTA was prepared by dissolving 0.5 M EDTA solution (pH 8.0) (100 mL per 1 L of dd-H_2O) and adjusted to pH 7.0.*

[c] *DTPA extracting solution consisted of 0.005 mol L^{-1} DTPA, 0.01 mol L^{-1} $CaCl_2$, and 0.1 mol L^{-1} TEA (1.967, 1.109, and 14.92 g per 1L, respectively) buffered at pH 7.30.*

[d] *The extracting solutions were prepared by adding 25 mL, 1.109 g, and 80.04 g of CH_3COOH, $CaCl_2$, and NH_4NO_3, respectively into 1 L of dd-H_2O.*

[e] *Soil:solution (w/v) ratio was modified according to Yara Italia S.p.a. protocol.*

[f] *Ammonium acetate was prepared by mixing 70 mL of NH_4OH and 57 mL of acetic acid per 1 L and the pH was adjusted to pH 7 with diluted NH_4OH or acetic acid.*

Two to three replicates were performed for each sample (Table 11) and each procedure. Blanks (reagent blank, procedural blank) were measured in parallel for all extracting reagents. K, Mg, Fe, and Zn were determined in the extracts following the determination procedures described in the next section. Before metal analysis the extracts were shaken to re-homogenize the content.

The moisture content of soil samples was determined in ten independent aliquots by drying 1 g and 5 g of pot soil samples (different treatments) in an oven (105 °C) until constant weight (approximately 3 hours) was attained. From this, a correction to dry mass was obtained which was applied to all analytical concentrations reported.

2.4.6 Determination of K, Mg, Fe, and Zn in leaf, grape, and soil samples by FAAS

FAAS measurements of leaf, grape and soil samples were carried out on diluted (especially in case of K and Mg) or non-diluted samples of wet digestion procedure. When needed (to match the calibration range for each element) we diluted them only with dd-H_2O and no HNO_3 was additionally added, since they were analysed on the day of preparation. Soil extracts analysis was performed within a few days to prevent adsorption of the analites to centrifugal tubes polyethylene and growth of bacteria (Pueyo et al., 2004). However, the samples exposed to wet digestion were very stable (even after one year) and so all analyses were done at the end of one set of samples (*e.g.* petioles at beery set in 2009).

2.4.6.1 Method optimisation

The method was tested to assess for sensitivity, LOD and LOQ.

During the measurements, the instrument was washed with dd-H_2O. Since a long series of samples were analysed, calibration on every 10–20 samples was performed in order to control the sensitivity of the instrument.

As dilution of samples was a regular sample preparation step, we have calculated the error of dilution as well. This error was calculated by Mg and K analysis of many samples, which were diluted (v/v) three times in the ratio of 1:100 (most used dilution in our analysis): 1) by mixing 0.5 mL of sample in 50 mL of H_2O or 2) by mixing 0.1 mL of sample in 10 mL of H_2O. All dilutions were performed using adjustable micropipettes. Each dilution was measured three times for each element by FAAS.

Doubly deionised water acidified to 2% (v/v) with suprapure HNO_3 was daily prepared and used as a blank. By measuring the blank, LOD (1) and LOQ (2) were calculated using the following equations:

$$LOD = 3s_b k^{-1} \quad (1)$$

$$LOQ = 10s_b k^{-1} \quad (2)$$

where k is the calibration sensitivity and s_b is the blank standard deviation (Skoog et al., 2004).

For each batch of mineralization or soil extraction procedure, a procedural blank (*i.e.* a vessel with no leaves, grapes or soil) was carried through the complete procedure in order to control the contamination of the whole procedure (reagents and material used). Additionally, a reagent blank was prepared (*i.e.* an aliquot of each extractant) and analysed for K, Mg, Fe and Zn content.

2.4.6.2 Quality control and quality assessment measures

Leaf analysis

Leaf mineralization procedure was confirmed using four plant SRMs (1572–citrus, 1547–peach, 1573 and 1573a–tomato leaves) exposed to the same method as described in sections 2.4.1.2. For each extraction, 0.25 g of sample and 2.5 mL of HNO_3 was used. Recovery (%; n > 4) values have been calculated according to the

certified (recommended) values by the producer (NIST). Precision of the whole procedure (wet digestion, sample dilution and FAAS measurements) was calculated as well.

To compare the impact of HF addition on elements recovery, two SRMs (1572 and 1573a, n = 3) were digested in two steps, starting with the same procedure (HNO_3/H_2O_2 digestion) as described above. After nitric acid evaporation and cooling, 1.5 ml of HNO_3 and 2.5 ml of HF were added (second step) and the samples were slowly heated for 2 hours on a sand-bath (T_{sand} 130–150 °C) to dryness (Hoenig et al., 1998). The residues were then re-dissolved as previously described.

In addition to SRMs analysis, the accuracy was checked with recovery assays by addition of known amounts of K, Mg, Fe, and Zn AAS standard solutions to petiole (0.3 g) and blade (0.5 g) samples (n = 4) prior to the digestion step (Kristl et al., 2003; Skoog et al., 2004). The final concentrations of added K, Mg, Fe, and Zn after the whole wet digestion procedure were 100.0; 20.0; 1.0; and 0.15 mg L^{-1} of the final extract (25 ml), respectively. Simultaneously, samples with no standard additions were also subjected to the same wet digestion and FAAS measurements.

Grape analysis

Since the SRMs for grape are not available, the accuracy was checked only with recovery assays by addition of known amounts of K, Mg, Fe, and Zn AAS standard solutions to grape (0.5 g) samples (n = 4) as described before for leaf samples. The final concentrations of added K, Mg, Fe, and Zn after the whole wet digestion procedure were the same as for leaves for Mg, Fe and Zn, while the K content in the final extract was 50.0 mg L^{-1}.

Soil analysis

Total metal content in soil samples was confirmed by the means of three soil SRMs (2709–San Joaquin, 2710–Montana I, and 271–Montana II soil) exposed to the same acid mixture procedure as described in section 2.4.5.1. Sample quantity, recovery assay and precision was the same as described above for leaf SRMs.

A sewage sludge amended soil CRM 483 was used for quality control purposes of EDTA and acetic acid-extractable Zn using the same procedure as described in Table 12. Same extraction procedures (*e.g.* EDTA-extracted Fe, Zn and NH_4NO_3-extracted K, Mg) were evaluated by comparing our results with the results obtained by one certified reference laboratory (YARA Italia S.p.a., Italy). Two soil samples sampled in 2011: #1-Control and 2#-NPK soil, were analysed in both laboratories and the obtained values were compared.

2.4.7 Comparison of potted grapevines with the ones grown in the vineyards

We selected four vineyards planted with 'Rebula' (*Vitis vinifera* L.) grapevines located nearby the pot experiment in Goriška Brda in order to compare the results obtained in the pot experiment with the grapevines grown in 'natural' environment. The description (rootstock, year of planting and other characteristics) of the vineyards are presented in Table 13.

Table 13: *Description of four selected 'Rebula' vineyards located nearby the pot experiment.*

Vineyard	*Rootstock*	*Year of planting*	*Landform (vine spacing)*	*Vineyard management*
A	*1103 Paulsen*	*1997*	*Terraces (0.8 m)*	*Integrated*
B	*SO4*	*2004*	*Plateaus (0.8 m)*	*Integrated*
C	*1103 Paulsen*	*1990*	*Terraces (1.0 m)*	*Organic*
D	*SO4*	*2009*	*Plateaus (0.8 m)*	*Organic*

Vineyard B represented grapevines of the same cultivar and rootstock planted in the vineyard soil which we have used for the pot experiment. All four vineyards were grass-grown following a sustainable agricultural practise (vineyards A and B are involved in the integrated vineyard management, a system that favours the use of natural alternatives to fertilize and to prevent and control pests and diseases (Rules on integrated ..., 2002); vineyards C and D are following the directives of organic farming, which relies on a number of objectives and principles, as well as common

practices designed to minimise the human impact on the environment, while ensuring the agricultural system operates as naturally as possible (Organic farming, 2013). Sampling data (date and sample material) and analysis performed (in triplicate) are presented in Table 14.

Table 14: *Sampling and analysis performed in four selected vineyards.*

Sample	*Sampling (Vineyard)* [a]	*Analysis (method description; section)*
Leaves (50 leaves per vineyard)	*Berry set and veraison: 2009 (A, B) 2011 (A, B, C, D)*	*K, Mg, Fe and Zn content determination (see 2.4.1.2)*
Grapes (10 clusters per vineyard)	*Harvest: 2009 (A, B) 2011 (A, B, C, D)*	*K, Mg, Fe and Zn content determination (see 2.4.1.2); Grape quality analysis (see 2.4.3); HCAs determination (see 2.4.4.2)*
Soil (20 cores per vineyard (0-30 cm; W-sheme)	*September 2011 (A, B, C, D)*	*Soil pH and redox potential (see 2.4.5); Total K, Mg, Fe, and Zn content (see 2.4.5.1); Extractable K, Mg, Fe, and Zn (see 2.4.5.2)*

[a] *Vineyard notification is refereeing to Table 13.*

2.5 STATISTICAL ANALYSIS

Data analysis was conducted by STATGRAPHICS Centurion XVI software package (Statpoint Technologies, Warrenton, Virginia, U.S.). General linear model (GLM) was applied in order to ascertain the significance of nutrient treatment (fixed factor), growing season (random factor) and their interaction on the element content in petioles and grape berries. Means were separated according to Student-Newman-Keuls´s test ($P < 0.05$). The relationships between elements themselves (K, Mg, Fe,

and Zn) and between elements (in leaves and grapes) *vs*. grape quality paramenters/HCAs/soil extractions were analysed by simple correlation analysis (determination of the Pearson`s correlation coefficient).

Principal component analysis (PCA) with varimax rotation (selected on eigenvalue 1 criterion) conducted in order to see the intrinsic variation in the data set (micro- and macro-elements and grape quality parameters). With PCA, the data arranged in tables can be reduced to a set of new latent variables called principal components (PCs). The loadings of the PC define the direction of the greatest variability and the score values represent the projection of each object onto PC. The first PC is the linear combination of the original variables, which explains the greatest variability. The second PC has been defined to be orthogonal to the first one and explain the second greatest amount of variability. The analysis proceeds until all PCs are obtained, the number of which is typically much smaller than the variables (Liu et al., 2006).

3 RESULTS AND DISCUSSION

3.1 CONCENTRATION OF K, Mg, Fe, AND Zn IN PLANT TISSUE

3.1.1 FAAS method optimisation

The method was tested to assess for sensitivity, LOD and LOQ. The working conditions for K, Mg, Fe, and Zn are summarized in Table 15.

Table 15: *Working conditions in FAAS for K, Mg, Fe, and Zn measurements.*

Parameter	*K*	*Mg*	*Fe*	*Zn*
Optimum working range (mg L^{-1})	*0.25–4.0*	*0.1–0.4*	*0.25–5.0*	*0.05–0.6*
Sensitivity (L mg^{-1}) [a]	*0.39*	*1.42*	*0.10*	*0.86*
LOD (mg L^{-1})	*0.006*	*0.003*	*0.038*	*0.002*
LOQ (mg L^{-1})	*0.022*	*0.011*	*0.127*	*0.008*
Dilution factor (DF):				
Leaves and grape	*100-200*	*50-100*	*1*	*1-2*
Soil (total)	*100*	*100*	*50*	*1-2*
Soil extracts EDTA	*20-50*	*50*	*3*	*3*
DTPA	*10-20*	*20*	*3*	*1*
CH3COOH	*10-20*	*100*	*2*	*1*
$CaCl_2$	*10-20*	*20*	*1*	*1*
NH_4NO_3	*50-100*	*100*	*1*	*1*
CH_3COONH_4	*20*	*20*	*1*	*1*
H2O	*10-20*	*3*	*2*	*1*

[a] *Mean value (n=10).*

The coefficient of variations (CV) of instrument measurements of all four elements were up to 2% (< 1 on the average) or in the cases of very low absorbance (*e.g.* Fe in petioles or Zn in soil extracts) up to 10% (on the average 5%) and were determined

by measuring the same sample for each element for at least three times (with three replicates each) with FAAS. Once the measurement uncertainty has been reduced to one third or less of the sampling uncertainty (that is, $s_m < s_s\ 3^{-1}$; where s_m is standard deviation of the method and s_s standard deviation of the sampling process), further improvement in the measurement uncertainty is fruitless (Skoog et al., 2004).

As dilution of samples was a regular sample preparation step (Table 15), we have calculated the error of dilution as well. The calculated CV of dilutions in the ratio 1:100 (0.5/50 ml *vs*. 0.1/10 ml of sample/water; most used dilution) was < 1.5% for K and Mg. Since there were no differences in CVs and absorbance values, the preparation of dilution 0.1 ml/10 ml seemed to be easier and faster to handle and therefore used in all measurements where needed.

The analysis of blank samples (wet digestion) did not revealed any contamination of the samples during the whole extraction procedure and measurements, since the measured concentrations of K, Mg, Fe, and Zn were very low (in majority of cases were under the LOQ) for HNO_3/H_2O_2 and $HNO_3/H_2O_2/HClO_4/HF$ digestion procedures (all used chemicals were of suprapure grade quality, except the perchloric acid was p.a.). The blank correction was not needed even in the case of Mg and Fe (for soil samples), since the soil samples were diluted in the ratio 1:100 and the blank correction represented less than 0.1% mistake.

In the contrast, the blanks of extraction procedures were taken into account in calculation of K, Mg, and Zn (not for Fe) soil extracts concentrations. The reagent blanks differed from the procedural ones, which meant that the extracts contamination occurred during the extraction procedure. The filtration through a filter paper seemed to be responsible (along with the reagent itself) for the high element content in blank samples (we analysed the soil extracts before and after filtration). Rinsing the filter paper with dd-H_2O and discarding the first 10 mL of filtrate were important steps to minimise the contamination of the soil extracts.

3.1.2 Quality control and quality assessment measures

3.1.2.1 Leaf analysis

Leaf mineralization procedure was confirmed using four plant SRMs (1572–citrus, 1547–peach, 1573 and 1573a–tomato leaves). The quantification results have shown very good agreement with all certified values for K, Mg and Zn (Table 16); except for Fe, which was in 51 and 60% recovered from SRMs 1573 and 1573a (tomato leaves). Fortunately, our wet digestion method gave good results of Fe concentrations in citrus (SRM 1572) and peach leaves (SRM 1547) (88 and 82% recovery, respectively), since those SMRs are the most comparable to grapevine leaves in the concentrations ranges of studied elements. The precision (%) obtained with plant SRMs was satisfactory with values up to 10%.

In agreement with Hoenig et al. (1998) the recovery of Fe depends on particular plant matrix, probably in relation to the binding of analite with the insoluble residue, which remained after wet digestion procedure. Hydrofluoric acid (HF) attack on less soluble inorganic compounds of the sample, followed by evaporation to dryness is known to volatize silicon and to release the associated elements (*e.g.* Fe). However, in our case, the Fe extraction yield was improved on the average for only 4-8% in the case of SRMs 1572 and 1573a. In the contrast, HF complementation did not have any influence on K and Zn content in these samples measured by FAAS, and Mg content was even lowered compared to non-HF treated samples of SRMs. Since the enhancement of Fe extraction was not higher than an error of extraction from leaf samples (determined to be up to 10%), we decided not to include the HF step in our procedure. Moreover, the additional step is time consuming (additional 2 hours), increases the costs of analysis and HF is known as very harmful acid as well.

In addition to good recoveries of studied elements from CRMs (except Fe in tomato leaves), the recoveries of grapevine blades and petioles ‘spiked’ with known amounts of the standard elements (K, Mg, Fe, and Zn) and subjected to the whole wet

digestion procedure and FAAS measurements were close to 100% for all four studied elements (Table 17).

Table 16: *Results for the sand-bath HNO_3/H_2O_2 digestion using various plant SRMs.*

Reference material	*Found value* [a]	*Recommended value* [a]	*% Recovery*
	Potassium *(g kg^{-1})*		
SRM 1572	*17.7 ± 0.4*	*18.2 ± 0.6*	*97 ± 2*
SRM 1573	*41.0 ± 1.6*	*44.6 ± 0.3*	*92 ± 4*
SRM 1573a	*26.0 ± 2.0*	*27.0 ± 0.5*	*95 ± 8*
	Magnesium *(g kg^{-1})*		
SRM 1572	*5.30 ± 0.17*	*5.70 ± 0.40*	*93 ± 3*
SRM 1547	*4.30 ± 0.07*	*4.32 ± 0.09*	*100 ± 2*
SRM 1573	*6.20 ± 0.40*	*7.00* [b]	*87± 5*
SRM 1573a	*9.70 ± 1.20*	*12.0* [b]	*81 ± 10*
	Iron *(mg kg^{-1})*		
SRM 1572	*79 ± 9*	*90 ± 10*	*88 ± 9*
SRM 1547	*180 ± 20*	*218 ± 13*	*82 ± 9*
SRM 1573	*353 ± 8*	*690 ± 25*	*51 ± 1*
SRM 1573a	*229 ± 19*	*368 ± 7*	*62 ± 5*
	Zinc *(mg kg^{-1})*		
SRM 1572	*29 ± 2*	*29 ± 2*	*100 ± 5*
SRM 1547	*18.4 ± 0.8*	*17.9 ± 0.4*	*103 ± 4*
SRM 1573	*58 ± 3*	*62 ± 6*	*93 ± 5*
SRM 1573a	*29 ± 1*	*30.9 ± 0.6*	*95 ± 5*

[a] *Mean ± standard deviation (n = 4-9 in case of found value).*

[b] *Non-certified value by producer.*

3.1.2.2 Grape analysis

Since the SRMs for grape are not available on the market was the accuracy checked only with recovery assays by addition of known amounts of K, Mg, Fe, and Zn AAS

standard solutions to grape samples. The recoveries were close to 100% for all four studied elements (Table 17).

Table 17: *Results for the sand-bath HNO_3/H_2O_2 digestion using standard addition method.*

	Recovery (%) [a]			
Sample	*K*	*Mg*	*Fe*	*Zn*
Blades	*99 ± 8*	*102 ± 3*	*91 ± 4*	*99 ± 5*
Petioles	*100 ± 9*	*99 ± 8*	*101 ± 3*	*94 ± 3*
Grape berries	*108 ± 10*	*97 ± 1*	*98 ± 4*	*96 ± 2*

[a] *Mean ± standard deviation (n = 4).*

In addition to grape berries analysis, we checked if removal of pedicels is a necessary step before mineralization procedure. The element contents were equal or very similar for K, Mg, and Fe; however Zn concentration was for 33% higher in berries with pedicels in comparison to berries without them (Table 18). Due to the results obtained we decided to remove the pedicels after oven-drying for the element content assessment.

Table 18: *Comparison of K, Mg, Fe, and Zn contents in berry samples with or without pedicels.*

Berry sample	*K (g kg^{-1})*	*Mg (g kg^{-1})*	*Fe (mg kg^{-1})*	*Zn (mg kg^{-1})*
With pedicels	*19.5 ± 0.2*	*0.481 ± 0.015*	*11.0 ± 0.4*	*6.6 ± 0.2*
Without pedicels	*18.9 ± 0.3*	*0.481 ± 0.032*	*11.5 ± 0.2*	*4.4 ± 0.1*

3.1.2.3 Soil analysis - total element determination

Total metal content in soil samples was confirmed by the means of three soil SRMs (2709–San Joaquin, 2710–Montana I, and 271–Montana II soil) exposed to $HNO_3/H_2O_2/HClO_4/HF$ acid mixture procedure. The results obtained for soil standard reference materials revealed that the whole procedure was satisfactory in terms of accuracy and repeatability. For all three SRMs (San Joaquine, Montana I,

and Montana II soil) the recovery of K, Mg, Fe, and Zn was close to 100% and very good precision (in most cases up to 2%) was obtained (Table 19).

3.1.2.4 Soil analysis – determination of soil fractions

Certified reference material (BCR 483) was used for quality control purposes of EDTA and acetic acid-extraction procedure (on a horizontal shaker operating at 200 rpm). Unfortunately, the certified values were among the studied elements given for Zn, only. However, the results of our laboratory were comparable (100% recovery) with the certified values and the good precision (approximately 5%) was obtained (Table 20).

Table 19: *K, Mg, Fe and Zn results for the sand-bath $HNO_3/H_2O_2/HClO_4/HF$ digestion using three soil SRMs.*

Reference material	*Found value* [a]	*Recommended value* [a]	*% Recovery*
	Potassium *(g kg^{-1})*		
SRM 2709	*21.3 ± 0.3*	*20.3 ± 0.6*	*102 ± 1*
SRM 2710	*22.9 ± 0.1*	*21.1 ± 1.1*	*104 ± 1*
SRM 2711	*26.3 ± 0.1*	*24.5 ± 0.7*	*104 ± 0.3*
	Magnesium *(g kg^{-1})*		
SRM 2709	*14.5 ± 0.2*	*15.1 ± 0.5*	*96 ± 1*
SRM 2710	*8.36 ± 0.19*	*8.53 ± 0.43*	*98 ± 2*
SRM 2711	*10.1 ± 0.6*	*10.5 ± 0.3*	*96 ± 6*
	Iron *(g kg^{-1})*		
SRM 2709	*33.7 ± 0.2*	*35.0 ± 1.1*	*96 ± 1*
SRM 2710	*32.6 ± 0.6*	*33.8 ± 1.0*	*97 ± 2*
SRM 2711	*28.0 ± 0.1*	*28.9 ± 0.6*	*97 ± 0.2*
	Zinc *(mg kg^{-1})*		
SRM 2709	*108 ± 0.8*	*106 ± 3.2*	*102 ± 1*
SRM 2710	*6724 ± 141*	*6952 ± 70*	*97 ± 2*
SRM 2711	*356 ± 8.2*	*350 ± 3.5*	*102 ± 2*

[a] *Mean ± standard deviation (n = 4 in case of found value).*

In addition, the EDTA-extracted Fe and Zn, and NH_4NO_3-extracted K and Mg were evaluated by comparing results obtained in our laboratory with the values obtained in one accreditated laboratory - Yara Italia S.p.a., Italy (Table 21). The results obtained for NH_4NO_3-extracted K and Mg, and EDTA-extractable Fe were very similar to those obtained by Yara laboratory (> 90%; not including K measured in the control soil sample where it was a bit lower). On the other hand EDTA-extractable Zn was more than two times lower comparing to the results obtained in Yara laboratory. However, 100% recovery was found using certified reference material (BCR 483; Table 20).

Table 20: *Certified and determined EDTA and CH_3COOH extractable concentrations for Zn in BCR 483 certified reference material.*

Extractant	*Determined value* [a]	*Certified value* [b]	*% Recovery*
	Zn *(mg kg^{-1})* [c]		
0.05 mol L^{-1} EDTA	*612 ± 25*	*612 ± 20*	*100 ± 4*
0.43 mol L^{-1} acetic acid	*628 ± 32*	*620 ± 24*	*101 ± 5*

[a] *Horizontal shaker operating at 200 rpm for 2 hours (EDTA) or for 16 hours (acetic acid) in a room at 22 ± 2 °C 2.*

[b] *End-over-end shaker operating at 30 ± 10 rpm for 1 hour (EDTA) or for 16 hours (acetic acid) in a room at 20 °C (Quevauviller et al., 1997).*

[c] *Mean ± standard deviation (n = 3 in case of determined value).*

Table 21: *EDTA-extracted Fe and Zn, and NH_4NO_3-extracted K and Mg as compared with reference laboratory Yara Italia S.p.a. (Milan, Italy).*

	Soil sample	*#1-Control*		*#2-NPK*	
Extractant	*Element*	*Results* [a] *(mg kg^{-1})*	*Yara lab* [b] *(mg kg^{-1})*	*Results (mg kg^{-1})*	*Yara lab (mg kg^{-1})*
NH_4NO_3	*K*	*143 ± 1*	*182*	*190 ± 8*	*205*
NH_4NO_3	*Mg*	*58.9 ± 0.1*	*60*	*35.8 ± 0.2*	*33*
EDTA	*Fe*	*113 ± 1*	*123*	*128 ± 11*	*132*
EDTA	*Zn*	*3.35 ± 0.1*	*6.7*	*3.74 ± 0.1*	*9.9*

[a] *Mean value ± standard deviation (n = 3).*

[b] *Mean value..*

3.2 LEAVES AND GRAPES ANALYSIS

Leaf analysis is widely recognized as the most reliable way to ascertain the grapevine nutritional status, and during the growing season the best periods to sample leaves are berry set and veraison (Christensen, 1984; Fregoni, 1997; Sinskey, 2009). Grape berry mineral composition must be considered equally important, since nutrients play a fundamental role in fruit development and they are involved in wine chemical composition and quality (Etchebarne et al., 2009).

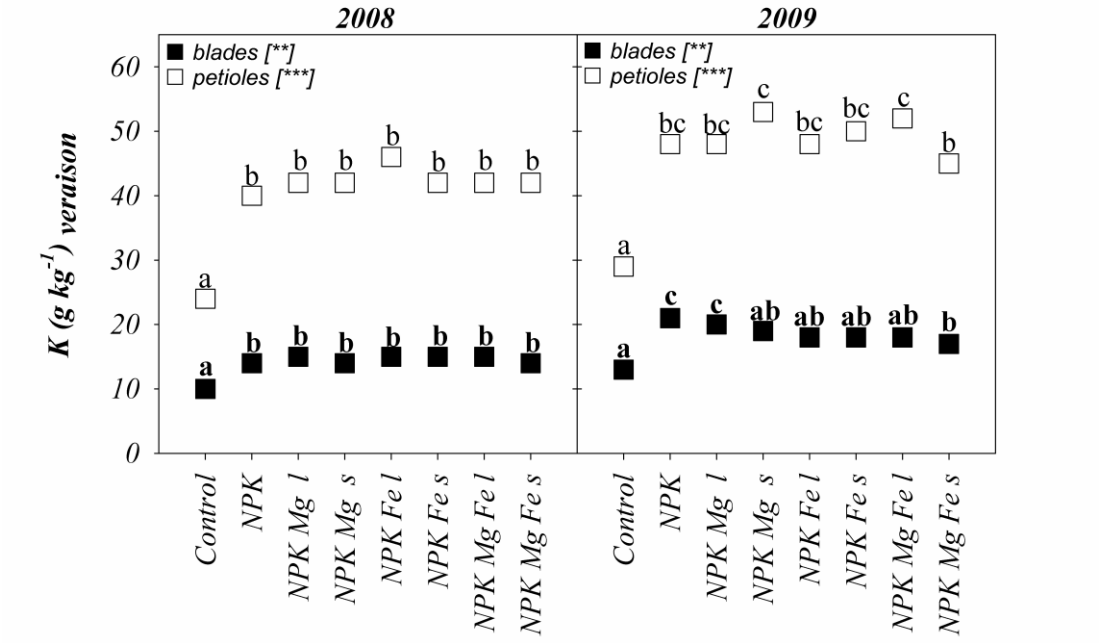

Figure 5: *Measured K contents (g kg^{-1}D.W.) in blades and petioles of differently treated grapevines at veraison 2008 and 2009. Within each graph, means (n = 3) followed by a different letter are significantly different at P < 0.05 using Duncan`s test. Asterisks indicate significance of ANOVA test (* P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant).*

In the first two years (2008 and 2009) we analysed mineral contents in both blades and petioles. From to the results obtained, statistical analysis and to the huge number of samples (cost and time consuming), we decided to determine the contents of the studied elements in the following years 2010 and 2011 in the petioles, only. Grape berries were analysed in the years from 2009 to 2011, since in 2008 the grapevines were only two-year old and they have no grape.

3.2.1 Comparison between blade and petiole analysis – preliminary analysis 2008 and 2009

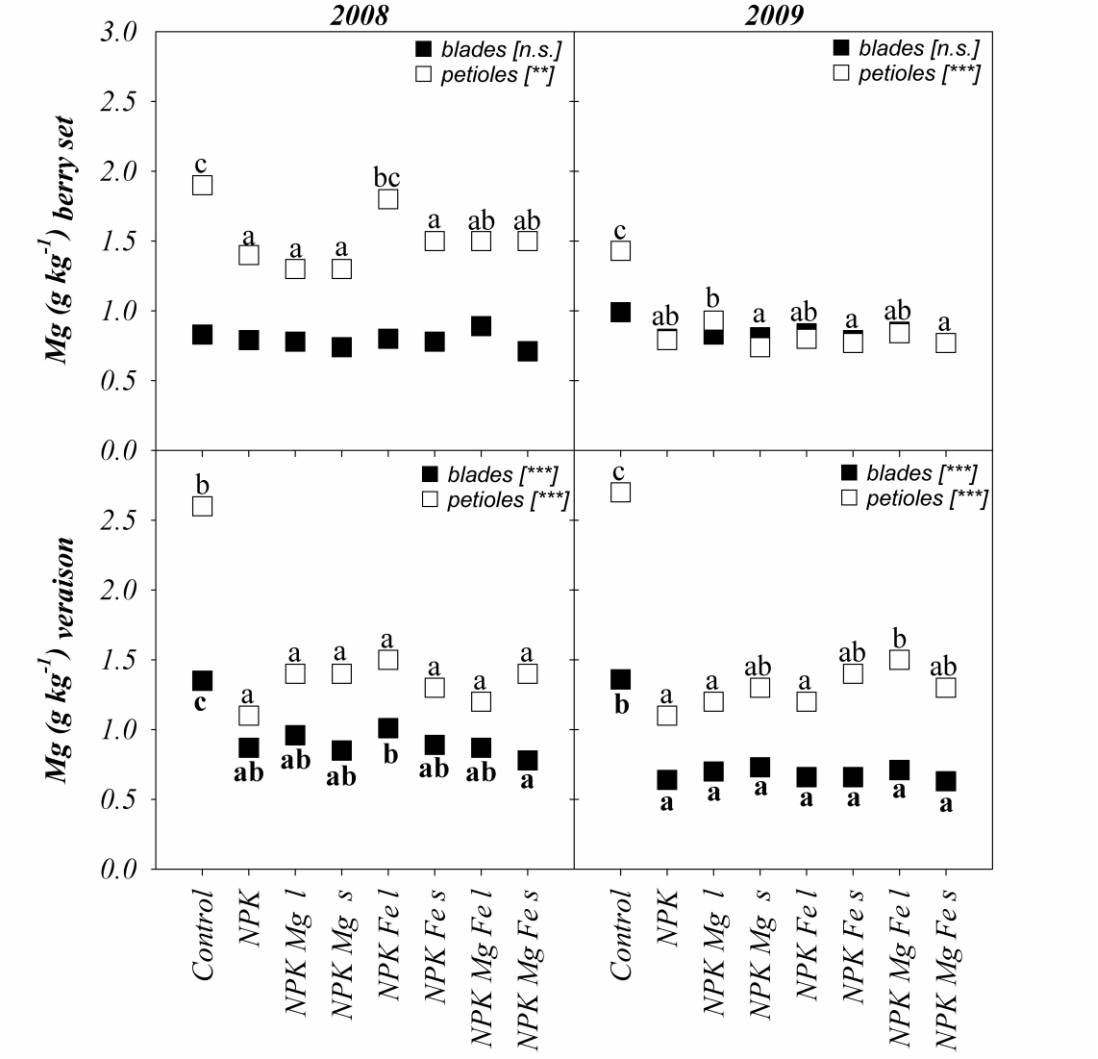

Figure 6: *Measured Mg contents (g kg^{-1}D.W.) in blades and petioles of differently treated grapevines at berry set and veraison 2008 and 2009. Within each graph, means (n = 3) followed by a different letter are significantly different at P < 0.05 using Duncan`s test. Asterisks indicate significance of ANOVA test (* P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant).*

As seen from Figures 5 and 6 pottasium and magnesium concentrations were higher in the petioles than in the blades at veraison as well as at fruit set in all treatments presented. Potassium contents were by 2.2-to-2.4 *vs*. 2.6-to-2.9–fold higher in the petioles than in the blades of control *vs*. NPK-treated vines in 2008 and 2009 at both

sampling times (data for berry set are not presented). A bit lower Mg contents (1.5-to-1.9-fold higher contents) were found in NPK-treated vines in 2008 (at berry set) and 2009 (at both phenophases), while in the control vines the differences between blades and petioles were similar as in the case of K (1.5-to-1.9-fold *vs*. 1.9-to-2.3-fold, respectively). However, at berry set 2009, the Mg contents were the same in blades and petioles of all NPK-treated vines, while in the control vines Mg contents were by 40% higher in the petioles than in the blades.

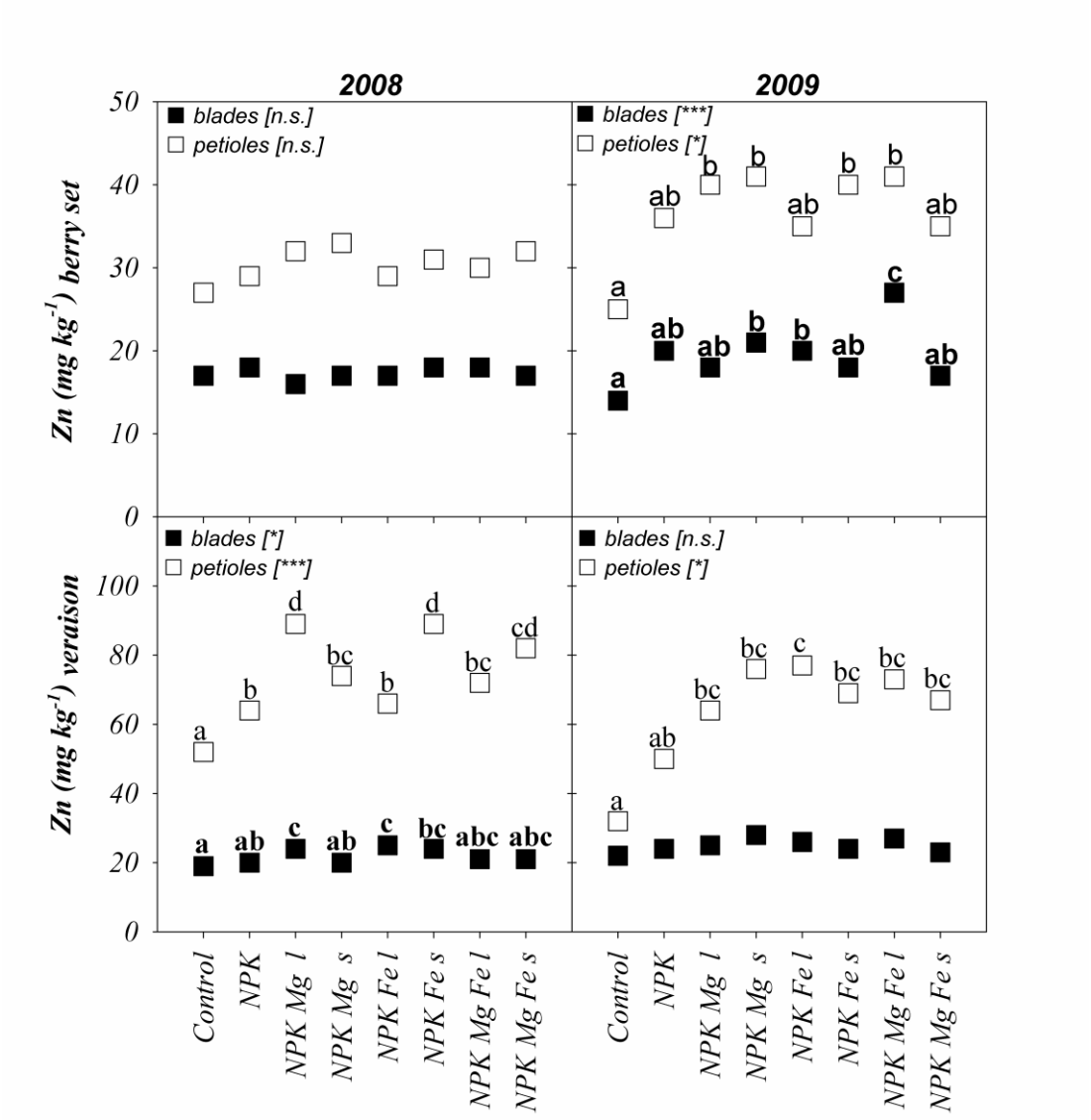

Figure 7: *Measured Zn contents (mg kg^{-1}D.W.) in blades and petioles of differently treated grapevines at berry set and veraison 2008 and 2009. Within each graph, means (n = 3) followed by a different letter are significantly different at P < 0.05 using Duncan`s test. Asterisks indicate significance of ANOVA test (* P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant).*

Moving on micro-elements, Zn contents were by 1.6-to1.9-fold higher in the petioles than in the blades of all experimental vines sampled at berry set in 2008 and 2009 (Figure 7). At veraison, the differences between blades and petioles were much higher especially in NPK-treated vines (by 3.5-fold (2008) and 2.7-fold (2009), while by 2.7-fold (2008) and 1.5-fold (2009) in the control vines). Higher K and Zn levels in petioles than in blades were in agreement with Christensen (1984) for many grapevine cultivars (as Barbera, Sauvignon blanc and others).

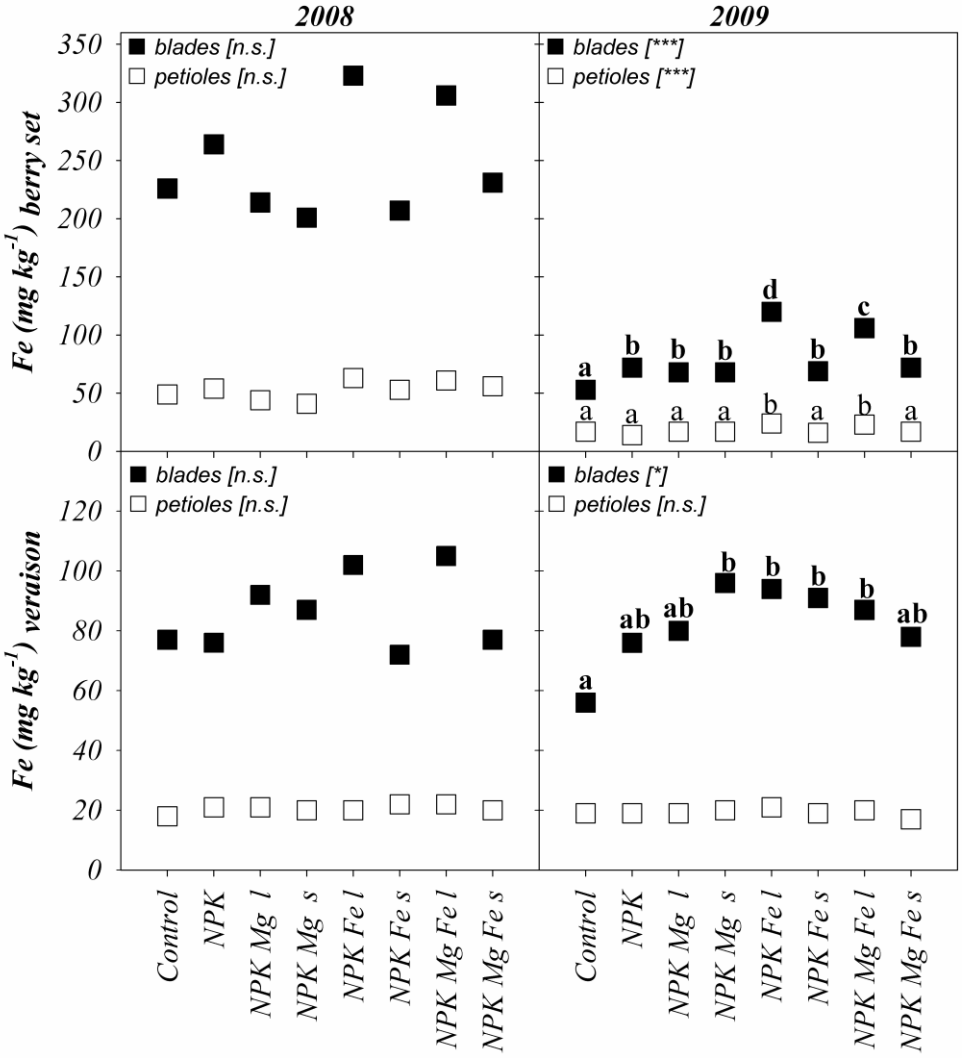

Figure 8: *Measured Fe contents (mg kg^{-1}D.W.) in blades and petioles of differently treated grapevines at berry set and veraison 2008 and 2009. Within each graph, means (n = 3) followed by a different letter are significantly different at P < 0.05 using Duncan`s test. Asterisks indicate significance of ANOVA test (* P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant).*

In the contrast to other three studied elements, Fe was the only element, with much higher concentration in leaf blades than in the petioles at both phenophases (Figure 8). The average difference was calculated to be 4.4–fold in NPK-treated vines in 2008 and 2009 (both phenophases) and in the control vines at berry set, while at veraison the difference between the blades and petioles (control vines) was approximately 3.0.

The statistically significant differences between the treatments were obtained in both blades and petioles; however the level of significance were for K, Mg and Zn higher in the petioles in comparison to the blades. In the contrast, Fe contents were very low in the petioles, but the significance between treatments was poor in both blades and petioles (except at berry set in 2009). Since the results (trends of the element contents) were similar in blades and petioles, we analysed in the years 2010 and 2011 petioles, only. Therefore, the results presented in the next sections referred on petiole analysis in the years 2009–2011.

3.2.2 Mineral content in grapevine as affected by fertilization treatment and year

The season-by-season effects on almost all elements studied (K, Mg, Fe, Zn) were highly significant (Table 22, 23). Fe in petioles at berry set was the only variable not influenced by the year. Similar results were found for treatment having significant effect on almost all parameters except on Fe content in whole grape berries. In addition for Mg (at berry set) and K and Fe contents in the petioles (at both phenophases) there was also a significant effect of the year x treatment interaction, suggesting that the effect of the fertilization treatment on these elements was different among seasons (2009–2011). In fact, for K, Mg, and Fe contents in the petioles there were some differences among years (Table 24). On the contrary, Zn content in grape berries and in the petioles at both sampling times seemed to be influenced by both year and fertilization treatment, without any interactive effect.

3.2.3 Potassium - Effect of soil NPK fertilization on K content in leaves and grapes

The average content of potassium in petioles of untreated vines was 27 g kg^{-1} in the years 2009–2011 regardless sampling times (Table 22). The amount of potassium was much higher in 2009 at both berry set and veraison. Looking in detail at interaction effects of season and treatment, in the year 2009 the concentration of potassium in the petioles of the treatments where K was added (#2–10) was 70% higher than in untreated, while the difference diminished in the following years (Table 24). Also in grape berries, the amount of potassium in grapes was significantly higher in 2009 as an average of all treatments, but the differences between untreated and NPK treated vines was more similar among seasons (no interaction effects), being the concentration of K 1.23–fold higher (as an overall average) in the grape berries of all treated vines.

The addition of potassium to the soil resulted in higher K concentration in both leaf petioles and grape berries, similarly to what was also reported by by Morris et al. (1987) and Poni et al. (2003). In any case, the statistical differences in K content in petioles between control and NPK treated vines (#2–10) steadily diminished in 2011 most probably as a consequence of lowered K availability (see Table 38) in the soil of NPK treated vines due to yearly removal for vine growth and grape harvesting (K fertilization was performed only in 2008). The content of K measured in petioles of control vines sampled at two phenophases was found within an optimal range (with mean values 24–30 g kg^{-1}), in agreement with what was reported by Fregoni (1997) and Sinskey (2009). The content of K in the petioles was enhanced in NPK treated vines (#2–10) reaching high values (> 35 g kg^{-1}; Fregoni, 1997) at both sampling times in 2009, while in the following season the values fitted within the optimal range, too. Water rate was the same for all the treatments, thus differences ascribed to water supply cannot be expected. Therefore, it is likely that greater K uptake in 2009 in NPK treated vines was due to higher soil K availability as a consequence of K fertilization.

3.2.4 Magnesium – effect of soil NPK and Mg fertilization on Mg content in leaves and grapes

Table 22: *Effects of fertilization treatments and season on K and Mg contents in petioles (sampled at berry set and at veraison) and whole grape berries.*

	K *(g kg^{-1} D.W.)*			***Mg*** *(g kg^{-1}D.W.)*		
Factor	*Petioles berry set*	*Petioles veraison*	*Grapes harvest*	*Petioles berry set*	*Petioles veraison*	*Grapes harvest*
Treatment						
1#Control	*27.5 a*	*27.0 a*	*12.9 a*	*1.40 c*	*2.39 b*	*0.60 b*
2#NPK	*36.8 ab*	*40.4 b*	*16.2 b*	*0.86 ab*	*1.14 a*	*0.49 a*
3#NPK Mg l	*35.3 ab*	*38.4 b*	*15.4 b*	*0.88 b*	*1.18 a*	*0.49 a*
4#NPK Fe l	*38.3 b*	*39.9 b*	*15.5 b*	*0.70 a*	*1.08 a*	*0.52 a*
5#NPK Mg Fe l	*34.3 ab*	*40.1 b*	*15.9 b*	*0.86 ab*	*1.24 a*	*0.52 a*
6#NPK Mg Fe hl	*44.6 b*	*45.3 b*	*15.8 b*	*0.76 ab*	*1.17 a*	*0.52 a*
7#NPK Mg s	*41.4 b*	*39.9 b*	*15.8 b*	*0.79 ab*	*1.22 a*	*0.51 a*
8#NPK Fe s	*39.0 b*	*39.6 b*	*15.7 b*	*0.72 ab*	*1.22 a*	*0.49 a*
9#NPK Mg Fe s	*35.9 ab*	*37.5 b*	*15.4 b*	*0.74 ab*	*1.18 a*	*0.49 a*
10#NPK Zn l	*35.8 ab*	*35.7 b*	*15.4 b*	*0.77 ab*	*1.15 a*	*0.50 a*
P-value	*	**	**	***	***	***
Year						
2009	*48.1 b*	*48.0 b*	*18.1 b*	*0.86 b*	*1.35 b*	*0.51 b*
2010	*30.0 a*	*35.0 a*	*14.0 a*	*0.64 a*	*1.11 a*	*0.54 c*
2011	*31.6 a*	*32.0 a*	*13.8 a*	*0.99 c*	*1.32 b*	*0.48 a*
P-value	***	***	***	***	***	***
Year x treatment						
P-value	*	**	*n.s.*	*	*n.s.*	*n.s.*

*Data were processed through GLM ANOVA (treatment, fixed factor; year, random factor): *P < 0.05; **P < 0.01; ***P < 0.001, n.s.: not significant. Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test. For treatment details refer to 2.1.3 Treatment layout: Table 8.*

The concentration of Mg was approximately 2–times higher in the leaf petioles of untreated (control) vines at both phenophases (Table 22, 24). At berry set, measured Mg concentrations in control vines was on the average 1.4 g kg^{-1} while in NPK

treated vines (#2–10) values varied from 0.70 to 0.88 g kg^{-1}. In the petioles of all experimental vines the concentration of Mg increased by approximately 1.6–fold from berry set to veraison. In grape berries Mg content was nearly 20% higher in the control vines than in K–added treatments in all three years. For grape berries no significant interaction effects between treatment and season were revealed.

Table 23: *Effects of fertilization treatments and season on Fe and Zn contents in petioles (sampled at berry set and at veraison) and whole grape berries.*

	Fe *(mg kg^{-1} D.W.)*			***Zn*** *(mg kg^{-1} D.W.)*		
Factor	*Petioles berry set*	*Petioles veraison*	*Grapes harvest*	*Petioles berry set*	*Petioles veraison*	*Grapes harvest*
Treatment						
1#Control	*13.8 a*	*16.1 a*	*9.70*	*29.6 a*	*41.4 a*	*4.01 a*
2#NPK	*14.1 a*	*15.7 a*	*10.1*	*36.8 ab*	*55.4 b*	*4.89 b*
3#NPK Mg l	*15.4 a*	*15.5 a*	*10.6*	*38.1 ab*	*61.6 bc*	*5.27 bc*
4#NPK Fe l	*25.6 c*	*17.2 ab*	*10.5*	*37.0 ab*	*75.7 c*	*5.50 bc*
5#NPK Mg Fe l	*23.1 bc*	*18.6 ab*	*11.2*	*40.6 ab*	*69.7 c*	*5.46 bc*
6#NPK Mg Fe hl	*22.2 bc*	*20.3 b*	*11.7*	*41.0 ab*	*66.5 c*	*5.51 bc*
7#NPK Mg s	*16.4 a*	*16.1 a*	*11.2*	*39.9 ab*	*72.7 c*	*5.90 c*
8#NPK Fe s	*16.1 a*	*15.8 a*	*10.5*	*41.7 ab*	*70.4 c*	*5.30 bc*
9#NPK Mg Fe s	*15.9 a*	*15.2 a*	*10.6*	*38.8 ab*	*70.0 c*	*5.25 bc*
10#NPK Zn l	*18.5 ab*	*15.5 a*	*9.90*	*47.2 b*	*61.6 bc*	*5.72 b*
P-value	***	*	*n.s*	**	***	***
Year						
2009	*17.9*	*19.2 c*	*12.2 b*	*38.7 b*	*64.0 a*	*4.80 a*
2010	*19.1*	*16.6 b*	*10.0 a*	*34.8 a*	*59.4 a*	*5.58 b*
2011	*17.3*	*14.2 a*	*9.40 a*	*43.5 c*	*71.4 b*	*5.52 b*
P-value	*n.s*	***	***	**	***	***
Year *x* ***treatment***						
P-value	**	***	*n.s.*	*n.s.*	*n.s.*	*n.s.*

*Data were processed through GLM ANOVA (treatment, fixed factor; year, random factor): *P < 0.05; **P < 0.01; ***P < 0.001, n.s.: not significant. Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test. For treatment details refer to 2.1.3 Treatment layout: Table 8.*

In petioles at berry set the K:Mg ratio was around 3.0, 2.8 and 1.9 times higher in NPK treated vines than in untreated, in the years 2009, 2010 and 2011, respectively. The same trend was shown also for petioles at veraison and berries at harvest, but the magnitude of difference with untreated vines was lower (Table 25).

Figure 9: *Basal leaf chlorosis (Brataševec, July 2009).*

As opposite to K, measured Mg concentrations were lower than optimal in petioles (Bigot et al., 2009; Fregoni, 1997; Sinskey, 2009), and in all years basal leaf chlorosis (Figure 9) was assessed in all treatments but not in control vines. Marchner (1995) reported that the addition of NPK yields a lower Mg content in petioles, and this fact could be explain since there is an antagonistic relationship with K. To support what the same researcher postulated, the correlation between Mg and K in our experiment revealed to be statistically significant (r_{max} = –0.85, P < 0.001). Another aspect that it is important to take into account is related to root absorption, since Stefanini et al. (1994) already reported that SO4 rootstock poorly uptakes Mg from the soil. The wide range of K petiole content (by 40% lowered from 2009 to 2010) during the 3–years experiment, could possibly support the high variations in Mg content, if only K–to–Mg antagonism is thought to be 'in charge' for the lower Mg uptake (approximately 20% variation from 2009 to 2010 and 2011; P < 0.001).

As reported by Bergmann (1992), nitrogen can also diminish the concentration of magnesium in the plant; these could explain the differences in leaf Mg content between the control and NPK treated vines and the trends observed over the three years. The amount of free amino nitrogen in grape berries was found 5–times lower in control vines as compared with NPK treated (see section 3.2.1, Table 28), thus we can confirm that control vines experienced N deficiency that was also ascertained from the pale green colour of the leaves.

According to Capps and Wolf (2000), Haefs et al. (2002) and Stefanini et al. (1994), foliar or soil application of $MgSO_4$ could be very effective in enhancing Mg levels in grapevine blades and petioles or on the other hand, no measurable effects could be obtained depending on experimental conditions (*e.g.* application rate and distribution timing and frequency).

3.2.5 Iron – effect of NPK and Fe fertilization on Fe content in leaves and grape

The content of Fe, irrespectively in grapes at harvest or in petioles at berry set or veraison was not consistently affected by soil application of NPK (#2; Table 22). In all three years, the concentration of Fe in petioles sampled at berry set was significantly enhanced by foliar fertilization with 'Foliacon Fe' (treatments #4, 5, and 6; Table 24). At veraison time, the differences among treatments were reduced as compared with berry set. Looking at the year by treatment interaction, higher content of Fe was still observed in 2010 and 2011, where the same element was added throughout foliar fertilization (0.45% 'Foliacon Fe'; #6). Comparing the years, Fe content in the petioles at berry set was not significantly different, but at veraison time was found much higher in 2009 and the lowest in 2011. The application of Fe or other nutrients was not profitable for a significant enhancement of Fe in grape berries at harvest.

During the vegetative period in years 2009–2011, iron concentrations in petioles of most treatments were in agreement with Fregoni (1997) and with Bigot et al. (2009) low-to-marginal (< 25 mg kg^{-1}). However, in the treatments where Fe was applied by

foliar spraying of ‘Foliacon Fe’ (#4–6), the Fe contents were statistically higher ($P < 0.001$) and close to the reference range. In the three–year experiment no differences were obtained in petiole Fe contents between 1#control and NPK treated vines (not including vines of treatments #4–6, where Fe was applied with foliar spraying), showing that application of NPK into the soil had no influence on Fe uptake. The results obtained at berry set 2010 and 2011 by foliar spraying (#4–6) are suggesting that Fe–ions in the commercial ‘Foliacon Fe’ solution could penetrate through the leaves significantly enhancing foliar Fe concentration, while the soil application of Fe (in spite of producers recommendations for vine soil applications) was not profitable for a significant increase of the same nutrient in veraison/fruit set petioles or in berries of ‘Rebula’ cv. at harvest. The availability of iron has known to be much affected by soil characteristics, and it is very poor in calcareous soils (Mengel, 1994). According to Tagliavini and Rombolà (2001), SO4 rootstock is moderately tolerant to iron chlorosis and despite low-to-marginal Fe concentration, young vines does not show chlorotic symptoms as reported also by Bergmann (1992).

Foliar spraying with Foliacon Fe – Fe complexed with amino acids could be an alternative to synthetic Fe–chelates, which are the most used and studied iron fertilizers (Díaz et al., 2010; Fernández and Ebert, 2005; Fernández et al. 2009; Tagliavini and Rombolà, 2001), and it can be used for organic farming, too.

3.2.6 Zn increasing as affected by NPK coupled with Mg and Fe fertilization

As opposite as described for iron, the amount of Zn in petioles sampled at veraison was significantly increased by NPK treatment alone and in combination when Mg and Fe were coupled through foliar treatments or added in the soil separately (41 mg kg^{-1} *vs*. 69 in the control *vs*. treatments #3–9; Table 23). Parallel results were highlighted in grapes with 37% higher Zn contents in treatments #3–9 than in the control vines, thus Mg and Fe fertilisation provided a positive evidence for higher content of zinc both in petioles and in grapes. In addition, possitive effect of Zn foliar fertilization (#10) could be seen in petioles at berry set (Table 23), although totally diminished at veraison. As regard season effect, the lowest values were shown in

petioles sampled in 2010 and the highest in 2011. In the contrast to K, Mg, and Fe, not significant effects of the year by treatment interaction were found in all three samples (petioles at both phenophases and grapes).

In all experimental vines, measured Zn contents showed values within the reference range of 26–150 mg kg^{-1} (Sinskey, 2009). As opposite to what discussed about Fe results, Zn uptake seemed to be affected by the addition of NPK (#2) and Mg and Fe fertilization (treatments #3–9; $P < 0.001$) in petioles at veraison and, what is more interesting, in the grape berries (Table 23). Aciksoz et al. (2011) and Kutman et al. (2011) noticed that high soil N application could elevate Zn (and Fe) content in wheat grains, while Peuke (2009) reported a negative influence of increased N–fertilization rate on Zn content in grapevines leaves. Moreover, Díaz et al. (2010) found that the application of Fe–chelate fertilizers and synthetic vivianite in one to three-years-old potted grapevines had no effect on Zn concentration or even a reduction was shown in the first year of application.

3.2.7 Changes in K, Mg, Fe and Zn leaf concentration over time

The contents of Mg and Zn in ʻRebulaʼ leaf petioles (*Vitis vinifera* L.) increased by approximately 60% (on the overall average of 3 years) from berry set to veraison. In the contrast, the contents of other two elements - K and Fe - were more or less stable during the vegetative period (the average ratio between berry set and veraison content was approximately 1.0 for both elements). In agreement with Christensen (1984) and Peuke (2009), the trends of the element contents depend on the grapevine cultivar. In our study, petiole K levels did not decline between berry set and veraison, as observed by Christensen (1984) for a wide range of cultivars. On the other hand, Peuke (2009) did not found a clear trend for K concentration and did not observe the Zn enhancement in the leaves of *Vitis vinifera* L. cv. ‘Riesling’ (SO4 rootstocks) during the vegetative period. In agreement with Peuke (2009), the time of collecting samples for leaf analysis must be taken into account for viticulture practice, especially when the concentration of elements change (*e.g.* for Mg, Zn) during the vegetative period, and also the grapevine cultivar must be considered.

Table 24: *K, Mg, and Fe content (mean values; n = 3) in petioles at berry set and veraison of differently treated 'Rebula' grapevines in years 2009, 2010 and 2011.*

Treatment / Year	***K*** *(g kg^{-1} D.W.)*		***Mg*** *(g kg^{-1} D.W.)*		***Fe*** *(mg kg^{-1} D.W.)*	
	Petioles berry set	*Petioles veraison*	*Petioles berry set*	*Petioles veraison*	*Petioles berry set*	*Petioles Veraison*
2009						
1#Control	*29.8 a*	*28.8 a*	*1.43 b*	*2.69 b*	*17.2 a*	*19.1*
2#NPK	*47.7 b*	*48.2 b*	*0.79 a*	*1.15 a*	*14.0 a*	*19.1*
3#NPK Mg l	*46.2 b*	*48.4 b*	*0.93 a*	*1.18 a*	*16.6 a*	*19.0*
4#NPK Fe l	*46.9 b*	*48.4 b*	*0.74 a*	*1.17 a*	*23.8 b*	*21.0*
5#NPK Mg Fe l	*48.9 b*	*52.2 b*	*0.84 a*	*1.50 a*	*22.9 b*	*20.2*
6#NPK Mg Fe hl	*51.6 b*	*52.4 b*	*0.79 a*	*1.09 a*	*15.7 a*	*19.6*
7#NPK Mg s	*52.0 b*	*52.6 b*	*0.74 a*	*1.32 a*	*17.4 a*	*19.5*
8#NPK Fe s	*52.5 b*	*49.9 b*	*0.77 a*	*1.36 a*	*15.6 a*	*19.2*
9#NPK Mg Fe s	*49.3 b*	*45.2 b*	*0.77 a*	*1.27 a*	*17.4 a*	*17.1*
10#NPK Zn l	*49.7 b*	*47.1 b*	*0.77 a*	*1.22 a*	*18.4 a*	*18.4*
P-value	**	***	***	***	***	*n.s.*
2010						
1#Control	*24.7 a*	*23.7 a*	*1.29 b*	*2.18 b*	*13.2 a*	*17.1 ab*
2#NPK	*29.3 ab*	*33.8 b*	*0.67 a*	*1.03 a*	*14.9 a*	*14.1 a*
3#NPK Mg l	*28.1 ab*	*34.8 b*	*0.65 a*	*0.92 a*	*15.6 a*	*14.3 a*
4#NPK Fe l	*34.6 ab*	*37.7 b*	*0.54 a*	*0.95 a*	*27.3 cd*	*17.7 ab*
5#NPK Mg Fe l	*28.0 ab*	*38.0 b*	*0.60 a*	*1.08 a*	*22.7 b*	*20.1 b*
6#NPK Mg Fe hl	*40.0 b*	*41.0 b*	*0.63 a*	*1.01 a*	*29.1 d*	*23.6 c*
7#NPK Mg s	*36.4 ab*	*34.9 b*	*0.58 a*	*1.09 a*	*17.3 a*	*14.3 a*
8#NPK Fe s	*31.4 ab*	*35.0 b*	*0.54 a*	*1.15 a*	*17.4 a*	*14.6 a*
9#NPK Mg Fe s	*25.0 a*	*35.0 b*	*0.56 a*	*0.95 a*	*15.1 a*	*13.9 a*
10#NPK Zn l	*26.6 a*	*31.4 b*	*0.50 a*	*1.00 a*	*23.6 bc*	*15.2 a*
P-value	*	***	***	***	***	***
2011						
1#Control	*27.9 a*	*28.5 ab*	*1.47 c*	*2.31 b*	*11.1 a*	*12.2 a*
2#NPK	*30.8 ab*	*35.3 b*	*1.05 ab*	*1.25 a*	*13.4 a*	*13.8 a*
3#NPK Mg l	*31.5 ab*	*31.8 ab*	*1.07 b*	*1.35 a*	*14.1 a*	*13.4 a*
4#NPK Fe l	*33.3 ab*	*33.7 b*	*0.77 a*	*1.12 a*	*25.8 b*	*14.1 a*
5#NPK Mg Fe l	*26.1 a*	*25.2 a*	*1.05 ab*	*1.23 a*	*23.7 b*	*15.5 a*
6#NPK Mg Fe hl	*38.6 b*	*41.2 c*	*0.83 ab*	*1.34 a*	*24.2 b*	*17.8 b*
7#NPK Mg s	*34.1 ab*	*32.2 ab*	*0.98 ab*	*1.25 a*	*14.5 a*	*14.4 a*
8#NPK Fe s	*30.5 ab*	*34.0 b*	*0.81 ab*	*1.15 a*	*15.3 a*	*13.1 a*
9#NPK Mg Fe s	*33.6 ab*	*32.2 ab*	*0.90 ab*	*1.31 a*	*15.3 a*	*14.5 a*
10#NPK Zn l	*31.0 ab*	*28.4 ab*	*1.04 ab*	*1.22 a*	*15.2 a*	*13.0 a*
P-value	*	**	***	***	***	***

*Data were subjected to ANOVA test (*P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant). Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test. For treatment details refer to 2.1.3 Treatment layout: Table 8.*

Table 25: *Ratio K:Mg (mean values ± standard deviation; n = 3) in petioles at berry set and at veraison, and in the whole grape berries at harvest of differently treated 'Rebula' grapevines in years 2009–2011.*

Treatment [a]	K:Mg		
Year	Petioles at berry set	Petioles at veraison	Whole grape berries at harvest
2009			
1#Control	21.0 ± 2.3	10.9 ± 2.9	24.6 ± 1.2
2#NPK	60.0 ± 4.3	42.8 ± 7.3	38.0 ± 2.9
3#NPK Mg l	50.1 ± 5.9	41.1 ± 4.2	37.9 ± 1.4
4#NPK Fe l	61.5 ± 12	41.3 ± 0.7	35.0 ± 1.8
5#NPK Mg Fe l	59.0 ± 6.8	33.1 ± 0.2	34.9 ± 1.1
6#NPK Mg Fe hl	65.1 ± 5.4	49.0 ± 4.8	36.2 ± 0.9
7#NPK Mg s	71.5 ± 15	40.0 ± 0.9	37.0 ± 3.7
8#NPK Fe s	69.8 ± 15	36.6 ± 0.3	37.4 ± 1.8
9#NPK Mg Fe s	64.4 ± 7.1	36.0 ± 3.7	38.4 ± 0.3
10#NPK Zn l	64.9 ± 9.7	40.1 ± 8.5	36.5 ± 1.0
2010			
1#Control	19.2 ± 0.6	11.0 ± 1.3	18.3 ± 1.9
2#NPK	39.2 ± 2.0	34.3 ± 9.1	28.6 ± 2.2
3#NPK Mg l	44.5 ± 16	38.1 ± 2.1	26.3 ± 1.3
4#NPK Fe l	59.6 ± 6.8	39.9 ± 2.2	27.4 ± 2.5
5#NPK Mg Fe l	50.2 ± 4.5	35.5 ± 6.0	27.9 ± 1.4
6#NPK Mg Fe hl	64.1 ± 13	43.3 ± 0.1	27.0 ± 0.8
7#NPK Mg s	62.5 ± 1.6	33.4 ± 9.7	27.1 ± 1.9
8#NPK Fe s	58.3 ± 1.9	30.9 ± 5.2	27.1 ± 0.8
9#NPK Mg Fe s	45.0 ± 8.0	37.4 ± 7.4	26.6 ± 1.5
10#NPK Zn l	53.0 ± 3.9	33.8 ± 3.6	26.7 ± 0.4
2011			
1#Control	19.1 ± 3.3	12.3 ± 1.1	21.7 ± 0.6
2#NPK	29.8 ± 4.8	27.4 ± 4.9	30.5 ± 2.7
3#NPK Mg l	29.6 ± 5.9	23.6 ± 2.6	29.7 ± 2.1
4#NPK Fe l	43.4 ± 1.3	30.7 ± 7.7	29.0 ± 2.5
5#NPK Mg Fe l	25.0 ± 2.6	20.3 ± 2.1	28.8 ± 2.7
6#NPK Mg Fe hl	47.0 ± 9.1	30.3 ± 5.6	26.6 ± 1.5
7#NPK Mg s	35.3 ± 6.1	25.9 ± 2.9	29.0 ± 1.3
8#NPK Fe s	40.3 ± 3.3	29.9 ± 6.2	31.2 ± 0.7
9#NPK Mg Fe s	37.5 ± 2.3	24.7 ± 2.9	27.0 ± 3.8
10#NPK Zn l	30.1 ± 3.5	24.0 ± 0.4	27.5 ± 0.8

[a] *For details refer to 2.1.3 Treatment layout: Table 8.*

3.2.8 Correlations between elements in the petioles and grape berries

Many positive and negative correlations for K, Mg, Fe and Zn between the petioles at berry set and veraison and grape berries at harvest were obtained during the three growing seasons (Annex D, E, F). Significant correlations ($P < 0.05$) were found between petioles at berry set and petioles at veraison for K ($r = 0.81$; 0.53; 0.73 in 2009, 2010 and 2011, respectively) and Mg content ($r \geq 0.86$ in all three years) and between petioles at veraison and grape berries (for K were $r = 0.52$; 0.65; 0.71 in 2009, 2010 and 2011, respectively; for Mg were $r = 0.81$; 0.65 in 2009, 2010, respectively). As expected, negative correlation between K and Mg were found in the petioles collected at the same sampling time (at berry set were $r = –0.81$; –0.62 in 2009 and 2011, respectively and at veraison were $r = –0.85$; –0.64 in 2009 and 2010, respectively). No detectable correlations were found in case of grapes.

In the case of micro-elements, not many correlations between contents in the petioles and in the grape berries were shown, occasionally only in one season, if any. Positive relationship ($P < 0.05$) was found between the content in Zn in petioles at berry set and in grape berries at harvest, in the years 2009 and 2011 ($r = 0.54$), and between the amount of Fe in petioles at berry set and at veraison ($r = 0.78$; 0.51 in 2010 and 2011, respectively).

In addition, some correlations between macro- and micro-elements were shown being the most evident between Mg (or K) and Zn. Significant negative correlations were found in all three years between Mg and Zn content in the petioles at veraison ($r = –0.56$; –0.64; –0.69 in 2009, 2010 and 2011, respectively) and between Mg in the petioles and Zn in the whole grape berries ($r = –0.68$; –0.75; –0.55 in 2009, 2010 and 2011, respectively). In grape berries and petioles at veraison synergism was obtained between Zn and K content (in grape was $r \geq 0.62$ in 2010 and 2011, in petioles were $r = 0.73$; 0.57 in 2009 and 2010, respectively).

Significant positive correlations observed for K and Mg between leaf petioles and grape berries, and between petioles sampled at two different vegetative stages are in agreement with high mobility of these two elements inside the plant tissues

(Marschner, 1995). The positive correlation in K and Mg content between grape berries and petioles confirmed the remobilization/transfer of K and Mg from mature leaves to the berries (sink-source relationship) during ripening via phloem tissue (Coombe, 1992; Etchebarne et al., 2009). As opposite to macro-elements, Fe and Zn are known to be plant immobile elements (Bergmann, 1992) and they need to be continuously absorbed by the plant during the vegetative growth. This could explain why the correlations in Fe and Zn contents between plant samples were poor, and if any, they were obtained in one season only. An exception was the correlations between Zn content in grape berries and petioles sampled at berry set in 2009 and 2011 and between Fe content in petioles of two different phenophases. In any case, the antagonism between these two micro-elements (Alloway, 1995) was not observed.

Beside the known antagonism between K and Mg (Marchner, 1995), which was showed also in our experiment, interesting correlations were found between K or Mg and Zn. Potassium content in grape and petioles sampled at veraison positively correlated with Zn in the same plant sample, in disagreement with Bergmann (1992) who reported that high concentrations of K could inhibit the uptake of Zn. As expected from the negative relationship between K and Mg, positive relationship was found between Mg and Zn in both plant organs - leaves and grapes. Best to our knowledge, these relationships have not been described yet.

3.2.9 Other macro- and micro-elements in grapevine leaves

Our samples were subjected to outsourcing lab to check the concentration of other important macro- and micro-elements like N, P, S, Ca, B, Mn, and Cu. The analysis performed by YARA Italia S.p.a. in 2011 revealed same additional differences in major element contents between untreated (#1) and NPK treated (#2) vines (Annex G). The main difference was observed in N content; low (13.9 g kg^{-1}) in leaves of control vines *vs*. high (31.1 g kg^{-1}) in NPK treated vines. Nitrogen application, which was according to the vineyard fertilization recommendations/practice (50 kg per hectare per year; Vršič and Lešnik, 2005) performed each year in all NPK treatments,

resulted in elevated N concentration in leaf blades. On the other hand, N deprivation of control vines was evident from the pale-green leaf color in all year (Figure 10) and decreased vegetative growth (especially in 2010 and 2011). The shoot length of control vines was in comparison to the NPK treated ones reduced by approximately 2–times (39 ± 13 cm *vs.* 76 ± 15 cm; on the overall average) in 2010 and 2011.

Both experimental vines (vines of treatments #1 and 2) contained optimal level of Ca, Mn, and Cu and low to marginal content of P, S and B.

Figure 10: *Comparison between control vine (left) and NPK treated vine (right) (Brataševec, July 2009).*

3.2.10 PCA analysis

A Principal Component Analysis (PCA) was then applied considering all data of micro- and macro-elements in grapes and petioles (both phenophases) during the three–year period of investigation to see, which of the element (and sample) was the major distinguishing factor among treatments and years (Table 26). The first three PCs (selected on eigenvalue 1 criterion) accounted for in total 80.2 of the variation in

the nutrient data. PC1 (37.8% of the total variance) was highly correlated with K in all three samples (grape, petioles at both phenophases), and with Fe in grapes at harvest and in the petioles at veraison. The second PC2 (27.6% of the total variance) was described mainly by Zn (in petioles), and had a negative correlation with Mg in grapes and in petioles at veraion. The third PC3 (14.7% of the total variance) included positive loadings for Fe in the petioles at berry set and Zn in grape berries and negative loadings for Mg in the petioles at both phenophases.

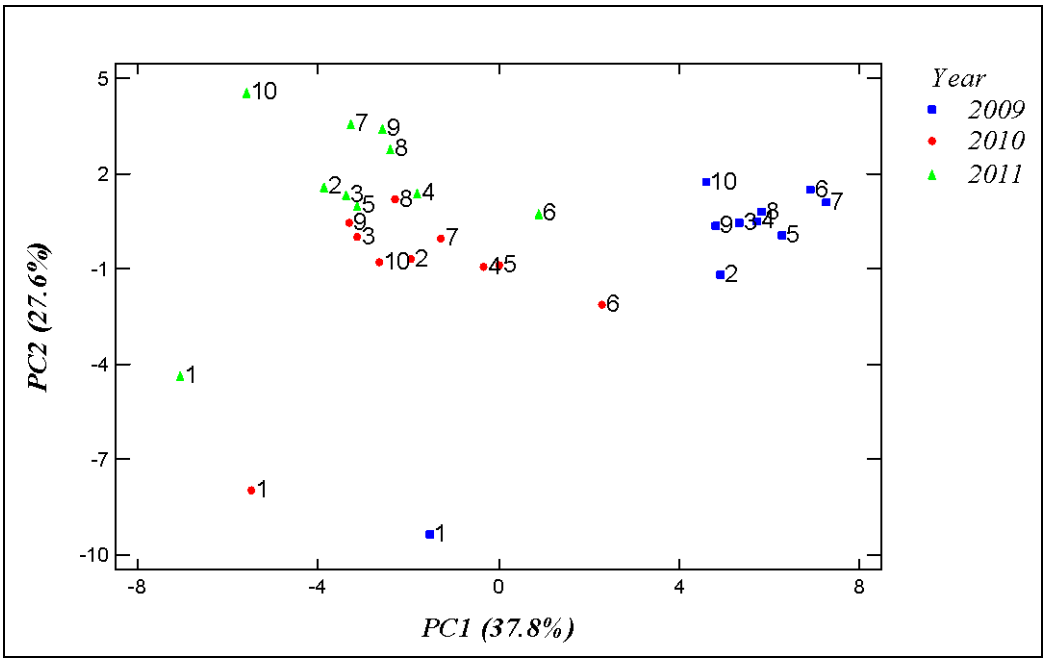

Figure 11: *Rotated PC1 x PC2 scatter plot of all analysed nutrients in grape berries at harvest and in petioles at berry set and veraison in the years 2009–2011. Treatments are represented by numbers 1–10 (for details refer to Table 8).*

In Figure 11 the score values for all micro-and macro-elements in grapes and petioles were projected onto PC1 and PC2 planes, and control treatments (#1) could be clearly separated from all nine NPK treatments (#2–10) by both PC1 (differences in K contents) and PC2 (differences in Mg and Zn contents). Moreover, the scatter plot clearly showed that the 2010 and 2011 data (NPK treatments) were closely grouped while 2009 ones were separated from them (similar K contents in 2010 and 2011). Additionally, all NPK treatments (#2–10) had similar PC2 (similar Zn contents in the petioles in all three years).

Table 26: *PCA after varimax rotation for the nutrient contents in grape and petioles at berry set and veraison: eigevalues, cummulative of the total variance, factor loading of the 2 factors, and communality estimates of the 12 parameters.*

		PC1	*PC2*	*PC3*	*Communality*
Eigenvalue		*4.54*	*3.32*	*1.77*	
Cummulative (%)		*37.8*	*65.5*	*80.2*	
K	*Grape*	*0.947*	*0.150*	*0.022*	*0.919*
	Berry set petioles	*0.944*	*0.206*	*-0.044*	*0.936*
	Veraison petioles	*0.942*	*0.163*	*0.132*	*0.930*
Mg	*Grape*	*-0.019*	*-0.873*	*-0.027*	*0.763*
	Berry set petioles	*-0.222*	*-0.165*	*-0.843*	*0.787*
	Veraison petioles	*-0.100*	*-0.516*	*-0.745*	*0.831*
Fe	*Grape*	*0.864*	*-0.102*	*0.105*	*0.768*
	Berry set petioles	*0.168*	*-0.249*	*0.769*	*0.682*
	Veraison petioles	*0.774*	*-0.406*	*0.235*	*0.819*
Zn	*Grape*	*-0.279*	*0.421*	*0.745*	*0.811*
	Berry set petioles	*0.041*	*0.830*	*0.052*	*0.693*
	Veraison petioles	*0.119*	*0.730*	*0.369*	*0.683*

3.3 GRAPE QUALITY ANALYSIS

3.3.1 Berry weight, sugar content, organic acids, and free amino nitrogen determination

In Tables 27 and 28, the main grape quality parameters measured in 2009, 2010 and 2011 are presented (organic acids content and FAN were not measured in 2009). Soluble solids (*i.e.* sugar content (°Brix) and specific weight) were not statistically higher (only in 2011 in terms of specific weight) in grape harvested on control vines in comparison to the grapes harvested on vines where N, P, and K have been added into the soil which is in agreement with results obtained by Conradie and Saayman (1989). However, mean values were 20.1 *vs*. 18.3; 18.5 *vs*. 16.2; and 21.2 *vs*. 17.6 in

control vines *vs*. NPK treated vines (#2–10) in 2009, 2010 and 2011, respectively (Table 27). A reduction in soluble solids in musts with increased nitrogen availability had been described by many researchers in other grape varieties (Christensen et al., 1994; Delgado et al., 2004; Hilbert et al., 2003; Spayd et al., 1994). Similarly, positive (Amiri and Fallahi, 2007; Kraic et al., 2008; Muthukrishnan and Srinivasan; 1974; Topalović et al., 2011) or no effect (Delgado et al., 2004; Morris et al., 1987; Poni et al., 2003) of K supply on sugar content has already been reported in the literature. Data from our experiment highlighted highly negative correlation (r_{max} = –0.82, $P < 0.001$) between K and sugar contents in the grapes (Annex H). Mpelasoka et al. (2003) reported that under conditions of low sugar production, more K may be accumulated in the berry possibly because K is highly mobile osmoticum, which may reduce the positive relationship between berry K and berry sugar.

The influence of Mg and Fe on sugar content could not be confirmed by Student-Newman-Keuls´s test (*P* was not significant), however, a strong positive correlation was found between sugar content and Mg in petioles at both phenophases in 2011 (r_{max} = 0.95, $P < 0.001$). Statistical correlations are also suggesting antagonism between soluble solids and Zn contents in grapes ($r = 0.50$ and $r = 0.65$, $P < 0.05$ in 2010 and 2011, respectively) and in the petioles at veraison ($r = –0.77$, $P < 0.01$) in 2011 as well.

In agreement with Christensen et al. (1994), Delgado et al. (2004), and Poni et al. (2003) no significant effects of both N and K nutrients on pH and berry weigh were revealed (Table 27). On the contrary, some other authors reported an enhancement in fruit pH due to N (Spayd et al., 1994) and/or K fertilization (Morris et al., 1987; Ruhl et al., 1992). In our experiment, no correlations between measured nutrients (K, Mg, Fe, and Zn) in grape and petioles with these two parameters were shown.

Free amino nitrogen (FAN), an indicator of nitrogen availability for yeast growth and fermentation (Manley et al., 2001), was found to be nearly five times lower ($P < 0.05$) in the control vines in comparison to NPK treated vines (#2). According to the Murat and Dumeau (2005) the control vines were really deficient in nitrogen, which was also evident from the leaf colour (pale-green; Figure 10) (Marschner, 1995).

Table 27: *Soluble solids (as sugar content and specific weight), berry weight and pH (mean values ± standard deviation; n = 3) of grape juice of differently treated 'Rebula' grapevines in years 2009–2011.*

Treatment / Year	Sugar content (°Brix)	Specific weight	Berry weight (g berry^{-1})	pH
2009				
1#Control	20.1 ± 1.3	1.084 ± 0.006	1.54 ± 0.10	3.56 ± 0.08
2#NPK	18.2 ± 0.3	1.076 ± 0.001	1.89 ± 0.28	3.64 ± 0.18
3#NPK Mg l	18.6 ± 0.4	1.077 ± 0.002	1.92 ± 0.34	3.80 ± 0.22
4#NPK Fe l	18.2 ± 2.1	1.076 ± 0.009	1.96 ± 0.09	3.72 ± 0.35
5#NPK Mg Fe l	17.8 ± 0.6	1.074 ± 0.003	1.80 ± 0.20	3.60 ± 0.07
6#NPK Mg Fe hl	18.6 ± 1.7	1.078 ± 0.007	1.68 ± 0.33	3.89 ± 0.45
7#NPK Mg s	17.1 ± 1.2	1.071 ± 0.005	1.57 ± 0.08	3.65 ± 0.10
8#NPK Fe s	18.3 ± 1.9	1.077 ± 0.008	1.94 ± 0.20	3.87 ± 0.37
9#NPK Mg Fe s	19.1 ± 1.9	1.080 ± 0.009	2.15 ± 0.13	4.00 ± 0.32
10#NPK Zn l	18.6 ± 2.1	1.078 ± 0.009	1.80 ± 0.23	3.76 ± 0.31
P-value	n.s.	n.s.	n.s.	n.s.
2010				
1#Control	18.5 ± 1.2	1.077 ± 0.005	1.36 ± 0.16	3.10 ± 0.09
2#NPK	15.6 ± 0.9	1.065 ± 0.004	1.46 ± 0.37	3.16 ± 0.05
3#NPK Mg l	16.1 ± 2.4	1.067 ± 0.011	1.51 ± 0.10	3.17 ± 0.08
4#NPK Fe l	16.3 ± 0.3	1.068 ± 0.002	1.65 ± 0.23	3.13 ± 0.02
5#NPK Mg Fe l	15.6 ± 1.2	1.065 ± 0.006	1.58 ± 0.13	3.14 ± 0.02
6#NPK Mg Fe hl	16.1 ± 1.3	1.067 ± 0.007	1.57 ± 0.13	3.15 ± 0.03
7#NPK Mg s	15.8 ± 0.7	1.065 ± 0.003	1.50 ± 0.25	3.17 ± 0.04
8#NPK Fe s	17.1 ± 0.5	1.072 ± 0.003	1.60 ± 0.11	3.18 ± 0.03
9#NPK Mg Fe s	16.9 ± 1.5	1.071 ± 0.007	1.65 ± 0.10	3.17 ± 0.03
10#NPK Zn l	16.7 ± 1.4	1.070 ± 0.007	1.65 ± 0.19	3.18 ± 0.05
P-value	n.s.	n.s	n.s.	n.s.
2011				
1#Control	21.2 ± 0.3	1.089 ± 0.001 b	1.19 ± 0.03	3.41 ± 0.08
2#NPK	17.2 ± 0.6	1.072 ± 0.003 a	1.46 ± 0.01	3.33 ± 0.04
3#NPK Mg l	18.7 ± 0.4	1.078 ± 0.001 a	1.42 ± 0.14	3.33 ± 0.01
4#NPK Fe l	17.3 ± 0.8	1.072 ± 0.004 a	1.48 ± 0.04	3.39 ± 0.09
5#NPK Mg Fe l	17.7 ± 1.1	1.074 ± 0.006 a	1.62 ± 0.01	3.35 ± 0.05
6#NPK Mg Fe hl	17.5 ± 0.3	1.073 ± 0.002 a	1.40 ± 0.16	3.36 ± 0.07
7#NPK Mg s	17.3 ± 0.1	1.072 ± 0.001 a	1.53 ± 0.29	3.34 ± 0.06
8#NPK Fe s	17.2 ± 0.2	1.072 ± 0.001 a	1.70 ± 0.26	3.35 ± 0.09
9#NPK Mg Fe s	17.6 ± 1.2	1.073 ± 0.006 a	1.55 ± 0.03	3.41 ± 0.13
10#NPK Zn l	nd [b]	Nd	nd	Nd
P-value	n.s.	*	n.s.	n.s.

*Data were subjected to ANOVA test (*P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant). Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test. For treatment details refer to 2.1.3 Treatment layout: Table 8.*

[b] *No data, since there was not enough grape to do all the analysis.*

Table 28: *Organic acid content (total acidity, tartaric and malic acids) and FAN (mean values ± standard deviation; n = 3) in whole grape berries at harvest of differently treated 'Rebula' grapevines in years 2009–2011.*

Treatment Year	TA ($g\ L^{-1}$) [b]	Tartaric acid ($g\ L^{-1}$)	Malic acid ($g\ L^{-1}$)	FAN ($mg\ L^{-1}$)
2010				
1#Control	10.6 ± 1.6	5.2 ± 0.7	5.8 ± 0.1 b	61 ± 14 a
2#NPK	8.6 ± 0.9	5.6 ± 0.4	3.9 ± 0.5 a	301 ± 48 b
3#NPK Mg l	8.9 ± 2.1	5.9 ± 0.9	4.1 ± 1.3 ab	254 ± 27 b
4#NPK Fe l	8.5 ± 0.2	6.0 ± 0.1	3.5 ± 0.2 ab	214 ± 16 b
5#NPK Mg Fe l	9.2 ± 0.6	6.0 ± 0.5	4.3 ± 0.8 a	289 ± 47 b
6#NPK Mg Fe hl	8.6 ± 1.2	5.6 ± 0.5	4.1 ± 1.2 a	258 ± 37 b
7#NPK Mg s	8.0 ± 0.2	5.6 ± 0.4	3.4 ± 0.4 ab	210 ± 32 b
8#NPK Fe s	8.0 ± 0.7	5.7 ± 0.3	3.5 ± 0.8 a	274 ± 18 b
9#NPK Mg Fe s	7.9 ± 0.6	5.9 ± 0.7	3.2 ± 0.4 a	276 ± 48 b
10#NPK Zn l	7.9 ± 0.1	5.8 ± 0.7	3.2 ± 0.5 a	277 ± 48 b
P-value	n.s.	n.s.	*	***
2011				
1#Control	6.1 ± 0.3	4.2 ± 0.1	3.3 ± 0.1	75 ± 20 a
2#NPK	6.4 ± 0.3	4.7 ± 0.2	3.4 ± 0.1	352 ± 15 b
3#NPK Mg l	6.4 ± 0.5	4.5 ± 0.1	3.4 ± 0.6	311 ± 46 b
4#NPK Fe l	5.9 ± 0.1	4.5 ± 0.1	3.1 ± 0.1	395 ± 82 b
5#NPK Mg Fe l	6.2 ± 0.2	4.6 ± 0.1	3.4 ± 0.2	328 ± 125 b
6#NPK Mg Fe hl	6.4 ± 0.4	4.4 ± 0.0	3.6 ± 0.4	404 ± 70 b
7#NPK Mg s	6.3 ± 0.1	4.4 ± 0.2	3.5 ± 0.1	324 ± 104 b
8#NPK Fe s	6.6 ± 0.8	4.5 ± 0.3	3.6 ± 0.4	306 ± 9 b
9#NPK Mg Fe s	6.1 ± 1.5	4.7 ± 0.1	3.1 ± 1.3	340 ± 64 b
10#NPK Zn l	nd [c]	Nd	nd	Nd
P-value	n.s.	n.s	n.s.	*

*Data were subjected to ANOVA test (*P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant). Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test. For details refer to 2.1.3 Treatment layout: Table 8.*

[b] As tartaric acid equivalent.

[c] *No data, since there was not enough grape to do all the analysis.*

With PCA analysis two significant factors (85.4% of the variation) of grape quality parameters were obtained (Table 29). The first PC1, accounting for 63.0% of total variance, included positive loadings for organic acids (TA and malic acid) and very negative loadings for pH. PC2 (22.3%) was described mostly by berry weight and tartaric acid, and high negative correlation was found with soluble solids. Figure 12 showed a PCA scatter plot with the first two PCs presented. The significant differences between the two growing seasons were explained by PC1 and also by

PC2. Total titratable acids and tartaric acids were much higher in 2010 than in 2011 while pH and sugar content reported an opposite behaviour (Table 27 and 28). In agreement with Jackson and Lombard (1993) the seasonal variability in grape quality might be explained by the climatic conditions during the growing period. The average August temperature was up to 2.6 °C higher in 2011 in comparison with 2010, which could explain lower TA and malic acid contents in grape juice. In addition high variability was shown between the two seasons, grape analysis revealed high diversity for control vines when compared with all NPK treatments (by PC1 and PC2) in 2010, while the differences were statistically confirmed only for malic acid content (Table 28).

Table 29: *PCA after varimax rotation for 'Rebula' grape quality parameters at harvest 2010 and 2011: eigevalues, cummulative of the total variance, factor loading of the 2 factors, and communality estimates of the 6 parameters.*

	PC1	*PC2*	*Communality*
Eigenvalue	*3.78*	*1.34*	
Cummulative (%)	*63.03*	*85.36*	
Berry weight	*-0.005*	*0.820*	*0.673*
Sugar content	*-0.178*	*-0.866*	*0.782*
pH	*-0.801*	*-0.564*	*0.959*
Total acidity	*0.943*	*0.329*	*0.996*
Tartaric acid	*0.585*	*0.717*	*0.856*
Malic acid	*0.915*	*-0.135*	*0.855*

3.3.2 Total phenols determination with Folin-Ciocalteu metod

For total phenol determination with FC metod, LOD and LOQ were calculated to be 0.73 and 2.43 ng L^{-1}, respectively. The total phenolic compound in juice and skins were calibrating against gallic acid and expressed as gallic acid equivalent (GAE) in mg L^{-1}. The extraction yield with gallic acid standard using Strata-X cartridges was calculated to be 97 ± 2%. The precision (expressed as CV) of the whole procedure (juice pressing, acid extraction, cartridge extraction), using two vineyard samples of

three sub-samples (50 berries; juice and skin extracts), was approximately 10% (n = 9). The CV of instrument measurements was under 0.2%, which allowed us to measure each sample solution only ones.

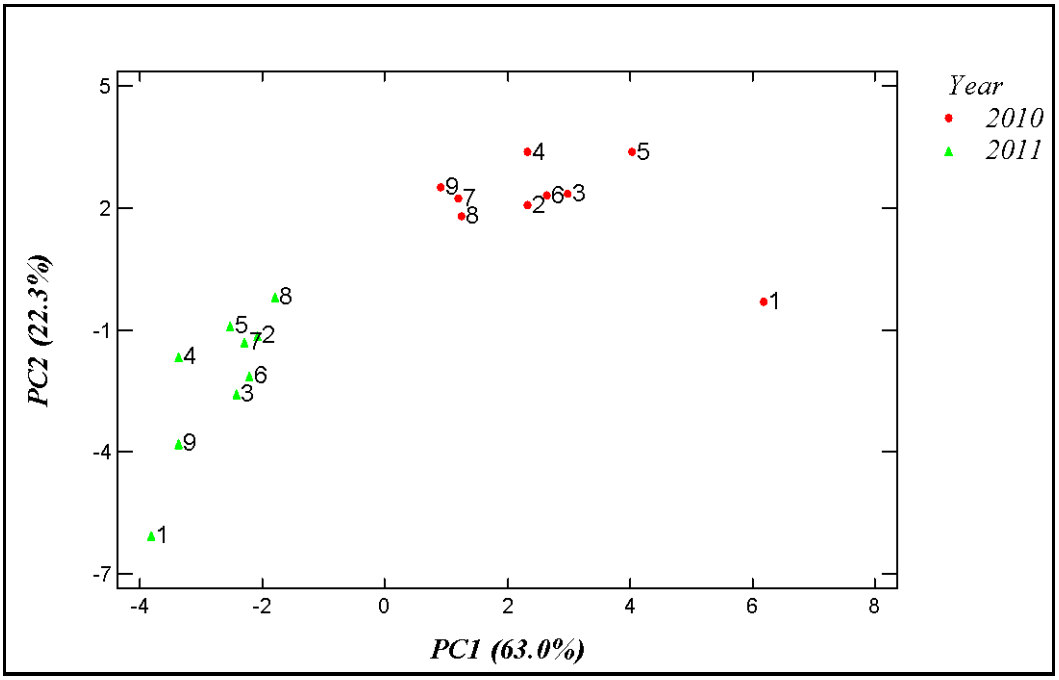

Figure 12: *Rotated PC1 x PC2 scatter plot of 'Rebula' grape quality parameters for 2010 and 2011. Treatments are represented by numbers 1–9 (for details refer to Table 8).*

3.3.3 HPLC-DAD quantification of hydroxycinnamic acids

3.3.3.1 Method optimisation for the quantification of grape phenolic compounds

The HPLC-DAD method was optimised in terms of selectivity (specificity), precision, linearity, and sensitivity (LOD and LOQ). To improve the selectivity, HCA compounds were quantified at their maximum absorbance (320 nm).

Sensitivity by means of the LOD and LOQ were in agreement with Mozetič et al. (2006) calculated to be 3.4 mg L^{-1} and 11.3 mg L^{-1}, respectively.

Table 30: *Total phenolic compound content (mean values; n = 3) in juice and skins and juice volume of grape samples harvested on differently treated 'Rebula' grapevines in 2009.*

Treatment	*Juice volume (mL)*	*Total phenolic compounds (mg L^{-1}) [a]*	
		in juice	*in skin*
1#Control	*30 a*	*184 b*	*408 b*
2#NPK	*45 ab*	*104 a*	*300 a*
3#NPK Mg	*55 b*	*124 a*	*260 a*
4#NPK Fe l	*47 ab*	*100 a*	*259 a*
5#NPK Mg Fe l	*45 ab*	*92 a*	*250 a*
6#NPK Mg Fe hl	*42 ab*	*121 a*	*262 a*
7#NPK Mg s	*46 ab*	*82 a*	*270 a*
8#NPK Fe s	*49 ab*	*98 a*	*271 a*
9#NPK Mg Fe s	*57 b*	*97 a*	*250 a*
10#NPK Zn l	*48 ab*	*99 a*	*289 a*
P-value	*	***	*

*Data were subjected to ANOVA test (*P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant). Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test. For treatment details refer to 2.1.3 Treatment layout: Table 8.*

[a] Expressed as GAE equivalents per L of juice.

3.3.3.2 Hydroxycinnamic acids contents in grape juice

Tartaric esters of HCAs represent the main phenols in white grapes and wines when made both with and without pomace contact (Singleton et al., 1986a). Total HCAs (sum of *trans*-CTA, CoTA, and FTA) and *trans*-CTA content were evaluated in grape juice in our experiment in 2009 and 2011. The levels of HCAs in grapes depend on many factors such as grape variety, growing conditions, climate, *etc*. (Rentzsch et al., 2009). By working with pots, all the factors were controlled; thus differences observed can be ascribed to fertilization treatments.

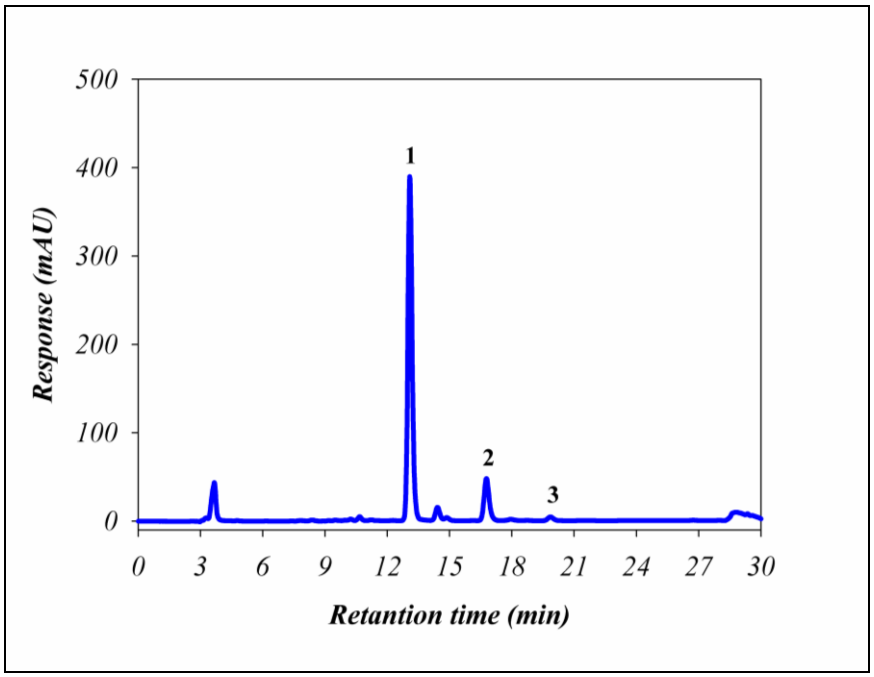

Figure 13: *HPLC-DAD separation of 'Rebula' white grape juice, monitored at 320 nm. Numbers denote the following tentative components (identified based on retention time and chromatographic properties): (1) trans-CTA; (2) trans-CoTA; (3) trans-FTA.*

Table 31: *Influence of grape juice matrix on slope of calibration curve of trans-CTA.*

Standard solution media	*Linear regression* [a]	*Correlation coefficient*	k^{c-d}/k^{b} *(%)*	k^{d}/k^{b} *(%)*
Water	$y = 54.222^{b} x$	*0.9987*		
Grape juice matrix	$y = 55.071^{c} x$	*0.9984*	*101 ± 3*	
Natural grape juice	$y = 52.13^{d} x + 3042.3$	*0.9999*	*95 ± 2*	*94 ± 2*

[a] *Linear regression is considering S.D. error as weight.*

k^{b} *– the slope of calibration curve for water standard solutions.*

k^{c} *– the slope of calibration curve for matrix standard solutions.*

k^{d} *– the slope of calibration curve obtained by the standard addition method.*

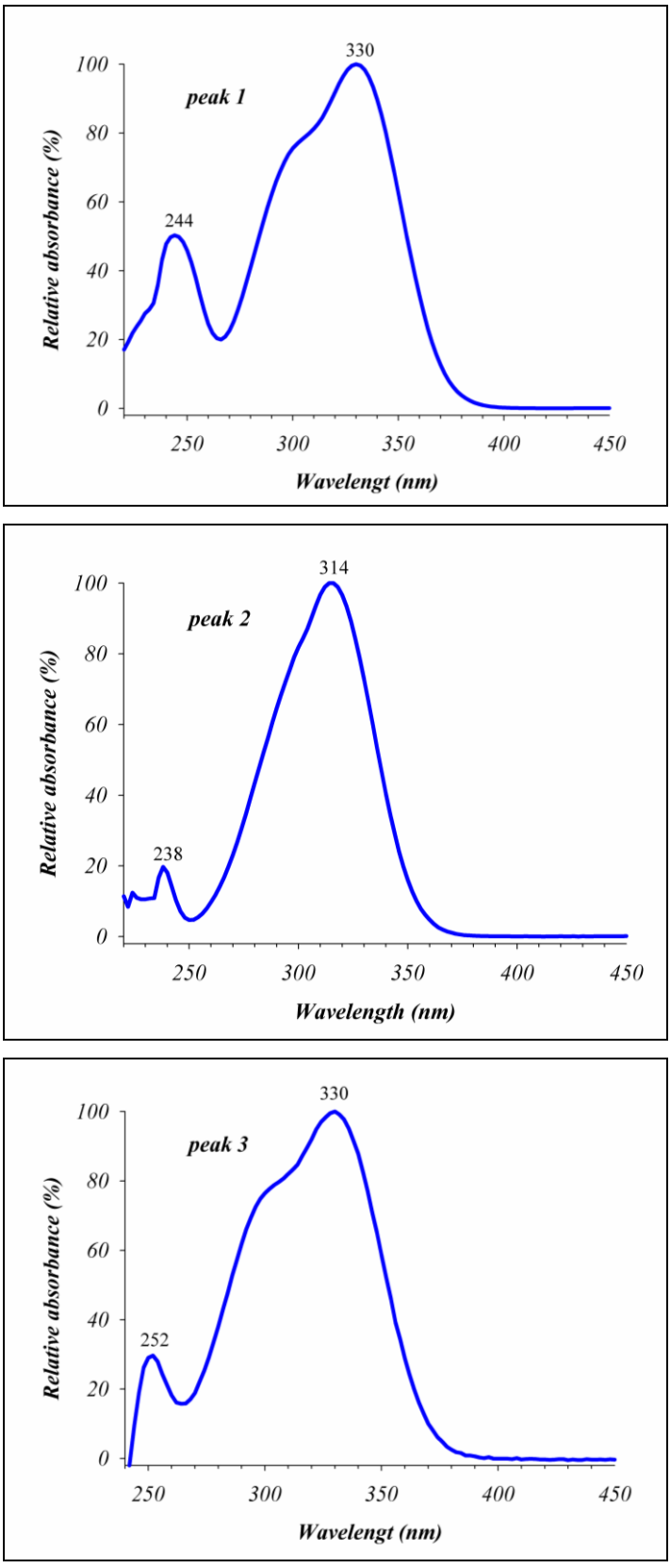

Figure 14: *UV-Vis spectra of monitored HCAs in the 'Rebula' grapes juice. The numbers 1, 2, 3 correspond to the peaks of trans-CTA, trans-CoTA and trans-FTA, respectively.*

Table 32: *Content of total HCAs, trans-CTA, and % trans-CTA, trans-CoTA and trans-FTA in grape juice of differently treated 'Rebula' grapevines in 2009, 2011.*

Treatment / Year	HCAs (mg L^{-1}) [a]	CTA (mg L^{-1}) [a]	CTA (%) [b]	CoTA (%)	FTA (%)
2009					
1#Control	100 b	87 b	86.8 ± 0.9	11.5 ± 0.8	1.6 ± 0.1
2#NPK	75 a	64 a	85.2 ± 0.9	11.9 ± 0.9	2.9 ± 0.2
3#NPK Mg l	69 a	58 a	84.2 ± 0.2	12.7 ± 0.5	3.1 ± 0.3
4#NPK Fe l	78 a	65 a	84.2 ± 0.9	12.7 ± 1.1	3.1 ± 0.3
5#NPK Mg Fe l	73 a	65 a	83.7 ± 0.4	13.3 ± 0.4	3.0 ± 0.2
6#NPK Mg Fe hl	77 a	61 a	84.2 ± 0.7	12.9 ± 0.8	3.0 ± 0.1
7#NPK Mg s	73 a	62 a	84.4 ± 0.2	12.7 ± 0.1	2.9 ± 0.2
8#NPK Fe s	80 a	67 a	84.4 ± 0.1	12.7 ± 0.3	2.9 ± 0.1
9#NPK Mg Fe s	72 a	61 a	84.6 ± 1.0	12.6 ± 0.9	2.8 ± 0.1
10#NPK Zn l	80 a	67 a	84.1 ± 0.4	13.0 ± 0.6	2.8 ± 0.2
P-value	*	*			
2011					
1#Control	82 b	71 b	87.1 ± 1.1	11.6 ± 1.5	1.3 ± 0.1
2#NPK	45 a	38 a	83.5 ± 1.1	13.5 ± 1.8	2.8 ± 0.4
3#NPK Mg l	55 a	46 a	84.8 ± 0.4	12.3 ± 0.1	2.8 ± 0.4
4#NPK Fe l	54 a	45 a	84.2 ± 1.1	12.7 ± 0.3	2.9 ± 0.8
5#NPK Mg Fe l	53 a	44 a	83.1 ± 0.4	13.7 ± 2.6	2.8 ± 0.1
6#NPK Mg Fe hl	53 a	44 a	83.3 ± 1.8	13.9 ± 0.3	3.0 ± 0.1
7#NPK Mg s	57 a	49 a	85.5 ± 1.7	11.6 ± 2.5	2.8 ± 0.1
8#NPK Fe s	50 a	43 a	85.3 ± 0.8	12.0 ± 0.9	2.7 ± 0.1
9#NPK Mg Fe s	57 a	48 a	85.2 ± 2.6	12.4 ± 3.1	2.4 ± 0.6
10#NPK Zn l	nd [c]	Nd	nd	nd	Nd
P-value	**	**			

*Data were subjected to ANOVA test (*P < 0.05; **P < 0.01; ***P < 0.001, n.s.: not significant) with treatment as the variability factor. For each column inside one year, Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls's test. For treatment details refer to 2.1.3 Treatment layout: Table 8.*

[a] *As trans-CTA equivalent (mean; n = 3).*

[b] *Percent of concentration of total HCAs measured (mean ± standard deviation; n = 3).*

[c] *No data, since there was not enough grape to do all the analysis.*

The total HCAs content was found 1.3–fold (in 2009) and 1.5–fold (in 2011) higher in the grapes of the control vines than on grapes harvested from NPK treated (#2–10) vines (100 *vs*. 75 mg L^{-1} and 82 *vs*. 53 mg L^{-1} on the average in 2009 and 2011, respectively). The same trend was observed also for *trans*-CTA content (87 *vs*. 63 mg L^{-1} and 71 *vs*. 48 mg L^{-1} on the average in 2009 and 2011, respectively; Table 32). The percentage of individual HCAs did not differ between two years and was 85 ± 1, 12.6 ± 0.7 and 2.7 ± 0.5% for *trans*-CTA, *trans*-CoTA and *trans*-FTA, respectively.

A negative correlation between *trans*-CTA in grape juice and K content in whole grape berries was found in 2009 and 2011 ($r = –0.69$, $P < 0.01$ and $r = –0.58$, $P < 0.05$ in 2009 and 2011, respectively; Table 33) while between *trans*-CTA and K content in the petioles only in 2009 ($r = –0.54$, $P < 0.05$ and –0.64, $P < 0.01$ at berry set and veraison, respectively). In addition, a positive correlation was obtained between *trans*-CTA in grape juice and Mg content in grape berries ($r = 0.69$, $P < 0.01$ in 2009) and in the petioles at both sampling times and in both years ($r_{max} = –0.72$, $P < 0.05$). Only one significant correlation between *trans*-CTA and Fe in grape berries at harvest was found ($r = 0.52$, $P < 0.05$ in 2009).

In our experiment, a negative correlation between K in grape berries/petioles and the *trans*-CTA content and a positive one with Mg was obtained in both years. It is well known that high N supply inhibited the synthesis of anthocyanins and might affect their composition in some red grapevine cultivars (Hilbert et al., 2003). Nitrogen deficiency has been reported to increase anthocyanin formation also in other crops like maize and apples (Awad and Jager, 2002). In addition, Delgado et al. (2004) concluded that there is a strong interaction effect of N and K on anthocyanin and total polyphenols content; an optimal nutritional N:K ratio may enhance the phenolic pattern of grape berries. Awad and Jager (2002) reported some poor negative correlations between the concentration of N and Mg (in whole fruit) and the concentration of anthocyanins and flavonoids in the skin of apples. Much was revealed about the relationship between nutrients and anthocyanins, while for hydroxycinnamic acids, to our knowledge, data are still missing. Within the growing season (2009 or 2011), the concentration of *trans*-CTA and total HCAs was much

higher in the control vines in comparison to the NPK treated ones, but the percentage of individual HCAs did not differ between two years. The results of our experiment provide interesting outcomes, since NPK fertilisation (treatments #2–9) was profitable for a reduction of *trans*-CTA in juice. In white wines, lower amounts of HCA are desirable since there is a lower probability of browning reactions that could compromise the overall quality of wines (Rentzsch et al., 2009). In addition, no further effects of the micro-elements (Fe and Zn) did prove to be affecting the concentration of HCA in juices, once again highlighting the leader importance of macro-elements in mineral nutrition and grape quality.

Table 33: *Pearson's correlation coefficients between grape total HCAs, trans-CTA and K, Mg, Fe, and Zn contents in grapes and petioles sampled in 2009 and 2011.*

Element/sample		*Year*	*Total HCAs*	*trans-CTA*
K	*g* [a]	*2009*	*–0.68**	*–0.69***
		2011		*–0.58**
	p1 [b]	*2009*	*–0.51**	*–0.54**
		2011		
	p2 [c]	*2009*	*–0.60***	*–0.64***
		2011		
Mg	*g*	*2009*	*0.66***	*0.69***
		2011		
	p1	*2009*	*0.67***	*0.70****
		2011	*0.69**	*0.71***
	p2	*2009*	*0.70****	*0.72****
		2011	*0.66**	*0.68**
Fe	*g*	*2009*	*–0.53**	*–0.52**
		2011		
	p1	*2009*		
		2011		
	p2	*2009*		
		2011		
Zn	*g*	*2009*		
		2011		
	p1	*2009*		
		2011		
	p2	*2009*		
		2011		

**P < 0.05; ** P < 0.01; *** P < 0.001; blank cells represent not significant correlations*

[a] *Grape.*

[b] *Petioles sampled at berry set.*

[c] *Petioles sampled at veraison.*

3.4 SOIL ANALYSIS

3.4.1 General characteristics of the pot soil

General chemical and physical characteristics of the soil examined in this study (pH, EC, OM, CEC, total N, and extractable major elements) were performed by outsourcing certified lab (YARA Italia S.p.a.) in 2007 and 2011 (Annex I). The soil consisting of carbonates (330 g kg^{-1} total $CaCO_3$, 90 g kg^{-1} active lime) had a clay loam texture (20.1, silt 47.2, and clay 32.7%) with normal EC (< 4.00 dS m^{-1}), good CEC and a relatively low level of soil organic matter (1.9% C) in 2007 (soon after planting). CEC and OM of the vineyard soil used in the pot experiment are in agreement with Rusjan et al. (2006) typical for the vineyards in the sub-Mediterranean winegrowing region.

At the begging (in 2007) and at the end of the experiment (in 2011), the measured total N concentration was low (< 2000 mg kg^{-1}), P content deficient and K content optimal (marginal in 2007). The availability of elements like S, Mg, Mn, Mo, Fe and Zn (in 2007) was poor, while B, Ca and Zn (in 2011) where in the reference range (YARA Italia S.p.a).

3.4.1.1 Soil pH and redox potential

The average soil pH in water (pH_{H2O}) was 8.1 ± 0.1. The $CaCl_2/H_2O$ and KCl/H_2O extracts were from 0.4 to 0.5 pH units (respectively) lower to aqueous extracts. According to Sinskey (2009) are the later results closely to the real field pH. The obtained soil pH values have not changed over the years (2007–2011) and have been the same in all pots regardless the treatment. Applications of fertilizers and agrochemicals did not influence the pH of the soil. Pot soil pH values were very similar to the ones from the selected vineyards in Brda region (pH_{H2O} = 8.1 ± 0.1; pH_{KCl} = 7.6 ± 0.1; pH_{CaCl2} = 7.7 ± 0.1) and characteristic for the sub-Mediterranean winegrowing region (Rusjan et al., 2006).

Redox potential (E_h, pE) is another important soil factor determining the availability of elements like Fe (Fe^{2+}/ Fe^{3+}). According to the measured redox potential (E_h = 0.3 ± 0.01 V or pE = 5.1 in the field) and determined soil pH (pH_{H2O} = 8.1 ± 0.1) was FeOOH (Fe^{3+}-containing mineral) probably the predominant state of iron present in the pot soil of our experiment (Figure 15). In agreement with Bohn et al. (2001) goethite (FeOOH) dissolves to Fe^{2+} (ferrous) under reduced and moderately acidic conditions. Ferrous is preferred form absorbed by green plants, therefore plants developed root strategies to cope with Fe insolubility based on acidification, extraction of reductants or chelators, and having an incersed root reductase activity. Most fruit tree species (including grapevines) belong to Strategy I – based plants (a reduction based strategy) (Römheld and Marschner, 1986). Calcareous and alkaline soils favour the occurrence of Fe deficiency–intervenial chlorosis of apical leaves, which is a wide-spread nutritional disorder of higher plants (Tagliavini and Rombolà; 2001).

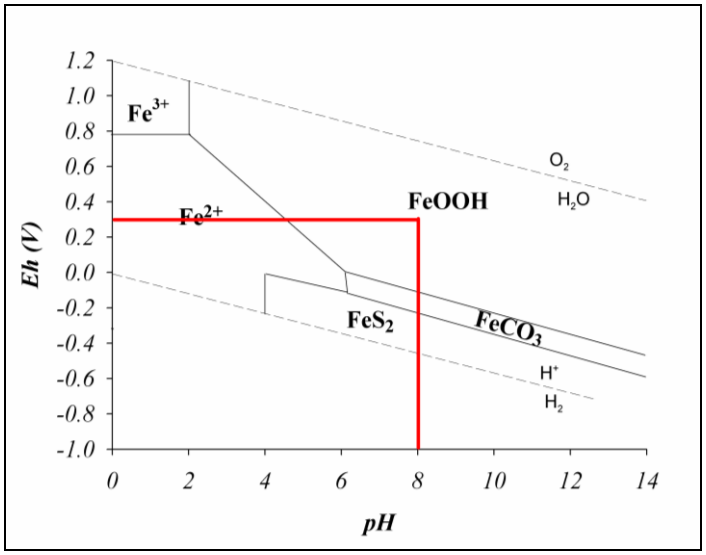

Figure 15: *The Eh-pH diagram of various iron ions and compounds (Bohn et al., 2001: 124). Red lines represent average pH and Eh in the pot soil.*

3.4.2 Total K, Mg, Fe, and Zn contents in the pot and vineyard soil

Table 34 shows the total element contents in the pot soil (control soil, soil of treatments #2 (addition of NPK) and #9 (addition of NPK, Mg and Fe) in 2009, 2010

and 2011) and in four vineyards (2011) from Brda region for comparison. Additionally, allowed values for Zn are given according to 'Directive of limited and critical values of dangerous heavy metals in the soils of Slovenia' (Directive of limited ... , 1996). Typical concentration ranges of K, Mg, Fe and Zn in soil mentioned by Alloway (1995) and Sparks (1996) are given, too.

Table 34: *Total K, Mg, Fe, and Zn contents (mean values ± standard deviation; n = 3) in pot soil during the experiment (2009–2011).*

Soil sample/Date	***K*** *(g kg^{-1})*	***Mg*** *(g kg^{-1})*	***Fe*** *(g kg^{-1})*	***Zn*** *(mg kg^{-1})*
Pot soil *June 2009*				
1#Control	*17.4 ± 0.2*	*10.5 ± 0.5*	*33.7 ± 1.0*	*101 ± 1*
2#NPK	*17.9 ± 0.2*	*10.3 ± 0.4*	*33.2 ± 0.8*	*101 ± 1*
9#NPK Mg Fe s	*17.4 ± 0.7*	*10.4 ± 0.5*	*33.0 ± 1.0*	*102 ± 2*
October 2010				
1#Control	*16.8 ± 0.6*	*10.2 ± 0.2*	*30.5 ± 3.1*	*102 ± 2*
2#NPK	*17.6 ± 0.2*	*10.4 ± 0.5*	*31.4 ± 2.4*	*103 ± 1*
9#NPK Mg Fe s	*17.9 ± 0.5*	*10.5 ± 0.5*	*33.1 ± 1.4*	*103 ± 3*
November 2011				
1#Control	*17.1 ± 0.2*	*10.6 ± 0.2*	*31.9 ± 1.8*	*107 ± 0.2*
2#NPK	*17.5 ± 0.1*	*10.6 ± 0.1*	*33.9 ± 0.5*	*110 ± 0.8*
9#NPK Mg Fe s	*17.8 ± 0.6*	*10.4 ± 0.5*	*33.4 ± 1.3*	*105 ± 2*
Vineyard				
A	*15.2 ± 0.7*	*9.90 ± 0.1*	*35.6 ± 2.0*	*112 ± 4*
B	*17.4 ± 0.1*	*10.6 ± 0.5*	*33.6 ± 0.1*	*105 ± 6*
C	*17.6 ± 0.8*	*9.70 ± 0.1*	*32.9 ± 1.9*	*102 ± 8*
D	*14.1 ± 0.4*	*8.00 ± 0.1*	*30.6 ± 0.1*	*80 ± 3*
Limited value [a]	*No data*	*No data*	*No data*	*200*
Range of value [b]	*0.4 - 30*	*0.4 – 30*	*<10 - 200*	*17 - 160*
(Common value) [b]	*(15)*	*(17.7)*	*(30)*	*(50)*

[a] *Limiting values of pollutants in soil according to Directive of limited (1996).*

[b] *Range of values (common value) in mineral soils mentioned by Alloway (1995) and Sparks (1996).*

Application of macro-elements N, P, and K had no measurable effect on macro- and micro-element content in the soil, as expected, since total element content analysis have not revealed any difference between treated (addition of fertilizers containing N, P, K, and Mg and Fe) and untreated pot soil (control) throughout the years of experiment (2009–2011). However, pot soil contained higher levels of Zn (103 mg kg^{-1}) and K (18 g kg^{-1}) and lower levels of Mg (10.4 g kg^{-1}), while Fe contents (33 g kg^{-1}) were in the reference range when compared to the common literature values (Alloway, 1995; Sparks, 1996). Total K, Mg, Fe and Zn contents in the pot soil were similar to those measured in the vineyards A, B and C. Vineyard D had lower content of all four measured elements in comparison to the pot soil and to the other three vineyards. The reason could lie in different parent material.

Among the studied elements, Zn can also be classified as heavy metal (Farago, 1994) and is known as possible soil pollutant, therefore its content in the soil should be controlled (Directive of limited ... , 1996). Our results showed that selected vineyards and pot soil used in the experiment can be classified as unpolluted, because the content levels of Zn were 2–fold lower than the regulated limits (Directive of limited ... , 1996). Similar results were obtained by Rusjan et al. (2006) who analysed 72 soil samples in Goriška Brda (24 sampling sites including vineyards of different age (< 5 to > 20 years) and landform as well as forests. Their results have proved that Zn content in Goriška Brda soils has not been increased by viticulture as the Zn contents measured in the vineyard soil were similar to the forest soil and has not increased by depth as well.

3.4.3 Soil extractions

A variety of single soil extractants are commonly used for estimation of plant nutrient availability (Rao et al., 2008; Zhang et al., 2002). Extracting reagents can be grouped into the four categories: (1) chelating agents, (2) diluted acids, (3) buffered and (4) un-buffered salt solutions. In our study, one or two extractants of each category have been used and evaluated for soil analysis considering two parameters; extraction pH and efficiency.

3.4.3.1 Extraction pH impact

The extraction procedures compared in preliminary study were composed of EDTA and DTPA as chelating agents, diluted acetic acid (CH_3COOH) as mild acids, buffered solution of amonacetate (CH_3COONH_4) and un-buffered salt solutions of $CaCl_2$ and NH_4NO_3. The pH of used extraction solvents ranged from acid (CH_3COOH and NH_4NO_3) to neutral (most of the solvents; Table 35).

Table 35: *pH value of extractants and soil extracts (mean value ± standard deviation).*

	pH	
Extractant	*Extractant solution*	*Soil extract*
0.05 mol L^{-1} EDTA	*7.0 ± 0.02* [a]	*7.9 ± 0.1*
0.005 mol L^{-1} DTPA/TEA	*7.3 ± 0.02* [a]	*7.4 ± 0.1*
0.43 mol L^{-1} CH_3COOH	*3.0 ± 0.1*	*4.2 ± 0.1*
0.01 mol L^{-1} $CaCl_2$	*7.0 ± 0.1*	*7.5 ± 0.1*
1 mol L^{-1} NH_4NO_3	*5.0 ± 0.1*	*7.5 ± 0.1*
1 mol L^{-1} CH_3COONH_4	*7.0 ± 0.02* [a]	*7.7 ± 0.1*
d-H2O	*6.9 ± 0.1*	*8.1 ± 0.1*

[a] *Adjusted pH.*

The extraction solutions have low effect on the soil pH and, therefore, the pH during the extraction process was mainly determined by the soil and not by the extractant (except in the case of acetic acid). The pH measured in majority of the extracts ranged between 7.4 and 8.1 (Table 35). The soil pH obtained with acetic acid extraction solvent was on contrary to the others in the acidic range, as expected. According to Menzies et al. (2007) acetic acid extraction gives poor representation of the true soil pH, hence resulting in changes to the soils characteristics and metal speciation. According to the results obtained (Table 35), acetic acid was employed for our soil analysis.

3.4.3.2 Extraction efficiency

The metal extraction efficiency obtained with the seven procedures studied is presented in the Table 36. The extractability of K obtained with the ammonium nitrate (5:25 soil to solvent ratio) and ammonium acetate procedures were comparable (approximately 180 mg kg^{-1}) and 2-fold higher than with $CaCl_2$ procedure. The ratios between different extraction solvents for K extractability were CH_3COOH < CH_3COONH_4 ~ NH_4NO_3 << EDTA < H_2O ~ $CaCl_2$ << DTPA. The NH_4NO_3 was a bit more efficient (10% higher) with 5:25 soil to solvent ratio than 10:25. In agreement with Pueyo et al. (2004) also our results have confirmed that $CaCl_2$ extracted medium (0.01 mol L^{-1}) is not very efficient for extraction of major elements in comparison to concentrated media NH_4NO_3 (1 mol L^{-1}). It is already known that ammonia ions (NH_4^+) are less effective as divalent cations (*e.g.* Ca^{2+}), but they can promote replacement of K ions in the interlayer exchanges sites of vermiculite (Gleyzes et al., 2002). According to Helmke and Sparks (1996) these ions are not truly 'exchangeable' and so K content in NH_4NO_3 or CH_3COONH_4 could be overestimated. The water-soluble K fraction was on the same level as in 0.01 mol L^{-1} $CaCl_2$ solution, confirming plant available K 'nature' of $CaCl_2$ fraction. Acetic acid gave the highest yields of K among all (223 ± 1 mg kg^{-1}), but according to the literature fraction recovered in such acid may be present as co-precipitated with minerals or as some specifically sorbed on the clay surface (Gleyzes et al., 2002; Helmke and Sparks (1996).

The same extraction solvents were tested also for Mg and gave the following results CH_3COOH < EDTA ~ CH_3COONH_4 < NH_4NO_3 ~ $CaCl_2$ ~ DTPA << H_2O. The NH_4NO_3 extractability of Mg was similar when using 5:25 or 10:25 ratio. Mg could be present in either the extracting reagents or in the soil matrix itself. The highest magnesium concentration was quantified in the NH_4NO_3 solution (approximately 18 mg L^{-1}), however the concentration of Mg in the NH_4NO_3 soil extract was higher (*e.g.* 35 mg L^{-1}) and so possible to quantify. Much smaller Mg contents were measured in $CaCl_2$ and EDTA solutions themselves, too, but still accounted to the final extracted amount of Mg. High Mg extractability obtained with acetic acid can

be attributed to the low pH of extraction procedure (≤ 5 pH) as described for K (Gleyzes et al., 2002).

Table 36: *Mean values ± standard deviation (n = 3; mg kg^{-1}) of single soil extracted fractions of K, Mg, Fe, and Zn in the vineyard soil.*[a]

Extractant (method) [b]	*K* (*mg kg^{-1}*)	*Mg* (*mg kg^{-1}*)	*Fe* (*mg kg^{-1}*)	*Zn* (*mg kg^{-1}*)
0.05 mol L^{-1} EDTA(5:50)	*106 ± 6*	*71 ± 7*	*117 ± 9*	*2.51 ± 0.22*
0.005 mol L^{-1} DTPA(10:20)	*37 ± 1*	*29.3 ± 0.1*	*20.5 ± 0.5*	*1.42 ± 0.04*
0.43 mol L^{-1} CH_3COOH(1:40)	*223 ± 1*	*87.4 ± 33*	*206 ± 13*	*6.53 ± 0.32*
0.01 mol L^{-1} $CaCl_2$ (5:50)	*77 ± 8*	*31.2 ± 1.6*	*8.6 ± 2.3*	*BQL* [c]
1 mol L^{-1} NH_4NO_3 (10:25)	*159 ± 1*	*31.7 ± 1.8*	*3.53 ± 0.59*	*0.06 ± 0.01*
1 mol L^{-1} NH_4NO_3 (5:25)	*176 ± 1*	*33.3 ± 1.3*	*Nm* [d]	*Nm*
1 mol L^{-1} CH_3COONH_4 (5:50)	*183 ± 5*	*65 ± 3*	*13.1 ± 3.8*	*BQL*
H_2O (5:50)	*82 ± 4*	*5.0 ± 0.1*	*30.8 ± 0.9*	*0.19 ± 0.01*

[a] *Extraction efficiency was tested in soil sampled in the vineyard from Goriška Brda.*

[b] *For details refer to 2.4.5.2 Extraction procedures – determination of plant available fraction, Table 12.*

[c] *Below quantification limit.*

[d] *Not measured.*

Extraction yields were for Fe and Zn very much affected by the low pH (similar as found for K and Mg), the highest with acetic acid: CH_3COOH << EDTA << H_2O < DTPA < CH_3COONH_4 < $CaCl_2$ < NH_4NO_3 and CH_3COOH << EDTA < DTPA << H_2O < NH_4NO_3 < CH_3COONH_4 ~ $CaCl_2$, respectively. The efficiency of EDTA extraction was much lower (approximately 40%) (117 ± 9 and 2.5 ± 0.2 mg kg^{-1} for Fe and Zn, respectively) than the CH_3COOH extraction, but still much higher (up to 80%) as comparing to other studied extractants (DTPA, $CaCl_2$, CH_3COONH_4 and NH_4NO_3). Both EDTA and DTPA complex reagents are according to literature widely used to access the phytoavailability of trace metals (Menzies et al., 2007). Because of it complexing ability, this reagents are less specific than *e.g.* acetic acid solution and are more able to extract metal ions that were bound to organic matter (Gleyzes et al., 2002). On the contrary, the Zn concentration quantified in the $CaCl_2$ and CH_3COONH_4 and NH_4NO_3 extracts were not much different than the procedural blank (approximately 0.01; 0.1 and 0.02 mg L^{-1}, respectively), indicating that these

extracting solvents are not suitable for predicting plant Zn availability for such soils, as used in our experiment. The use of these extractants may become effective for Zn contaminated soils (Pueyo et al., 2004).

On the bases of the result obtained and handling of the extraction procedures (*e.g.* extractant preparation), we decided to use four different exractants to assess the soil fractions: EDTA, $CaCl_2$, NH_4NO_3 and H_2O for pot soil extractions.

3.4.4 Estimation of plant available metal soil fraction

3.4.4.1 Correlations between metal contents of soil fractions and different parts of vines

Similarly, as for other results already discussed about plant macro-nutrients, more correlations were found for K and Mg than for Zn or Fe (Table 37). K content in petioles sampled at berry set was found to correlate well with EDTA, $CaCl_2$, and NH_4NO_3 fractions of the soil sampled in May or June 2009-2010 ($r^2 \geq 0.78$; $P < 0.001$). In addition, high statistical correlation was obtained among all three extractants ($CaCl_2$ *vs*. EDTA and NH_4NO_3 fractions; EDTA *vs*. NH_4NO_3; $r^2 = 0.99$; $P < 0.001$; Annex I). No correlations could be obtained between soil K and veraison petioles K, while EDTA-extractable K positively correlated with K content in grape berries ($r = 0.60$, $P < 0.05$). Relatively strong relationships ($r = 0.51$, $P < 0.05$) were obtained between H_2O- and $CaCl_2$-extractable K ($r = 0.51$, $P < 0.05$ in May/June; $r = 0.75$, $P < 0.01$ in October/November) and also between H_2O- and NH_4NO_3-fraction in soil sampled in October/November ($r = 0.75$, $P < 0.01$).

As found for K, Mg contents in petioles (sampled at veraison) resulted to correlate well with $CaCl_2$ and NH_4NO_3-extractable Mg ($r^2 = 0.77$, $P < 0.01$ and $r^2 = 0.94$, $P < 0.001$; respectively), but not with EDTA. Soil $CaCl_2$ fraction correlated well ($r^2 = 0.75$, $P < 0.01$) with Mg content in grape berries, too. Mg of NH_4NO_3 fraction was in positive linear and statistically significant relationship with $CaCl_2$ ($r^2 = 0.78$, $P <$

0.01) and EDTA (r^2 = 0.85, P < 0.001) extractable Mg (soil sampled in October/November).

Table 37: *Correlation between EDTA, $CaCl_2$, NH_4NO_3 and H_2O-soil extractable K, Mg, Fe and Zn and metal contents in plant tissues - grapes and petioles.*

Elem.	*Sample*	*EDTA*	*$CaCl_2$*	*NH_4NO_3*	*H_2O*
K	*Grape* [a]	*0.60**			
	Petioles at berry set [b]	*0.78****	*0.79***	*0.79****	
	Petioles at veraison [a]				
Mg	*Grape*		*0.75***		
	Petioles at berry set				
	Petioles at veraison		*0.94****	*0.77***	
Fe	*Grape*				
	Petioles at berry set	*-0.52**	*0.59**		
	Petioles at veraison				
Zn	*Grape*				
	Petioles at berry set				*0.72***
	Petioles at veraison	*0.57**			

[a] *Correlations were done comparing element contents in the soil extracts (soil sampled in October 2010 and November 2011) and in grape berries or in petioles sampled at veraison (n = 13-15).*

[b] *Correlations were done comparing element contents in the soil extracts (soil sampled in May and June 2009 and June 2010) and in petioles sampled at berry set (n = 13-15).*

In the case of micro-nutrients, negative correlations were obtained between Fe in the petioles sampled at berry set and EDTA-extractable Fe, while positive correlations were found for $CaCl_2$-extracted Fe (r^2 = -0.52, P < 0.05 and r^2 = 0.59, P < 0.05; respectively).

Correlation studies among the exctration reagents, revealed relatively good correlations between $CaCl_2$- and H_2O-extractable Fe in the soil sampled in October/November (r^2 = 0.64, P < 0.01), while a negative relationship was found between EDTA- and NH_4NO_3-extractable Fe in the soil sampled in May/June (r^2 = -0.59, P < 0.05). In the contrast to Fe, relatively strong positive correlation was obtained between petioles sampled at veraison and EDTA-extracted Zn (r^2 = 0.57, P

< 0.05) and even higher between petioles sampled at berry set and H_2O-extracted Zn (r^2 = 0.72, P < 0.05). No correlations were found between Zn-extracts of different extractants.

The complexing reagents (*e.g.* EDTA and DTPA) are a common approach for assessment of many trace metals phytoavailability (*e.g.* Zn, Cd, Ni). Concentrations of DTPA- and EDTA-extractable trace metals have been reported to poorly correlate ($r^2 \leq 0.50$) with plant uptake when compared across a wide range of soil types (see Menzies et al., 2007 and references therein). For speciation analysis of trace elements and evaluation of bioavailability Wang et al. (2001) recommended using wet rhizosphere soils rather than air-dried rhizosphere or bulk soil in order to simulate realistic conditions close to those in the environment. Good correlation ($r^2 > 0.80$; P < 0.01) between EDTA-extractable Zn in wet rhizosphere soil and Zn concentration in wheat roots has been confirmed by Zhang et al. (2002), too. In spite of this, Menzies et al. (2007) concluded, based on the analysed data set of different single soil test studies, that complexing reagents (*e.g.* DTPA and EDTA), and acid extractants (*e.g.* HCl) give poor correlation of extracted Zn to plant uptake. He concluded that, of all the extractant types examined, neutral salt solutions ($NaNO_3$) tended to provide the best relationship between soil-extractable trace metal and plant tissue accumulation, however only limited data sets are available and further research is required. In fact, each of the different extractants (*e.g.* 0.01 M $CaCl_2$, 0.1 M $NaNO_3$, 1.0 mol L^{-1} NH_4NO_3) have been reported to provide various benefits when compared to the others (Menzies et al., 2007) and so it is really important to test different extractants and to found the most suitable for the region and soil type.

In summary our results have shown that 0.01 mol L^{-1} $CaCl_2$ and 1 mol L^{-1} NH_4NO_3 solution seemed to be suitable for determination of K and Mg, since this two extractant gave good correlations between their contents in the soil extracts and concentrations in the leaf petioles. But, the NH_4NO_3 extraction efficiency was 1.8–fold higher (on the overall average of all measurements of pot soil) than $CaCl_2$ in the case of K most probably due to the differences in the concentration of salts ion (Gleyzes et al., 2002). NH_4NO_3 solution is known to have the affinity of extracting K in not ‘truly’ exchangeable sites in the soil (in ‘wedge’ zones of weathered

vermiculites) and therefore exchangeable K could be overestimated with this extractant (Helmke and Sparks, 1996). On the other hand, similar extracting efficiencies were obtained for Mg, but the correlations between Mg contents in soil extracts and plant organs was statistically higher in case of $CaCl_2$. For K, the correlations obtained between these two extractants were very high and statistically significant as well as between soil extracts and K, Mg concentrations in leaf petioles. In the further studies (described in the next section 3.4.4.2) $CaCl_2$-extracted K and Mg were used.

For the micro-elements, 0.05 mol L^{-1} EDTA (or water) was the only suitable for extracting Zn from the non-contaminated vineyard soils of Goriška Brda with good extractability and precision (< 10%) obtained for both micro-nutrients, Zn and Fe (only EDTA). EDTA-extractable Fe resulted negative correlation with Fe in leaves, while $CaCl_2$–extractable Fe was in positive relation with Fe content in petioles, but unfortunately unsatisfactory extraction precision (in some cases approximately 40%) was obtained. For that reason EDTA-extracted Fe and Zn were selected for further studies (section 3.4.4.2).

3.4.4.2 Changing of nutrient plant availability over years

As described in the previous section, $CaCl_2$-extracted K and Mg, and EDTA-extracted Fe and Zn were used for further analysis. Firstly, we wanted to evaluate the differences among the years (from 2009 to 2011) and secondly, among the treatments. We compare five treatments: 1#Control, 2#NPK (application of N, P, and K into the soil), 5#NPK Mg Fe l (foliar spraying of nutrients), 6#NPK Mg Fe hl (foliar spraying, higher concentration of nutrients), and 9#NPK Mg Fe s (fertirigation, application of nutrients into the soil).

In general, extractable amounts K, Mg, Fe, and Zn contents were very low (in relation to total element content measured), being under 1% for K, Mg, and Fe and a bit higher for Zn (3% on the average).

Table 38: *$CaCl_2$-extractable K concentration (mean values, n = 3) in the soil exposed to different treatments in years 2009–2011.*

	$CaCl_2$-extractable **K** (mg kg^{-1})				
Treatment [a]	June 2009	July 2010	October 2010	November 2011	P-value
1#Control	47 **A** a	48 **A** a	66 **B** a	83 **C** a	**
2#NPK	222 **C** d	121 **B** b	100 **A** b	125 **B** c	***
5#NPK Mg Fe l	188 **B** b	Nm [b]	114 **A** b	111 **A** b	**
6#NPK Mg Fe hl	200 **B** c	Nm	105 **A** b	104 **A** ab	***
9#NPK Mg Fe s	220 **B** d	Nm	106 **A** b	103 **A** ab	***
P-value	***	***	**	***	

Data were subjected to ANOVA test with either treatment or sampling date as the variability factor. For each column (a, b...) or for each row (A, B...), mean values followed by the same letter are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test.

[a] *For details refer to 2.1.3 Treatment layout, Table 8.*

[b] *Not measured.*

Table 39: *$CaCl_2$-extractable Mg concentration (mean values, n = 3) in the soil exposed to different treatments in years 2009–2011.*

	$CaCl_2$-extractable **Mg** (mg kg^{-1})				
Treatment [a]	June 2009	July 2010	October 2010	November 2011	P-value
1#Control	35.7 c	46.7	49.6 b	40.7 e	n.s.
2#NPK	38.3 **C** c	34.3 **BC**	25.1 **A** a	21.0 **AB** a	**
5#NPK Mg Fe l	32.6 **B** b	Nm [b]	24.8 **A** a	25.5 **A** b	***
6#NPK Mg Fe hl	26.9 **A** a	Nm	27.1 **A** a	33.0 **B** d	*
9#NPK Mg Fe s	36.6 **C** c	Nm	26.3 **A** a	30.0 **B** c	***
P-value	***	n. s.	***	***	

Data were subjected to ANOVA test with either treatment or sampling date as the variability factor. For each column (a, b...) or for each row (A, B...), mean values followed by the same letter are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test.

[a] *For details refer to 2.1.3 Treatment layout, Table 8.*

[b] *Not measured.*

The K extractability was 5–times lower in control soil (#1) in comparison to NPK treated soil in June 2009 and was also statistically confirmed ($P < 0.001$; Table 38).

However the difference diminished throughout the years and $CaCl_2$-extracted K was only 1.5–fold higher in NPK treated vines in October 2010 and November 2011. Additionally, extractable K was higher in October 2010 and November 2011 than in July 2010 or in June 2009 in the soil of control vines, while the situation was reversed in the soil of NPK treated vines (the highest extractability was measured in 2009). The same trend was observed by measuring K content in the leaf petioles of NPK treated vines at berry set and veraison (Table 24, section 3.2.3). By soil analysis ($CaCl_2$ extracting procedure) we can confirm that lower K uptake during the seasons from 2009 to 2011 was the consequence of lower K availability in the soil matrix.

Mg extractability was more or less constant (43 mg kg^{-1} on the overall average) in the control soil, while in the treated soil same statistically significant differences were observed from 2009 to 2011 (Table 39). $CaCl_2$-extracable Mg in the soil exposed to treatments #2, 5, and 9 was lower in October 2010 and November 2011 in comparison to June 2009 (or July 2010). The differences between control (#1) and NPK treatments (#2, 3, 6, 9) were observed in the soil sampled in October 2010 and November 2011, while in June 2009 the highest Mg extractability was not measured in the control soil. The differences observed in 2010 and 2011 can be attributed to N-fertilization with ammonium sulphate which was performed each year. In the contrast, no differences were obtained between soil where only NPK was added (#2) and soil where Mg fertilizer was applied into the soil (#9). The ratio between $CaCl_2$ extractable K and Mg was quite constant from 2009 to 2011 in the control soil being between 1.0 and 2.0, while in the soil of NPK treatment was approximately 6.2 in June 2009 and for 35% lower in 2010 and 2011 (4.0 on the overall average; Table 40). On the other hand, leaf analysis (Table 24, section 3.2.4) showed high statistical difference between control vines and NPK treated vines (#2-10) in all three years and not only in 2010 and 2011. The Mg content was by approximately 2–folds higher in the control petioles than in the petioles of NPK treated vines (#2). On the basis of the results obtained, we can say that the Mg uptake is much more affected by the ratio between K and Mg, than on available Mg (extractable Mg) in the soil matrix. In addition to K, nitrogen fertilization (NH_4^+ ions) negatively influenced Mg availability and its uptake. Therefore, application of fertilizers containing potassium and/or nitrogen negatively influences the uptake of Mg consequently leading to Mg

deprivation in grapevines, which is very often observed also in the vineyards of our region.

Table 40: *Ratio K:Mg (mean values ± standard deviation; n = 3) in the soil exposed to different treatments in years 2009–2011. Ratio was determined between $CaCl_2$ extractable K and Mg.*

	*Ratio **K:Mg***			
Treatment [a]	*June 2009*	*July 2010*	*October 2010*	*November 2011*
1#Control	*1.3 ± 0.1*	*1.0 ± 0.2*	*1.3 ± 0.1*	*2.1 ± 0.2*
2#NPK	*5.6 ± 0.2*	*3.5 ± 0.1*	*4.0 ± 0.7*	*4.2 ± 0.4*
5#NPK Mg Fe l	*5.6 ± 0.1*	*Nm* [b]	*4.6 ± 0.3*	*4.4 ± 0.3*
6#NPK Mg Fe hl	*7.5 ± 0.4*	*Nm*	*3.9 ± 0.2*	*3.2 ± 0.1*
9#NPK Mg Fe s	*6.0 ± 0.1*	*Nm*	*4.1 ± 0.1*	*3.4 ± 0.2*

[a] *For details refer to 2.1.3 Treatment layout, Table 8.*

[b] *Not measured.*

Table 41: *EDTA-extractable Fe concentration (mean values, n = 3) in the soil exposed to different treatments in years 2009–2011.*

	*EDTA-extractable **Fe** (mg kg^{-1})*				
Treatment [a]	*June 2009*	*July 2010*	*October 2010*	*November 2011*	*P-value*
1#Control	*121 **C** d*	*102 **A***	*102 **A** a*	*113 **B** a*	***
2#NPK	*108 **A** c*	*98 **A***	*104 **A** a*	*128 **B** ab*	*
5#NPK Mg Fe l	*84 **A** a*	*Nm* [b]	*106 **B** a*	*137 **C** b*	***
6#NPK Mg Fe hl	*104 **A** c*	*Nm*	*104 **A** a*	*127 **B** ab*	**
9#NPK Mg Fe s	*88 **A** b*	*Nm*	*134 **B** b*	*143 **C** b*	*
P-value	***	*n. s.*	***	*	

Data were subjected to ANOVA test with either treatment or sampling date as the variability factor. For each column (a, b...) or for each row (A, B...), mean values followed by the same letter are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test.

[a] *For details refer to 2.1.3 Treatment layout, Table 8.*

[b] *Not measured.*

Fe-extractability was statistically different among years and treatments (Table 41). In the pot soil of NPK treated vines (#2, 5, 6 and 9) Fe extractability increased from 2009 to 2011, while in the control soil the Fe availability was higher in 2009. In 2009, the highest Fe-content was measured in the control soil, while in 2010 and 2011 was the highest in the soil of treatment #9, where 'Foliacon Fe' was applied by fertirigation. On the other hand, no differences among years and these treatments were obtained by foliar analysis. Since negative correlation was obtained between EDTA-extractable Fe and Fe content in leaves, it is impossible to discuss results only on the basis of Fe-extraction.

Table 42: *EDTA-extractable Zn concentration (mean values, n = 3) in the soil exposed to different treatments in years 2009–2011.*

	*EDTA-extractable **Zn** (mg kg^{-1})*				
Treatment [a]	*June 2009*	*July 2010*	*October 2010*	*November 2011*	*P-value*
1#Control	*2.23 **A** a*	*3.03 **B** b*	*2.92 **B** b*	*3.35 **C** a*	***
2#NPK	*2.44 **A** d*	*2.58 **A** a*	*2.96 **B** b*	*3.74 **C** ab*	***
5#NPK Mg Fe l	*2.38 **A** c*	*Nm* [b]	*3.59 **B** c*	*4.07 **B** b*	*
6#NPK Mg Fe hl	*2.31 **A** b*	*Nm*	*2.75 **B** ab*	*3.06 **C** a*	**
9#NPK Mg Fe s	*2.45 **A** d*	*Nm*	*2.63 **B** a*	*3.18 **C** a*	**
P-value	***	*	***	*	

Data were subjected to ANOVA test with either treatment or sampling date as the variability factor. For each column (a, b...) or for each row (A, B...), mean values followed by the same letter are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test.

[a] *For details refer to 2.1.3 Treatment layout, Table 8.*

[b] *Not measured.*

More clear results were obtained for zinc. Zn extractability increased from June 2009 to November 2011, which was also statistically confirmed ($P < 0.05$) in all five treatments (#1, 2, 5, 6, and 9; Table 42). The statistical differences were obtained also among treatments; however they were not consistent during the years. On the contrary, Zn concentration has been increasing in the petioles of control (#1) and NPK (#2) treated vines (at veraison) from 2009 to 2011 (from 32-to-38-to-53 mg kg^{-1} and from 40-to-51-to-69 mg kg^{-1}, respectively). In addition the difference between control and NPK treated vines was also statistically confirmed (Table 44, section

3.2.2). In other three treatments (#5, 6 and 9) Zn content increased only from 2010 to 2011 not from 2009 to 2010 (data not showed). Moreover, it seems that application of NPK did not influence Zn availability (EDTA-extractable Zn), since Zn extracted in NPK treated soil was not higher comparing to the control soil during the experiment. On the basis of the results obtained, we can conclude that soil application of NPK does not have much effect on soil Zn-availability (or Zn-extractability) as it had it on Zn uptake (Zn concentration was much higher in the petioles of NPK treated vines in comparison to the control vines in all three years).

3.5 COMPARISON BETWEEN POTTED GRAPEVINES AND VINEYARD SAMPLES

Four vineyards planted with 'Rebula' (*Vitis vinifera* L.) grapevines located nearby the pot experiment in Goriška Brda were selected in order to compare the results obtained in the pot experiment with the grapevines grown in 'natural' environment. Vineyard B represented grapevines of the same cultivar and rootstock planted in the vineyard soil which we have used for the pot experiment.

Table 43: *Total K, Mg, Fe, and Zn contents (mean values; n = 3) in the vineyard and pot soil in 2011.*

Soil sample	*K (g kg^{-1})*	*Mg (g kg^{-1})*	*Fe (g kg^{-1})*	*Zn (mg kg^{-1})*
Untreated pot soil	*17.1 c*	*10.4 cd*	*32.0*	*103 b*
Treated pot soil	*17.7 c*	*10.4 cd*	*32.7*	*104 b*
Vineyard A	*15.2 b*	*9.9 bc*	*35.6*	*112 b*
Vineyard B	*17.4 c*	*10.6 d*	*33.6*	*105 b*
Vineyard C	*17.6 c*	*9.7 b*	*32.9*	*102 b*
Vineyard D	*14.1 a*	*8.0 a*	*30.6*	*80 a*
P-value	***	***	*n. s.*	**

*Data were subjected to ANOVA test (*P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant). Mean values followed by the same letters are not significantly different at 0.05 probability level in accordance with the Student-Newman-Keuls´s test.*

3.5.1 Soil analysis

The obtained soil pH values for all four vineyards were very similar to the pH values of the pot soil (pH_{H2O} = 8.1 ± 0.1; pH_{KCl} = 7.6 ± 0.1; pH_{CaCl2} = 7.7 ± 0.1) and in agreement with Rusjan et al. (2006) characteristic for the sub-Mediterranean winegrowing region. Total K, Mg, Fe and Zn contents in the vineyards A, B, and C were similar to the contents measured in the pot soil (Table 44). However, vineyard D contained lower ($P < 0.01$) content of K, Mg, and Zn in comparison to the pot soil and to the other three vineyards, while Fe content was not statistically different. Measured Zn values (from 80 to 112 mg kg^{-1}) in selected vineyards were in the same range as found by Rusjan et al. (2006) for these region.

Table 44: *$CaCl_2$-extractable K and Mg and EDTA-extractable Fe and Zn concentrations (mean values ± standard deviation; n = 3) in the vineyard and pot soil in 2011.*

	K *(mg kg^{-1})*	***Mg*** *(mg kg^{-1})*	***Fe*** *(mg kg^{-1})*	***Zn*** *(mg kg^{-1})*
Sample	*$CaCl_2$*		*EDTA*	
Vineyards				
A	*65.6 ± 0.4*	*39.6 ± 0.2*	*154 ± 5*	*4.00 ± 0.13*
B	*128 ± 0.8*	*53.5 ± 0.2*	*133 ± 6*	*6.01 ± 0.25*
C	*101 ± 2*	*39.5 ± 0.5*	*207 ± 4*	*5.21 ± 0.02*
D	*56.6 ± 5*	*18.9 ± 0.3*	*163 ± 1*	*1.25 ± 0.01*
Average value	*88 ± 32*	*38*	*164*	*3.4*
(CV %)	*(32)*	*(33)*	*(16)*	*(43)*
Pot soil				
1#Control	*83 ± 8*	*40.7 ± 0.2*	*113 ± 1*	*3.35 ± 0.08*
2#NPK	*125 ± 4*	*29.6 ± 0.3*	*128 ± 10*	*3.74 ± 0.10*

$CaCl_2$-extractable K and Mg, and EDTA-extractable Fe and Zn in the vineyard soils are presented in Table 45. Element extractability differs among vineyards for all four elements. The average values of extractable K, Mg and Zn were similar to the control pot soil, while EDTA-extractable Fe was a bit higher than in the studied soil. The variability (CV %) among vineyards was high for K, Mg, and Zn. In 2011, similar

extractable quantity of K and Fe were found in NPK treated pot soil (#2) and in the Vineyard B, where soil for pots was taken few years ago (in 2007). However, $CaCl_2$-extractable Mg was 1.8–fold lower in NPK treated vines than in the Vineyard B, while in the control soil the extractability was more close to the value measured in the vineyard sample (40.7 *vs*. 53.5 mg kg^{-1}).

3.5.2 K, Mg, Fe, and Zn contents in leaf petioles and grape berries

As in the pot experiment, we measured K, Mg, Fe, and Zn content in the leaf petioles (at berry set and veraison) and in the whole grape berries (at harvest). In 2009 and 2011, the average K content in the leaf petioles were 21 ± 7 g kg^{-1} at both sampling times (from 12.4 to 31.8 g kg^{-1}), while the average Mg content was 2.7 ± 0.6 g kg^{-1} at berry set and from 2.6 to 8.8 g kg^{-1} at veraison. Increasing in Mg contents from berry set to varaison was seen also in the pot experiment. K and Mg contents in the leaf petioles sampled at veraison 2011 are presented in the Table 46.

Table 45: *K, Mg, Fe and Zn contents (mean values ± standard deviation; n = 3) in the petioles of 'Rebula' grapevine grown in four vineyards and in the pot soil at veraion 2011.*

	Petioles (D.W.) at veraison 2011				
Sample	***K*** *(g kg^{-1})*	***Mg*** *(g kg^{-1})*	***K:Mg***	***Fe*** *(mg kg^{-1})*	***Zn*** *(mg kg^{-1})*
Vin A	*17.3 ± 0.5*	*5.55 ± 0.14*	*3.1 ± 0.1*	*13.6 ± 0.8*	*50.7 ± 0.4*
Vin B	*25.5 ± 0.7*	*2.82 ± 0.04*	*9.1 ± 0.2*	*13.7 ± 0.3*	*44.1 ± 0.4*
Vin C	*18.4 ± 0.3*	*3.87 ± 0.10*	*4.8 ± 0.1*	*16.0 ± 0.7*	*47.3 ± 1.9*
Vin D	*31.8 ± 0.1*	*2.64 ± 0.01*	*12.0 ± 0.1*	*15.5 ± 0.2*	*51.4 ± 2.5*
1#Control	*28.5 ± 2.8*	*2.31 ± 0.02*	*12.3 ± 1.1*	*12.2 ± 0.6*	*53.0 ± 3.2*
2#NPK	*35.3 ± 2.4*	*1.25 ± 0.13*	*27.4 ± 4.9*	*13.8 ± 0.6*	*68.6 ± 1.9*

The ratio K:Mg in the petioles sampled at veraison 2011 showed big differences between Vin A and Vin C (3.1 and 4.8, respectively) *vs*. Vin B and Vin D (9.1 and 12, respectively) (Table 46). The differences among vineyards in K and Mg leaf contents or K:Mg ratio can be explained by the differences in the vine rootstock.

Grapevines of Vin A and Vin C, grafted on the 1103 Paulsen rootstock, had in agreement with Stefanini et al. (1994) good Mg absorption capacity. On the contrary, grapevines of Vin B and Vin D grafted on SO4 rootstock are known to poorly absorb Mg (Stefanini et al., 1994), as already discussed for the potted grapevines in the Section 3.2.4. Ratio K:Mg in the leaf petioles of control vines sampled at veraison (11.4 on the overall average in 2009–2011) was similar as found in the Vin B and Vin D.

In the contrast to macro-elements, lower variability was observed by measuring Fe and Zn content in the leaf petioles. Fe content was similar at berry set and veraison in the leaf petioles sampled in four vineyards being 15.3 ± 1.5 mg kg^{-1} (on the average), while Zn content increased from berry set to varaison (from on the average 27.3 to 49.1 mg kg^{-1} in the Vin A and Vin B, in 2009) as seen in the pot experiment, too. Fe and Zn contents in the leaf petioles sampled at veraison 2011 are shown in the Table 46.

Table 46: *K, Mg, Fe and Zn contents (mean values ± standard deviation; n = 3) in the whole grape berries of 'Rebula' grapevine grown in four vineyards and in the pot soil at harvest 2011.*

	Grapes (D.W.) at harvest 2011				
Sample	***K*** *(g kg^{-1})*	***Mg*** *(g kg^{-1})*	***K:Mg***	***Fe*** *(mg kg^{-1})*	***Zn*** *(mg kg^{-1})*
Vin A	*9.5 ± 0.1*	*0.44 ± 0.02*	*21.5 ± 0.9*	*7.5 ± 0.01*	*3.17 ± 0.05*
Vin B	*10.9 ± 0.2*	*0.44 ± 0.01*	*24.6 ± 0.9*	*7.1 ± 0.7*	*3.01 ± 0.15*
Vin C	*12.9 ± 0.2*	*0.48 ± 0.01*	*26.8 ± 0.4*	*5.9 ± 0.7*	*2.91 ± 0.23*
Vin D	*10.1 ± 0.2*	*0.41 ± 0.02*	*24.4 ± 1.1*	*8.8 ± 0.01*	*3.73 ± 0.20*
1#Control	*12.0 ± 0.3*	*0.55 ± 0.03*	*21.7 ± 0.6*	*6.8 ± 0.3*	*3.80 ± 0.08*
2#NPK	*14.2 ± 0.6*	*0.47 ± 0.06*	*30.5 ± 2.7*	*7.6 ± 0.6*	*5.09 ± 1.04*

Element contents of the whole grape berries harvested in 2011 on grapevines grown in the vineyards were similar as measured in grapes harvested on the potted vines (Table 47). K and Zn contents (11.6 ± 2 g kg^{-1} and 3.38 ± 0.45 mg kg^{-1}, respectively) of the vineyard samples were more close to values found in the control vines, while Mg contents were more similar to NPK treated vines.

3.5.3 Grape quality parameters

The main grape quality parameters measured in selected 'Rebula' vineyards in 2009 (Vin A, B), 2010 (Vin A, B), and 2011 (Vin A, B, C, D) are summarised in Table 48. Berry weight was on the average 33% higer in the grape harvested in the vineyard than in the grape samped in the pot experiment and was, as asspected, the most affected parameter by the growing conditions in the pots. The trend of organic acids was similar as faund in the pot experiment (Table 28) with higher total acidity and tartaric acid content in 2010 in comparison to 2011 (7.5 *vs*. 5.5 and 5.2 *vs.* 3.9 on the averge, respectively).

Table 47: *Main grape quality parameters (mean values) of 'Rebula' vineyards in 2009, 2010 and 2011.*

Year	*Sugar content (°Brix)*	*Berry weight (g berry^{-1})*	*pH*	*Total acidity (g L^{-1})*	*Tartaric acid (g L^{-1})*	*Malic acid (g L^{-1})*
2009	*18.4*	*2.82*	*3.3*	*5.9*	*4.5*	*2.9*
2010	*20.5*	*2.50*	*3.1*	*7.5*	*5.2*	*3.3*
2011	*20.1*	*2.22*	*3.2*	*5.5*	*3.9*	*2.1*

Total HCAs (sum of *trans*-CTA, CoTA, and FTA) and *trans*-CTA content were evaluated in juice of grape harvested in the vineyards in 2009 (Vin A, B) and in 2011 (Vin A, B, C and D). The average total HCAs content and *trans*-CTA content did not differ between years 2009 and 2011 (total HCAs: 67 ± 11 and 68 ± 7 mg L^{-1}, respectively; *trans*-CTA: 59 ± 10 mg L^{-1} in both years). Total HCAs and *trans*-CTA contents were approximately 10% lower than measured in the NPK treted vines (#2) in 2009 (75 ± 8 and 64 ± 6 mg L^{-1}, respectively), while in 2011 hydroxicinnamates were approximately 30% higher than in potted grapevines (45 ± 2 and 38 ± 2 mg L^{-1}, respectively). However, *trans*-CTA content was in both years lower in the vineyard samples than in the grape of control vines (see Table 32; Section 3.3.3.2). The percentage of individual HCAs (% *trans*-CTA, CoTA, and FTA) did not differ between two years (on the averge 87.9, 10.3, 1.8% in 2009 and 86.9, 11.6, 1.5% in

2011) and was similar as found in the grape juice of potted grapevines (Table 32; Section 3.3.3.2).

Our data showed that potted grapevines behaved similar (similar trends) as grapevines grown in the natural environment – in the vineyard.

4 CONCLUSIONS

Our results showed that NPK soil fertilization positively influenced K and Zn contents in leaves and grape berries, and negatively Mg, while the uptake of Fe seemed to be regulated by the soil type/characteristics (calcareous soil). In contrast, foliar spraying with Fe-fertilizer could increase the element content in the leaf petioles at berry set and veraison. The obtained results also suggested that application of NPK in the soil coupled with Mg and Fe fertilization was 'profitable' for Zn increase in grape berries and that K (synergistic activity) and Mg (antagonistic activity) could have some influence on Zn uptake.

$CaCl_2$-extractable K and Mg presented good correlations with the same element contents in the leaf petioles. The difference in K availability between the control and NPK-treated soils diminished throughout the years as the consequence of K uptake and removal by leaf and grapes development during the seasons (K was applied only in 2008). It seems that K uptake in the 'Rebula' vines is regulated by the available vine fraction ($CaCl_2$-extractable) in the soil matrix, since K was much higher in 2009 than in 2011 in NPK-treated petioles and not determined only by plant needs. Therefore, adequate K application is of great importance and should not be overestimated since it influences also Mg uptake which is affected by the ratio between K and Mg in the soil matrix. Therefore, application of fertilizers containing potassium negatively influences the uptake of Mg, consequently leading to Mg deprivation in grapevines, which is very often observed as chlorosis in the vineyards of our region. However, Mg uptake is highly influenced also by the grapevine rootstock and negatively influenced by the nitrogen fertilization, observed in our study. Unfortunately, the positive effect of Mg fertilization (Haefs et al., 2002) could not be observed in our experiment neither by foliar spraying nor by fertirigation, most probably due to high K uptake or an inadequate fertilization rate (Mg fertilization may be repeated several times during the vegetative period).

Our results suggested that NPK application does not have much effect on soil Zn availability (or EDTA Zn extractability) as it has it on Zn uptake (In all three years Zn concentration was much higher in the petioles of NPK-treated vines in

comparison to the control vines). These facts are really important from the environmental point of view, suggesting that application of basic macro-nutrients could elevate the Zn uptake in grapevines, thus 'diminishing' the accumulation of this heavy metal in the soil matrix. Our observations are in agreement with the results obtained by Rusjan et al. (2006) analysing many vineyard samples in the sub-Mediterranean winegrowing region where no Zn soil contaminations have been found. On the other hand, Zn is essential nutrient and its availability is very poor in alkaline soils, typical for this winegrowing region. Therefore, higher Zn uptake in NPK-treated vines is important for reaching an adequate Zn content in the grapevine leaves.

Among the studied elements, Fe is the only one whose uptake is much more regulated by the soil properties (pH, organic matter and carbonates) than by its content in the soil. EDTA-soil extractions were not a good indicator of Fe uptake. On the other hand, our results showed that foliar spraying of 'Foliacon Fe' elevates Fe content in leaves; however, fertilization should be repeated several times to ensure an adequate Fe concentration in leaves.

To sum up the results, the seasonal variations and the nutrients applied provided a relatively high modification of grapes quality parameters. Out of the experiment here described with 'Rebula' potted grapevines, the application of nutrients (NPK) affected FAN and HCAs with a higher magnitude than seasonal variability, while organic acids and pH in grape juice seemed to be more influenced by the season. Our results highlighted a highly negative correlation between K and sugar contents in the grapes, which is the opposite to the positive effect of K reported in the literature (Amiri and Fallahi, 2007; Topalović et al., 2011). Statistically significant correlations were obtained between sugar content and Zn content in grape (antagonism) and Mg content in the petioles (synergism), too. Moreover, our findings suggested that NPK supply lowered the amount of *trans*-CTA in grape juice, thus decreasing numerous reactions that occur during wine-making. A negative correlation between K in grape berries/petioles and the *trans*-CTA content and a positive one with Mg was found. The effect of fertilisation on HCAs occurrence in wine grapes has never been studied before.

The element leaf analysis showed the plant nutritional status and reflected the ‘actual’ nutrient uptake by a plant species. However, routine laboratories bring much higher attention to the soil analysis rather than to the leaf analysis, which are ‘left to the customer's request’. Nevertheless, higher prices and sometimes missing knowledge of the importance of leaf analysis in plant nutrition could be the main factor for vine growers and winemakers to not perform such analyses in greater extent. Our results suggested that soil analyses should not be the only data on which fertilization in viticulture is based on and that leaf analysis (petioles) should be included more in the sustainable viticulture practise in the future. Monitoring leaves (petioles) element content offers a better control over the actual consumption of nutrients and the plant needs for fertilization treatments. With the rational combination of soil analysis (before planting) and leaf analysis (at least four years after planting a new vineyard) we could ensure adequate element contents in the grapevines and, on the other hand, avoid the unnecessary application of elements and their accumulation in the environment.

Best to our knowledge, our work is the first multi-level study on ‘Rebula’ grapevines, including grapevines nutritional status determination by the element leaf and grape analyses, nutrient soil availability and, finally, the impact on grapes quality parameters taking into account also seasonal variations. With the results obtained in our work, we expect to contribute to a better understanding of plant nutrient uptake, especially for *Vitis vinifera* L. cv ʻRebula’, which is a study organism of this research. Our results revealed some new facts about the uptake of nutrients from flysh and marlstone soil by a plant organism, which is very well adapted to such soil and represents the natural heritage of Slovenian viticulture as well. The statistical processing of data obtained in the multi-annual pot experiments allowed us to compare data on the various nutrient applications and ʻRebula’ vine nutrient uptake along with grapes quality. The information obtained in our research will be useful for winegrowers and winemakers as well, since it is worth nothing that a balanced vine growth is of crucial importance for the grape/wine quality. Knowledge of the leaf elemental analysis and soil extractions will supplement or even ‘improve’ routinely used methods already performed by national laboratories in agricultural and other institutes.

5 SUMMARY

Grapevine nutrient oversupply as well as its shortage can both result in an unbalanced vine growth and poor grape production, thus mineral fertilization is a powerful tool also in viticulture to increase the yields and improve grapes quality. On the other hand, a repeated use of metal-enriched chemicals, such as fungicides and pesticides, farm manures and chemical fertilizers can contribute variable amounts of trace elements, especially heavy metals, in the environment. However, the uptake of one mineral does not depend only on the nutrient availability in the soil, since interactions among elements – synergism and/or antagonism – can influence the plant nutrient uptake. Interaction between K and Mg is one of the widely known antagonisms in grapevine, and often leads to Mg deficiency. Alkaline (calcareous) soils limit the uptake of low mobile micro-elements, such as Fe and Zn, which are important grapevine nutrients.

An experimental trial was carried out in Podsabotin (Goriška Brda, West Slovenia) on a local white variety named 'Rebula' (Vitis vinifera L.), grafted on SO4 rootstock, during four consecutive seasons (2008-2011). The effects of different fertilization treatments - application of basic nitrogen, phosphorus and potassium, in combination with the foliar or soil application of fertilizers containing Mg, Fe, and Zn - were investigated as related to the nutrient status and grapes quality parameters. The uptake study was conducted in pots to minimize factors affecting the plant uptake (pH, moisture, soil, location, etc.) as all vines were grown in the same, sieved soil, collected in the vineyard of Goriška Brda. Nutrient uptake was determined by measuring the concentrations of K, Mg, Fe and Zn in the leaf petioles and whole-grape berries using the flame atomic absorption spectrometry. Grapes quality parameters (soluble solids, pH, organic acid contents, and free amino nitrogen) were determined by WineScan spectrometer. Hidroxycinnamic acids in grape juice were quantified by HPLC–DAD method. Different single soil extractants (EDTA, $CaCl_2$, NH_4NO_3 and H_2O) were used to estimate the nutrient availability of grapevines and to find out the correlations between element contents in the grapevine organs and plant-available fraction in the soil. All results have been statistically evaluated with appropriate tests (ANOVA, Student-Newman-Keuls´s test, simple correlation

analysis) to weigh up the effect of fertilizing (foliar spraying and fertirigation) on the concentrations of studied elements in the plant`s organs and on grapes quality.

Our results showed that NPK soil fertilization positively influenced K and Zn contents in leaves and grape berries, and negatively Mg, while the uptake of Fe seemed to be regulated by the soil type/characteristics (calcareous soil). In contrast, foliar spraying with Fe-fertilizer could increase the element content in the leaf petioles at berry set and veraison. In addition to K and N fertilization impact, Mg uptake is highly influenced by the grapevine rootstock.

$CaCl_2$-extractable K and Mg presented good correlations with the same element contents in the leaf petioles. Our results suggested that NPK application does not have much effect on soil Zn availability (or EDTA Zn extractability) as it has it on Zn uptake (in all three years Zn concentration was much higher in the petioles of NPK-treated vines in comparison to the control vines). These facts are really important from the environmental point of view, suggesting that application of basic macro-nutrients could elevate the Zn uptake in grapevines, thus 'diminishing' the accumulation of this heavy metal in the soil matrix.

Our results showed that the vintage and fertilization treatments have a relatively high influence on grapes quality parameters. Moreover, our findings suggested that NPK supply lowered the amount of trans-CTA in grape juice, thus decreasing numerous reactions that occur during wine-making. A negative correlation between K in grape berries/petioles and the trans-CTA content and a positive one with Mg were found. The effect of fertilisation on HCAs occurrence in wine grapes has never been studied before.

The element leaf analysis showed the plant nutritional status and reflected the 'actual' nutrient uptake by a plant species. Our results confirmed the fact that cultivar and rootstock are, in addition to soil properties, a very important parameter determining the nutrient uptake. Our results suggested that soil analyses should not be the only data on which fertilization in viticulture are based on and that leaf

analysis (petioles) should be included more in the sustainable viticulture practise in the future.

Keywords: Grapevine, 'Rebula', Fertilization, Potassium, Magnesium, Iron, Zinc, Grape quality, Hidroxycinnamic acids, Single soil extraction.

6 POVZETEK

DOLOČITEV DEJANSKEGA PRIVZEMA ESENCIALNIH HRANIL V RAZLIČNE DELE VINSKE TRTE SORTE REBULA (*Vitis Vinifera* L.)

Znano je, da lahko tako pomanjkanje kot presežek hranil povzročita neuravnoteženo rast vinske trte in s tem vplivata na produkcijo grozdja. Gnojenje, uporabljeno v skladu s priporočili dobre vinogradniške prakse, je tako lahko pomembno orodje za izboljšanje donosa in kakovosti grozdja. Po drugi strani pa lahko z uporabo umetnih pripravkov kot so fungicidi, pesticidi, organska in mineralna gnojila vnesemo v okolje elemente v sledovih, zlasti težke kovine. Zavedati se moramo, da privzem hranil ni odvisen le od razpoložljivosti hranil v tleh, ampak tudi od pozitivnih oz. negativnih interakcij med elementi (sinergizem oz. antagonizem). Med najbolj znane interakcije pri vinski trti spada antagonizem med kalijem in magnezijem, ki pogosto vodi k pomanjkanju magnezija v rastlini. Na privzem hranil vplivajo tudi fizikalno-kemijske značilnosti tal. Alkalna (karbonatna) tla omejujejo zlasti privzem mikroelementov kot sta železo in cink.

Poskus je bil izveden na lokalni beli sorti Rebula (Vitis vinifera L.), cepljeni na podlagi SO4, in sicer v kraju Podsabotin (Goriška Brda, Zahodna Slovenija) v letih 2008–2011. Z lončnim poskusom smo zmanjšali vpliv nekaterih dejavnikov (pH, vlaga, zemlja in lokacija), ki lahko vplivajo na privzem hranil v rastlino. Trse smo posadili v presejano zemljo iz izbranega vinograda v Goriških Brdih. Proučevali smo vpliv osnovnega NPK (dušik – fosfor – kalij) gnojenja v kombinaciji z listnim škropljenjem ali fertirigacijo s sredstvi na osnovi magnezija, železa in cinka na prehranski status rastline in kakovost grozdja. Vsebnost kalija, magnezija, železa in cinka v listnih pecljih in grozdnih jagodah smo določali s plamensko atomsko absorpcijsko spektrometrijo. Parametre kakovosti grozdja kot so vsebnost sladkorjev in organskih kislin, vrednost pH in prosti aminokislinski dušik smo določali s pomočjo WineScan spektrometra; hidroksicimetne kisline v grozdnem soku pa smo kvantificirali z metodo HPLC-DAD. S pomočjo različnih ekstraktantov (EDTA, $CaCl_2$, NH_4NO_3 in H_2O) smo pripravili ekstrakcije tal in vsebnost elementov v zemeljski frakciji (s pomočjo korelacij) primerjali z vsebnostjo elementov v listih.

Vse rezultate smo statistično ovrednotili z ustreznimi testi (ANOVA, test Student-Newman-Keuls in korelacijska analiza) in tako ovrednotili vpliv gnojenja na vsebnost preučevanih elementov v rastlini in na kakovost grozdja.

Pridobljeni rezultati štiriletne študije (2008–2011) so pokazali, da dodajanje hranil NPK pozitivno vpliva na vsebnost kalija in cinka v listih in grozdnih jagodah, negativno pa na vsebnost magnezija. Privzem železa je v večji meri odvisen od tipa tal, medtem ko je privzem magnezija poleg odvisnosti od negativnega vpliva kalija in dušika odvisen tudi od podlage vinske trte. Rezultati so pokazali tudi statistično značilne korelacije med vsebnostjo kalija in magnezija ekstrahiranega s $CaCl_2$ ter vsebnostjo le-teh elementov v listnih pecljih. Rezultati kažejo, da dodajanje NPK ne vpliva na dostopno obliko cinka v tleh (ekstrakcija z EDTA), ampak na sam privzem cinka v rastlino (koncentracija cinka je bila v vseh treh letih veliko večja v listnih pecljih tretitranih (NPK) trsov v primerjavi s netretiranimi trsi). Pridobljeni podatki so pomembni tudi z okoljskega vidika, saj kažejo na to, da uporaba osnovnih makro-hranil lahko poveča privzem cinka v vinsko trto in tako zmanjša kopičenje te težke kovine v tleh.

Rezultati so pokazali, da imata tako vpliv sezone kot dodajanje hranil velik vpliv na parametre kakovosti grozdja. Z dodajanjem hranil NPK lahko zmanjšamo vsebnost trans-kaftarne kisline v grozdnem soku. Pokazala se je negativna korelacija med kalijem v grozdnih jagodah/pecljih in vsebnostjo trans-CTA ter pozitivna korelacija z magnezijem in vsebnostjo trans-CTA. Vpliv gnojenja na hidroksicimetne kisline še ni bil raziskan.

Z analizo elementov v listih določamo prehranski status rastline in dejanski privzem hranil v rastlino. Rezultati so potrdili dejstvo, da sta tako sorta kot tudi podlaga vinske trte pomembna parametra, ki vplivata na privzem hranil. Za dosego uravnoteženga in trajnostnega pristopa, naj bi dodajanje hranil temeljilo na povezavi med analizo tal in listov.

Ključne besede: Vinska trta, ‘Rebula’, Gnojenje, Kalij, Magnezij, Železo, Cink, Kakovost grozdja, Hidroksicimetne kisline, Ekstrakcija tal.

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ANNEXES

Annex A

Distribution of monthly rainfall (rain) and average temperatures (T_{avr}) during the experiment (2008-2011).

Year	***2008*** [a]		***2009*** [b]		***2010*** [b]		***2011*** [b]	
Month	*Rain (mm)*	*T_{avr} (°C)*	*Rain (mm)*	*T_{avr} (°C)*	*Rain (mm)*	*T_{avr} (°C)*	*Rain (mm)*	*T_{avr} (°C)*
January	*145*	*5.9*	*84*	*6.1*	*74*	*2.6*	*45*	*4.0*
February	*53*	*5.4*	*132*	*5.3*	*246*	*5.2*	*74*	*6.5*
March	*110*	*8.4*	*259*	*9.0*	*40*	*8.4*	*168*	*9.7*
April	*143*	*12.3*	*89*	*15.3*	*59*	*13.8*	*44*	*15.8*
May	*161*	*18.1*	*41*	*19.9*	*224*	*16.5*	*133*	*19.4*
June	*146*	*21.5*	*162*	*20.2*	*4*	*21.4*	*144*	*21.7*
July	*243*	*23.2*	*108*	*23.3*	*458*	*21.9*	*219*	*22.6*
August	*111*	*23.9*	*27*	*25.6*	*105*	*22.6*	*11*	*25.2*
September	*65*	*18.0*	*76*	*21.0*	*354*	*17.8*	*120*	*22.8*
October	*90*	*14.8*	*113*	*14.3*	*97*	*13.3*	*214*	*14.2*
November	*248*	*9.6*	*134*	*10.0*	*389*	*10.2*	*26*	*10.4*
December	*342*	*5.6*	*256*	*5.4*	*0*	*2.0*	*99*	*14.1*
Sum	***1857***		***1481***		***2050***		***1297***	

[a] *Weather Station of Capriva del Friuli (ARPA-OSMER FVG, Italy).*

[b] *Weather Station of Biljana (WMR 200, Oregon Scientific, Oregon, U.S.).*

Annex B

Ammonium lactate-extractable K_2O and P_2O in soil taken in the pots with planted vine prior to application of fertilizers (analysis performed by internal Al-method by Agricultural and Forestry Institute of Nova Gorica, 2007).

Parameter	*P_2O_5 (mg 100^{-1} g^{-1})*	*K_2O (mg 100^{-1} g^{-1})*
Value	*< 2*	*10*
Guide	*13-20*	*16-25*

Annex C

Measured K, Mg, Fe, and Zn concentrations in agrochemicals, used in the pot experiment. The results are given in g kg^{-1} (n = 2).

Fertilizer (nutrient)	*K*	*Mg*	*Fe*	*Zn*
Ammonium sulphate (N)	*< 0.1*	*< 0.01*	*≤ 0.01*	*< 0.01*
Potassium sulphate (K)	*430 ± 50*	*< 10*	*≤ 0.1*	*< 0.01*
Phosphorus oxide (P)	*< 10*	*< 10*	*< 0.01*	*< 0.01*
Bittersalz (Mg)	*< 10*	*94 ± 2*	*< 0.1*	*< 0.01*
Foliacon Fe (Fe)	*< 1*	*< 0.1*	*52 ± 3*	*< 0.01*
Zinc 25 (Zn)	*< 1*	*< 0.1*	*< 0.1*	*270 ± 30*
Mikal (fungicide)	*< 1*	*< 0.1*	*< 0.1*	*≤ 0.01*
Kumulus DF (fungicide)	*< 1*	*≤ 0.01*	*< 0.1*	*≤ 0.01*

Annex D

Pearson's correlation coefficients between macro-elements (K, Mg) and in grapes and petioles sampled in 2009-2011.

Element/ sample		*Year*	***K*** *g* [a]	*p1* [b]	*p2* [c]	***Mg*** *g*	*p1*	*p2*
K	*g*	*2009*	*x*					
		2010	*x*					
		2011	*x*					
	p1	*2009*	*0.51**	*x*				
		2010		*x*				
		2011	*0.52**	*x*				
	p2	*2009*	*0.65***	*0.81****	*x*			
		2010	*0.71***	*0.53**	*x*			
		2011		*0.73***	*x*			
Mg	*g*	*2009*		*-0.72****	*-0.66***	*x*		
		2010				*x*		
		2011				*x*		
	p1	*2009*	*-0.62***	*-0.81****	*-0.83****	*0.74****	*x*	
		2010			*-0.58**	*0.82****	*x*	
		2011	*-0.73****	*-0.62**			*x*	
	p2	*2009*	*-0.65***	*-0.72****	*-0.85****	*0.81****	*0.86****	*x*
		2010	*-0.57**		*-0.64***	*0.60**	*0.88****	*x*
		2011	*-0.67***				*0.86****	*x*

**P < 0.05; ** P < 0.01; *** P < 0.001; blank cells represent not significant correlations*

[a] *Grape.*

[b] *Petioles sampled at berry set.*

[c] *Petioles sampled at veraison.*

Annex E

Pearson's correlation coefficients between micro-elements (Fe, Zn) in grapes and petioles sampled in 2009-2011.

Element/ sample		*Year*	***Fe***			***Zn***		
			g [a]	*p1* [b]	*p2* [c]	*g*	*p1*	*p2*
Fe	*g*	*2009*	*x*					
		2010	*x*					
		2011	*x*					
	p1	*2009*		*x*				
		2010		*x*				
		2011	*0.55**	*x*				
	p2	*2009*			*x*			
		2010	*0.54**	*0.78****	*x*			
		2011		*0.51**	*x*			
Zn	*g*	*2009*		*0.57***	*0.65***	*x*		
		2010				*x*		
		2011	*0.63**			*x*		
	p1	*2009*			*0.44**	*0.54**	*x*	
		2010	*-0.53**				*x*	
		2011				*0.53**	*x*	
	p2	*2009*					*0.79****	*X*
		2010						*X*
		2011		*0.59**		*0.65***		*X*

**P < 0.05; ** P < 0.01; *** P < 0.001; blank cells represent not significant correlationsx*

[a] *Grape.*

[b] *Petioles sampled at berry set.*

[c] *Petioles sampled at veraison.*

Annex F

Pearson's correlation coefficients between micro- and macro-elements in grapes and petioles sampled in 2009-2011.

Element/ sample		*Year*	***K***			***Mg***		
			g [a]	*p1* [b]	*p2* [c]	*g*	*p1*	*p2*
Fe	*g*	*2009*						
		2010					*0.56**	*0.54**
		2011	*0.60**	*0.55**	*0.56**		*-0.64***	*-0.57**
	p1	*2009*						
		2010			*0.61**			
		2011		*0.67***	*0.62**		*-0.59**	
	p2	*2009*						
		2010						
		2011		*0.55**	*0.59**			
Zn	*g*	*2009*						
		2010	*0.65***		*0.65***		*-0.53**	*-0.68***
		2011	*0.62**	*0.60**			*-0.64***	*-0.75***
	p1	*2009*		*0.44**	*0.72****		*-0.48**	*-0.55***
		2010						
		2011						*-0.53**
	p2	*2009*	*0.47**	*0.59***	*0.73****		*-0.61***	*-0.56***
		2010			*0.57**	*-0.63***	*-0.79****	*-0.64***
		2011		*0.67***			*-0.73***	*-0.69***

**P < 0.05; ** P < 0.01; *** P < 0.001; blank cells represent not significant correlations*

[a] *Grape.*

[b] *Petioles sampled at berry set.*

[c] *Petioles sampled at veraison.*

Annex G

Average element content in leaf blades of control and NPK treated 'Rebula' potted vines at veraison 2011 (analysed performed by YARA Italia S.p.a., Italy).

Element	*Content in leaf blades*	
	#1 Control	*#2 NPK*
N (g kg^{-1}) [a]	*13.9*	*31.1*
P (g kg^{-1}) [b]	*2.8*	*1.5*
K (g kg^{-1}) [b]	*10.9*	*16.1*
S (g kg^{-1}) [b]	*1.4*	*1.6*
Ca (g kg^{-1}) [c]	*18.7*	*20.3*
Mg (g kg^{-1}) [c]	*1.2*	*0.9*
B (mg kg^{-1}) [b]	*22.0*	*10.3*
Fe (mg kg^{-1}) [c]	*57*	*88*
Mn (mg kg^{-1}) [c]	*32.3*	*59.7*
Zn (mg kg^{-1}) [c]	*12.1*	*19.5*
Cu (mg kg^{-1}) [c]	*4.5*	*8.4*

[a] *Kjeldahl procedure with HF/H_2SO_4 and catalyst; determination of liberated ammonia by 0.1 M HCl titration (N measurements could not be correct, since leaves were oven dried at 105°C).*

[b] *Dry oxidation at 400 °C, ash digestion in HCl; spectrophotometer or Inductively Coupled Plasma Analyser (ICP) determination*

[c] *Dry oxidation at 400 °C, ash digestion in HCl; AAS or ICP determination*

Legend (results interpretation):

Deficient	*Low to marginal*	*Adequate*	*High to excessive*

Annex H

Pearson's correlation coefficients between grape compositional parameters and K, Mg, Fe, Zn contents in grapes and petioles sampled in 2010 and 2011.

Element/ sample		*Year*	*Soluble solids*	*Total acidity*	*Tartaric acid*	*Malic acid*	*Free amino nitrogen*
K	*g* [a]	*2010*	*–0.76****		*0.56**		
		2011	*–0.82****				*0.81***
	p1 [b]	*2010*	*–0.62***				
		2011					*0.75***
	p2 [c]	*2010*	*–0.62**			*–0.55**	
		2011					*0.67**
Mg	*g*	*2010*				*0.73***	*–0.73***
		2011					
	p1	*2010*		*0.59**	*–0.57**	*0.91****	*–0.85****
		2011	*0.91****				*–0.77***
	p2	*2010*	*0.57**		*–0.66***	*0.88****	*–0.74****
		2011	*0.95****				*–0.84****
Fe	*g*	*2010*				*0.56**	
		2011					*0.67**
	p1	*2010*					
		2011					*0.65**
	p2	*2010*					
		2011					
Zn	*g*	*2010*	*–0.50**			*–0.63***	
		2011	*–0.65**				*0.70**
	p1	*2010*					
		2011					*0.62**
	p2	*2010*		*–0.51**	*0.52**	*–0.76***	*0.69***
		2011	*–0.77***				*0.84****

**p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; blank cells represent not significant correlations*

[a] *Grape.*

[b] *Petioles sampled at berry set.*

[c] *Petioles sampled at veraison.*

Annex I

Average chemical characteristics of the pot soil sampled in 2007 and 2011 (analyzed by YARA Italia S.p.a., Italy).

Parameter (unit; method) [a]	*2007 (All pots)* [b]	*2011 (#1-control)* [c]	*2011 (#2-NPK)* [d]
pH *(in water 1:1.25)*	*8.0*	*7.9*	*7.8*
EC *(dS m^{-1})*	*0.80*	*1.23*	*0.98*
OM *(%; Walkley-Balck procedure)*	*1.9*	*4.8*	*5.2*
CEC *(cmolc kg^{-1})*	*24.9*	*21.7*	*19.5*
N total *(mg kg^{-1}; Sulphuric and orthophosphoric acid digestion)*	*1039*	*1200*	*1540*
P *(mg kg^{-1}; Olsen procedure)*	*8*	*7*	*6*
K *(mg kg^{-1}; 1 M NH_4NO_3)*	*174*	*182*	*205*
S *(mg kg^{-1}; Calcium tetrahydrogen diorthophosphate)*	*7*	*21*	*6*
Na *(mg kg^{-1})*	*19*	*21*	*19*
Ca *(mg kg^{-1}; 1 M NH_4NO_3)*	*4793*	*4850*	*4307*
Mg *(mg kg^{-1}; 1 M NH_4NO_3)*	*43*	*60*	*33*
B *(mg kg^{-1}; water, 80°C)*	*1.34*	*1.08*	*1.11*
Cu *(mg kg^{-1}; 0.05 M EDTA)*	*17.5*	*13.2*	*14.2*
Fe *(mg kg^{-1}; 0.05 M EDTA)*	*143*	*123*	*132*
Mn *(mg kg^{-1}; 1 M ammonium acetate with quinol)*	*104*	*97.3*	*106.1*
Mo *(mg kg^{-1}; ammonium acetate and oxalic acid)*	*0.05*	*0.02*	*0.03*
Zn *(mg kg^{-1}; 0.05 M EDTA)*	*3.9*	*6.7*	*9.9*
Ratio ***K:Mg***	*4.0*	*3.0*	*6.2*

[a] *Analysed by AAS/ICP, spectrophotometer, or other instruments.*

[b] *Pot soil sampled in all pots before the trial setup.*

[c] *Soil sampled in pots of control vines (#1).*

[d] *Soil sampled in pots of NPK treated vines (#2.)*

Legend (results interpretation):

Deficient	*Low to marginal*	*Adequate*	*High to excessive*

Annex J

Correlations between EDTA, $CaCl_2$, NH_4NO_3 and H_2O-extractable K, Mg, and Fe in the pot soil. [a]

	EDTA	*$CaCl_2$*	*NH_4NO_3*	*H_2O*
Potassium				
EDTA	*X*			
$CaCl_2$	*0.99****	*X*		
NH_4NO_3	*0.99****	*0.99****	*X*	
H_2O		*0.51**		*X*
		*0.75***	*0.75***	
Magnesium				
EDTA	*X*			
$CaCl_2$		*X*		
NH_4NO_3			*X*	
	*0.85****	*0.78***		
H_2O				*X*
Iron				
EDTA	*X*			
$CaCl_2$		*X*		
NH_4NO_3	*-0.59**		*X*	
H_2O		*0.64***		*X*

[a] *Correlations (n = 13-15) between metal fractions obtained with different extractants in the soil sampled in May/June 2009 and June 2010 (in black colour) and in October 2010 and November 2011 (in blue colour).Zn is not presented, since no correlations were obtained.*

**P < 0.05; **P < 0.01; ***P < 0.001, n.s.: not significant*