DETERMINATION OF HEAVY METALS AND LEAD-STRONTIUM ISOTOPE CHARACTERIZATION FROM MERLOT SOIL SAMPLES, DEALU BUJORULUI VINEYARD

**FLORIN DUMITRU BORA[a*], ALINA DONICI[a], ANAMARIA CALUGAR[b],
IOAN VALENTIN PETRESCU MAG[c], EMESE GÁL[d],
CLAUDIU IOAN BUNEA[b]**

ABSTRACT. Contamination of vineyard soils with heavy-metals has been a worldwide concern, determination of these elements it is important for the wine industry. The aim of this research was to determine the concentration of different heavy metals (Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr) from vineyard soil on different depths in an area of vineyard cultivation from Dealu Bujorului Galati County. In order to highlight the combination of different sources, natural or anthropogenic of heavy metals the isotopic ratios for $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{207}Pb$ and $^{87}Sr/^{86}Sr$ from soil were established. The highest concentrations for heavy metals were recorded on the surface, with increasing depth of the soil these concentrations decrease. Regarding of Pb sources (lithogenic or anthropogenic), the average $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ ratios in soil profile increase horizons followed the order: 1.13526 (0-10 cm $^{206}Pb/^{207}Pb$) > 1.13427 (10-20 cm $^{206}Pb/^{207}Pb$); 2.12626 (10-20 cm $^{208}Pb/^{206}Pb$) > 2.12472 (10-20 cm $^{208}Pb/^{206}Pb$) and 17.36201 (10-20 cm $^{206}Pb/^{204}Pb$) > 17.36128 (0-10 cm $^{206}Pb/^{204}Pb$). Statistically, in the case of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ there are very significant differences between the analyzed variants while $^{206}Pb/^{204}Pb$ does not show any differences with in terms of distribution on the depth of the soil profile. Our results confirm that the

[a] *Research Station for Viticulture and Enology Targu Bujor, 65 G-ral Eremia Grigorescu, RO-805200, Galați Country Romania*

[b] *University of Agricultural Sciences and Veterinary Medicine, Department of Horticulture and Landscaping, 3-5 Mănăştur Street, RO-400372 Cluj-Napoca, Romania*

[c] *University of Agricultural Sciences and Veterinary Medicine, Department of Agriculture, 3-5 Mănăştur Street, RO-400372 Cluj-Napoca, Romania*

[d] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos str., RO-400028, Cluj-Napoca, Romania*

* Corresponding author boraflorindumitru@gmail.com

F. D. BORA, A. DONICI, A. CALUGAR, I. V. PETRESCU MAG, E. GÁL, C. I. BUNEA

^{207}Pb/^{206}Pb, ^{208}Pb/^{206}Pb, ^{204}Pb/^{206}Pb and ^{87}Sr/^{86}Sr isotope ratio can be used to track the geographical origin of wine, discriminate between wine production regions, and be used to characterize wine *terroirs* for forensic purpose.

INTRODUCTION

The soil conservation is an important and fundamental factor for sustainable development and preservation, biodiversity and balance of ecosystems. The main sources of different elements in soil are natural background (elements derived from parent rocks) and anthropogenic, which include agrochemicals, amendments, mineral fertilizer, irrigation, sewage sludge and also, the industrial wastes [1, 2]. Nevertheless, the concentration, the distribution and the bioavailability of chemical elements in the environment are influenced mainly by the climatic conditions, soil type, topography, geology, and erosive process [3, 4].

Between anthropic activities, agriculture is the main pollutant and contaminant of soil. Viticulture is an intensive practice, so the phytopharma-ceuticals and chemical fertilizers are the main source of elemental pollution [5]. In vineyard areas, the use of fungicide based on Cu in their composition pollute the soil by accumulation in high concentrations in worldwide grapevine areas [6-9]. Vines and other plants have the capacity to tolerate Cu in excess. Plants differ in terms of their capacity to accumulate heavy metals depending on genotype, on the soil conditions and on the microorganism associated to the soil, which play the role of pollutants absorbers [10]. The most of the cooper and other applied chemical tends to remain on the surface of soils [11]. Some European countries have banned the use or restricted the amount of copper-based fungicides, in response to environmental concerns over the accumulation of copper in agricultural soils and the potential impact on soil ecology [12].

Lead is an element found in the Earth's crust and soil and may be taken by plants (some species absorbing more than others, especially in certain parts) and grazing animals. The lead is evacuated in the atmosphere by different sources and can get directly into organism within the inspired air, or indirectly, after the depositing on the surface or soil, then water and food [13]. Nickel is an element found in soil and is highly dependent on the content of the parent rock material and climatic conditions, meaning that, in

soil of arid and semiarid regions, its amounts are higher [14]. The total content of Ni showed significant positive correlation with the organic matter from soil surface (0-20 cm depth) which indicate that the organic matter on the first layer of the cultivated soil contribute to Ni retention in the soil [9]. Nickel may be removed by some cultures or by leaching to deeper layer in the soil profile and this could justify the reduction of this element in some areas given its high mobility [15, 16]. Arsenic is a metalloid well-known for its toxicity and it is found in the environment from natural and anthropogenic sources (represented by mining, the use of pesticides in agriculture, industrial and municipal wastes discharging, burning of fossil fuels) [17].

Evaluation of natural abundance isotope ratios provides information on plant type or animal diet (carbon ratios) and geographical origin (lead, strontium, deuterium and oxygen isotopic ratios) [18]. Strontium is found in nature as three abundant isotopes: ^{86}Sr, ^{87}Sr, ^{88}Sr and ^{84}Sr as less abundant isotope [19–20]. Since the content of ^{87}Sr in soil varies with geological age and geographical location, the ^{87}Sr/^{86}Sr isotopic ratio can be used as a tracer for determining the geographical origin of grapes and wine [21]. Lead is found in nature as four abundant isotopes: ^{206}Pb, ^{207}Pb, ^{208}Pb and ^{204}Pb as less abundant isotope [22]. The Pb isotope of ore deposits and anthropogenic sources has their distinct isotopic ratios or signatures [23]. The Pb isotope ratio did not change in industrial or environmental processing and it's retained its characteristic ratio from source ore [24]. Each geologic substratum of vineyards is liable to heave its own Sr isotope composition, which can potentially represent a fingerprint to trace the wine production provenance [25]. The use of ^{87}Sr/^{86}Sr in tracking wine regional provenances was among the most pioneering application of isotope geology to other sciences [26]. In most of the cases, however, the analytical uncertainty observed in Sr isotopes analyses of wines from literature is larger than most of the soil/rock isotopic variability, giving strong difficulties in matching data of wines with those from geologic substrata of the vineyards. Recently, high precision analytical method for determining ^{87}Sr/^{86}Sr has been provide enabling then direct comparison between data on wines with those of the pedological and geological substrata [27,28]. Long lived isotope ratios of heavy metals of geological interest, such as ^{87}Sr/^{86}Sr, ^{206}Pb/^{204}Pb, ^{207}Pb/^{204}Pb, ^{208}Pb/^{204}Pb, have in the last decades gained importance in tackling the issues of geographical food traceability as well as in solving issues related with archaeological, environmental, medical and also forensic sciences [29].

The purpose of this study was to determine the elemental composition (Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr) from vineyard soil, on different depths (0-120cm) using the ICP-MS technique (method approved by

L'Organisation International du Vie et Vin) and also to complete the knowledge of the large-scale distribution of strontium (^{87}Sr/^{86}Sr) and lead (^{206}Pb/^{204}Pb, ^{207}Pb/^{204}Pb, ^{208}Pb/^{204}Pb) isotope ratios in soil from Dealu Bujorului Vineyard (Galati county).

RESULTS AND DISCUSSION

Heavy metals content from Merlot soil samples

In Table 1 are summarized the total contents of Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr on the depth of the soil profile. The mean contents of Cd and Pb were 0.33±0.05 mg/kg and 6.20±0.57 mg/kg, in case of Cd the lowest concentrations were recorded in the surface of the soil profile [0.19±0.02 mg/kg (10-20 cm)], while the highest concentration was recorded on the depth of the soil profile [0.61±0.05 mg/kg (90-100 cm)]. Pb content in sol ranged from [10.46±1.28 mg/kg (10-20 cm)] and [2.61±0.73 mg/kg (110-120 cm)], the highest concentration was recorded on the surface of the soil profile. The results agree with other scientific papers [30] 0.30 mg/kg (Cd), 11.50 mg/kg (Pb); [31] 0.18 mg/kg (Cd) and significantly lower than those obtained by some authors [32] 7.38 mg/kg (Cd), 132.30 mg/kg (Pb) and [31] 36.00 mg/kg (Pb). The U concentration from soil was between [0.51±0.10 mg/kg (0-10 cm)] and [0.25±0.10 mg/kg (110-120 cm)] with an average of 0.35±0.06 mg/kg. Regarding the distribution of U concentration on the depth of the soil profile, it can be observed that with increase of the depth the U concentration from soil decreases, as in the case of Pb. The results agree with Moragues-Quiroga et al. [33] 0.03 mg/kg (U); and significantly lower than those obtained by Saat et al. [34] 2.21 mg/kg (U).

The content of Hg, As Sr and Co found in Dealu Bujorului Vineyard agreed with literature data [33, 35-37]. The average values of these metals 0.076±0.012 mg/kg (Hg), 1.31±0.11 mg/kg (As), 34.44±1.62 mg/kg (Sr) and 3.45±0.25 mg/kg (Co) do not indicate soil pollution in Dealu Bujorului vineyard (Table 1).

In terms of Ni and Cr the highest concentration was recorded in the surface of the soil profile for both metals, Ni [9.17±0.99 mg/kg (0-10 cm); 8.18±1.00 mg/kg (10-20 cm)] and Cr [13.80±1.33 mg/kg (30-40 cm); 13.08±0.83 mg/kg (20-30 cm)] with an average value of 5.61±0.23 mg/kg (Ni) and 10.81±0.72 mg/kg (Cr) (Table 1). The results agree with literature data [30-37].

Concerning Cu concentration in soil, at the surface of the soil profile were recorded the highest concentration [546.01±9.15 mg/kg (0-10 cm); 521.37±4.59 mg/kg (10-20 cm)] with an average value of 371.25±2.01 mg/kg,

these concentrations from far exceed the maximum allowed by the legislation (20 mg/kg). The data demonstrate strong pollution of vineyard soil by copper. Copper concentrations in the topsoil of Dealu Bujorului vineyard was between the values of 546.01±9.15 mg/kg (0-10 cm) and 120.59±1.15 mg/kg (80-90 cm) with an average value of 356.03±4.36 mg/kg. The copper enrichment in different vineyard soil types reflects the period of copper-based pesticide application [39]. We consider the climate and application of elevated volumes of fungicide as the circumstantial factor for the high copper contamination in vineyards soil. Copper concentration in soil is significantly lower than the maximum value reported in literature (1500 mg/kg) [40]. Regarding the distribution of copper concentration on the depth of the soil profile, it can be observed that with increase of the depth the copper concentration from soil decreases. We assume that total copper distribution between vine lines mainly depends on certain technical operations, such as implemented spraying technical operations, foliage removing from midway zones or simply foliage and other organic debris redistribution in horizontal directions by winds, machine wheels, animals and/or humans [39].

The copper sulphate actually are used in all wine counties and also in Bordeaux, according to Mirlean et al. [39] mixture preparation contains: Zn 1309 mg/kg, Pb 95 mg/kg, Cr 19 mg/kg Ni 10 mg/kg and Cd 1.4 mg/kg. Therefore, we considered that copper-based pesticide is the principal source of established soil enrichment by heavy metals. Similar conclusions have reached also some authors in Serbia [32], Brasil [39], Italy [41], Romania [42], France [43] and Spain [44]. The results agree with Alagić et al. [32] 315.00 mg/kg (Cu), Couto et al. [45] 602.23 mg/kg (Cu), Romić et al. [46] 586 mg/kg (Cu), Chaignon et al. [47] 398 586 mg/kg (Cu) and significantly higher than those obtained by Rusjan et al. [48] 88.00 mg/kg (Cu).

Concerning factor which influences the distribution of metals on the soil profile, it can be seen as the depth factor had influenced very significant distribution Cd, Pb, U, As, Sr, Co, Cu, Ni and Cr, in case of Hg distribution on the soil profile depth factor had a lesser influence (Table 1).

Reporting the obtained results [Cd average 0.33±0.05 mg/kg (1mg/kg M.A.L = (maximum limit allowed); Pb average 5.82±0.75 mg/kg (20 mg/kg M.A.L); Hg average 0.076±0.012 mg/kg (0.1 mg/kg M.A.L); As average 1.31±0.11 mg/kg (1 mg/kg M.A.L); Co average 3.45±0.25 mg/kg (15 mg/kg M.A.L); Ni average 5.61±0.23 mg/kg (1 mg/kg M.A.L) and Cr average 10.81±0.72 mg/kg (30 mg/kg M.A.L)] to national and international legislation we can say that the soil from Dealu Bujorului vineyard falls within the limits set by the law, except the Cu average 356.03±4.36 mg/kg concentration which exceeds this limit (20 mg/kg) (Table 1).

Table 1. Variation of the metal content in Merlot soil samples from Dealu Bujorului Vineyard (mg/kg)

Area / Type of soil	Depth (cm)	Cd M.A.L.*	Pb M.A.L.	U M.A.L.	Hg M.A.L.	As M.A.L.	Sr M.A.L.	Co M.A.L.	Cu M.A.L.	Ni M.A.L.	Cr M.A.L.
*Normal Values		1 mg/kg	20 mg/kg	-	0.1 mg/kg	5 mg/kg	-	15 mg/kg	20 mg/kg	20 mg/kg	30 mg/kg
Alert threshold — Susceptible		3 mg/kg	50 mg/kg	-	1 mg/kg	15 mg/kg	-	30 mg/kg	100 mg/kg	75 mg/kg	100 mg/kg
Less Susceptible		5 mg/kg	250 mg/kg	-	4 mg/kg	25 mg/kg	-	100 mg/kg	250 mg/kg	200 mg/kg	300 mg/kg
Intervention threshold — Susceptible		5 mg/kg	100 mg/kg	-	2 mg/kg	25 mg/kg	-	50 mg/kg	200 mg/kg	150 mg/kg	300 mg/kg
Less Susceptible		10 mg/kg	1.000 mg/kg	-	10 mg/kg	50 mg/kg	-	250 mg/kg	500 mg/kg	500 mg/kg	600 mg/kg
Chernozem	0-10	0.34±0.07 c	7.52±1.05 b	0.51±0.10 a	0.045±0.013 b	0.44±0.11 f	40.43±0.72 ab	2.66±0.53 de	546.01±9.15 a	9.17±0.99 a	12.67±0.77 ab
	10-20	0.19±0.02 d	10.46±1.28 a	0.51±0.05 a	0.076±0.019 a	0.58±0.44 f	41.02±2.13 a	2.94±0.94 d	521.37±4.59 b	8.18±1.00 ab	12.02±1.59 b
	20-30	0.25±0.07 cd	10.25±0.89 a	0.33±0.07 bcde	0.075±0.018 a	0.87±0.08 e	39.10±1.37 abc	3.16±0.16 d	516.10±7.85 bc	7.68±0.68 b	13.08±0.83 ab
	30-40	0.27±0.08 cd	9.41±0.26 a	0.35±0.09 bcde	0.064±0.017 ab	1.44±0.19 b	39.71±1.52 abc	4.11±0.08 c	510.79±3.35 c	5.31±0.15 cde	13.80±1.33 a
	40-50	0.28±0.06 cd	8.03±0.51 b	0.42±0.07 ab	0.046±0.014 b	1.28±0.07 bc	36.18±1.76 abc	4.13±0.02 c	328.79±2.84 f	4.45±0.39 defg	12.48±0.44 ab
	50-60	0.18±0.06 d	4.18±0.89 cd	0.40±0.01 abc	0.092±0.012 a	1.38±0.14 b	32.18±1.04 d	4.41±0.13 bc	357.46±4.73 e	4.17±0.03 efg	9.86±0.39 cd
	60-70	0.24±0.07 cd	2.66±0.78 de	0.38±0.06 bcd	0.091±0.004 a	0.99±0.02 de	37.65±1.80 bc	5.39±0.27 a	400.73±1.93 d	5.20±0.10 cdef	9.82±0.39 cd
	70-80	0.53±0.03 ab	3.70±0.86 cde	0.34±0.08 bcde	0.080±0.016 a	2.42±0.10 a	36.70±1.18 c	4.75±0.06 b	319.52±6.65 g	3.67±0.93 g	10.53±0.52 c
	80-90	0.44±0.05 b	2.76±0.35 de	0.26±0.07 cd	0.086±0.004 a	2.22±0.08 a	37.16±1.55 c	2.35±0.19 e	120.59±1.55 f	3.96±0.07 fg	10.35±0.75 c
	90-100	0.61±0.05 a	3.71±0.26 cde	0.28±0.04 cde	0.083±0.007 a	1.47±0.27 b	26.49±2.01 e	2.36±0.36 e	121.16±3.47 i	4.47±0.12 defg	7.56±0.55 e
	100-110	0.32±0.04 c	4.5±1.16 c	0.27±0.04 cde	0.070±0.022 ab	1.31±0.03 bc	21.82±1.99 f	2.23±0.11 e	315.28±6.19 g	6.32±1.47 c	8.75±0.52 de
	110-120	0.33±0.06 c	2.61±0.73 e	0.25±0.10 d	0.085±0.005 a	1.14±0.10 cd	22.81±2.38 f	2.95±0.51 d	213.41±0.31 f	5.35±0.79 cde	8.58±0.52 de
Average		0.33±0.05	5.82±0.75	0.35±0.06	0.076±0.012	1.31±0.11	34.44±1.62	3.45±0.25	371.25±2.01	5.61±0.23	10.81±0.72
Minimum Values		0.18±0.06	2.61±0.73	0.25±0.10	0.045±0.013	0.44±0.11	21.82±1.99	2.23±0.11	120.59±1.15	3.96±0.07	7.56±0.55
Maximum Values		0.61±0.05	10.46±1.28	0.51±0.10	0.092±0.012	2.42±0.10	41.02±2.13	5.39±0.27	546.01±9.15	9.17±0.99	13.80±1.33
Sig.		***	***	***	**	***	***	***	***	***	***
Depth F		18.320	21.562	4.356	2.987	58.951	114.565	54.123	2531.546	21.546	18.152
Sig.		***	***	***	*	***	***	***	***	***	***
[1]		2.20	36.60						368.00	52.70	67.60
[2]		3.14±0.03	42.80			10.70±0.01			293.00	16.67±0.09	
[3]			16.18±5.20				241.88±493.40	3.60	10.87±5.10		
[4]		26.00	264.90						165.30	9.00	27.80
[5]		0.40	7.10						211.40		54.10

Average value ± standard deviation (n = 3). Different letters are significantly different for $p \leq 0.05$ between depths. The difference between any two values, followed by at least one common letter, is insignificant. Order of the Ministry of Waters, Forests and Environmental Protection No.756/3 November 1997, approving the regulation on the assessment of environmental pollution, Bucharest, Romania; 1997. M.L.A.* (maximum limit allowed) = Normal Values.

$^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratio from Merlot soil samples

The Pb isotope ratio for the selected soil (Table 2) varies in range between 1.13142-1.14205 ($^{206}Pb/^{207}Pb$), 2.12065-2.14153 ($^{208}Pb/^{206}Pb$) and 17.29604-17.42010 ($^{206}Pb/^{204}Pb$) with average 1.13418 ($^{206}Pb/^{207}Pb$), 2.12906 ($^{208}Pb/^{206}Pb$) and 17.36398 ($^{206}Pb/^{204}Pb$). The wide range of isotope ratio obtained suggest that Pb content in the soil is a product of the combination of different sources and not from a sole origin, natural or anthropogenic, and that it has also been accumulating over time since the massive use of Pb in fuel, pain [49]. The $^{206}Pb/^{207}Pb$ ratio is most commonly used in environmental studies because it can be determined precisely, and the abundances of these isotopes are relatively important [50]. The isotope ration $^{206}Pb/^{207}Pb$ revealed a different behaviour between soil profiles at each sampling site.

Regarding $^{206}Pb/^{207}Pb$ isotope ration based on analyses it can be concluded that the vineyard soil from Dealu Bujorului with an average values of 1.13418 $^{206}Pb/^{207}Pb$ come from automobile emissions (if $^{206}Pb/^{207}Pb$=1.1000-1.1400 [automobile emissions]) [51]. The values of $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ isotope ratio are between the ranges from 2.12065 to 2.14153 ($^{208}Pb/^{206}Pb$) and 17.29604 to 17.42010 ($^{206}Pb/^{204}Pb$) with an average values of 2.12906 ($^{208}Pb/^{206}Pb$) and 17.36398 ($^{206}Pb/^{204}Pb$). The highest values of $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ isotope ratio were registered on the depth of the soil profile from 70-80 cm [2.14153 ($^{208}Pb/^{206}Pb$)] followed by 80-90 cm [2.13417 ($^{208}Pb/^{206}Pb$)] and values recorded at 50-60 cm [17.42010 ($^{206}Pb/^{204}Pb$)] followed by 90-100 cm [17.41431 ($^{206}Pb/^{204}Pb$)] and 110-120 cm [17.39252 ($^{206}Pb/^{204}Pb$)]. The lowest values of $^{08}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ isotope ratio were recorded also on the depth of the soil profile 30-40 cm [2.21065 ($^{208}Pb/^{206}Pb$)] followed by 110-120 cm [2.12311 ($^{208}Pb/^{206}Pb$)], 10-20 cm [2.12472 ($^{208}Pb/^{206}Pb$)], 60-70 cm [2.12443 ($^{208}Pb/^{206}Pb$)], 90-100 cm [2.12512 ($^{208}Pb/^{206}Pb$)], 100-110 [2.12457 ($^{208}Pb/^{206}Pb$)].

Regardless of Pb sources (lithogenic or anthropogenic), the average $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ ration in soil profile horizons followed the order: 1.13526 (0-10 cm $^{206}Pb/^{207}Pb$) > 1.13427 (10-20 cm $^{206}Pb/^{207}Pb$); 2.12626 (10-20 cm $^{208}Pb/^{206}Pb$) > 2.12472 (10-20 cm $^{208}Pb/^{206}Pb$) and 17.36201 (10-20 cm $^{206}Pb/^{204}Pb$) > 17.36128 (0-10 cm $^{206}Pb/^{204}Pb$). Statistically, in the case of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ there are very significant differences between the analyzed variants while $^{206}Pb/^{204}Pb$ does not show any differences with in terms of distribution on the depth of the soil profile.

These results demonstrate that Pb isotopic ratios were derived mainly from weathered parent material, except the $^{206}Pb/^{207}Pb$ uppermost horizons of soil profiles which come from automobile emissions. The more radiogenic $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{207}Pb$ ratio in soil probably reflects the Pb derived from

weathered bedrock, and the isotopic composition of Pb is mostly influenced by the decay of U and Th content in the soil, weathering processes and original rock age, which provide a fingerprint used for different forensic and archeological purposes [52].

Concerning $^{87}Sr/^{86}Sr$, isotope ratio the values are between the ranges from 0.72316 to 0.72701, with an average value of 0.72514. The highest values were registered on the depth of the soil surface profile 60-70 cm (0.72701) followed by values recorded at 40-50 cm (0.72653) and 50-60 cm (0.72616), the lowest value of $^{87}Sr/^{86}Sr$ isotope ratio was registered on the depth of the soil at 70-80 cm (0.722316) (Table 2). In this case, these are no significant differences between the $^{87}Sr/^{86}Sr$ isotope ratio. These obtained values of $^{87}Sr/^{86}Sr$ isotope ratio can be attributed to a larger proportion of radiogenic (K and Rb rich) mineral due to the weathering of the most weatherable mineral, i.e. Ca-plagioclase, which is promoted by a strong decrease of the soil pH [53].

CONCLUSIONS

In this work the heavy metals concentration from Merlot plantation was studied in order to highlight the heavy metals composition of soil. In case of Cd (1 mg/kg), Pb (20 mg/kg), Hg (0.1 mg/kg), As (5 mg/L), Co (15 mg/kg), Ni (20 mg/kg) and Cr (30 mg/kg) metals in analysed soil samples were under Maximum Permissible Limits (MPL). Cu concentration in the soil exceeds the maximum admissible limit (20 mg/kg) having the average value of 371.25 mg/kg, this value is a common one for vineyards soils.

Regardless of Pb sources (lithogenic or anthropogenic), the average $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ ration in soil profile horizons followed the order: 1.13526 (0-10 cm $^{206}Pb/^{207}Pb$) > 1.13427 (10-20 cm $^{206}Pb/^{207}Pb$); 2.12626 (10-20 cm $^{208}Pb/^{206}Pb$) > 2.12472 (10-20 cm $^{208}Pb/^{206}Pb$) and 17.36201 (10-20 cm $^{206}Pb/^{204}Pb$) > 17.36128 (0-10 cm $^{206}Pb/^{204}Pb$). Statistically, in the case of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ there are very significant differences between the analyzed variants while $^{206}Pb/^{204}Pb$ does not show any differences with in terms of distribution on the depth of the soil profile.

Our results confirm that the $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ and $^{87}Sr/^{86}Sr$ isotope ratio can be used to track the geographical origin of wine, discriminate between wine production regions, and be used to characterize wine *terroirs* for forensic purpose.

Table 2. The $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratios obtained from soil samples on Dealu Bujorului Vineyard

Area	Type of soil	Depth (cm)	$^{206}Pb/^{207}Pb$	SD	RSD (%)	$^{208}Pb/^{206}Pb$	SD	RSD (%)	$^{206}Pb/^{204}Pb$	SD	RSD (%)	$^{87}Sr/^{86}Sr$	SD	RSD (%)	Pb (mg/kg)	Sr (mg/kg)
Dealu Bujorului Vineyard (Merlot plantation)	Chernozem	0-10	1.13526 b	0.00081	0.07094	2.12626 cde	0.00450	0.21164	17.36128 ab	0.00372	0.02144	0.72402 a	0.00127	0.17510	7.52±1.05 b	40.43±0.72 ab
		10-20	1.13427 bc	0.00179	0.15741	2.12472 de	0.00245	0.11508	17.36201 ab	0.04304	0.24790	0.72551 a	0.00300	0.41403	10.46±1.28 a	41.02±2.13 a
		20-30	1.13367 bcd	0.00244	0.19737	2.13431 bcd	0.00143	0.06723	17.36131 ab	0.01093	0.06295	0.72526 a	0.00203	0.28009	10.25±0.89 a	39.10±1.37abc
		30-40	1.13514 b	0.00267	0.23493	2.12065 e	0.00664	0.31299	17.36118 ab	0.04951	0.28516	0.72420 a	0.00205	0.28306	9.41±0.26 a	39.71±1.52 abc
		40-50	1.13142 d	0.00114	0.10101	2.13304 bc	0.00231	0.10842	17.36735 ab	0.09673	0.55696	0.72653 a	0.00522	0.71860	8.03±0.51 b	36.18±1.76 abc
		50-60	1.13299 bcd	0.00048	0.04237	2.13010 bcd	0.00629	0.29552	17.42010 a	0.03343	0.19188	0.72616 a	0.00065	0.08994	4.18±0.89 cd	32.18±1.04 d
		60-70	1.14205 a	0.00051	0.04482	2.12443 de	0.00118	0.05573	17.29604 b	0.07263	0.41993	0.72701 a	0.00673	0.92558	2.66±0.78 de	37.65±1.80 bc
		70-80	1.13260 bcd	0.00016	0.01376	2.14153 a	0.00521	0.24325	17.33670 ab	0.03654	0.42661	0.72316 a	0.00260	0.35885	3.70±0.86 cde	36.70±1.18 c
		80-90	1.13375 bcd	0.00132	0.11655	2.13417 b	0.00153	0.07163	17.34712 ab	0.04841	0.27906	0.72498 a	0.00218	0.30032	2.76±0.35 de	37.16±1.55 c
		90-100	1.13330 bcd	0.00149	0.13186	2.12512 de	0.00264	0.12429	17.41431 a	0.01073	0.06160	0.72511 a	0.00353	0.48734	3.71±0.26 cde	26.49±2.01 e
		100-110	1.13222 cd	0.00040	0.03513	2.12457 de	0.00037	0.01750	17.34786 ab	0.03338	0.19243	0.72451 a	0.00227	0.31268	4.50±1.16 c	21.82±1.99 f
		110-120	1.13354 bcd	0.00166	0.14682	2.12978 cbd	0.00465	0.21854	17.39252 a	0.02353	0.13528	0.72541 a	0.00360	0.49581	2.61±0.73 e	22.81±2.38 f
		Average	1.13418	0.00122	0.10775	2.12906	0.00327	0.15348	17.36398	0.03855	0.22211	0.72514	0.00293	0.40245	5.82±0.75	34.44±1.62
		Minimum Values	1.13142	0.00016	0.01376	2.12065	0.00037	0.01750	17.29604	0.00372	0.02144	0.72316	0.00127	0.08994	2.61±0.73	21.82±1.99
		Maximum Values	1.14205	0.00267	0.23493	2.14153	0.00664	0.31299	17.42010	0.09673	0.55696	0.72701	0.00673	0.92558	10.46±1.28	41.02±2.13
		Sig.	***			***			in			in			***	***
		[6]	1.17190	0.00004		2.09943	0.00013		18.31684	0.00160					9.00	
		Tyszka et al. [88]	1.17300	0.01400		2.09500	0.01700								36.00	
		Ayuso et al. [89]	1.19550						18.67900	0.86000		0.72238			11.00	1.74
		dos Santos et al. [91]	1.17000	0.05000					17.98000						13.65	
		Reza et al. [92]	1.18390			2.46240						0.71008			6.20	
		Sherman et al. [93]	0.84380			2.06200										
		Vorster et al. [94]										0.71270				

Average value ± standard deviation (n = 3). Romans letters represent the significance of the variety difference ($p \le 0.05$). The difference between any two values, followed by at least one common letter, is insignificant. SD-standard deviation, RSD %-relative standard deviation. in = insignificant.

EXPERIMENTAL SECTION

Study area

Soil samples was harvested from Dealu Bujorului vineyard (45°52′10″ N, 27°55′8″E), the scattered spreading territory of this vineyard corresponds almost entirely to the geographical subunit known as Colinele Covurluiului, in whose area is also the Dealu Bujorului vineyard. The vineyard is crossed by the parallel 46° latitude north, intersected by the 28° longitude meridian. Dealu Bujorului vineyard belongs to Galați country. The specificity of the transition area is highlighted by the predominance of deposits of clays and sands. Versants were made from clay deposits and sandy sands.

Soil sample collection

Soil sampling was carried out on the depth of the soil profile (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50-60 cm, 60-70 cm, 70-80 cm, 80-90 cm, 90-100 cm, 100-110 cm and 110-120 cm). Soil samples were collected using stainless steel shovels and were stored in individual black plastic bags (darkness). All samples were taken in triplicates from the defined experimental plot. Soil samples have been brought first to sand-size material (< 2 mm) using a jaw crusher then mechanically split to obtain a representative samples and eventually pulverized to powder-size, grain-size smaller than 100 µ (< 400 mesh), using a ball mill. Agate ball mill is used in place of any other pulverization metal device to avoid possible trace element contamination [54]. Soil samples before splitting and pulverisation have been dried at 60 ^{0}C.

Reagents and solutions

Ten elements (Cd, Pb, U, Hg, As, Sr, Co, Cu, Ni and Cr) were determined in order to assess their ability to discriminate wines by geographical origin. The analysis was made using multielement analysis and ICP-MS technique, after an appropriate dilution, using external standard calibration method. The calibration was performed using XXICertiPUR multielement standard, and from individual standard solution of Cr and Hg. The working standards and the control sample were prepared daily from the intermediate standards that were prepared from the stock solution. The intermediate solutions stored in polyethylene bottles and glassware was cleaned by soaking in 10% v/v HNO_3 for 24 hours and rinsing at least ten rimes with ultrapure water (18.2 MΩ cm^{-1} ultrapure water-Types 1). The

accuracy of the methods was evaluated by replicate analyses of fortified samples (10 µL-10 mL concentrations) and the obtained values ranged between 0.8-13.1 percent, depending on the element. The global recovery for each element was estimated and the obtained values were between 84.6-100.9% [21].

For quality control purpose, blanks and triplicates samples (n = 3) were analyzed during the procedure. The variation coefficient was under 5% and detection limits (ppb) were determined by the calibration curve method. Limit of detection (LoD) and Limit of quantification (LoQ) limits were calculated according to the next mathematical formulas: LoD = 3SD/s and LoQ = 10 SD/s (SD = estimation of the standard deviation of the regression line; s = slope of the calibration curve).

Table 3. Instrumental conditions for the determination of each element (ICP-MS technique)

Element	Correlation coefficient	LoD* (µg/L)	LoQ*** (µg/L)	BEC** (µg/L)
Cd	0.9999	0.0202	0.0673	0.027
U	0.9999	0.0253	0.0842	0.005
As	0.9999	0.2335	0.7776	0.538
Co	0.9999	0.0365	0.1215	0.152
Ni	0.9999	0.0591	0.1968	0.091
Pb	0.9999	0.0003	0.0010	0.002
Hg	0.9999	0.0417	0.1379	0.128
Sr	0.9999	0.1434	0.4775	0.955
Cu	0.9999	0.0402	0.1339	0.237
Cr	0.9999	1.6630	5.5378	0.636

*Detection limit; **Background equivalent concentration; ***Quantification limit.

For calibration and also to verify the achieved accuracy and precision, ten NIST-SRM 987 and NIST-SRM 982 analysis results were pooled together with the calculated relative standard deviation presented in Table 4. Based on the obtained results, it was verified that, applying quadrupole ICP-MS, relative standard deviation and reproducibility of approximately 0.5% for $^{87}Sr/^{86}Sr$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ are feasible. The results were in agreement with those reported by [21, 52].

Table 4. Lead isotopic ration and Lead isotopic ration determination precision and accuracy based on the NIST SRM 982 (Lead) NIST SRM 987 (Strontium) (n=10)

Replicate	$^{207}Pb/^{206}Pb$ (a)	RSD (%)	$^{208}Pb/^{206}Pb$ (b)	RSD (%)	$^{204}Pb/^{206}Pb$ (c)	RSD (%)	$^{87}Sr/^{86}Sr$ (d)	RSD (%)
1	0.46483	0.51	0.99891	0.67	0.00271	0.32	0.70493	0.31
2	0.47891	0.48	0.99452	0.61	0.00272	0.41	0.72046	0.45
3	0.46978	0.32	0.99794	0.55	0.00275	0.28	0.70325	0.63
4	0.47123	0.64	0.99688	0.64	0.00273	0.51	0.70634	0.48
5	0.46987	0.56	0.99726	0.48	0.00246	0.14	0.71478	0.36
6	0.46154	0.37	0.99647	0.56	0.00258	0.39	0.71245	0.59
7	0.47362	0.70	0.99969	0.34	0.00279	0.47	0.70987	0.46
8	0.45641	0.43	0.99744	0.58	0.00278	0.51	0.72326	0.42
9	0.41562	0.36	0.99576	0.59	0.00273	0.49	0.70845	0.68
10	0.45612	0.45	0.99874	0.61	0.00278	0.36	0.10789	0.47
Average	0.46179	0.48	0.99736	0.56	0.00270	0.41	0.71117	0.49

[a]Certified value=$^{207}Pb/^{206}Pb$ (0.46707±0.00020);
[b]Certified value=$^{208}Pb/^{206}Pb$ (1.00016±0.00036);
[c]Certified value=$^{204}Pb/^{206}Pb$ (0.027219±0.00027);
[d]Certified value=$^{87}Sr/^{86}Sr$ (0.71034±0.00026); RSD (%) = relative standard deviation.

Sample preparation for determination of heavy metals and isotopic ration from soil using ICP-MS

For the determination of heavy metals from soils samples were used an amount of 0.5 g soil and adjust 8 mL (7 mL HNO_3 65%+1 mL H_2O_2) were placed in a clean Teflon digestion vessel, after 15-30 minutes the mineralization was performed using a microwave system Milestone START D Microwave Digestion System set in three steps: step I (time 10 min., temperature 220ºC), step II (time 15 min., temperature 220ºC) and step III (time 60 min., ventilation - temperature 35ºC). After mineralization, samples were filtered through a 0.45 mm filter and brought to a volume of 50 mL. The Pb and Sr isotope ration in the analysed soil samples ($^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$,) were determined according to the methodology indicated by Mihaljevič *et al*. [51]; Geana et al. [21].

In order to confirm the best chosen conditions for soil digestion standard additions for checking accuracy of the microwave digestion and recoveries were calculated (Table 5). The digestion seemed visually completed in all of the combinations, but the spiked recoveries showed significant differences for total elements content (p = 0.005).

Table 5. Standard additions for checking accuracy of the microwave digestion ICP-MS method (n = 3)

Element	Certified Concentration (mg/kg)	Measured Concentration (mg/kg)
Cd	0.371±0.002	0.369±0.006
U	3.15±0.05	3.17±0.03
As	10.50±0.30	10.26±0.18
Co	130.00±9.00	129.32±0.36
Ni	85.00±2.00	83.65±1.89
Pb	0.00173±0.00001	0.00169±0.0002
Hg	0.90±0.20	0.91±0.21
Sr	239.00±6.00	238.02±1.56
Cu	33.90±0.50	33.96±0.53
Cr	130.00±9.00	130.15±0.78

Instrumentation

The determination of metals was performed on mass spectrometer with inductively coupled plasma, (ICP-MS) iCAP Q Thermo scientific model, based polyatomic species before they reach the quadrupole mass spectrometer, using a PFA micro flow concentric nebulizer. The argon used was of 99.99% purity (Messer, Austria). The instrument was daily optimized to give maximum sensitivity for M^+ ions and the double ionization and oxides monitored by the means of the rations between Ba^{2+}/Ba^+ and Ce^{2+}/CeO^+, respectively, these always being less than 2%. The experimental conditions were: argon flow on nebulizer (0.82 L/min.), auxiliary gas flow 0.80 L/min., argon flow in plasma 15 L/min., lens voltage 7.30 V; RF power in plasma 1100 W, spray chamber temperature (2.50±1.00^{o}C). Accuracy was calculated for the elements taken into consideration (0.5-5.0%).

Statistical analysis

The statistical interpretation of the results was performed using the Duncan test, SPSS Version 24 (SPSS Inc., Chicago, IL., USA). The statistical processing of the results was primarily performed in order to calculate the following statistical parameters: average and standard deviation. This data was interpreted with the analysis of variance (ANOVA) and the average separation was performed with the DUNCAN test at $p ≤ 0.05$.

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REFERENCES

1. Li, J.L., He, M., Han, W., Gu, Y.F., *Journal of Hazardous Materials*, **2009**, *164*, 976-981.
2. L.R.F. Alleoni, R.B. Borba, O.A. Camargo, *Topicos em Ciencia do Solo*, **2005**, 4, 1–42.
3. J.F.G.P. Ramalho, N.M.B. Amaral Sobrinho, A.C.X. Velloso, *Pesquisa Agropecuaria Brasileira*, **2000**, *35*, 1289–1303.
4. A. Kabata-Pendias, "Trace elements in soils and plants". 4th ed. Boca Raton: Chemical Rubber Company Press, **2011.**
5. A. Facchinelli, E. Sacchi, L. Mallen, *Environmental Pollution*, **2001**, *114* (3), 313–324.
6. M.C. Ramos, M. L´opez-Acevedo, *Advances in Environmental Research*, **2004**, *8* (3-4), 687-696.
7. M. Kom´arek, E. ˇCadkov´a, V. Chrastn´y, F. Bordas, J. Bollinger, *Environment International*, **2010**, *36* (1), 138–151.
8. G.C.G. dos Santos, G.S. Valladares, C.A. Abreu, O.A. de Camargo, C.R. Grego, *Applied and Environmental Soil Science*, **2013**, Article ID790795, 1-10.
9. W. Preston, Y.J.A.B. da Silva, C.W.A. do Nascimento, K.P.V. da Cuhna, D.J. Silva, H.A. Ferreira, *Geoderma Regional*, **2016**, *7*, 357-365.
10. C. Nicula, A. Peter, L. Mihaly-Cozmuta, A. Mihaly-Cozmuta, *Carpathian Journal of Food Science and Technology*, **2013**, *5* (1-2), 1-8.
11. G.R. Nachtigall, R.C. Nogueirol, L.R.F. Alleoni, M.A. Cambri, *Brazilian Archives of Biology and Technology*, **2007**, *50* (6), 941–948.
12. Q-Y. Wanga, D-M. Zhoua, L. Canga, *Soil Biology and Biochemistry*, **2009**, *41*, 1504-1509.
13. L. Bretan, O. Ketney, F. Boltea, *Carpathian Journal of Food Science and Technology*, **2010**, *2* (2), 8-12.
14. A. Kabata-Pendias, H. Kabata, "Trace elements in soils and plants", 3rd edition CRC Press, Boca Raton, **2001**.
15. V. Antoniadis, C.D. Tsadilas, *Applied Geochemistry*, **2007**, *22*, 2375-2380.
16. E.V. Mellis, M.P. Cruz, J.C. Casagrande, *Scientia Agricola*, **2004**, *61* (2), 190-195.

17. M. Senila, T. Kotsev, E. Levei, M. Roman, V. Mladenov, Z. Cholakova, L. Senila, STUDIA UBB CHEMIA LXI, **2016**, *3*, Tom II, 333-344.
18. S. Kelly, K. Heaton, J. Hoogewerff, *Trends in Science and Technology*, **2005**, *16*, 555–567.
19. M. Berglund, M.E. Wieser, *Pure and Applied Chemistry*, **2011**, *83* (2), 397–410.
20. P. Martins, M. Madeira, F. Monteiro, *OIV*, **2013**, *48* (1), 21–29.
21. E.I. Geana, A. Marinescu, A.M. Iordache, C. Sandru, R.E. Ionete, C. Bala, *Food Analytical Methods*, **2017**, *10* (1), 63-73.
22. K.J.R. Rossman, P. D. P. Taylor, *International Union of Pure and Applied Chemistry*, **1998**, *70* (1), 217–235.
23. H. Cheng, Y. Hu, *Environmental Pollution*, **2010**, *158* (5), 1134-1146.
24. W.U. Ault, R.G. Senechal, W.E. Erlebach, *Environmental Science and Technology*, **1970**, *4*, 305-313.
25. S. Marchionni, A. Buccianti, A. Bollati, *Food Chemistry*, **2016**, *190*, 777-785.
26. R.D. Di Paola-Naranjo, M. V. Baroni, N. S. Podio, *Journal of Agricultural and Food Chemistry*, **2011**, *59*, 7854-7865.
27. M.R. Mercurio, E. Grilli, P. Odierna, V. Morra, T. Prohaska, E. Coppola, C. Grifa, A. Buondonno, A. Langella, *Geoderma*, **2014**, *230-231*, 64-78.
28. C. Baschieri, L. Bertacchini, D. Bertelli, M. Cocchi, A. Marchetti, D. Manzini, G. Papotti, S. Sighinolfi, *Food Chemistry*, **2015**, *173*, 557-563.
29. S. Voerkelius, G.D. Lorenz, S. Rummel, C.R. Quéte, G. Heiss, M. Baxter, C. Brach-Papa, P. Deters-Itzelberger, S. Hoelzl, J. Hoogewerff, E. Ponzevera, M. van Bocxstaele, H. Ueckermann, *Food Chemistry*, **2010**, *118*, 933-940.
30. E. Mahmoudabadi, F. Sarmadian, R.N. Moghaddam, *International Journal of Environmental and Science Technology*, **2015**, *12* (10), 3283-3298.
31. F. Curran-Cournane, G. Lear, L. Schwendenmann, J. Khin, *Soil Research*, **2015**, *53*, 306-315.
32. S.Č. Alagić, D.B. Tošić, M.D. Dimitrijević, M.A. Antonijevic, M.M. Nujikic, *Environmental Science and Pollution Research*, **2014**, *22* (9), 7155-7175.
33. C. Moragues-Quiroga, J. Juilleret, L. Gourdol, E. Pelt, T. Perrone, A. Aubert, G. Morvan, F. Chabaux, A. Legout, P. Stille, C. Hissler, *Catena*, **2017**, *149* (1), 185-198.
34. A. Saat, A.S. Kamsani, W.N.A.N. Kamri, N.H.M. Talib, A.K. Wood, Z. Hamzah, *AIP Conference Proceeding*, **2015**, *1659* (1), 1-6.
35. H. Kailing, S. Zehanf, H. Yuanan Z. Xiangying; Y., Zhiqiang; C., Hefa, *Environmental Science and Pollution Research*, **2017**, *24*, 9387-9398.
36. M. Laghlimi, *European Journal of Scientific Research*, **2015**, *129* (2), 167-178.
37. R.T. Ottesen, M. Birke, T.E. Finne, et al., *Applied Geochemistry*, **2013**, *33*, 1-12.
38. M. Çolak, *Journal of Environment and Earth Science*, **2012**, *67*, 695-712.
39. M. Mirlean, A. Roisenberg, J.O. Chies, *Environmental Pollution*, **2007**, *149*, 10-17.
40. L.M. Flores-Veles, J. Ducaroir, A. M. Jaunet, M. Robert, *European Journal of Soil Science*, **1996**, *47*, 523-532.

41. F. Pinamonti, G. Nicolini, A. Dalpiaz, et al*., Communications in Soil Science and Plant Analysis*, **1999**, *30*, 1531-1549.
42. F.D. Bora, C.I. Bunea, T. Rusu, et al., *Chemistry Central*, **2015**, *9*, 1-13.
43. E.I.B. Chopin, B. Marin, R. Mkougafoko, et al., *Envrionmental Pollution*, **2008**, *156*, 1092-1098.
44. S. Bravo, J.A. Amorós, C. Pérez-de-los-Reyes, et al., *Journal of Geochemical Exploration*, **2015**, *173*, 79-83.
45. R.R. Couto, L. Benedet, J.J. Comin, et al., *Environmental Earth Sciences*, **2014**, *73* (10), 6379-6386.
46. M. Romić, D. Romić, G. Ondrašek, *Agriculturae Conspectus Scientificus*, **2004**, *2-3*, 35-41.
47. V. Chaignon, I. Sanchez-Neira, P. Herrmann, et al., *Environmental Pollution*, **2003**, *123*, 229-238.
48. D. Rusjan, M. Strlič, D. Pucko, et al., *Geoderma*, **2006**, *136*, 930-936.
49. I. Galušková, M. Mihaljevič, L. Borůvka, et al., *Journal of Geochemical Exploration,* **2014**, *147*, 215-221.
50. M. Komárek, V. Ettler, V. Chrastny, et al., *Environmental International*, **2008**, *34*, 562-577.
51. M. Mihaljevič, V. Ettler, O. Šebek, et al., *Journal of Geochemical Exploration*, **2006**, *88*, 130-133.
52. C.M.S. Almeida, A.C. Almeida, M.L.D.P. Godoy, et al., *Journal of the Brazilian Chemical Society*, **2016**, *27* (6), 1-6.
53. T. Drouet, J. Herbauts, W. Gruber et al., *European Journal of Soil Science*, **2007**, *58*, 302-319.
54. A. Takamasa, S. Nakai, *Geochemical Journal*, **2009**, *43*, 389-394.