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Effect of ozonated water treatment on inorganic components of Saperavi vines from the Bolnisi region of Georgia

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Abstract

In recent years, much attention paid to the influence of ozone on the organic components of grapes and wine, while the influence on the inorganic macro- and micro-components of soil, vines and wine has been practically unstudied. In this work studied the effects of spraying grapevines with ozonated water on inorganic elements in composition of branches, leaves, seeds and skins, as well as the resulting juice and red wines from the Bolnisi region of Georgia. Found that ozone increases the effectiveness of virus and bacterial killing and helps reduce sulfite consumption. This study also demonstrated the advantage of using ozone in reducing heavy metal levels in both grapes and wine, improving quality and enhancing stability by reducing the risk of iron and copper "casse" formation. This study demonstrates a general trend toward a reduction in inorganic component content in soil and grapes treated with ozone, compared to vineyards treated with traditional methods, throughout grape growth, ripening, fermentation in the following sequence: soil → trunk → bunch (leaves) → skin → pulp → seeds → juice → wine. However, these changes do not affect the quality of the grapes or wine—organoleptic properties - taste, color, aroma and others are preserved.

1. Introduction

Grapevine is one of the most important agricultural crops in the world, covering a vast area of approximately 8 million hectares. It is one of the most widely grown fruit crops in the world and a leading consumer of pesticides (Segade et al. 2018) due to its high pathogen load of all tree species, its lack of resistance to diseases and pests, and therefore its need for constant and meticulous care.

Every year, up to 30% of crop, yields are lost due to diseases and damage, and their quality reduced. If protective measures nor taken in a timely manner, losses can reach 50% or more, potentially leading to plant death. Preventive measures and intensive pesticide treatment are essential (Peres et al. 1999). However, the environmental and health risks associated with chemical pesticides necessitate their careful use and the exploration of other approaches to sustainable pest control.

The use of ozonated water against pests is a modern method offering a number of advantages and enormous potential for eliminating the adverse effects of pesticides on agricultural produce (Chen et al. 2020). The effectiveness of ozonated water in pesticide removal depends on the concentration and duration of treatment, the properties of the pesticides, and the surface characteristics of the products being treated. With prolonged exposure, pesticides steadily diffuse from the surface of vegetables into the innermost layers.

To reduce pesticide use and improve the control of fungal diseases (mildew, oidium and grey mould), ozonated water has been widely used as an alternative phytosanitary treatment in vineyards in the last two decades (Garsia-Martines et al. 2020; Carbone et al. 2015; Tzortzakis et al. 2016), which has been shown to reduce fungal and bacterial populations on grapevines (Ge Sanctis et al. 2015).

A bluish gas with a pungent odor, ozone is a triatomic allotrope of oxygen and formed by the rearrangement of oxygen atoms under high energy. Ozone has a redox potential of 2.07 V, making it a powerful, versatile oxidizer capable of cracking numerous organic components via free-radical mechanisms, thereby inactivating a wide range of bacteria, fungi, viruses, protozoa, and bacterial and fungal spores (Modesti et al.2019) Given its low stability and spontaneous decomposition to a non-toxic product, ozone considered environmentally safe. This gas is partially soluble in water, and its stability in the aqueous phase depends on many factors, such as the partial pressure of the gas, temperature, water purity and pH, and hydrodynamic conditions (Soja et al. 1997). When dissolved in water, ozone is even more unstable than in the gas phase (Mittler. 2002). Upon entering the body's cells, ozone immediately converted into reactive oxygen species and causes oxidative stress, which in turn leads to changes in secondary metabolism. Ozone, as an elicitor, induces a protective mechanism. It has a dual effect on grapes and red wine: in small, controlled doses, it improves wine quality and protects against pathogens, while in higher concentrations, it causes vine burn. According to various sources, ozone treatment can either worsen or improve the chemical composition of wines (Botondi et al. 2015; Bellincontro et al. 2017; Shan et al. 2019; Allende et al. 2007; Jia et al. 2020; Goffi et al. 2018). Preferably, ozone treatment should be performed before fermentation, i.e., during the growth and maturation of the vines (to remove pesticides and microflora), but not on the wine itself.

Scientists García-Martínez M.M. and others evaluated the effect of ozonated water treatment on grape composition using a combination of irrigation and spraying (Campayo et al. 2019). Ozonated water was found to significantly improve grape color, as well as the concentration of phenols and aromatic compounds. Other studies have also been conducted on the use of ozonated water for grapevine treatment to improve grape quality (Campayo et al. 2020). Numerous publications have examined in detail various aspects of the use of gaseous ozone in scientific research and its practical application in food production (Burak, 2022; Edmundo Sitoe et al. 2025; https://www.postharvest.biz/post-harvest-treatment-of-grapes-with-ozone-preserves-their-quality). Many scientific publications demonstrate the advantages and benefits of using ozonated water compared to gaseous ozone (Campayo et al. 2019; Mostashari et al. 2022). However, a systematic review of the various ozonated water treatment methods and their practical application is still lacking. Moreover, existing studies on the effects of ozone on the chemical composition of wine materials and wine focus primarily on organic components—flavonoids, antioxidant polyphenols, anthocyanins, VOCs, organic acids, etc. However, there is virtually no information in the literature on the effect of ozone on changes in mineral composition.

Plant-based substances—micro- and macroelements—that are non-reproducible by the human body determine the daily micronutrient requirement in the human diet, affecting the functioning of all organs and systems. Heavy metals enter the plant through the nutrient medium, and their accumulation in the soil can cause toxic effects. There is virtually no data in the literature on the effect of ozonated water on the content of inorganic components in grapes and wines. Therefore, it is interesting to study the dynamics of heavy metal levels and macroelements under the influence of ozonation.

It should be noted that inorganic components make up a small portion of the total composition of grapes and wine (usually 0.2–0.5% in wine, no more than 15% in vine composition), but they play a particularly important role in vine physiology, shoot growth, photosynthesis and bunch ripening, fermentation processes and the formation of taste, color, aroma and other organoleptic properties of wine.

The main inorganic components include potassium, sodium, calcium, iron, copper, zinc, lead, magnesium, manganese, aluminum and trace elements (arsenic, molybdenum, titanium, vanadium, etc.), as well as phosphorus, chlorine, bromine, fluorine, sulfur, etc., and, in addition, heavy metals contained in the soil and migrating into grapes and wine (Heli Siren et al. 2015). Important aspects include both the identification of mineral components in the final product and the recording of their formation and migration in the following sequence: soil → root → stem → leaf → skin/pulp → juice → wine. Such detailed analyses are rare and fragmentary, and such studies will significantly benefit Georgian winemaking. They will make the winemaking process more controlled and manageable. These analyses and monitoring are particularly interesting and necessary for wines made using the unique qvevri method.

The aim of this study is to investigate the effects of multiple ozonated water treatments of Saperavi grapes in the Bolnisi region of Georgia on the chemical composition of wine and its components, specifically the content of micro- and macroelements and known heavy metals.

It should be noted that the chemical composition of wines from Bolnisi, one of Georgia's oldest region, in common, has been poorly studied, and this study examines the impact of the environmentally friendly ozonated water method of protecting grapes from diseases on the main components for the first time. In this study, we examined the effects of spraying grapevines with ozonated water on the chemical composition of branches, leaves, pulp, and berry skins, as well as the resulting juice and red wines from the Bolnisi region of Georgia, over two consecutive growing seasons in 2024–2025. The results were compared with the compositional data for the same samples obtained after pesticide treatment.

2. Objectives and methods

The objects were Saperavi wine from the Bolnisi region of Georgia, which were provided by the winery "Dzmebis Marani" and its manager Guram Avkopashvili. We were provided too with parts of the grape vine and juice.

Ozonation was carried out by spraying method directly in the vineyards, where ozone generators (to generate the ozonated water immediately prior to the grapevine treatments) and water containers were installed. The ozonated water treatment was carried out seven times a season from April to September, with a duration of 15 minutes per treatment, with concentration 2–3 mg/L early in the morning at temperature 18-20^{0}C. The procedure was performed by winegrowers periodically before flowering, fruit set, during ripening, three weeks before harvest, and after prolonged rainfall. Sampling and sorting were done manually. Saperavi red wine was produced using the ancient Kakhetian method of qvevri (24 https://mildiani.ge/en/production/106).

The analyzes were carried out using flame atomic absorption spectrophotometry (spectrophotometer of firm Perkin-Elmer AAnalyst 800), photocolorimetry, spectrophotometry, conventional chemical analysis methods according to ISO [25–27 ISO 8288 – 1986; ISO 6058 – 1984; ISO 9964-3-1993], GOST [28–30 GOST 31861 – 2012; GOST 5180 – 2015; GOST 26726 – 2019] and others.

Acid digestion of soil material was carried out in a sample decomposition system Microwave Pressure Digestion Labstation MWS-3, BERGHOF. Strong acid 1N HNO_3, ammonium acetate buffer solution with pH = 4.8 and water was used as extractant.

Wine and juice samples were treated by the method of wet mineralization. A sample of 50 ml red wine was put into a glass, 5 ml con. HNO_3 was added and heated on a sand bath. If the sample was not discoloration for 1 hour, it was cooled and then 2 ml con. HNO_3 was added. Heating continued until the solution discoloration, after which the sample was transferred into a graduated 10 ml cylinder and brought to volume.

3. Results and discussion

The mineral composition of grapes and wine varies greatly, and their content depends on a number of factors: grape variety, soil and climate, maturity, agricultural practices, and grape processing technology (Panasyuk et al. 2004). The migration of key inorganic components from the soil along the vine trunk to the juice and wine also requires study, as they often influence the formation and overall structure of wine. Therefore, we began our study of the effect of ozonation on inorganic components by analyzing the soil, grapevine parts (bunch, leaves, berry skins, pulp, and seeds), and then the juice and wine.

Table 1
Some physical parameters of the studied soils' sites, measured in spring

N	Site, deepness	Humidity,%	Humus, %	pH	loss on heating, %
1	Non-ozonated site, 2–3 cm	2.88	4.78	6.86	12.52
2	30 cm	7.58	2.50	7.87	14.43
3	50 cm	8.02	2.40	7.32	11.75
4	Ozonated site, 2–3 cm	7.36	5.75	7.00	19.06
5	30 cm	5.39	3.80	7.12	13.98
6	50 cm	7,00	2.17	6.80	11.37
7	Non-ozonated site, 2–3 cm	3.75	3.69	6.75	13.05
8	30 cm	4.35	2.55	6.89	13.09
9	50 cm	6.84	2.01	6.99	14.15

According to soil analysis data, the soil in the studied vineyards is dry (Table 1), with moisture content not exceeding 8%, while the optimal soil moisture for vineyards should be above 20%. However, total moisture loss is 15–20%, which is consistent with sandy soils. Humus and pH values are within the normal range for vineyards: 2–4% and pH 6.75–7.87, respectively (a pH of 6.5 to 7.5 is considered ideal for grapes). Apparently, the reaction of ozone with organic acids in the soil slightly increased the pH of the environment.

The content of the main inorganic components in the ozonated plot is slightly (10–20%) higher than in the non-ozonated vineyard. Apparently, ozone, as a strong oxidizer, breaks down organic soil complexes, thereby releasing cations and facilitating their penetration into the grapevine. Thus, ozone increases the availability of nutrients (especially phosphorus, nitrogen, and sulfur) to the grapes and promotes soil aeration.

It is known that the content of potentially mobile, exchangeable, and readily soluble forms of an element decreases with increasing soil depth (Baranov. 2023). However, in the ozonated area, the Ni content increased with depth. With depth, the distribution of forms of heavy metals in the soil profile has multidirectional dynamics: the amounts of Ni, Cd, and Co decrease; the contents of the remaining cations pass through a minimum. It can be assumed that Ni, Cd, and Co easily transform into more mobile soluble forms and penetrate deeper into deeper layers. Because of increasing soil density with depth, the remaining cations cannot migrate to the deepest layer, since their total content is tens of times higher than the amounts of Ni, Cd, and Co. Their soluble forms saturate the soil and become “stuck” in the depths. Exchangeable forms of Ni, Co, as well as Zn, Cu, Pb, and Mn in soils pass into solution as a result of ion exchange with H^+, Na^+ in the soil-absorbing complex. When assessing the effect of ozonation on the distribution of inorganic components in parts of the vine, found that in the bunches and leaves of the ozonated area the amount of basic ions was higher (Table 2), and the amount of heavy metal ions was lower than in the control area, with the exception of Pb (Table 3); no pattern observed in the distribution of elements in the skin and seeds. The leaves contain the highest amount of potassium among the elements, and with ozonation the concentration increases by another 32%, the amount of magnesium also increases, and calcium and iron decreases (Fig. 1).

Table 2
Effect of ozonation on the migration of the main inorganic components from the soil into the bunch, leaves, skins and seeds of Saperavi vine variety.

N	Name	Mg, mg/kg	Al, mg/kg	K, mg/kg	Si, mg/kg	P, mg/kg	S, mg/kg	Ca, mg/kg	Fe, mg/kg
1	Soil (ozonated site)	9633.6	79200	16812	263786,7	1640	938.6	14272	32150
2	Soil (non-ozonated site)	8563.2	69840	14008	255406.6	1616	768.3	14272	27354
3	Bunch (ozonated site)	18092.7	1972.5	12170	690.7	1520	343.7	6345.6	517.9
4	Bunch (non-ozonated site)	12154.5	1367.2	7968	457.3	2160	712.8	6087.2	565.9
5	Leaf (ozonated site)	27830.4	460.8	2977	10056.7	128	505.3	4709.7	379
6	Leaf (non-ozonated site)	23192.0	460.8	1532.5	9225.3	120	723.5	3211.2	283
7	Skin (ozonated site)	299.6	< 0.01	6362	0	**373.7**	463.7	1141.6	811.2
8	Skin (non-ozonated site)	342.4	< 0.01	4334	0	**375**	554.9	1284.3	1075.2
9	Grape seed (ozonated site)	733.7	< 0.01	1860.5	733.3	955	232.4	2242.4	1673.3
10	Grape seed (non-ozonated site)	611.4	< 0.01	4027.5	1193.3	844,57	206.8	2446.3	1467.5

The migration of the main inorganic elements across the vine during growth and ripening is unidirectional – a gradual decrease in the total element content along the following chain: soil (∑=154,646.2 mg/kg) → combs (∑=42,450 mg/kg) → leaves (∑=42,315 mg/kg) → skin (∑=11,551 mg/kg) → seeds (∑=8,428 mg/kg). A similar trend observed for heavy metals (Table 3), with the exception of Mn, Pb, and Zn: Mn and

Pb contents sharply increased in the skin and seeds of grapes, while zinc is almost four times lower in the woody bunch.

Table 3
Effect of ozonation on heavy metal content in soil, bunches, leaves, skin, and seeds of Saperavi vines.

N	Name	Ni, mg/kg	Cd, mg/kg	Cu, mg/kg	Pb, mg/kg	Mn, mg/kg	Co, mg/kg	Zn, mg/kg
1	Soil (ozonated site)	32.9	1.3	3334312.7	15	820.8	6.1	80.3
2	Soil (non-ozonated site)	32.6	2.1	297.2	46.9	840.4	9,4	283.2
3	Bunch, (ozonated site)	6.9	< 0.001	10.60	1.9	3.65	0	8.88
4	Bunch (non-ozonated site)	7.2	< 0.001	18.68	1.9	5.6	0	31.73
5	Leaf (ozonated site)	4.7	< 0.001	8.14	0.64	9.71	0.06	3.76
6	Leaf (non-ozonated site)	4.2	< 0.001	8.94	0.67	16.6	0.04	3.43
7	Skin (ozonated site)	0	< 0.001	1.42	4.52	85.3	0.14	7.55
8	Skin (non-ozonated site)	0	< 0.001	5.89	4.06	127.7	0.13	8.21
9	Grape seed (ozonated site)	08.88	< 0.001	10.81	3.12	104.6	0.11	14.2
10	Grape seed (non-ozonated site)	-	< 0.001	30.0	2.63	62.2	0.08	4.5

In Saperavi juice and wine, the levels of detectable elements in the ozonated area were lower than in the control area (Table 4). Ozonated water does not alter the oxidation state of the main macrocomponents of wine—K, Na, Ca, Mg—but reduces their concentrations. Ozone, due to the oxidation of tartaric acid, can accelerate the precipitation of "tartar" (potassium hydrogen tartrate and calcium tartrate). Therefore, after filtration, the concentrations of alkaline and alkaline earth ions, as well as the total concentration of essential ions, decrease—3255 mg/L in the must from the non-zonated area and 1165 mg/L in the ozonated area.

Ozonated water converts soluble forms of heavy metals into insoluble oxides and hydroxides according to the following pathways: $Fe^{2+} \rightarrow Fe^{3+} \rightarrow Fe(OH)_3$; $Mn^{2+} \rightarrow Mn^{4+} \rightarrow MnO_2$; $Cu^{2+} \rightarrow CuO$. Sediments removed by filtration. Reducing the content of these elements (Table 5) reduces the likelihood of black and copper "casse" (wine cloudiness) appearing in wine.

Table 4
Effect of ozonation on the content of major inorganic components in Saperavi juice and wine.

N	Name	Mg	Al	K	Si	P	S	Ca	Fe
1	Wine “Saperavi”, ozonated site	112.72	57.6	262.6	120.4	161.1	54.0	100	18.18
2	Wine “Saperavi”, non-ozonated site	136.96	72	814.2	132.5	172,	66.6	114.16	23.38
3	Juice “Saperavi”, ozonated site	111.3	14.4	525.4	133.3	105,	50.65	100	35.5
4	Juice “Saperavi”, non-ozonated site	348	14.4	577.6	138.13	114	51.08	371.2	38.3

Table 5
Effect of ozonation on the content of heavy metals in Saperavi juice and wine.

N	Name	Ni	Cd	Cu	Pb	Mn	Co	Zn
1	Wine “Saperavi”, ozonated site	-	-	0.78	0.12	0.469	0.06	0.50
2	Wine “Saperavi”, non-ozonated site	-	-	1.42	0.14	1.076	0.07	0.82
3	Juice “Saperavi”, ozonated site	-	-	1.90	0.26	1.19	0.06	0.81
4	Juice “Saperavi”, non-ozonated site	-	-	5.14	0.2	2.11	0.10	0.96

A comparison of diagrams (Figs. 2 and 3) showing the distribution of the main inorganic components in the ozone-treated and control grapevines reveals minor differences in their content, which do not affect the quality or organoleptic properties of Saperavi wine from the Bolnisi vineyards.

Our data support the view that the mineral content of the various parts of the grape bunch and pulp is the same as in other parts. The most important of these is potassium, which accounts for 50% of the mineral content of the ash residue. Calcium is next, and is always present in greater quantities than magnesium. However, in wine, the ratio changes because of the precipitation of calcium tartrate (https://vinograd.info/stati/stati/uvologiya.html). In the studied wines, the distribution of the elements is uneven: the bunches and especially the leaves contain the greatest amounts of magnesium. In the finished wine, C(Mg) exceeds C(Ca) too. Thus, this study demonstrates a general trend toward a reduction in inorganic component content in soil and grapes treated with ozone, compared to vineyards treated with traditional methods, throughout grape growth and ripening, and then during juice and wine fermentation in the following sequence: soil → trunk → bunch (leaves) → skin → pulp → seeds → juice → wine. However, these changes do not affect the quality of the grapes or wine—organoleptic properties (taste, color, aroma, etc.) are preserved. Ozone promotes partial destruction of skin cells, thereby stimulating the stress response and facilitating the extraction of inorganic components throughout the entire process.

4. Conclusion

Consumption of ozonated water, in addition to the environmental benefits (significant reduction or elimination of pesticides), does not cause the formation of toxic ozone breakdown products. On the contrary, ozone breakdown saturates plant roots and soil with oxygen. Compared to other oxidizing agents, it increases the effectiveness of virus and bacterial killing and helps reduce sulfite consumption. This study also demonstrated the advantage of using ozone in reducing heavy metal levels in both grapes and wine, improving quality and enhancing stability by reducing the risk of iron and copper "casse" formation. This study demonstrates a general trend toward a reduction in inorganic component content in soil and grapes treated with ozone, compared to vineyards treated with traditional methods, throughout grape growth, ripening, fermentation in the following sequence: soil → trunk → bunch (leaves) → skin → pulp → seeds → juice → wine. However, these changes do not affect the quality of the grapes or wine—organoleptic properties - taste, color, aroma and others are preserved.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contribution

L. Akhalbedashvili, G. Maisuradze wroth the manuscript;S. Jalaghania, A. Mskhiladze and G. Cotadze provide the experimental materials;B. Kvirkvelia prepared the samples for the analyses;G. Avkopashvili provide the materials.

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Figures

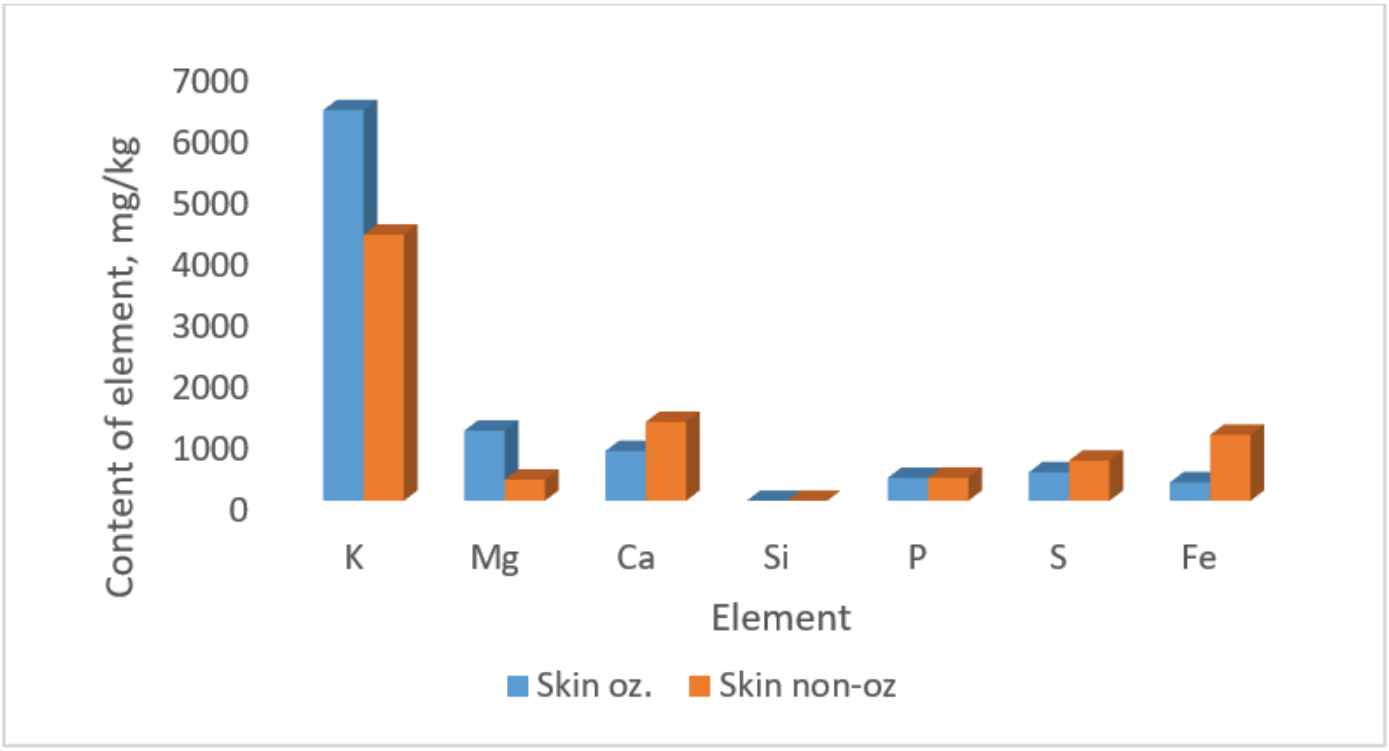


Figure 1

Changes in the ratio of the main inorganic elements depending on ozonation

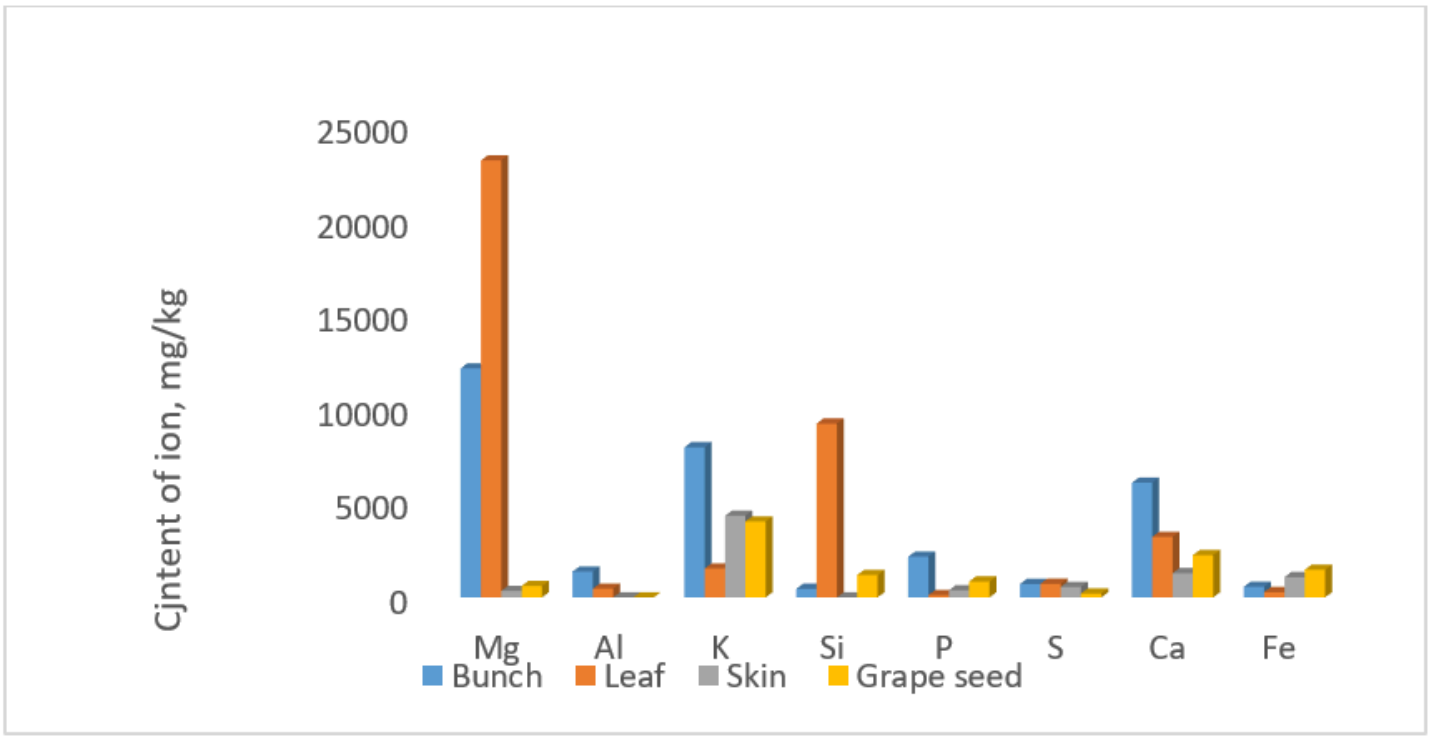


Figure 2

Distribution of main inorganic elements in parts of ozonated vine

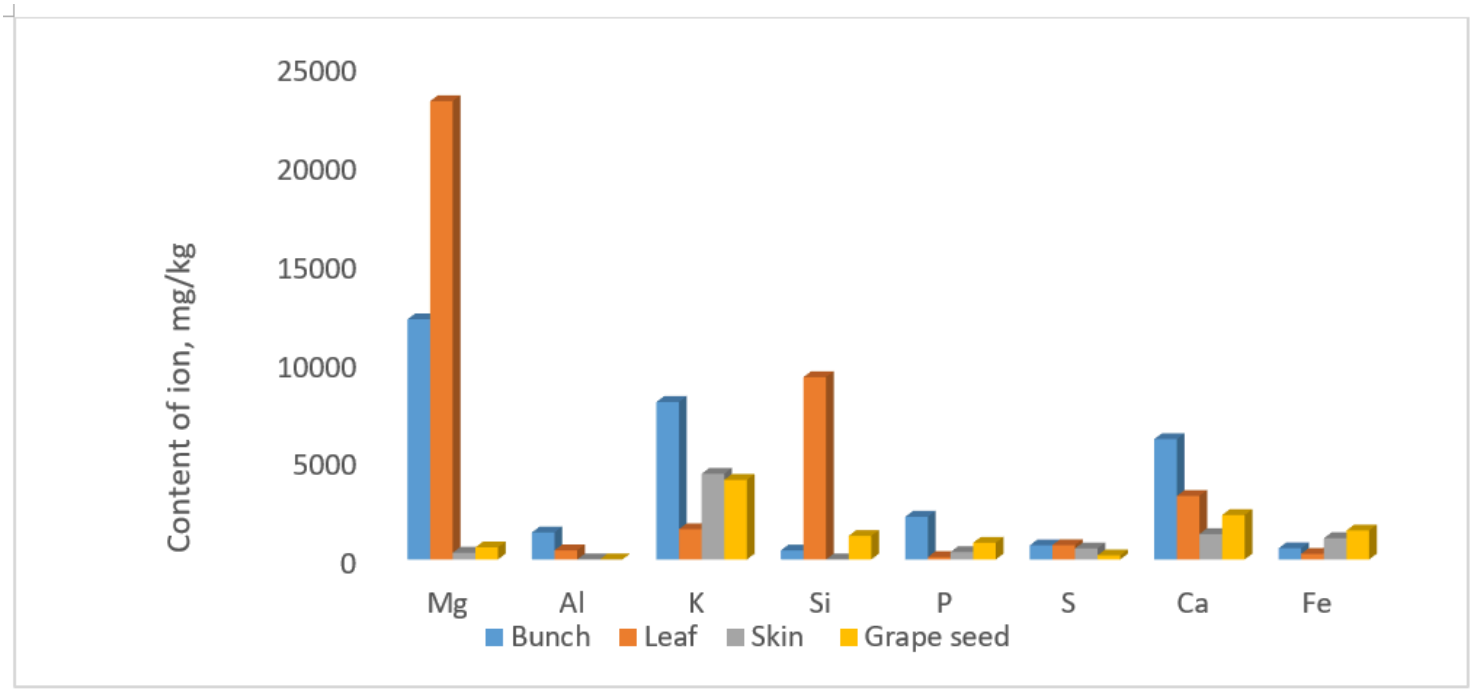


Figure 3

Distribution of main inorganic elements in parts of non-ozonated vine