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ОДРЕДУВАЊЕ НА ИЗОТОПИ НА ОЛОВО ВО ВИНО И МАСЛО ЗА ЈАДЕЊЕ СО ПРИМЕНА НА ИНДУКТИВНО СПРЕГНАТА ПЛАЗМА СО МАСЕНА СПЕКТРОМЕТРИЈА

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Апстракт: Преку определувањето на односот на изотопите на олово може да се добие информација за изворот на загадување со олово во примероци од животната средина. Од друга страна, овие анализи можат да обезбедат корисна информација за географското потеклото на анализираните примероци. Многу мали варијации во изотопскиот однос се јавуваат во природата и тоа најчесто зависат од некои локални извори на загадување. Индуктивно спрегната плазма со масена спектрометрија (ICP-MS) беше користена како работна техника, со цел да се утврди дали оваа техника е соодветна во практично справување со полиатомските интерференци при анализата на органски матрикси. Овој труд опфаќа оптимизација на инструменталната метода за мерење на изотопите на олово во дигестирани примероци на вино и масло за јадење. Покрај тоа, изотопските односи на олово беа одредени во примероци од исто и различно географско подрачје. Вкупната концентрација на олово во испитуваните примероци на вино се наоѓа во опсегот од 3,74-16,3 µg/L, не надминувајќи ги максимално дозволените концентрации за олово во вино (200 µg/L). Вредностите за односите на концентрациите на изотопите $^{207}\text{Pb}/^{206}\text{Pb}$ и $^{208}\text{Pb}/^{206}\text{Pb}$ се наоѓаат во опсег од 0,98 – 1,12 и 2,22 – 2,99, соодветно. Вкупната содржина на олово во примероците на масло за јадење се наоѓа во опсегот од 18,3 до 29,6 µg/kg, не надминувајќи ги максимално дозволените концентрации за олово во масло за јадење. Статистички значајна разлика помеѓу вредностите за односите на концентрациите на изотопите $^{207}\text{Pb}/^{206}\text{Pb}$ и $^{208}\text{Pb}/^{206}\text{Pb}$ е утврдена за примероците со различно географско потекло.

Клучни зборови: *изотопи на олово, вино, масло за јадење, ICP-MS.*

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ISOTOPIC LEAD MEASUREMENTS IN WINE AND EDIBLE OIL USING INDUCTIVELY COUPLED PLASMA WITH MASS SPECTROMETRY

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Abstract: Lead isotope ratios provide analytical information related to the source of lead contamination in naturally occurring samples. On the other hand, these measurements can provide useful information for routine means of “fingerprinting” the components grown in different habitats. Small Pb isotope abundance variation occurs in nature and the isotopic composition of lead in the environment is dependent on the local pollution sources. In this study, inductively coupled plasma with mass spectrometry (ICP-MS) was used to investigate whether this chemical application can offer a reliable and practical solution to the problem of the polyatomic overlap in the presence of organic based matrix samples. The study summarizes the instrument optimization procedure for Pb isotope measurements in wine and edible oil samples. Also, the isotopic ratios were presented for samples from same and from different geographical region. The total lead concentration in wine samples ranges from 3.74-16.3 µg/L, not exceeding the maximum allowable concentrations of lead in wine (200 µg/L). Values for the isotopes concentration ratios $^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ ranges from 0.98-1.12 and 2.22-2.99, respectively. The total lead content in edible oil samples ranges from 18.3 to 29.6 µg/kg, not exceeding the maximum allowable concentrations of lead in edible oil (100 µg/kg). Statistically significant differences between the values for the isotopes concentration ratios $^{207}\text{Pb}/^{206}\text{Pb}$ и $^{208}\text{Pb}/^{206}\text{Pb}$ were determined for samples from different geographic origin.

Kew words: *lead isotope, wine, edible oil, ICP-MS.*

1. Introduction

Due to the complex environmental chemistry, transport patterns, meteorological conditions, the mixing of emissions from multiple sources, and uncertainties associated with receptor modeling, it remains difficult to quantify the relative impact of emissions from different sources on metal accumulation in different food stuffs [1, 2]. The emission and deposition of hazardous trace metals such as lead (Pb) is of significant concern because it can affect human and environmental health [3, 4]. Lead isotope ratio analysis is important as it is used for Pb-Pb dating in geochronology, and to trace the

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origin of artifacts, precious metals and even foodstuffs [5, 6]. Lead isotope ratio measurement can provide analytical information related to the source of lead contamination in naturally occurring samples [7]. Studies of the isotopic composition of lead are therefore commonly used in the environmental, geological and anthropological studies [8]. Small Pb abundance variations occur in nature and the isotopic composition of lead in the environment is dependent on the local pollutant source. If lead is present in the soil, a plant will take up small amounts and subsequent isotope ratio studies might provide unique means of differentiating between different plant sources of origin [6]. Local lead level may become mixed with external source of contamination that vary with time depending on the anthropogenic activity. There are four stable isotopes of Pb with the following approximate abundances: ^{208}Pb (52.4%), ^{207}Pb (24.1%), ^{206}Pb (22.1%), and ^{204}Pb (1.4%). ^{204}Pb is non-radiogenic and was not measured in this study because of its low relative abundance. ^{208}Pb , ^{207}Pb , and ^{206}Pb are formed by the radioactive decay of ^{232}Th (half-life = 14 billion years), ^{235}U (half-life = 0.7 billion years), and ^{238}U (half-life = 4.5 billion years), respectively [9]. Inductively coupled plasma mass spectrometry (ICP-MS) has been increasingly used in isotope ratio measurements in recent years [6, 9, 10]. The main purpose of this work was to test the sensitivity of inductively coupled plasma with mass spectrometry (ICP-MS) for measurements of stable lead isotopes (^{206}Pb , ^{207}Pb and ^{208}Pb). The lead isotope ratios were also calculated for Vranec wine samples from Tikveš wine region and edible oils produced from same seed variety as well as from same and different geographical origin.

2. Materials and methods

2.1. Sample preparation

Wine and edible oil samples, before the isotopic measurements, were totally digested with application of a closed digestion system (Model MARS 5, CEM Corporation, USA). A representative sample (0.5 g) was placed in a digestion vessel. For digestion of vine samples 5 mL nitric acid (HNO_3 , 65% w/w, ultra-trace purity) was used for total dissolution of organic compounds [11]. For digestion of oil samples 5 mL of nitric acid and 2 mL of hydrogen peroxide (H_2O_2 , 30% w/w, ultra-trace purity) were added in order to achieve total dissolution of the organic compounds. The vessels were closed and settled in the microwave system and the four step program was applied for total dissolution of the organic matrix samples (Table 1).

**Table 1.** Microwave digestion program for digestion of oil and wine samples

Step	Initial <i>T</i> (°C)	Final <i>T</i> (°C)	Power (W)	Time (min.)
1	25	150	600	15
2	150	150	600	5
3	150	180	800	10
4	180	180	800	10

2.2. ICP-MS measurements

The single quadrupole inductively coupled plasma mass spectrometer (ICP-MS) was used for all isotopic measurements (model 7500cx, Agilent technologies, USA). The instrument was tuned for standard robust plasma conditions, equipped with micro-mist nebulizer and octopole reaction system (ORS). The operating conditions of the ICP-MS instrument were optimized before the analysis was performed. The integration time of analysis was set for 0.5 s per one point, with 5 replications for one measurement. First ionization was set at -3 V and -150 V for the second ionization. The CeO/Ce ratio was 0.58% (reference value <0.65%) and for the double charge ions (Ce⁺⁺/Ce ratio) was obtained a satisfactory value of 2.05% (reference value <3%). For the quantitative analysis of the digested wine samples, external calibration curves were built at different concentration levels: 5, 10, 50 and 100 µg/L. Ten sample blanks (ultra-pure water with 2.5% w/w HNO₃) were run to determine instrument detection limits (DL). Background equivalent concentration (BEC) was calculated as an indicator for the calibration offset expressed as a concentration, due to elemental contamination of the blank.

3. Results and discussion

3.1. Method validation

Whichever methodology is selected, the natural or “common lead” ²⁰⁴Pb isotope is measured as a reference to calculate the original (primordial) level of the other (mainly radiogenic) Pb isotopes, so accurate measurement of ²⁰⁴Pb is essential. Unfortunately, for measurements made using inductively coupled plasma mass spectrometry (ICP-MS), ²⁰⁴Pb suffers an isobaric interference from ²⁰⁴Hg (an isobaric overlap is where isotopes of different elements occur at the same nominal mass) meaning that any mercury present as a contamination or as a component of the sample would bias the measurement of ²⁰⁴Pb. Very good sensitivity was obtained for ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb isotopes concentration measurements (Figure 1). Satisfactory linearity (R) was obtained in the range from 5 to 100 µg/L (Figure 1). The instrumental detection limits for the three isotopes (²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb) were calculated as 0.35, 2.86 and 0.85 µg/L, respectively.

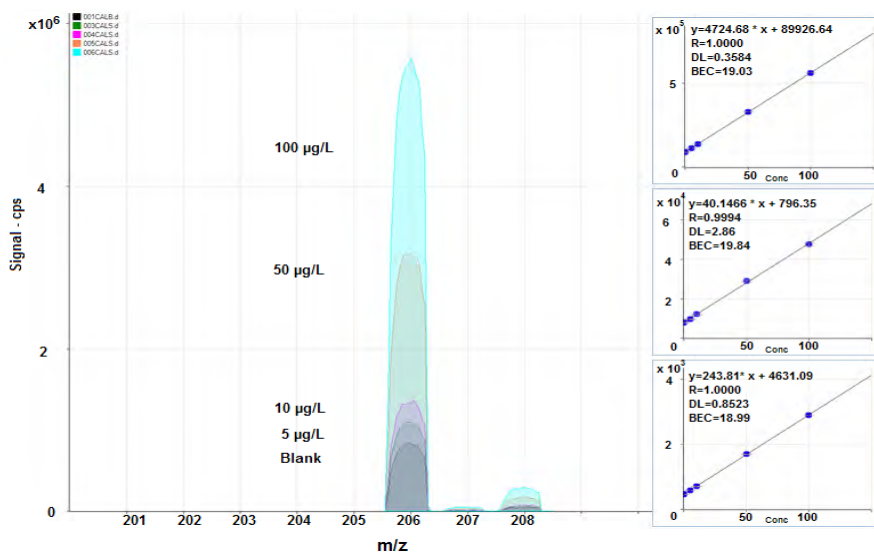


Figure 1. Mass spectrum and linearity for ^{206}Pb , ^{207}Pb and ^{208}Pb calibration curves

To confirm that Pb does not undergo a charge-transfer reaction (and to check if any cluster ions are formed), 20 µg/L Pb solution was aspirated and a mass scan was performed. The sum of the signal for these clusters amounted to <0.5% of the total Pb signal (the sum of the unreacted precursor and reacted product Pb ions); at this level the product ions can be considered negligible and ignored. Pb did not appear to undergo any charge transfer reaction, as the Pb signal with argon gas in the cell remained at practically the same level as with no reaction gas.

Table 2. Short-term stability of Pb isotope ratio determination (20 µg/L lead standard solution)

Acquisition time	1 replica	2 replica	3 replica	4 replica	5 replica	RSD (%)
	10:22 AM	10:26 AM	10:29 AM	10:31 AM	10:34 AM	
^{206}Pb (µg/L)	0.241	0.241	0.214	0.241	0.241	0.05
^{207}Pb (µg/L)	0.221	0.220	0.220	0.220	0.220	0.05
^{208}Pb (µg/L)	0.523	0.524	0.523	0.522	0.523	0.03
$^{207}\text{Pb}/^{206}\text{Pb}$	0.916	0.914	0.914	0.914	0.914	0.08
$^{208}\text{Pb}/^{206}\text{Pb}$	2.169	2.168	2.169	2.167	2.165	0.08



The obtained signal expressed as counts per seconds for all three lead isotopes showed very strong stability among the time measurements. The median value for ^{206}Pb was 79260 cps (RSD=5.5%), for ^{207}Pb was 67500 cps (RSD=8.2%), for ^{208}Pb was 157330 cps (RSD=8.9%).

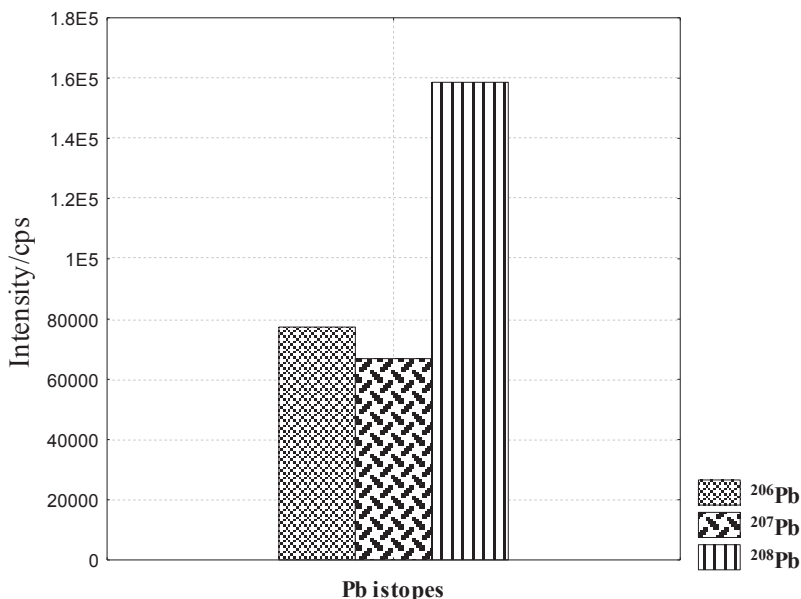


Figure 2. Intensities of measurements for ^{206}Pb , ^{207}Pb and ^{208}Pb

3.2. Lead isotopes measurements for wine samples

Ten different Vranec wines samples fermented with different yeasts were analysed for total lead concentration and lead isotopes ratio. The total Pb concentration ranged from 3.74-16.3 $\mu\text{g/L}$ [11]. Maximal acceptable limits for total lead content in wine samples is 200 $\mu\text{g/L}$ [12]. In none of the analysed wines values above the critical value for total lead concentration were obtained. The calculated isotope ratios ranged: a) $^{207}\text{Pb}/^{206}\text{Pb}$: from 0.985 to 1.122 with standard deviation of data distribution of 0.038 and b) $^{208}\text{Pb}/^{206}\text{Pb}$: from 2.221 to 2.998 with standard deviation of data distribution 0.21 (Table 3) presenting no significant variation, probably because of the same geographical origin of the grapes. In fact, Vranec wines were produced from Vranec grapes (*Vitis vinifera* L.) grown in Tikveš wine region in R. Macedonia [11]. Similar analyses were conducted with wine samples from different geographical regions in Italy [13]. For the Italian wines the isotopes concentration ratio $^{208}\text{Pb}/^{206}\text{Pb}$ ranges from 2.09 to 2.14, while the $^{207}\text{Pb}/^{206}\text{Pb}$ concentration ratio ranges from 1.14 to



1.19 [13]. These data were also compared to data obtained for the Australian and South Africa wines [13]. Significant variation occurs for the isotopes concentration ratio $^{208}\text{Pb}/^{206}\text{Pb}$.

Table 3. Lead concentration and lead isotopic ratio for wines

Wine samples	Lead isotopes concentration ratio		Total Pb concentration ($\mu\text{g/L}$) [1]
	$^{207}\text{Pb}/^{206}\text{Pb}$	$^{208}\text{Pb}/^{206}\text{Pb}$	
Vranec 1	0.998	2.569	6.35
Vranec 2	1.011	2.356	8.79
Vranec 3	1.122	2.578	3.74
Vranec 4	1.055	2.356	3.81
Vranec 5	0.985	2.345	9.75
Vranec 6	1.012	2.221	11.2
Vranec 7	1.055	2.365	10.2
Vranec 8	1.036	2.998	16.3
Vranec 9	0.997	2.457	9.58
Vranec 10	1.022	2.345	10.4

3.3. Lead isotopes measurements for edible oil samples

Total lead contents and lead isotope concentration ratios in sunflower and oilseed rape oil were determined (Table 4). The total lead content in sunflower oil from R. Macedonia (N=22, cold press) ranges between 18.3-29.6 $\mu\text{g}/\text{kg}$, while the lead content in sunflower oil produced sunflower seed (N=10, refined) ranged from 6.25-15.4 $\mu\text{g}/\text{kg}$ (Table 4). For none of the analyzed oil samples values above the maximum allowed content 100 $\mu\text{g}/\text{kg}$ [12] for total lead in edible oils were obtained. Therefore, the pollution factor in the analyzed samples has not been considered. Very similar values were obtained for the lead isotopes concentration ratio $^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$, for sunflower and oilseed rape oil produced from seeds planted in R. Macedonia. For the sunflower oil produced from seed with foreign origin 0.859 for $^{207}\text{Pb}/^{206}\text{Pb}$ ratio and 2.044 for $^{208}\text{Pb}/^{206}\text{Pb}$ ratio were obtained. Furthermore, these isotopic ratios probably could be correlated with the origin of the seeds.



Table 4. Lead isotopic ratio for sunflower and oilseed rape oils

Oil sample seed variety	Geographical origin of seed used for oil production	N	Lead isotopes concentration ratio		Range for Pb total content (µg/ kg)
			$^{207}\text{Pb}/^{206}\text{Pb}$	$^{208}\text{Pb}/^{206}\text{Pb}$	
Sunflower oil	RM	22	1.078 ± 0.30	2.769 ± 0.11	18.3-29.6
Sunflower oil	Unknown origin	10	0.859 ± 0.18	2.044 ± 0.28	6.25-15.4
Oilseed rape oil	RM	6	1.09 ± 0.25	2.596 ± 0.08	11.5 -33.6

RM - oil produced from seed planted in R. Macedonia; Unknown origin - oil produced from seed imported in R. Macedonia

The intensities of the isotopic measurements are presented by using bar-plots, in order to visualize the chemical abundance of the single lead isotopes, as mean value, for all three groups of edible oils (Figure 3). For the sunflower oil (produced from seed planted in R. Macedonia) the relative abundance of the Pb isotopes is $^{206}\text{Pb} < ^{207}\text{Pb} < ^{208}\text{Pb}$, the same sequence follows the oilseed rape oil (produced from seed planted in R. Macedonia); while for the sunflower oil (produced from seed imported in R. Macedonia) the relative abundance of the Pb isotopes is $^{206}\text{Pb} < ^{208}\text{Pb} < ^{207}\text{Pb}$ (Figure 3). Kelly et al. in 2005 introduce very similar approach in tracing the geographical origin of various food [14]. No significant variation occurs in values obtained for the isotopes concentration ratio $^{207}\text{Pb}/^{206}\text{Pb}$. However, the $^{208}\text{Pb}/^{206}\text{Pb}$ isotopes concentration ratio, has been shown to vary significantly depending on the geographical origin for numerous varieties of plant foods, including olive oil [14].

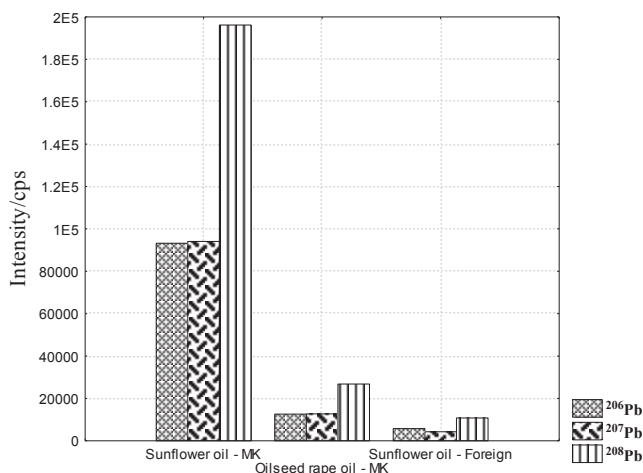


Figure 3. Intensities of measurements for ^{206}Pb , ^{207}Pb and ^{208}Pb in edible oil samples



4. Concluding remarks

The use of ICP-MS for measuring the isotopic composition to identify the origin of the anomalous lead content in wine and edible oil samples proved to be a powerful technique. The precision and accuracy of ICP-MS analysis made it possible to satisfactorily discriminate among the likeliest lead sources in the studied samples. The performance of the applied techniques allowed measuring three lead isotopes (^{206}Pb , ^{207}Pb and ^{208}Pb) with very good sensitivity. For the ^{204}Pb , a very low sensitivity was obtained due to the isobaric interference from ^{204}Hg . The total lead contents in all of the analysed samples did not exceed the maximum allowed concentration for that kind of samples. The isotope ratios for wine samples ($^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$) didn't show any significant variation, probably because the wines were produced from grapes with identical geographical origin. For the edible oil samples, significant variation occurred for the same variety of the seed taken from different geographical region for both isotope ratios. Therefore, these isotopic analyses can provide useful information correlated with the geographical origin of wine and edible oil.

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