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ÜNİVERSİTESİ FEN BİLİMLER

ENSTİTÜSÜ DERGİSİ

Determination of Cr, Cu, Fe, Ni, Pb and Zn by ICP-OES in mushroom samples from Sakarya, Turkey

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ABSTRACT

Russula cyanoxantha, Russula delica, Lactarius salmonicolor, Lactarius deliciosus, Pleurotus eryngii, Pleurotus ostreatus, Agaricus bisporus, Suillus luteus, Pleurotus spp and Boletus edulis were collected from Sakarya-Turkey respectively. Also canned food in the form of the <i>Pleurotus eryngii, Pleurotus ostreatus, and Lactarius salmonicolor mushrooms were used for the examination. Trace metal concentrations found in these mushrooms were determined inductively using coupled plasma optic emission spectrometry microwave processes. The results were obtained for (Cr) 0.3-26.65, (Cu) 17.38-132.75, (Fe) 26.3-225.40, (Ni) 2.57-39.28, (Pb) 11.52-185.20, and (Zn) 22.86-126.84 mg/kg. The accuracy of the method was checked by the standard reference material; tea leaves (INCY-TL-1) and tomato leaves (1573a).

Keywords: Mushroom, ICP-OES, Heavy Metal, Sakarya

Türkiye, Sakarya'dan toplanan mantar numunelerindeki Cr, Cu, Fe, Ni, Pb ve Zn ICP-OES ile tayini

ÖZ

Bu çalışma'da Türkiye'nin Sakarya ilinden *Russula cyanoxantha*, *Russula delica*, *Lactarius salmonicolor*, *Lactarius deliciosus*, *karakulak*, *Pleurotus ostreatus*, *Agaricus bisporus*, *Suillus luteus*, *Pleurotus spp*, orman mantarları ayrı ayrı toplanmıştır. Ayrıca *Malgadın*, *Pleurotus ostreatus*, *Lactarius salmonicolor* mantarlarının konserve türleri alınmıştır. Bu mantarların eser metal konsantrasyonları mikrodalga işleminden sonra indüktif eşleşmiş plazma optik emisyon spektroskopisi (ICP-OES) ile tespit edilmiştir. Sonuçlar (Cr) için 0,3-26,65, (Cu) için 17,38-132,75, (Fe) için 26,3-225,40, (Ni) için 2,57-39,28, (Pb) için 11,52-185,20, (Zn) için 22,86-126,84 mg/kg olarak tespit edilmiştir. Prosedürün doğruluğu (INCY-TL-1) kodlu çay yaprakları ve (1573a) kodlu domates yaprakları sertifikalı referans malzeme ile doğrulanmıştır.

Anahtar Kelimeler: Mantar, ICP-OES, ağır metal, Sakarya

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1. INTRODUCTION

Mushrooms that grow in the wild are consumed in the countries of at East and Middle Europe and in Turkey. This is because of the taste, aroma and heterogeneous specie of mushrooms[1]. Most of the existing mushroom species are edible. Mushrooms are part of the gourmet tradition throughout the world. They are also used as traditional pharmaceuticals in the countries such as China, Japan, Nigeria, Tibet etc. [2-4]. Mushrooms are also reported as beneficial in terms of preventing hypertension, hypercholesterolemia and cancer]. Mushroom, having thousands of species found throughout the world, is a high nutritional value food. In particular, it has high protein and iron content. In addition, they contain A, B, D, P and K vitamins and calcium, potassium, phosphor and copper minerals. Mushrooms are the best vegetable protein resource ever known [5, 6]. 88-94% of the mushroom is water. The remaining 6-12% consists of 15-42% protein, 2-6% raw oil, 42 -71% carbohydrate and 6 - 13% ash [7].

Trace elements such as iron, zinc, copper and manganese are basic elements and as they have important roles to play in biological systems. Aluminum and lead are not basic metals but they demonstrate toxic effect, even at trace amounts [8]. Required metals can have toxic effects, when the metal intake is exceedingly high [9-10]. Mushrooms have a very effective mechanism against heavy metal accumulation from the environment. For this reason, mushroom can be used in order to identify the level of environmental pollution [11]. On the other hand several studies have been conducted to evaluate the effect of consuming mushrooms containing heavy metals on human health [12-13].

Turkey can be separated into seven geographical regions. The Marmara region is one of them. In this region, it is dry in summer and rainy in winter. Seasons are generally sultry. Climatic conditions, especially in the fall, are ideal for growth of mushrooms throughout the year. In this study, the chrome (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), and zinc(Zn) content in the samples of mushroom collected from the provinces of Sakarya city was determined by inductively coupled plasma optic emission spectrometry after solubilized using a microwave system. In this study trace element analysis was performed at the 10 mushroom and 3 canned mushroom samples from Sakarya city between September 2014 and February 2015.

2. MATERIALS AND METHODS

2.1. Instrument

Inductively coupled plasma optic emission spectrometry (ICP-OES, Spectro Arcos, SPECTRO model Analytical Devices, Kleve, Germany) was used. This device is an effective technique for measuring almost all metals including refractor metals and non-metals. For this that reason, the ICP-OES systems has become a preferred analytical method for a wide range of applications, and is preferred by most research centers. The ICP-OES operation parameters are determined by the producer ICP-OES operating conditions were given in Table 1. The reaction vessels were cleaned using 5 mL of concentrated nitric acid before each digestion.

Table 1. The operating parameters of determination of elements by ICP-OES

Device	SPECTRO ARCOS				
Instrumental Parameter	Value				
Height Scanning (mm)	12				
Wave Length	Nm				
RF Power (W)	1450				
Spray Division	Cyclonic				
Nebulizer	Modified Lichte				
Nebulizer Flow (L/min)	0.8				
Plasma Torch	Quartz, Stable, 3.0 mm Injector Pipe				
Plasma Gas Flow (L / min.)	13				
Auxiliary Gas Flow (mL / min.)	0.7				
Sample Aspiration Speed (mL / min.)	2.0				
Sample Pump Speed (rpm)	25				

2.2. Reactive and Solution

HNO₃ and H₂O₂ were used with supra pure quality (E. Merck, Darmstadt). All plastic and glass wares were sopped in diluted HNO₃ (10 %) and cleaned by washing demineralized water. During the preparation of the standard solutions for the calibration of the elements, ultra high quality deionized (UHQ, chemical resistance 18 MΩ.cm) water (Millipore, Bedford, MA, USA) was used for dilution of the 1000 mg L⁻¹ stock solutions.

2.3 Samples

In this study trace element analysis was performed at the 10 mushroom and 3 canned mushroom samples from provinces of Sakarya city between September 2014 and February 2015. All samples were washed with ultra-pure water after collection and dried for 24 hours. Then then

have remained in the drying oven at 60°C for 24 hours. Samples were taken from drying oven, put into polyethylene container and soaked for analysis.

2.4. Microwave Method

For microwave digestion, the certified reference materials and mushroom samples were accurately weighed out to 1 g. The solution was put into microwave containers and placed into the microwave. Samples were digested by making use of HNO_3/H_2O_2 (3:1) acid mixtures in a microwave digestion system and diluted to 10 mL with double deionized water (UHQ water, chemical resistivity 18 M Ω cm) 6 mL HNO₃ (65 %) and 2 ml H₂O₂ (35 %) were added on it. The conditions of microwave system were heated 5 min 1000 W 90 °C, 4 min 1000 W 90 0C, 5 min 1000 W 180 °C, 16 min 1000 W 180 °C respectively and cooling procedure was applied 1000 w for 8 min. and then analyzed in ICP-OES by diluting 25mL.

2.5. Chemical Analysis

Standard addition method was used to remove matrix effect. Elemental analyses were held by using inductively coupled plasma optic emission spectrometry (ICP-OES) device. Suitable calibration standards were conducted to monitor the efficiency loss periodically for standard samples. ICP-OES data quality control was evaluated with blank solutions and calibrations standards prepared by present data. Accuracy of the ICP-OES measurements were determined by using various certified reference materials.

2.6. Quality Control and Quality Assessment

Quality of the analytic procedures was assured by using certified reference materials. Certified reference material (CRM) analysis element concentrations were provided accuracy and sensitivity assessment on a wide range. Values which were observed in the concentration values drawn against certified values were in good correlation for Cr, Cu, Fe, Ni, Pb, and Zn determination in the certified reference material. Three repetitions were performed for each sample in CRM. Three measurements were performed for each sample. Results were given in the table 2 and table 3.

Table 2. Certificate	for th	ne v	values	of th	e CRM	and	the
results of tea leaves	(INC)	Y-T	L-1)				

Element	Certified values	Determined values	Recovery	
	(mg kg ⁻¹)	(mg kg ⁻¹)	(%)	
Cr	1.91	1.7	89.00	
Cu	20.4	20	98.04	
Fe	432	460	106.48	
Ni	6.12	6	98.04	
Pb	1.78	1.74	97.75	
Zn	34.7	34	97.98	

Table 3. Certificate for the values of the CRM and the results of tomato leaves (1573a)

Tomato leaves (1573a)						
Element	Certified values	Determined values	Recovery			
	(mg kg ⁻¹)	(mg kg ⁻¹)	(%)			
Cr	1.99	1.89	94.98			
Cu	4.70	4.47	95.10			
Fe	368	363.5	98.77			
Ni	1.59	1.61	101.25			
Pb	-	-	-			
Zn	30.9	30.5	98.70			

3. CONCLUSIONS AND DISCUSSION

In this study, trace element analysis was performed at the 10 mushroom and 3 canned mushroom samples from Sakarya city. The determination of trace elements in the samples was performed using ICP-OES. The accuracy of the method was tested with tea leaves standard reference material and tomato leaves standard reference material (CRM). Three measurements were handled for each sample and each mushroom in CRM.

Trace element levels of mushroom samples were found as 0.3-26.65 mg/kg (Cr); 17.38-132.75 mg/kg (Cu); 26.30-225.40 mg/kg (Fe); 2.57-39.28 mg/kg (Ni); 11.52-182.50 mg/kg (Pb); 22.86-126.84 mg/kg (Zn). In Table 4 the places where the mushrooms were collected and heavy metal levels amounts of mushrooms were specified.

Chrome is a transition element with various industrial uses. It is an element which cannot be used raw form [14]. Chromite is the most found and processed chrome mineral. Chromite mineralizes as the result of the

magmatic fractionation during the phase of crystallization beginning at magmatic stage. Chromium can be found in most industrial wastes as compounds of maximum +3 and +6 valences. As the result of industrial oxidation of chrome containing minerals and incineration of fossil fuel, wood and paper products, hexavalent chromium compound is formed. Chrome has a recycle as from rocks and soil to water, ecosystem, air and again soil [15]. The lowest and the highest zinc values (Lactarius detterimus) 0.3 mg/kg and (Russula delica) 26.65 mg/kg. Normal chrome content is in the range of 0.5 - 5 mg/kg for many species. It is rarely reported between 5-10 mg/kg [12, 16, 17, 18, 19, 20, 21]. Also the lowest chrome content is reported 1.1-9 mg/kg [22-23] and the highest chrome content 22-24.3 mg/kg [19]. These chrome values are in harmony with our values.

Copper is a required element for the creatures [24]. Also copper is one of the metals that is used by the human and it is naturally found in the nature. The effect of the copper on the plant and creatures varies according to chemical form and size. As it demonstrates toxic effect for small and simple creatures, it is essential for bigger creatures [25]. Copper, being around 50-120 mg in the adults, is the key element of the reactions of amino acids, fat acids and vitamins in the metabolism [24]. The lowest and the highest zinc values (*Lactarius detterimus*) 17.38 mg/kg and (*Pleurotus eryngii*) 126,84 mg/kg. Copper content of the mushroom samples in the literature is reported as 4.71-51.00 mg/kg [26], 505 mg/kg [27], 2 mg/kg [28] respectively. These results are seemed same as our results.

Iron is an important element that enters the structure of the creatures especially oxygen transport of the vertebrate. Iron is one of the most commonly find metals in the nature. While iron is encountered as compound in the nature, it is not encountered as element form. Element for iron is only encountered in the structure of the meteors. Compound form iron is encountered with oxide, sulfur and carbonate compounds [29, 30]. The lowest ant the highest iron value lactarius salmonicolor 26.30 mg/kg and canned oyster mushroom 225.40 mg/kg. Iron content in the results of the reports which are released until 1999 is 30-150 mg kg-1. The lowest iron content of mushroom samples in the literature is 76.3 mg/kg [17], and the highest value is 568-3904 mg/kg [19]. These iron values are similar to our results.

Nickel is found in the nature in solid, shiny, metallic form and silver color. It is resistant to external effect because of the paramagnetic features [29]. If nickel concentration at the external ambient air is accepted as 10-20 ng/m³, daily respiration capacity 20 m³, daily metal intake of human is calculated as 0.2 μ g for the rural zone and 0.4 μ g for the city. Tobacco use increases this amount. It is possible to take 3-15 μ g nickel for the ones who consumes two packs of cigarettes a day. Daily allowable nickel amount via respiration varies between 0.05–5 mg limits. Absorbance of the nickel from the lungs occurs fast and particles accumulate in the lungs were absorbed here. If there is 5 μ g nickel in the drinking water, allowable nickel amount for the person is 10 μ g for 2 consumption of water [26]. The highest and the lowest cadmium value cultivated mushroom 2.57 mg/kg and *Russula delica* mushroom 39.28 mg/kg. Nickel content was reported 15 mg/kg for many species [12, 17, 31, 32, 33]. These nickel values in the *Lactarius salmonicolor*, , *Agaricus bisporus*, *Suillus luteus* and *Pleurotus spp* mushrooms are proper with our values.

Any form of lead either metal or compound released to the atmosphere demonstrate toxic feature. Lead is a harmful element for the ecosystem as the result of the human activities. Estimated lead amount in human body is around 125-200 mg. Human body remove lead 1-2 mg/day under normal conditions [34-35]. Lead element is not absolutely necessary for plants; exist in the soil 15-40 ppm dose. It doesn't cause hazard for human and plant health, unless it exceeds 150 ppm. In the cases it exceeds 300 ppm it is accepted as potential danger for human health [36]. The highest and the lowest lead value cultivated mushroom 11.52 mg/kg and Russula Cyanoxantha mushroom 122.43 mg/kg. Lead content is 1-10 mg kg-1 in the report results until 1999. The lowest lead content in the literature is 0.1-40 mg/kg-1 [11] and the highest value is 20 - 40 mg/kg-1 [37]. Also tolerable limit is given as 25 mg/kg for lead [38]. These lead values are proper to our values.

Zinc is an important metal to be taken into human body with determined amounts. It can be found most of the body. It is an important effect for the catalytic parts of the proteins and enzymes required for the body. Also zinc has important role in carbohydrates and fats. It is an important organizer of immune and nervous system in the mammals. Changes in the zinc level of blood cause mental disorders. Daily zinc intake is determined as 5-22 mg in different regions the studies performed. Allowed zinc intake level is determined as 15 mg/day for male, 12 mg/day for adult female, 5 mg/day for babies and 10 mg/day for preadolescence [39]. Zinc is a necessary element for biochemical reactions. This element is main element of various proteins. The lowest and the highest zinc values (Lactarius detterimus) 22.86 mg/kg and (canned oyster mushroom) 126.84 mg/kg. Zinc content in the mushroom varies between 25-250 mg/kg range. Zinc content of the mushroom samples in the literature is reported as 9.90-118 mg/kg [40], 35.8-410 mg/kg [3]. These zinc values are almost same with our values. Metal concentrations for each mushroom sample are seen in the graphics in Figures 1 to 6.

As the highest heavy metal accumulation 132 .75 mg/kg copper, the lowest accumulation is 14.625 mg/kg chromium in the *Russula cyanoxantha* sample.

Metal sequence is Cu>Pb>Fe>Zn>Ni>Cr

As the highest heavy metal accumulation 182. 5 mg/kg lead, the lowest accumulation is 26.65 mg/kg chromium in the *Russula delica* mushroom sample.

Metal sequence is Pb>Cu>Fe>Zn>Ni>Cr>

As the highest heavy metal accumulation 34.12 mg/kg lead, the lowest accumulation is 0.45 mg/kg chromium in the *Lactarius salmonicolor* mushroom sample.

Metal sequence is Pb>Zn>Fe>Cu>Ni>Cd>Cr

As the highest heavy metal accumulation 34.30 mg/kg lead, the lowest accumulation is 0. 30 mg/kg chromium in the *Lactarius deliciosus* mushroom sample.

Metal sequence is Pb>Fe>Zn>Cu>Ni>Cr

As the highest heavy metal accumulation 149. 90 mg/kg iron, the lowest accumulation is 14.72 mg/kg in chromium the *Pleurotus eryngii* mushroom sample.

Metal sequence is Fe>Pb>Zn>Cu>Ni>Cr

As the highest heavy metal accumulation 117. 22 mg/kg lead, the lowest accumulation is 13.97 mg/kg chromium in the *Pleurotus ostreatus* mushroom sample.

Metal sequence is Pb>Zn>Cu>Fe>Ni>Cr

As the highest heavy metal accumulation 66.18 mg/kg zinc, the lowest accumulation is 0.94 mg/kg chromium in the *Agaricus bisporus* mushroom sample.

Metal sequence is Zn>Cu>Fe>Pb>Ni>Cr

As the highest heavy metal accumulation 123. 98 mg/kg iron, the lowest accumulation is 1.77 mg/kg chromium in the *Suillus luteus* mushroom sample.

Metal sequence is Fe>Cu>Zn>Pb>Ni>Cr

As the highest heavy metal accumulation 138.66 mg/kg iron, the lowest accumulation is 1.12 mg/kg chromium in the *Pleurotus Spp* mushroom sample. Metal sequence is Fe>Zn>Cu>Pb>Ni>Cr As the highest heavy metal accumulation 74.12 mg/kg lead, the lowest accumulation is 8.04 mg/kg chromium in the *Boletus Edulis* mushroom sample.

Metal sequence is Pb>Cu>Fe>Zn>Ni>Cr As the highest heavy metal accumulation 225. 4 mg/kg iron, the lowest accumulation is 2.55 mg/kg chromium in the canned *Pleurotus eryngii* mushroom sample.

Metal sequence is Fe>Zn>Cu>Pb>Ni>Cr

As the highest heavy metal accumulation 81.57 mg/kg zinc, the lowest accumulation is 1.74 mg/kg chromium in the canned, *Lactarius salmonicolor* mushroom sample.

Metal sequence is Zn>Cu>Fe>Pb>Ni>Cr

As the highest heavy metal accumulation 81. 10 mg/kg iron, the lowest accumulation is 1.68 mg/kg chromium in the canned *Lactarius Deliciosus* mushroom sample.

Metal sequence is Fe>Zn>Cu>Pb>Ni>Cd>Cr

Sample Collecting Location	Mushroom Kind	Code	Fe	Ni	Pb	Zn	Cr	Cu
Karapürçek	Russula Cyanoxantha	M1	83.60	23.68	122.43	71.25	14.63	132.75
Karapürçek	Russula Delica	M2	99.68	39.28	182.5	61.23	26.65	122.02
Geyve	Lactarius Salmonicolor	M3	26.30	5.16	34.12	32.06	0.45	23.90
Geyve	Lactarius Deliciosus	M4	32.64	3.36	34.30	22.86	0.30	17.38
Hendek	Pleurotus eryngii	M5	149.90	24.66	120.47	80.6	14.72	74.16
Akyazı	Pleurotus ostreatus	M6	86.90	25.13	117.22	53.47	13.97	76.00
Adapazarı	Agaricus Bisporus	M7	34.78	2.57	11.52	66.18	0.94	40.10
Karasu	Suillus Luteus	M8	123.98	3.35	11.8	49.69	1.77	57.64
Akyazı	Pleurotus Spp	M9	138.66	2.94	12.65	69.34	1.125	25.89
Taraklı	Boletus Edulis	M10	47.16	13.67	74.12	39.38	8.04	68.22
Hendek	Pleurotuseryngii(Canned)	M11	225.4	3.49	15.73	126.84	2.55	40.72
Pamukova	Lactarius salmonicolor (Canned)	M12	59.53	3.59	16.03	81.57	1.74	71.89
Geyve	Lactarius Deliciosus	M13	81.10	4.35	15.78	78.15	1.68	36.99

Table 4. Metal Concentration in the Moushroms (mg/kg)



Figure 1. Cr concentrations of Mushroom Samples (mg/kg)



Figure 2. Cu concentrations of Mushroom Samples (mg/kg)



Figure 3. Fe concentrations of Mushroom Samples (mg/kg)



Figure 4. Ni concentrations of Mushroom Samples (mg/kg)



Figure 5. Pb concentrations of Mushroom Samples (mg/kg)



Figure 6. Zn concentrations of Mushroom Samples (mg/kg)

Metal concentrations for each mushroom sample are seen in the graphics in Figures 1 to 6. It can be clearly seen by the graphics that different metal levels in each mushroom sample, metal levels varies according to the mushroom kind, region and soil.

4.CONCLUSION

A mushroom generally causes element content changes in their structures and environments they are connected. According to the result of this study, it can be seen that heavy metal contents of mushroom samples are at different ranges for some elements as compared to the tolerable value ranges in the literature. However, the copper, zinc, iron and chrome content are in compliance with the values in the literature. It is seen that the values in the Lactarius deliciosus, Lactarius salmonicolor, Agaricus bisporus, Suillus luteus and Pleurotus spp mushroom samples are the same as the levels in the literature. . Metal levels in some edible mushroom species were found to be over the legal limits. The results obtained by the study indicate that because mushrooms contain heavy metals that have a toxic effect that are used in the structure with anthropogenic effects related to soil structure, ecological factors (vegetation), this is in opposition to the guidance in terms of medical or nutritional usability. Consequently, it is considered that overconsumption of naturally collected mushrooms may have a harmful effect.

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