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Elemental Composition of Various Apple Cultivars Grown in Serbia

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Metals are essential for important biochemical and physiological functions and are necessary for maintaining health throughout life. In order to assess the impact of human activity on the food chain, monitoring of trace metals in a apple samples has been focus of this study. Trace levels of metals such as Na, Mg, Ca, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and Cd were determined in five different varieties of apples purchased from local market of Niš city of Serbia. Metal levels analyzed by using inductively coupled plasma optical emission spectrometry (ICP-OES). Among the 12 elements analyzed, sodium was the most abundant element distributed throughout all analyzed apple samples. Iron was the predominant minor element constituents. The accuracy of the results was evaluated by spike recovery tests. Analysis of variance was used to establish the metals with significant difference in mean content between the cultivars from apples.

Key Words: Minerals, Apples, ICP-OES.

INTRODUCTION

Numerous investigations of whole fruits of various types, including apples, have been carried out because of the nutritional importance of these fruits, mainly regarding their acids, amino acids, minerals and vitamins content[1,2]. It is a well-known fact that fruits and their juices constitute a rich source of vitamins and minerals. Due to recent heavy metal contamination of the environment, the analysis of trace elements in seasonal fruit samples as well as in their products has gained considerable importance because of health considerations[3]. Approximately 30 elements are recognized as essential to life. Whereas some are required in macronutrient amounts, such as Ca, K, Mg and Na, others occur in trace or ultra quantities. Cu, Fe, Ni, Zn and Mn are at the top and of this trace scale and play an important role in biological systems. Also, Cr, Co and Se are essential for normal development and function of human cells. But when the elemental intake is excessively elevated even essential elements can elicit toxic effects. Some elements (*e.g.* Pb and Cd) are non-essential elements and toxic even in trace amounts[1].

Trace minerals consistency of fruits and vegetables depends on different factors *e.g.* genetic, weather, soil and the harvesting stage of maturity and their supply to the markets. Mineral contents reduced in plants by the climatic conditions due to rain, mist, fog, light and temperature and then further loss takes place due to loss suitable storage conditions. If the cultivated soil is different in certain trace metals then the cultivated crop would ultimately be deficient for those metals[4].

Due to the importance of the minor and major elements present in fruits, several studies have been carried out to determine their levels. Tokglioglu and Gurbuz[5] proposed flame atomic absorption spectrometry for the determination of copper and iron in various food samples. The same technique was used for the determination of the trace heavy metals in some beverages and food drinks[6].

Inductively coupled plasma optical emission spectrometry (ICP-OES) has proved to be a rapid and accurate technique for the determination of minor and major element contents in fruits[7]. ICP-OES is attractive for trace analysis, owing to the satisfactory sensitivity coupled with the advantage of simultaneous determinations of several metals at different spectral lines. ICP-OES and exploratory analysis were used for the determination metals in apples and apple juice[8].

The aim of the present work was the determination of the minor and major element compositions in five apple cultivars as basis for evaluating the health effect by metals through apple consumption. Five apple cultivars purchased from local market were analyzed for their minor and major element contents after optimization and validating an appropriate analytical method using ICP-OES. The study is focused on those essential elements which can be easily determined by ICP-OES, namely Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn.

EXPERIMENTAL

Ultra-scientific (USA) ICP multi-element standard solutions of about 20.00 ± 0.10 mg L^{-1} were used as a stock solution for

calibration. The plastic containers used for storing the samples were cleaned to avoid contamination of the samples with traces of any metals. Containers were treated with 5 % nitric acid and washed with ultra-pure water 0.05 μS cm^{-1} (MicroMed high purity water system, TKA Wasseraufbereit-ungssysteme GmbH).

All analysis were carried out on iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK) which use an Echelle optical design and a change injection device solid-state detector. The operating conditions are shown in Table-1. Dry aching method was carried out in a VIMS electric (Serbia) furnace equipped with microprocessor program of temperature IVIGOS3123 (±1º).

TABLE-1
OPERATIONAL PARAMETERS FOR ICP-OES MEASUREMENTS

Parameters	
Flush pump rate	100 rpm
Analysis pump rate	50 rpm
RF power	1150 W
Nebulizer gas	0.7 L min^{-1}
Coolant gas flow	12 L min^{-1}
Auxiliary gas flow	0.5 L min^{-1}
Plasma view	Dual mode

Fruit samples: The apple (n = 5) samples were purchased from local markets in Nis. All apples analyzed are frequently consumed in Serbia.

All apple samples were washed thoroughly and separately. Running tap water was employed to remove dust and adhered particles. The samples were later rinsed thrice with deionized water and subsequently dried in oven at 60-80 ºC. Firstly, apples were removed from each bunch and samples (500 g) with skins, seeds and pulps in blender were homogenous. Prior to ICP-OES analysis, 10 g of homogenized apple samples was placed in porcelain vessels and heated for 12 h. The vessels with the residues obtained after the vaporization of the water and of the most organic compounds were then ashed in a furnace for 24 h. The furnace was programmed to raise temperature starting from 50 to 450 ºC in the first 16 h, after which it was kept at constant 500 ºC until the end of the process. The residues were treated with 1 mL conc. HNO_3 and heated in a furnace again until a complete mineralization of the sample was attained (*ca.* 10 h). The white obtained ashes were dissolved in 5 % (v/v) HNO_3 to a total volume of 50 mL. Table-2 lists the emission line selected for each of the element, based upon tables of known interferences and baseline shifts and empirical observations made during the sample preparation. Also, the correlation coefficients for the calibration straight line and the detection and determination are presented in Table-2.

Results are expressed as milligrams of metal per liter of working solution. The detection and quantification limits, given by LOD = 3 SD m^{-1} and LOQ = 10 SD m^{-1}, respectively, where SD is the standard deviation of reagent blank and m is the slope of the calibration graph.

TABLE-2
EMISSION WAVELENGTH, LINEAR WORKING RANGE, LOD, LOQ AND CORRELATION COEFFICIENT OF THE CALIBRATION FOR EACH MINERAL DETERMINATION

Mineral	Wavelength (nm)	LOD[1] (mg L^{-1})	LOQ[2] (mg L^{-1})	Correlation coefficient
Na	818.326	0.4047	1.3489	1.00000
Ca	393.366	0.480	1.600	0.99040
Mg	279.553	0.360	1.201	0.99894
Fe	259.940	0.0539	0.1799	0.99992
Cu	324.754	0.1326	0.4421	0.99955
Zn	202.548	0.1138	0.3794	0.99967
Mn	257.610	0.1985	0.6619	0.99900
Cr	284.325	0.0723	0.2412	0.99987
Cd	226.502	0.0826	0.2755	0.99983
Co	230.786	0.1021	0.3405	0.99973
Pb	220.353	0.2325	0.7751	0.99864
Ni	231.604	0.1138	0.3794	0.99969

[1]LOD-limits of detection, [2]LOQ-limits of quantification

Statistical analysis: Data were reported as mean ± SD for triplicate determinations. Significance of inter-group differences was determined by analysis of variance. A p value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The ICP-OES was employed to determine 12 (Ca, Mg, Na, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in apples. The best are chosen and at the same time, the working wavelengths on

TABLE-3
MEAN CONCENTRATION (mg kg^{-1} f.w.) AND ASSOCIATED STANDARD DEVIATION (mean ± SD) OF MAJOR ELEMENT (Na, Ca AND Mg) IN APPLE CULTIVARS WITH SPIKE RECOVERY TEST (n = 3)

Metal	Cultivar	Found (mean ± SD)[a]	Added	Found (mean ± SD)[a]	Recovery (%)
Na	GD	142.10 ± 1.52[b]	50.00	192.90 ± 1.32[b]	103
	GS	132.25 ± 1.38[b]	50.00	183.05 ± 1.05[b]	102
	Fu	26.65 ± 0.42[d]	50.00	76.15 ± 0.58[d]	96
	Mu	104.00 ± 1.03[c]	50.00	153.17 ± 0.92[c]	98
	Id	160.75 ± 2.72[a]	50.00	202.38 ± 1.52[a]	93
Ca	GD	54.30 ± 0.32[c]	50.00	100.34 ± 0.78[c]	94
	GS	48.30 ± 0.28[d]	50.00	100.07 ± 0.63[c]	103
	Fu	65.05 ± 0.52[b]	50.00	111.02 ± 0.39[b]	94
	Mu	43.80 ± 0.43[e]	50.00	94.59 ± 0.42[d]	101
	Id	117.80 ± 0.82[a]	50.00	163.87 ± 1.93[a]	96
Mg	GD	68.60 ± 0.37[b]	50.00	122.87 ± 1.13[b]	105
	GS	59.60 ± 0.42[c]	50.00	111.47 ± 1.28[c]	102
	Fu	40.91 ± 0.21[d]	50.00	89.49 ± 0.99[d]	98
	Mu	56.50 ± 0.29[c]	50.00	103.34 ± 0.83[c]	95
	Id	96.40 ± 0.38[a]	50.00	151.67 ± 1.67[a]	106

GD-Golden Delicious, GS-Granny Smith, Fu-Fuji, Mu-Mutsu, Id-Idared; [a]Mean values in the same column with different subscripts (a-e) significantly different ($p < 0.05$) between the cultivars

the bases of the next criteria: relative intensity of signal as a function of sensitivity of method; relative error and relative standard deviation of standard solution' signal; and interfering effect of other elements also present in that real samples. The results for five apple cultivars are summarized in Tables 3-5. Two groups of elements were established for all the data obtained. One group contained elements known to be major and to be present in all fruits, *e.g*. Na, Ca and Mg. The median

TABLE-4
MEAN CONCENTRATION (mg kg^{-1} f.w.) AS ASSOCIATED STANDARD DEVIATION (MEANS ± SD) OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS (Fe, Cu, Zn, Mn, Cr, Cd, Co, Pb AND Ni) IN APPLE CULTIVARS WITH SPIKE RECOVERY TEST (n=3)

Metal	Cultivar	Found (mean ± SD)[a]	Added	Found (mean ± SD)[a]	Recovery (%)
Fe	GD	4.226 ± 0.161[b]	3.00	7.113 ± 0.105[b]	98
	GS	3.591 ± 0.132[c]	3.00	6.703 ± 0.117[c]	102
	Fu	3.201 ± 0.160[d]	3.00	6.411 ± 0.203[c]	105
	Mu	4.140 ± 0.118[b]	3.00	6.806 ± 0.192[b]	94
	Id	5.290 ± 0.193[a]	3.00	8.124 ± 0.232[a]	97
Cu	GD	0.661 ± 0.021[c]	1.00	1.635 ± 0.058[e]	98
	GS	0.572 ± 0.023[d]	1.00	1.540 ± 0.042[d]	97
	Fu	0.827 ± 0.040[b]	1.00	1.858 ± 0.053[b]	102
	Mu	0.542 ± 0.052[d]	1.00	1.569 ± 0.018[d]	103
	Id	1.743 ± 0.090[a]	1.00	2.836 ± 0.052[a]	105
Zn	GD	0.185 ± 0.025[d]	0.20	0.392 ± 0.010[c]	103
	GS	0.277 ± 0.073[b]	0.20	0.455 ± 0.009[b]	94
	Fu	0.229 ± 0.058[c]	0.20	0.436 ± 0.015[b]	102
	Mu	0.154 ± 0.056[e]	0.20	0.318 ± 0.019[d]	98
	Id	0.390 ± 0.077[a]	0.20	0.572 ± 0.025[a]	95
Mn	GD	0.445 ± 0.032[d]	0.20	0.668 ± 0.012[d]	106
	GS	0.523 ± 0.070[b]	0.20	0.712 ± 0.023[bc]	98
	Fu	0.285 ± 0.025[e]	0.20	0.493 ± 0.018[e]	102
	Mu	0.487 ± 0.017[c]	0.20	0.710 ± 0.032[c]	105
	Id	0.897 ± 0.068[a]	0.20	1.046 ± 0.058[a]	94
Cr	GD	0.070 ± 0.003[d]	0.10	0.162 ± 0.009[d]	94
	GS	0.082 ± 0.002[c]	0.10	0.176 ± 0.015[c]	95
	Fu	0.121 ± 0.009[a]	0.10	0.217 ± 0.021[a]	98
	Mu	0.122 ± 0.011[a]	0.10	0.212 ± 0.017[a]	94
	Id	0.111 ± 0.010[b]	0.10	0.201 ± 0.019[b]	94
Cd	GD	0.001 ± 0.000[a]	0.01	0.011 ± 0.003[a]	102
	GS	0.001 ± 0.000[a]	0.01	0.011 ± 0.002[a]	102
	Fu	ND	-	-	-
	Mu	ND	-	-	-
	Id	0.001 ± 0.000[a]	0.01	0.010 ± 0.005[a]	98
Co	GD	0.141 ± 0.010[b]	0.05	0.197 ± 0.012[b]	105
	GS	0.003 ± 0.000[c]	0.05	0.054 ± 0.008[c]	102
	Fu	0.001 ± 0.000[d]	0.05	0.052 ± 0.007[c]	102
	Mu	0.004 ± 0.000[c]	0.05	0.053 ± 0.010[c]	98
	Id	0.154 ± 0.008[a]	0.05	0.211 ± 0.038[a]	105
Pb	GD	0.023 ± 0.001[c]	0.05	0.071 ± 0.009[c]	95
	GS	0.025 ± 0.001[c]	0.05	0.073 ± 0.009[c]	95
	Fu	0.033 ± 0.002[b]	0.05	0.084 ± 0.012[b]	102
	Mu	0.012 ± 0.007[d]	0.05	0.061 ± 0.007[d]	97
	Id	0.079 ± 0.008[a]	0.05	0.131 ± 0.015[a]	103
Ni	GD	0.052 ± 0.004[b]	0.05	0.104 ± 0.013[b]	102
	GS	0.017 ± 0.003[d]	0.05	0.069 ± 0.008[d]	106
	Fu	0.019 ± 0.002[d]	0.05	0.068 ± 0.008[d]	97
	Mu	0.026 ± 0.003[c]	0.05	0.077 ± 0.007[c]	103
	Id	0.109 ± 0.010[a]	0.05	0.162 ± 0.015[a]	102

GD = Golden delicious, GS = Granny Smith, Fu-Fuji, Mu-Mutsu, Id-Idared; [a]Mean values in the same column with different subscripts (a-e) significantly different ($p < 0.05$) between the cultivars

TABLE-5
METAL CONCENTRATION IN APPLE SAMPLES (n=5)

Element	Mean ± SD (mg kg^{-1} f.w.)	Range of quantified values (mg kg^{-1} f.w.)	Element	Mean ± SD (mg kg^{-1} f.w.)	Range of quantified values (mg kg^{-1} f.w.)
Na	113.15 ± 1.41	26.65 - 160.75	Mn	0.527 ± 0.042	0.258 - 0.897
Ca	65.85 ± 0.47	43.80 - 117.80	Cr	0.101 ± 0.007	0.070 - 0.122
Mg	58.40 ± 0.33	40.91 - 96.40	Cd	0.001 ± 0.000	≤ 0.001
Fe	4.089 ± 0.153	3.201 - 5.290	Co	0.060 ± 0.003	0.001 - 0.154
Cu	0.869 ± 0.045	0.542 - 1.743	Pb	0.034 ± 0.004	0.012 - 0.079
Zn	0.247 ± 0.058	0.154 - 0.390	Ni	0.044 ± 0.004	0.017 - 0.109

concentrations of these metals for each apple sample are summarized in Table-3. Calcium, magnesium and sodium are the most abundant elements; the minimum value was observed for apple cv. Mutsu, while largest amounts of those elements were found in apple cv. Idared. Looking at values in the Table-3, sodium was the element with a major content in all samples between analyzed elements. Na^+ contents in apple cv. Idared was approximately 6.0 as high the levels than apple cv. Fuji. Sodium is one of the essential elements required in appropriate amount in daily diet to regulate the blood pressure and in nervous system for transmitting signaling pathway of the body as well as stabilizes the water in our cells. Lower level of Na can results in confusion, seizures *etc*. Sodium is an important extracellular cation and stabilizes the extracellular fluid and is regulated by the kidney which is influenced by hormonal and neutral stimulation. The Na daily recommended range in developing countries is between 2400-5175 mg per day[9].

Content of Ca^{2+} and Mg^{2+} in analyzed samples were also determined and their average value are 65.85 and 64.40 mg kg^{-1} f.w. respectively. Significant differences in Ca and Mg concentration in apples were observed. Calcium is known in human nutrition for the development and growth of skeletal *e.g.* bones teeth as well as coenzyme in metabolic regulations of biomolecules. Most of the Ca is stored in the bones and the rest on the remaining is utilized in the multiple functioning as in nerve excitation and museles contraction[10]. In sufficiency of Mg in fruits and vegetables is seen through out the world, specially the areas where the soil is acidic. Magnesium plays an important role in nervous system stability, muscles contraction as activator of alkaline phosphatase as well as it is used alternative to calcium in the body[11].

The second group of elements known to be essential to life, namely Fe, Zn, Cu and Mn and non-essential elements, namely Co, Ni, Pb and Cd, reflect an exogenous influence that may be related to environmental pollution[12]. These elements are referred to as toxic elements and their concentrations for the sample studies are displayed in Table-4. Metals such as iron, copper, zinc and manganese are essential metals since they play an important role in biological system whereas non-essential metals, such as Cr, Ni, Pb and Cd are toxic even in trace amounts. The essential metals can also produce toxic effects at higher concentrations.

Iron is a necessary metal and is core component of the red blood cells, its deficiency can cause anemia. According to a study, Fe is found in two oxidative states ferrous and ferric, through the intestinal track only ferrous state is absorbed and ferric state is reduced by the food constituents. The daily intake limits is 8-11 mg/day[4]. Iron concentration ranged from 3.590 mg metal kg^{-1} f.w. in the apple cv. Granny Smith to 5.290 mg metal kg^{-1} f.w. in the apple cv. Idared. Significant differences were found in Fe concentration when comparing between apple cultivars. These values are higher than those measured in different apple cultivars by other authors. Hegedus *et al*.[13] obtained Fe values in the order of 1.7, 1.3 and 1.0 mg kg^{-1} f.w. in the cv. Golden Delicious, Granny Smith and Jonathan, respectively. Mean iron concentration (4.089 mg kg^{-1} f.w.) found in apple samples in this study was lower than that detection in Turkish apple cultivars (15.6 mg kg^{-1} f.w., n = 3)[5].

Copper concentration in apple samples resulted in 0.572 mg/kg f.w. in cv. Granny Smith and 1.743 mg kg^{-1} f.w. in cv. Idared. A significantly higher ($p < 0.05$) copper concentration was found in apple cv. Idared. The similar copper concentrations of apples samples (1.33 mg kg^{-1} f.w., n = 3) were detected by Tokalioglu and Gurbuz[5]. Copper is an essential micronutrient for living organisms, including humans, who required it for normal metabolic processes along with amino and fatty acids as well as vitamins. However, as the body cannot synthesize copper, the human diet must supply regular amounts. Copper deficiency leads to an increased risk of developing coronary heart disease whereas high levels of copper causes the nausea, vomiting and abdominal and muscle pain[14].

Zinc is an essential element for plants and is take up in the form of Zn^{2+}. Zinc is reported as a coenzyme for over 200 enzymes involved in immunity, new cells growth, acid base regulation *etc*.[15] The zinc contents in the apple samples ranged from 0.154 mg kg^{-1} f.w. in cv. Mutsu to 0.276 mg kg^{-1} f.w. in cv. Granny Smith. Significant differences were found in Zn concentration in comparisons between apple cultivars. Available literature has shown that the level of Zn in apples is 0.6, 1.36 and 2.05 mg kg^{-1} f.w.[13,16,17] Hegedus *et al*.[13] have also reported Zn levels 1.0 mg kg^{-1} for strawberry, 2.9 mg kg^{-1} for raspberry, 1.9 mg kg^{-1} for red currant and 2.2 mg kg^{-1} for black currant, respectively.

Manganese is one of the important essential element required in carbohydrates metabolism as well as an antioxidant in superoxide dismutases enzymes it is required in very little quantity and its deficiency is rarely occur. Manganese toxicity is reported in alcoholic and liver diseases[15]. Manganese has been reported in the range of 0.9 to 3.2 mg kg^{-1} f.w. of different fruits13 and 0.535 to 0.716 mg kg^{-1} in different sour cherry cultivars[7]. Manganese was detected in almost all the apple samples and the concentration ranged from 0.285 to 0.896 mg kg^{-1} f.w.

Chromium is one of the essential trace elements in the human body, as it appears to play a role in the metabolism of glucose and some lipids (mainly cholesterol)[18]. However, adverse effects may occur at higher concentrations. Excessive amounts of Cr, particularly in the more toxic Cr(VI) valence state, may be involved in the pathogenesis of some diseases such as lung and gastrointestinal cancer[19]. Chromium contents ranged from 0.070 to 0.122 mg kg^{-1} f.w. in apple samples. Chromium levels likely reflected differences in apple cultivars and environmental factors (*e.g.* soil, climate). The value is within the range reported for fruits by other research. For example, in Serbian table grape and sour cherry samples, chromium levels ranged from 0.053 to 0.105 mg kg^{-1} f.w.[7].

As known, same areas where there is a human activity have heavy metal pollution due to different sources such as home wastes, straw and traffic wastes. Plant which grown under to effects of those pollutants can contain different range of heavy metals. Also, the aim of this study is determination of cadmium, nickel and lead content of apple cultivars were produced in Serbia and investigation of potential health risks for humans.

Cadmium is a non-essential element in foods and natural waters and it accumulates principally in the kidneys on liver[20]. Various sources of environmental contamination have been

TABLE-6
COMPARISON OF SERBIAN APPLES WHICH OTHER REGIONS REGARDING MINERAL LEVELS (MILLIGRAMS PER KILOGRAM FRESH WEIGHT)

Grown region	Na	Ca	Mg	Fe	Cu	Zn	Mn	Cr	Cd	Co	Pb	Ni	Reference
Serbia	113.15	65.85	58.40	40.89	0.869	0.247	0.527	0.101	0.001	0.060	0.034	0.444	Current
Hungary	36.7	105.8	62.7	1.3	1.1	0.6	0.9	–	–	–	–	–	Hegedus *et al.*[13]
Poland	–	–	–	–	0.414	1.185	–	–	0.006	0.068	–	–	Kreipcio *et al.*[23]
Canada	31	45	38	0.4	–	0.2	0.2	–	–	–	–	–	Rupasinghe *et al.*[24]
Spain	6.0	42.6	50.2	0.94	0.24	0.18	0.3	–	–	–	–	–	Gorinstein *et al.*[25]

implicated for its presence in foods. Cadmium was not detected in all the samples. Golden delicious, Granny Smith and Idared apple cultivars contained 0.001 mg kg^{-1} f.w.

The results of the analysis showed that the levels of Pb in all apple samples where between 0.012 mg kg^{-1} in cv. Mutsu and 0.079 mg kg^{-1} in cv. Idared. An approximately 6.58-fold difference in Pb content was found between highest and lowest ranged cultivars Idared and Mutsu. Nickel content ranged from 0.017 mg kg^{-1} f.w. in cv. Granny Smith and 0.109 mg kg^{-1} f.w. in cv. Idared.

As a results, the findings of the present study indicate that the mean intake of heavy metals due to consumption of apple cultivars grown in Serbia is generally well below the tolerable levels.

The results of heavy metal concentrations were compared with the values recommended by the World Health Organization. Evaluation of certain food additives and contaminants, technical report series *et al*. 1993) and EU Directives (Commission of the European Communities: Commission Regulation (EC) No.221/2002 of the 6 February 2002) for fruits. Based on the WHO health criteria and EU Directive (milligram per kilogram wet weight) for Pb (0.1-1.0), Cd (0.05), Cr (0.03), Ni (0.5) and Cu (0.2-1.2), there are no health risks with respect to the concentrations of lead, nickel, copper, cadmium and chromium in fruits analyzed in this study.

Principal component analysis is a bilinear modeling method which gives an interpretable overview of the main information in a multi-dimensional data table. The information carried by the original variables is projected onto a smaller number of underlying (latent) variables called principal components[21]. Principal component analysis was applied to classify analyzed elements in five apple cultivars according to their contents.

The distribution of the metals in the plot was defined by taking two principal components as the coordinate's axes. Each principal component (PC) is associated with an eigenvalue, PC1 has the largest eigenvalue and carries the largest variance of the original data and subsequent principal components carry variance in a decreasing order. Scree test proposed by Cattell[22] suggests that only the first two principal components (PCs) contained significant information.

The relation between PC1 which explained 58.41 % of the total variance and PC2 which explained a further 27.34 % based on metal content is shown in Fig. 1. Two principal components were shown to be the most significant ones accounting together for 85.75 % of the total variance associated with metal concentrations in all samples. Elements at the right-hand side of the scores plot are in higher concentrations than those at left-hand side. At the upper-right region of the bi-plot are the most abundant elements (Na, Ca, Mg) in all apple

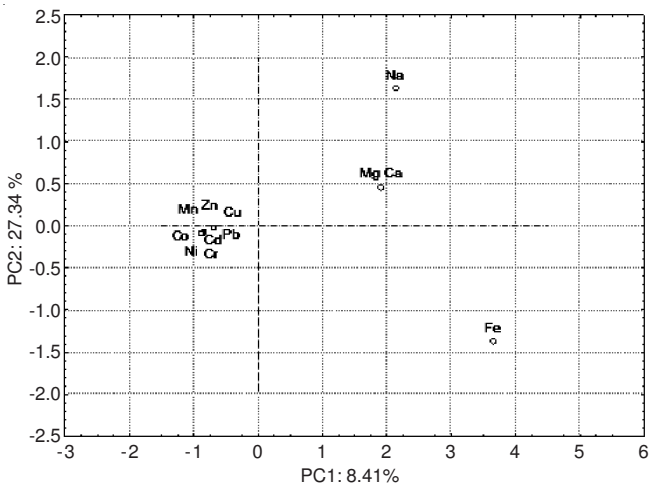

Fig. 1. Principal component score plot (PC1 and PC2) of the studied apple samples based on metals content

cultivars followed by Fe. On the other hand, Ni, Cd, Pb, Co and Cr with negative values in PC1 and PC2 are associated with lower concentrations. Cu, Zn and Mn are elements with positive values in PC2 and negative values in PC1.

Statistical analysis: Data were reported as mean ± SD for triplicate determinations. Significance of inter-group differences was determined by analysis of variance. Ap value of $p < 0.05$ was considered statistically significant. Principal component analysis is used to establish the relationships among variable. Principal component analysis analysis was performed using a statistical package running on a computer (Statistica 8.0, StatSoft, Inc, Tulsa, OK, USA).

Conclusion

The present investigation indicates that ICP-OES technique is suitable for determination of Na, Ca, Mg, Fe, Cu, Zn, Mn, Cr, Cd, Co, Pb and Ni in apple samples grown in Serbia. Concentrations of the elements in the various apple cultivars are more or less different. The analytical values obtained for the analyzed metals where in good agreement with those reported by other researchers. Comparison of Serbian apples with other fruits, regarding mineral levels is given in Table-6. The levels of heavy metals in apple samples where assessed by comparing levels found in samples with requirements of the WHO and EU Directives. The results obtained from this study show that there are no health risks from consumption of apples when compared with levels stipulated by the health authorities.

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