International Conference on

Food Safety and Regulatory Measures

I.C.S.I. RM.VALCEA

Elemental profile and Sr isotope ratio as fingerprints for geographical traceability of Romanian Wines



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Birmingham, UK, 17-19 August 2015

Summary

- Background
- Chemometric applications in food chemistry
- Purpose
- Instrumental analytical techniques
- Results and discussions

I. Geographical origin identification of Romanian wines by ICP-MS elemental analysis

II. Differentiation of Romanian wines on geographical origin by elemental composition and phenolic components

III. Geographical traceability of wines based on $^{87}Sr/^{86}Sr$ isotopic ratio and multi-element composition



- 6. Conclusions

Background

- Assessments of wine traceability and authenticity become a prerequisite in many countries worldwide.
- The geographical traceability of wine is an important issue in the context of wine authentication
- Wine is a product widely consumed around the world and due to their high economic value (quality wines), wines adulteration is tempting, generally being motivated by maximizing profit using false declaration of origin, vintage year.
- Development of methods giving reliable information about wine authenticity is highly desirable for consumers, producers and administrative authorities.

NECESSITY !!!!!!

Suitable tools for characterization and classification of wines according to the geographical origin, grape variety and vintage year



Background

➢Wine is a complex matrix which contains many classes of compounds like sugars, alcohols, acids, tannins, polyphenols, minerals, proteins, amino acids.









Natural diffusion movement of elemental traces



➢Its composition is influenced by many factors related to the specific production area, like climate, soil, grape variety, ripening of the grapes and winemaking techniques.

Chemometric applications in food chemistry

❖**Building mathematical–statistical models based on quantitative and qualitative information about the natural wine constituents**

- Mineral and trace elements
- Isotopic ratio
- Volatile compounds
- Amino acids
- Sugars
- Organic acids
- Phenolics
- Anthocyanins

→

- Geographical origin
- Wine variety
- Vintage
- Technological characteristics
- Adulteration practices



Wine variety

Natural organic constituents

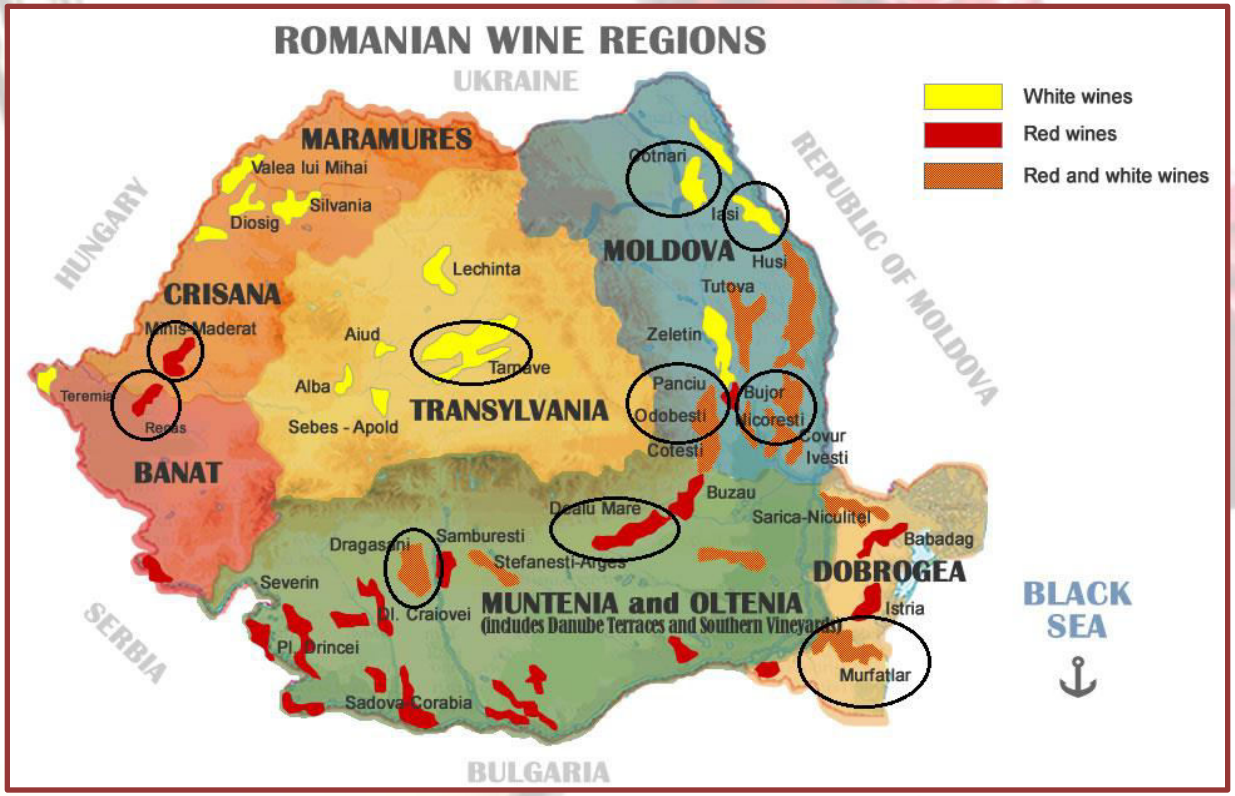
Vintage year

Geographical origin

Isotopic ratios ($^{13}C/^{12}C$, $^{18}O/^{16}O$ and D/H) and mineral determinations

Purpose

➢**To assess the potential of elemental wine composition for classification of wines produced in most important Romanian vineyards according to their geographical origin.**



- ❖ Dobrogea (Murfatlar vineyard),
- ❖ Oltenia (Dragasani vineyard)
- ❖ Muntenia (Valea Calugareasca vineyard)
- ❖ Banat (Recas and Minis vineyards)
- ❖ Moldova (Iasi, Cotnari, Panciu, Odobesti, Nicoresti and Bujoru vineyards)

Instrumental analytical techniques and equipments

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- **Inductively coupled plasma mass spectrometry (ICP-MS)**
- **Flame atomic absorption spectroscopy (FAAS)**
- **Graphite furnace atomic absorption** spectroscopy (**GF-AAS**)











➢Mars 5 Microwave System

Statistical techniques:
PCA, LDA, ANOVA

I. Results and discussions

ELSEVIER

Food Chemistry

Volume 138, Issues 2–3, 1 June 2013, Pages 1125–1134



Geographical origin identification of Romanian wines by ICP-MS elemental analysis

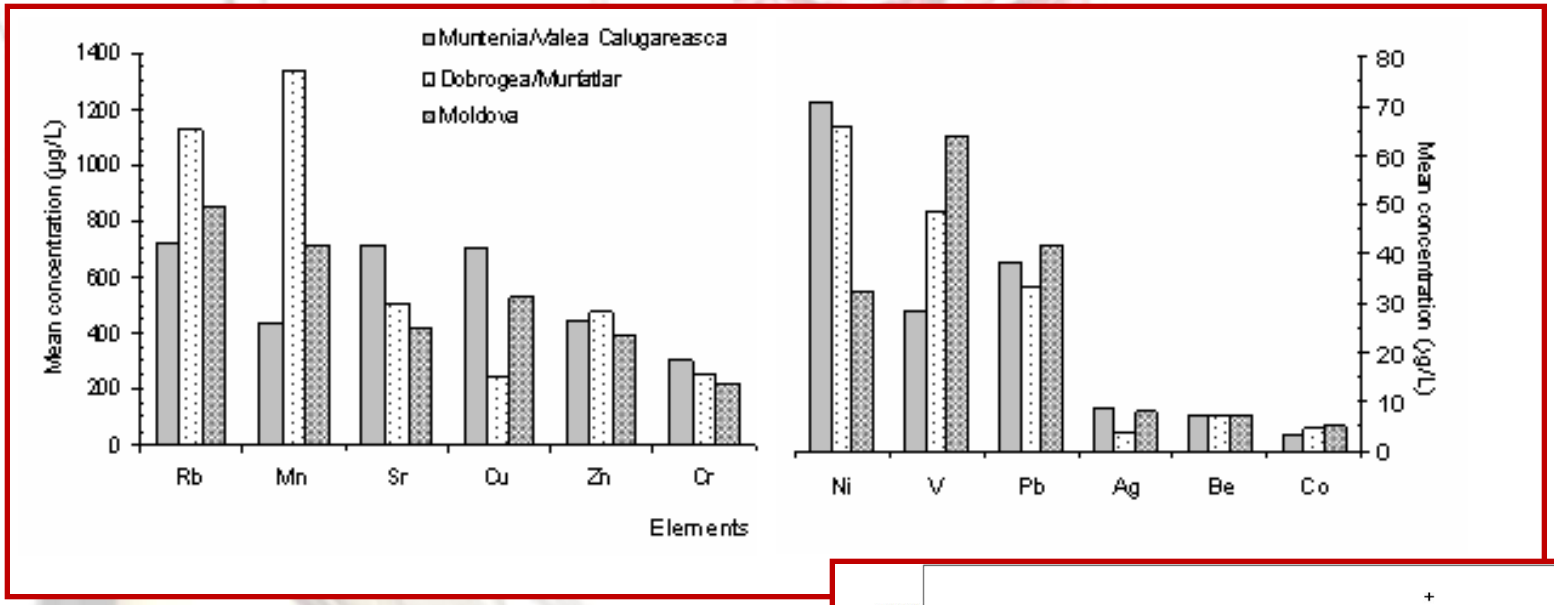
Irina Geana[a], Andreea Iordache[a], Roxana Ionete[a], Adrian Marinescu[a], Aurora Ranca[b], Monica Culea[c]

- 60 authentic Romanian wines / 2009 and 2010 harvests

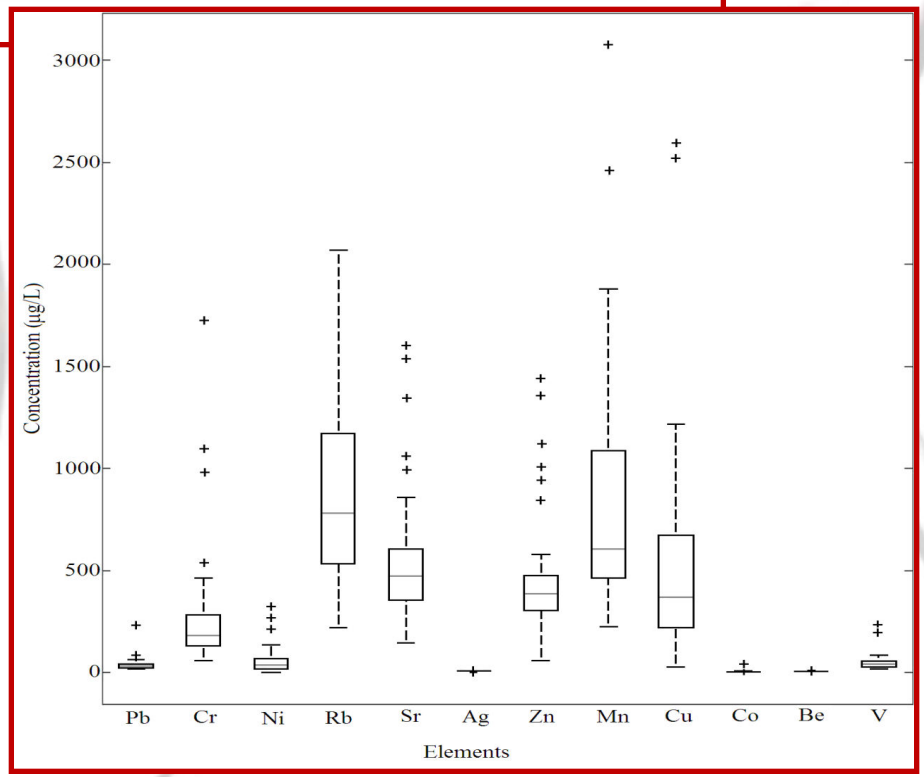
Cr, Ni, Rb, Sr, Ag, Zn, Mn, Cu, Co, V, Pb and Be

- Murfatlar
- Moldova
- Valea Calugareasca





Mean concentration of trace – elements across the wine producing areas

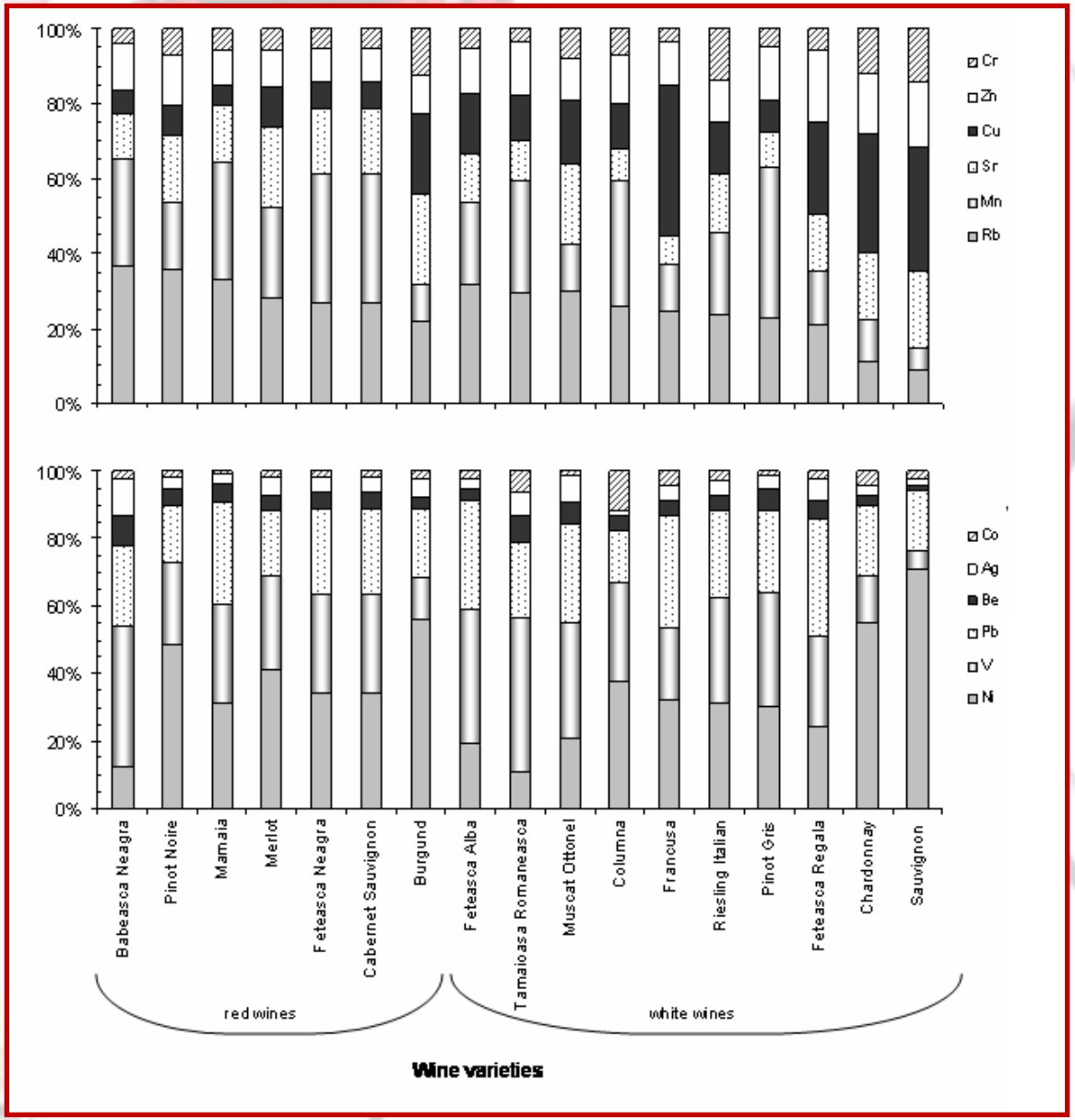




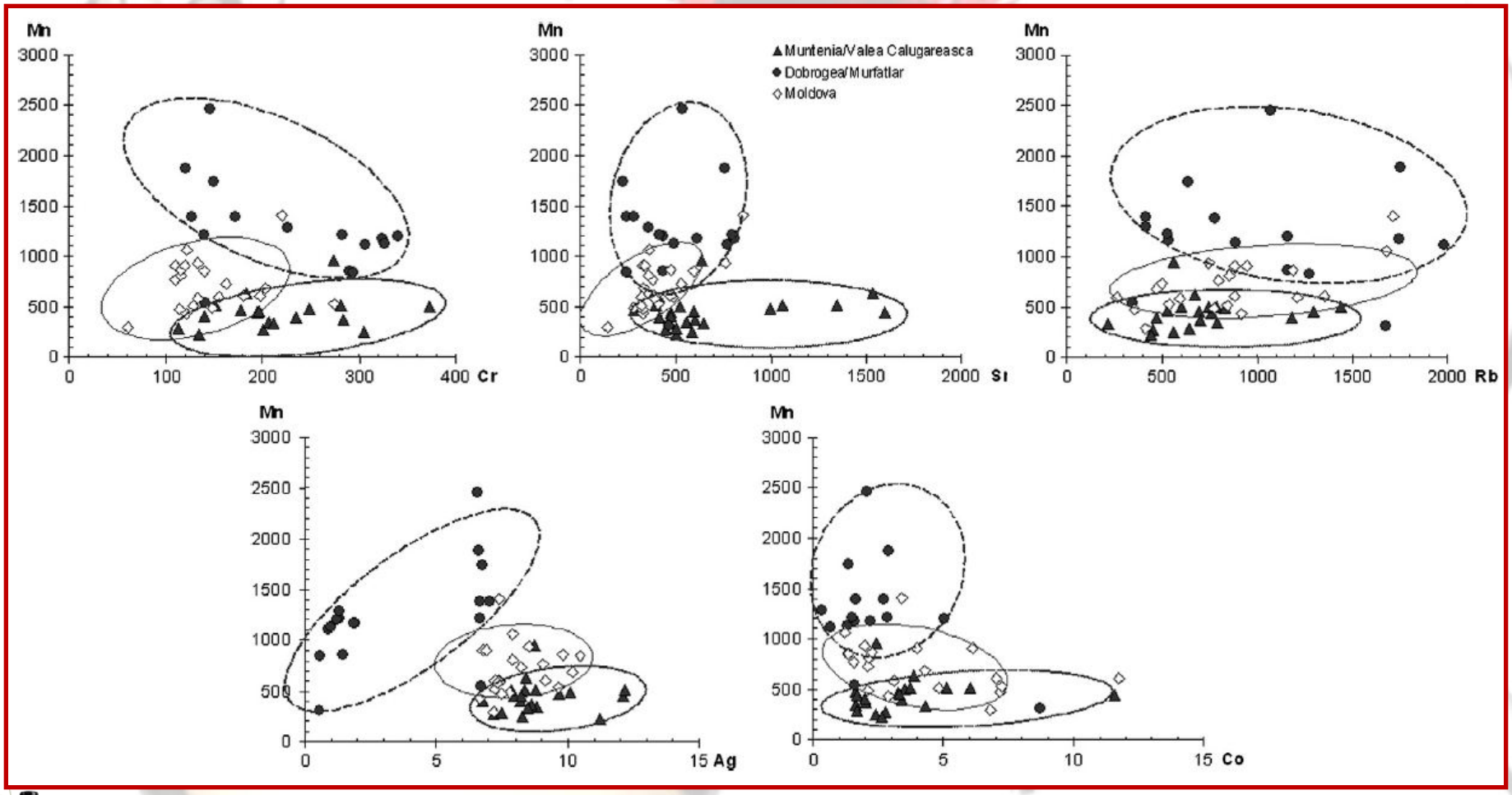
Concentration of elements in the 60 analyzed wine samples

The contribution of trace – elements concentration across the investigated wine varieties

- Feteasca Regala,FR
- Feteasca Alba, FA
- Columna, CO
- Tamaioasa Romaneasca, TR
- Grasa de Cotnari, GR
- Francusa, FC
- Saugvinon, S
- Pinot Gris, PG
- Chardonnay, C
- Riesling Italian, RI
- Muscat Ottonel, MO
- Cabernet Saugvinon, CS
- Pinot Noir, PN
- Merlot, M
- Burgund Mare, BM
- Mamaia, MM
- Babeasca Neagra, BN
- Feteasca Neagra, FN



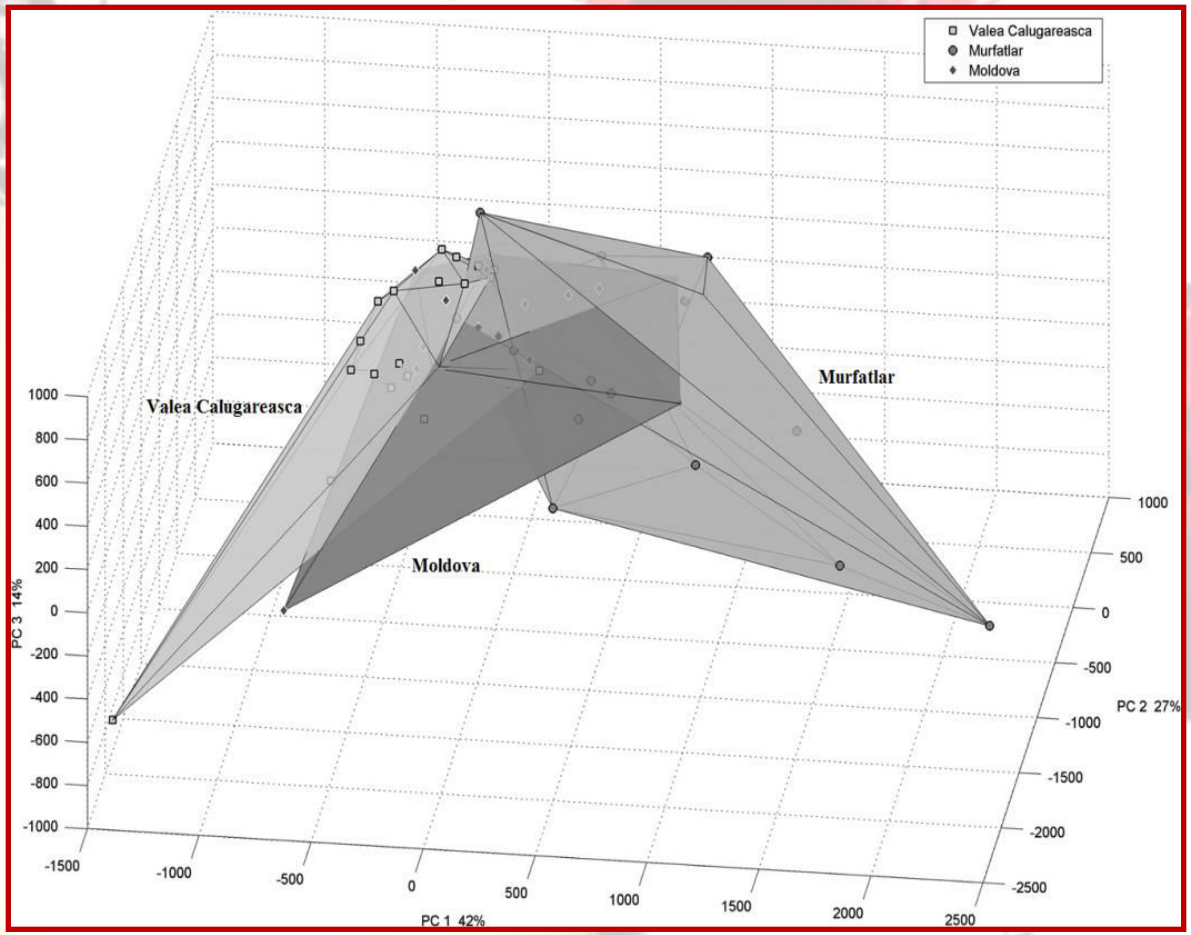
Correlation of Mn element with Cr, Sr, Rb, Ag and Co to differentiate the origin of wine samples





➢The correlation of Mn with Cr, Sr, Rb, Ag or Co content in wines shows a reasonable degree of discrimination of wines by geographical origin.

Geographical origin classification based on elemental profile



PC_1 - 42%
PC_2 - 27%
PC_3 - 14%

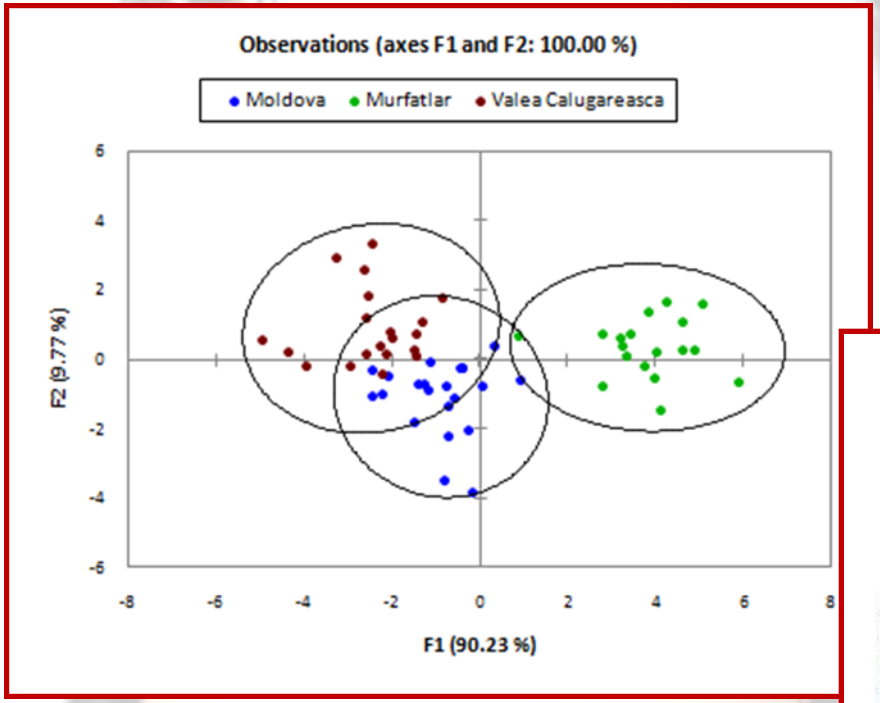
Score plot of the three principal components of trace-elements in the Romanian wine samples



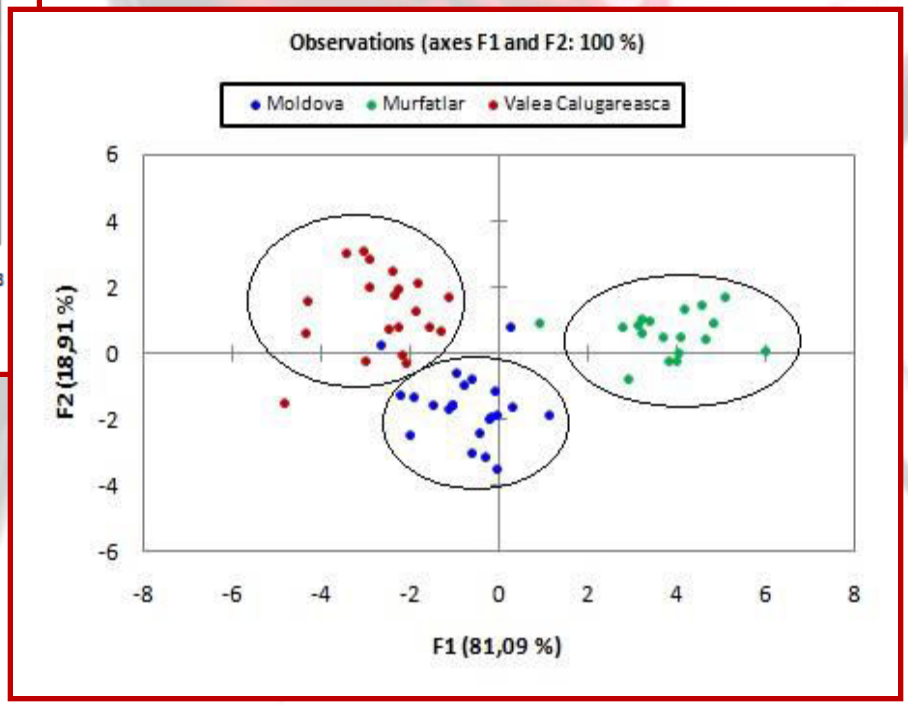
➢is possible to explain **83 %** of the variability among samples

Geographical origin classification

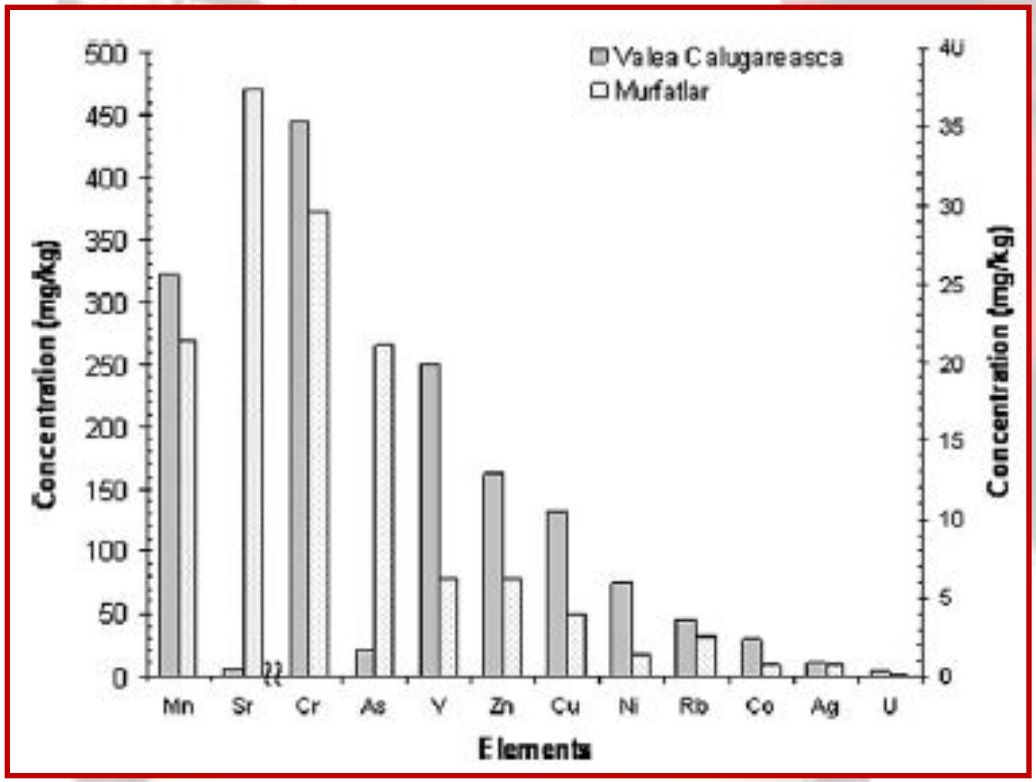
Elemental profile



Elemental profile + izotopic variables ($\delta^{13}C$ and $\delta^{18}O$)



Correlation of trace-elements from wines and their provenance soil



Graphical representation of elements average concentration in soil samples.

Soils	
Valea Calugareasca	Murfatlar
0-20 cm 20-40 cm 40-60 cm	0-20 cm 20-40 cm 40-60 cm 60-80 cm

Ni, Ag, Be, Cr, Zn, Pb, Co, Cu



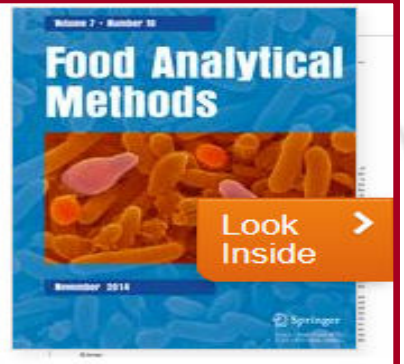
II. Results and discussions

Food Analytical Methods
November 2014, Volume 7, Issue 10, pp 2064-2074

Date: 04 Apr 2014

Differentiation of Romanian Wines on Geographical Origin and Wine Variety by Elemental Composition and Phenolic Components

Elisabeta Irina Geana, Adrian Marinescu, Andreea Maria Iordache, Claudia Sandru, Roxana Elena Ionete, Camelia Bala



22 authentic wine samples / 2008-2011 harvests

Li, Be, Co, Ni, Cs, U, Pb, V, As, Ba, Cr, Cu, Zn, Al, Mn, Rb, Sr, Fe, Ca,

gallic acid, (+)-catechin, (−)-epicatechin, p-coumaric acid, ferulic acid, trans-cinnamic acid, resveratrol



Recas

Dragasani S

Dragasani I

Experimental results of selected elements in studied wines (μg L^{-1})

Element	Dragasani vineyard				Recas vineyard			
	Mean	SD	Range	Median	Mean	SD	Range	Median
Li	10.47	5.05	1.29–19.80	10.69	25.94	15.13	13.09–50.99	22.88
Be	4.14	1.93	0.45–6.96	4.05	2.78	1.45	0.22–3.78	3.38
Co	2.08	1.19	0.51–5.31	1.80	1.75	0.89	0.70–3.02	1.86
Ni	18.39	12.20	2.46–37.71	17.81	28.49	17.17	5.95–52.60	31.26
Cs	3.05	2.76	0.37–8.27	1.72	4.59	2.63	2.07–8.84	4.23
U	0.86	0.55	0.20–2.17	0.70	0.94	0.58	0.40–1.91	0.79
Pb	38.91	29.09	3.92–115.55	30.46	23.08	11.96	7.95–40.74	22.97
V	43.44	20.51	3.81–69.39	48.43	55.65	35.57	32.09–117.67	41.29
Ba	169.80	150.02	17.80–550.96	110.99	137.18	62.19	49.72–211.67	160.51
Cr	146.18	70.96	9.78–211.09	178.21	124.31	62.64	16.84–178.20	147.73
Cu	154.39	73.45	46.53–307.17	160.47	215.99	151.01	95.60–474.03	160.52
Zn	563.08	474.01	72.21–1,567.73	340.62	534.98	274.71	249.04–936.04	567.12
Al	692.52	360.13	196.67–1,444.52	703.96	721.78	447.64	380.83–1,482.48	608.40
Mn	845.73	443.33	164.32–1,878.96	751.76	870.88	496.40	408.26–1,655.96	858.27
Rb	669.26	297.81	133.43–1,070.40	729.57	626.54	315.02	221.08–1,063.46	662.36
Sr	358.94	178.97	43.18–687.40	391.16	761.63	462.57	217.04–1,488.18	696.19
Fe[a]	1.22	0.66	0.1–2.28	1.45	1.74	0.86	0.56–2.74	2.10
Ca[a]	26.88	14.08	0.78–44.64	30.33	22.63	10.52	6.07–34.16	26.38
Mg[a]	46.40	25.10	2.87–76.18	54.33	55.44	27.05	13.37–78.48	67.97
Na[a]	10.50	8.78	0.55–27.79	7.43	23.45	12.32	10.80–40.80	25.57
K[a]	431.84	209.23	79.46–899.60	407.00	610.71	297.93	256.95–1,032.80	644.50

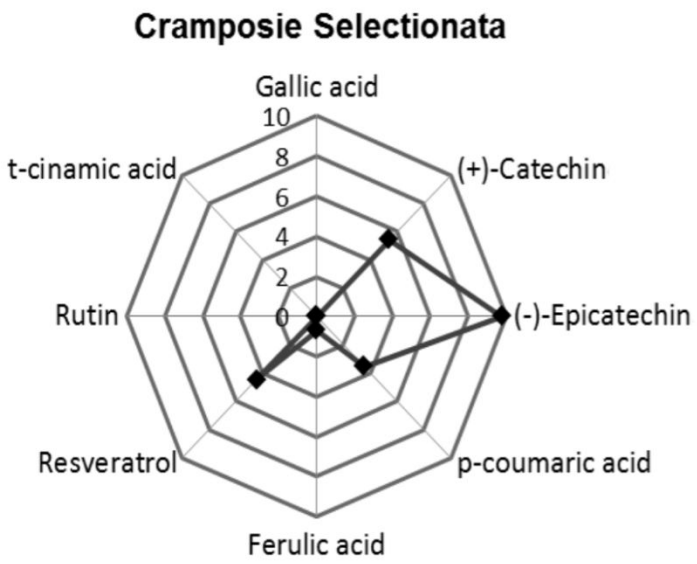
[a] Results expressed as milligrams per liter

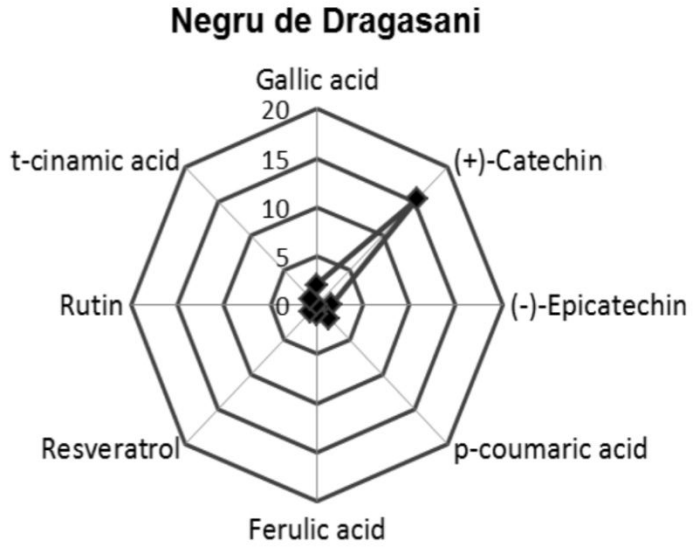


Concentration (mg L−1) of phenolic compounds in Romanian wines

Compound	Dragasani vineyard		Recas vineyard	
	Range (mg L^{-1})	Median values (mg L^{-1})	Range (mg L^{-1})	Median values (mg L^{-1})
Gallic acid	nd–2.06	1.81	nd–1.73	1.73
p-Coumaric acid	1.91–4.53	2.51	1.82–2.13	2.00
Ferulic acid	0.11–1.33	0.24	0.1–0.84	0.67
trans-Cinnamic acid	nd–0.87	0.48	nd–1.13	0.73
(+)-Catechin	nd–25.06	5.57	nd–21.62	6.01
(−)-Epicatechin	nd–10.99	4.50	1.43–4.61	2.60
Resveratrol	nd–13.09	3.39	nd–0.77	0.62

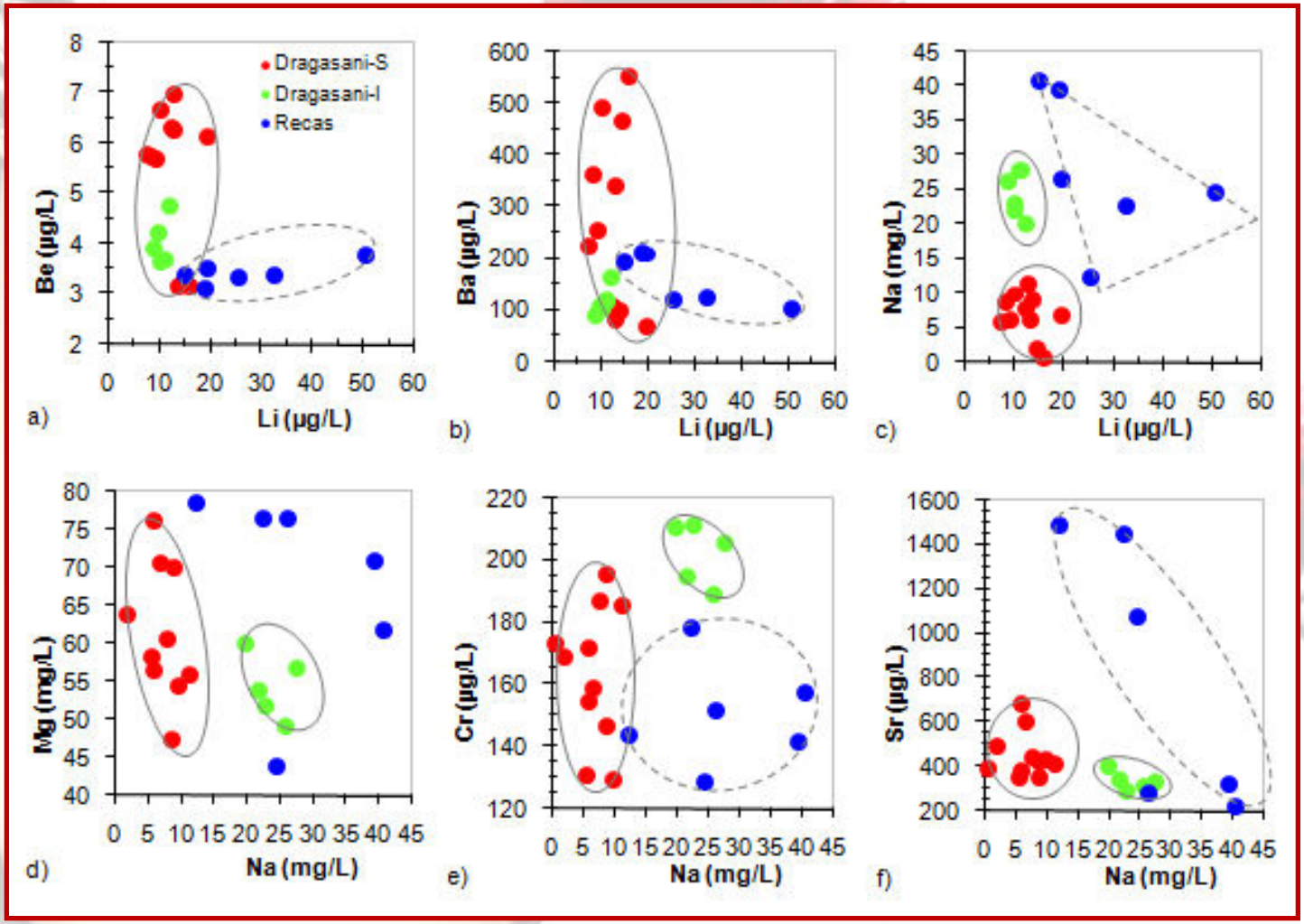
nd not determined



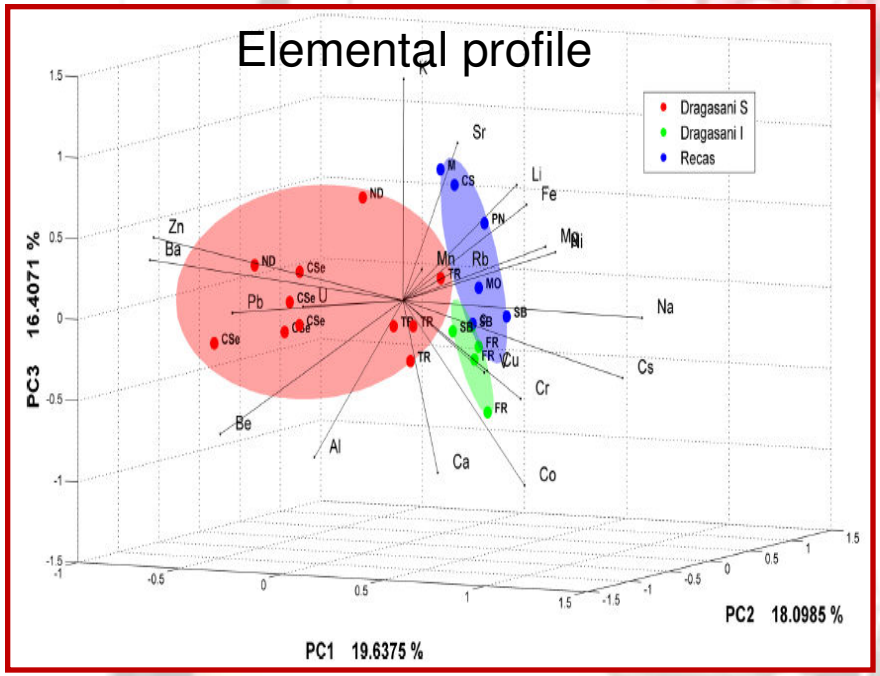


Radar plot of phenolic compounds (mg L^{-1}) in Dragasani wine native varieties

Correlation of elements to differentiate the origin of wine samples

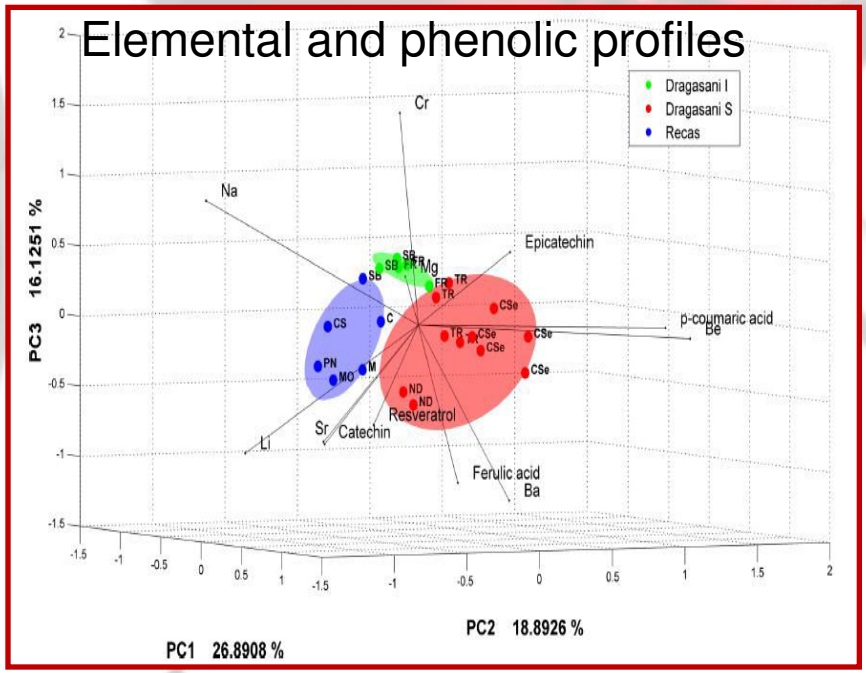


Wines geographical origin classification - PCA analysis



is possible to explain **54 %** of the variability among samples

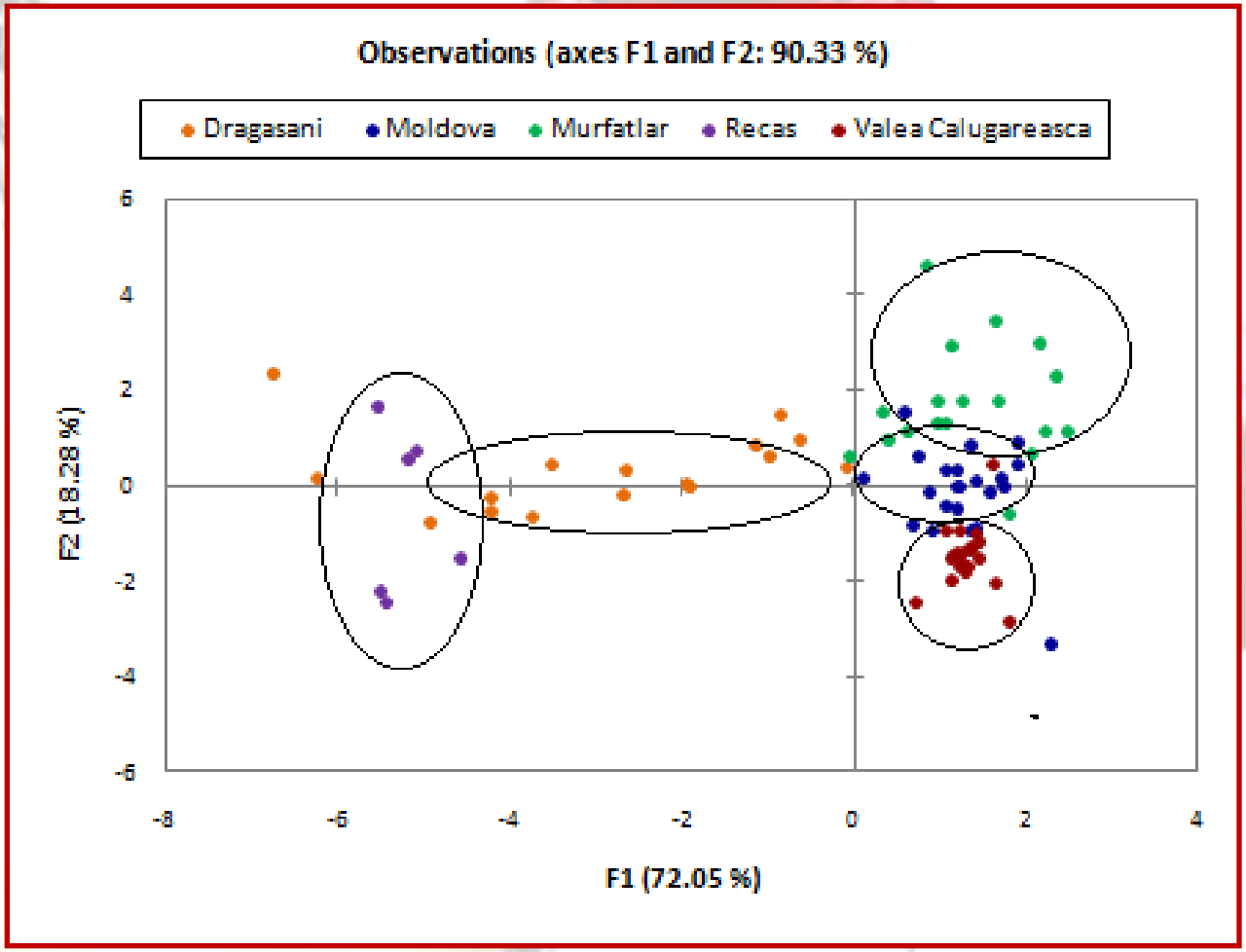
62 % classification was achieved



PCA score of wine from Dragasani S, Dragasani I, and Recas vineyards



Geographical Origin Classification based on elemental compositon



Ba
Be
Cr
Li
Mg
Na
Mn
Ag
Co
Sr

Discriminant analysis showing separation between geographical region

III. Results and discussions

Geographical traceability of wines based on $^{87}Sr/^{86}Sr$ isotopic ratio and multi-element composition

- **21 red wine (DOC and IG -** Dealurile Vrancei and Terasele Dunării) / **2012 and 2013 harvests -** Fetească Neagră and Merlot varieties

Samples preparation – microwave acid digestion

Aparatus:

- ICP-QMS – elemental (Li, Ga, Se, Ag, Tl, Pb, Ni, Cr, Ba, Zn, Sr, Rb) and isotopic determinations ($^{87}Sr/^{86}Sr$)
- F-AAS - Mn, Fe, Ca, Mg, Na and K
- GF-AAS – Al and Cu

Sr isotope analysis:

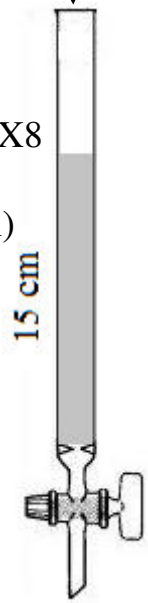
Rb/Sr separation – to eliminate the isobaric interferences of ^{87}Sr with ^{87}Rb

Optimization of instrumental parameters



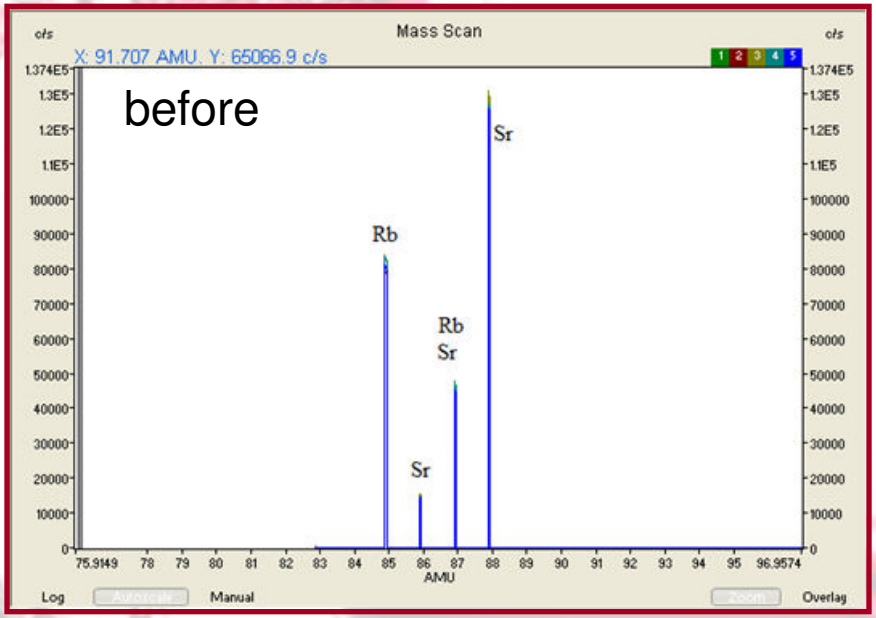
Rb/Sr separation - Ion exchange chromatography

Wine extract: Sr, Rb, Ca, Mg, K, Na, Fe

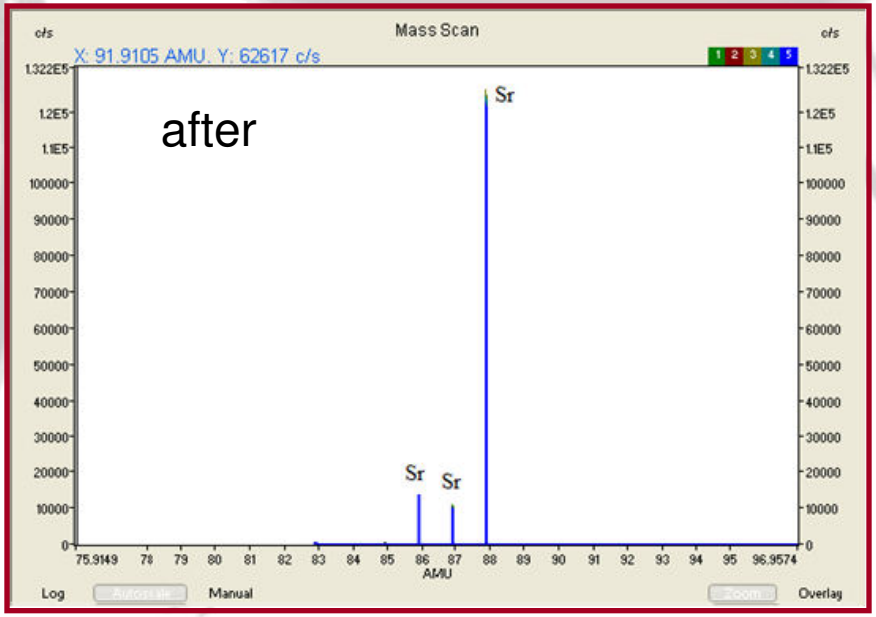


Ca, Mg - EDTA 0.02 M (pH=5.5)
Sr – EDTA 0.05 M (pH=7)

Vorster, C. et all., Analytical and Bioanalytical Chemistry, 2008, 392(1-2), 287–296.



Mass spectra for multielement standard solution

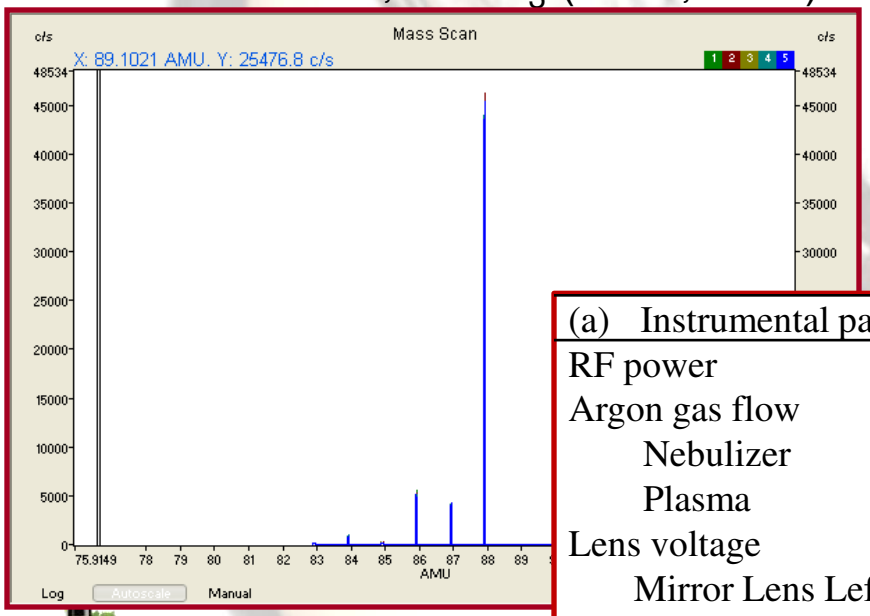


Optimization of instrumental parameters

Sr isotope determinations:

- Thermal Ionization Mass Spectrometer (**TIMS**)
- Multicollector-Inductively Coupled Plasma Mass Spectrometer (**MC-ICPMS**)
- Inductively coupled plasma sector field multicollector mass spectrometer (**ICP-SF-MC-MS**)
- Quadrupole Inductively Coupled Plasma Mass Spectrometer (**ICP-QMS**)

NIST SRM 987, $SrCO_3$ (NIST, USA)



Mass spectra

Instrumental (a) and data acquisition (b) parameters of ICP-QMS

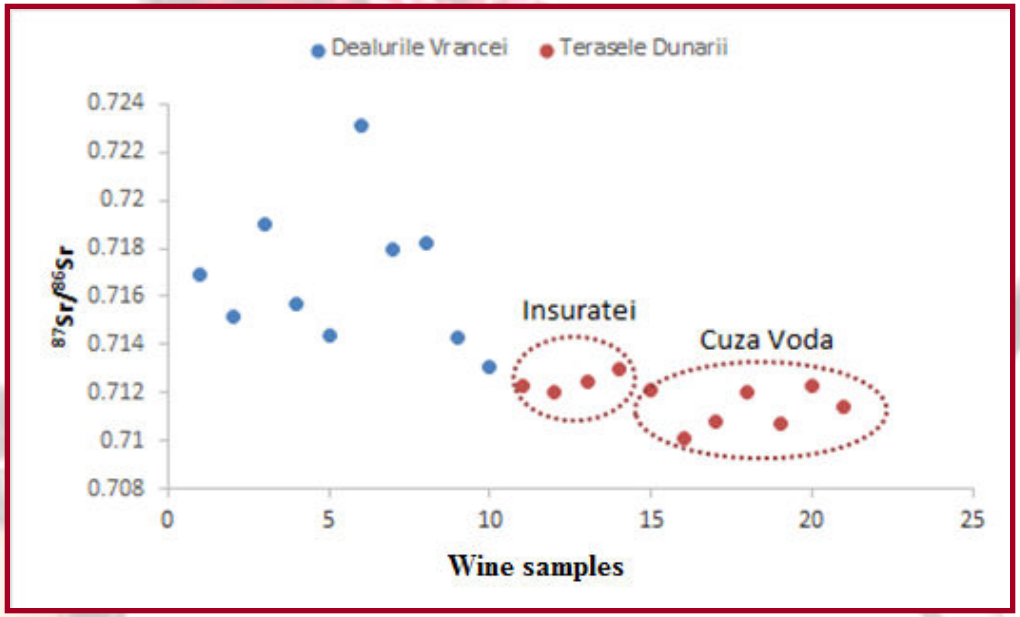
(a) Instrumental parameters		(b) Data acquisition parameters	
RF power	1.4 kW	Measuring mode	Peak hopping
Argon gas flow		Point per peak	5
Nebulizer	1.0 L/min	Scans/Replicate	10
Plasma	16.0 L/min	Replicate/Sample	10
Lens voltage		Dwell time (ms)	1
Mirror Lens Left	37 V		
Mirror Lens Right	31 V		
Mirror Lens Bottom	30 V		
Sample uptake rate	60 s	Integration time (s)	166.09 s

Sr isotopic determinations

Analytical results on standard material NIST SRM 987 – $^{87}Sr/^{86}Sr = 0.71034$

Nr.	$^{87}Sr/^{86}Sr$	RSD (%)	Acuracy (%)
1	0.70965	0.47	0.0971
2	0.71046	0.68	0.0169
3	0.70891	0.51	0.2013
4	0.71169	0.61	0.1900
5	0.70652	0.48	0.5378
6	0.70929	0.41	0.1478
7	0.71027	0.46	0.0099
8	0.71121	0.57	0.1225
9	0.70849	0.65	0.2604
10	0.71064	0.44	0.0422
Average	0.70971	0.53	0.1626

Isotope ratio $^{87}Sr/^{86}Sr$ of wine samples



- Strontium isotope ratios of the 21 Romanian wines ranged from **0.71015 - 0.72311**

0.70870 - 0.71300 – Modena, Italy
0.70762 - 0.71069 – 4 regions in Italy
0.7072 – 0.7104 – 3 regions in Argentina



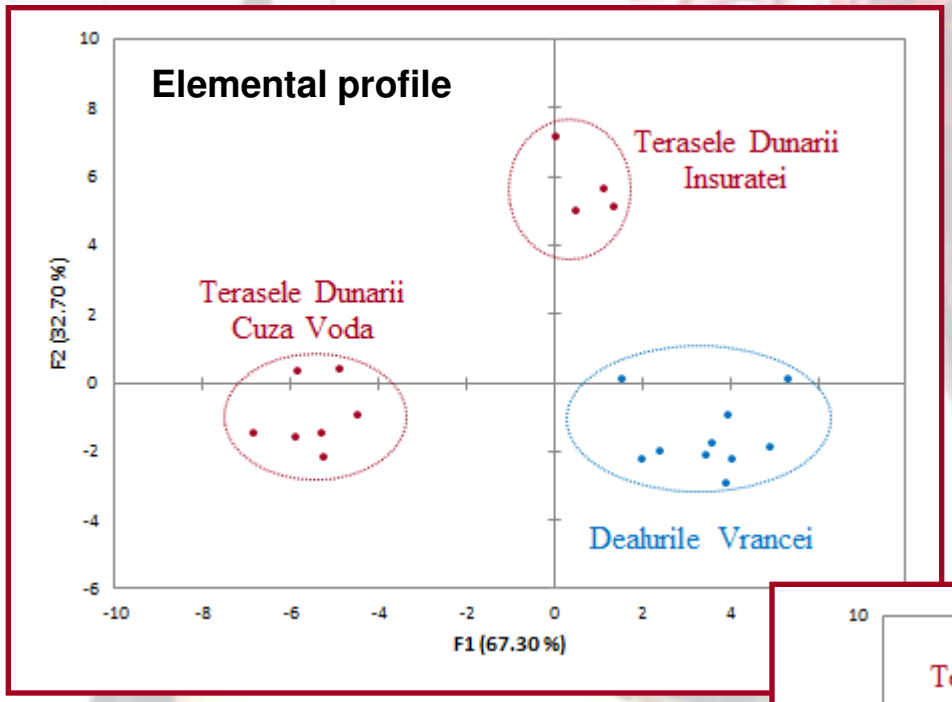
Durante et all., Food Chemistry 141 (2013) 2779–2787
Marchionni et all., J. Agric. Food Chem. 2013, 61, 6822−6831
Di Paola-Naranjo et all., J. Agric. Food Chem. 2011, 59, 7854–7865

Elemental content of wines, depending on the geographical origin (in µg/L or * mg/L)

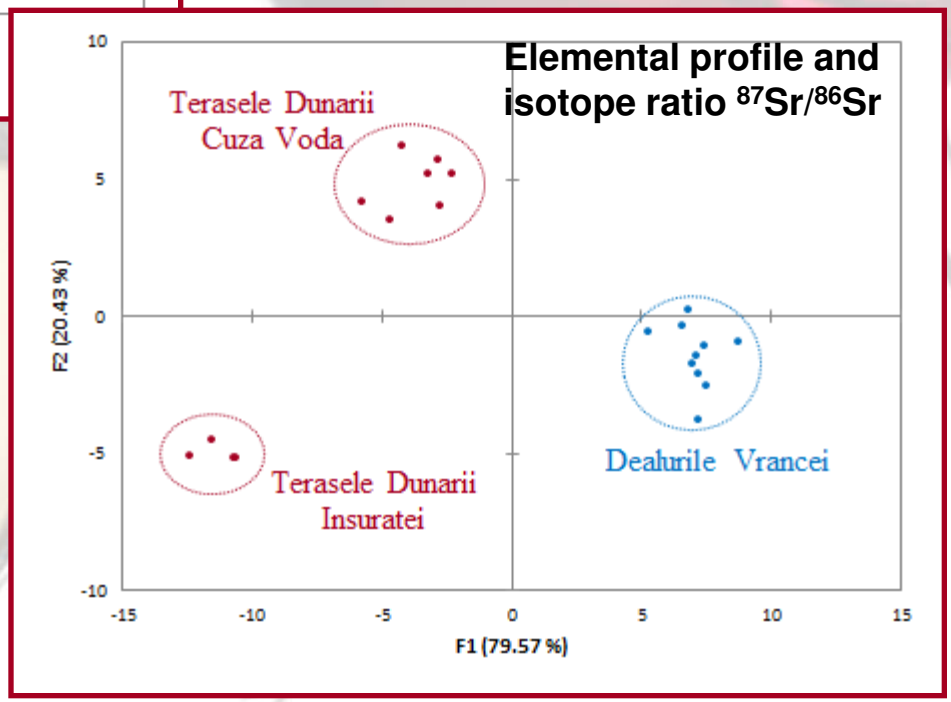
Element	Dealurile Vrancei (n=10)			Terasele Dunării (n=11)					
				Însurăței (n=4)			Cuza Vodă (n=7)		
	Average	*SD*	*Range*	*Average*	*SD*	*Range*	*Average*	*SD*	*Range*
Li	27,9a	40,3	7,6-141,6	34,7a	30,8	12,0-78,6	36,1a	27,1	12,7-78,7
Ga	7,2b	1,8	3,6-9,4	8,8a	1,1	7,9-10,3	5,0c	1,6	1,8-6,5
Se	62,9a	29,7	6,4-108,6	77,5a	23,5	43,9-96,3	56,0a	39,6	7,8-102,1
Ag	9,1a	24,4	0,9-78,4	1,2a	0,3	0,9-1,6	2,3a	1,9	1,1-6,5
Tl	1,3a	0,8	0,8-3,4	1,1a	0,2	0,8-1,2	1,2a	0,2	0,9-1,5
Pb	52,4a	77,1	9,1-261,6	15,5a	4,0	10,4-20,1	23,2a	20,9	11,1-69,9
Ni	189,6a	118,5	114,4-507,4	195,0a	67,1	130,6-271,4	91,8b	30,0	66,2-157,7
Cr	374,7a	363,3	138,8-1394,2	366,7a	305,7	134,2-816,9	265,8a	93,3	138,8-377,0
Ba	221,7a	24,0	198,4-276,6	203,6a	58,3	130,2-272,0	212,1a	21,3	186,5-238,5
Zn	369,0a	199,8	172,1-796,3	383,6a	257,2	200,1-763,7	314,4a	81,0	217,4-410,8
Sr	905,3ab	252,8	648,5-1406,8	793,9b	66,7	719,6-881,8	997,2a	200,1	795,3-1391,1
Rb	1798,7a	252,1	1314,2-2068,0	1918,8a	447,8	1302,1-2374,3	1656,8a	316,8	1140,9-2104,2
Al	442,7c	126,7	173,8-637,2	882,2a	465,4	294,6-1345,0	610,9b	176,8	312,2-843,8
Cu*	1,0a	0,2	0,6-1,3	0,9a	0,2	0,7-1,2	0,7b	0,3	0,3-1,1
Mn*	0,5a	0,4	0,1-1,1	0,4a	0,3	0,1-0,8	0,6a	0,5	0,1-1,1
Fe*	2,4a	1,3	0,1-5,2	1,7ab	1,2	0,1-3,0	0,9b	0,8	0,3-2,5
Ca*	11,2b	2,9	7,3-16,1	13,0ab	2,2	10,6-15,8	14,5a	3,9	8,8-19,9
Mg*	17,3a	4,7	14,6-30,2	16,2a	1,6	14,4-18,3	16,5a	2,8	14,3-21,4
Na*	33,5b	15,4	16,7-65,3	32,4b	7,3	23,1-39,8	45,9a	19,2	24,1-75,6
K*	1646,5a	111,2	1513-1904	1666a	177	1432-1859	1714a	93	1561-1834
K/Rb	938a	200	795-1449	911a	273	709-1312	1072a	253	853-1607
Ca/Sr	12,8b	3,6	8,5-19,2	16,5a	3,4	13,4-20,0	14,9ab	4,6	9,0-22,5



Wines geographical origin discrimination



- 100% successful classification of wines





CONCLUSIONS

- A correlation was observed between the elemental composition of the wine and its provenance soil.
- This premise is important for the application of the fingerprinting methodology based on multi-element data and statistical analysis for the classification of Romanian wines according to geographical origin, even for very close areas within the same region.
- Our results show that wine geographical origin may be distinguished based on elemental analysis and Sr isotope ratio.
- On the other hand, the use of Sr isotope ratio as tracer for wines geographical origin require performant instruments which are not accessible for many laboratories and in this way, elemental profile is an important tool for tracing the geographic authenticity and provenance of wines.
- We intend to improve our criteria for classification by analyzing a large number of samples of the same wine variety obtained from the studied geographical regions.
- The results of this study constitute the starting point in building a database for Romanian wines. These methodologies can be applied for classification of unknown wine according to geographical origin.



Acknowledgements

**Romanian Ministry of Education and Research,
National Authority for Scientific Research**

Thank you for your attention!

