UNIVERSITATEA DE ȘTIINȚE AGRICOLE ȘI MEDICINĂ VETERINARĂ CLUJ NAPOCA
FACULTATEA DE ZOOTEHNIE ȘI BIOTEHNOLOGII

Correlation between mineral content and free aminoacid profile in bee pollen from different geographical regions from Romania

Corelarea conținutului mineral cu profilul aminoacizilor liberi din polenul de albine din diferite regiuni geografice din România

Autors:

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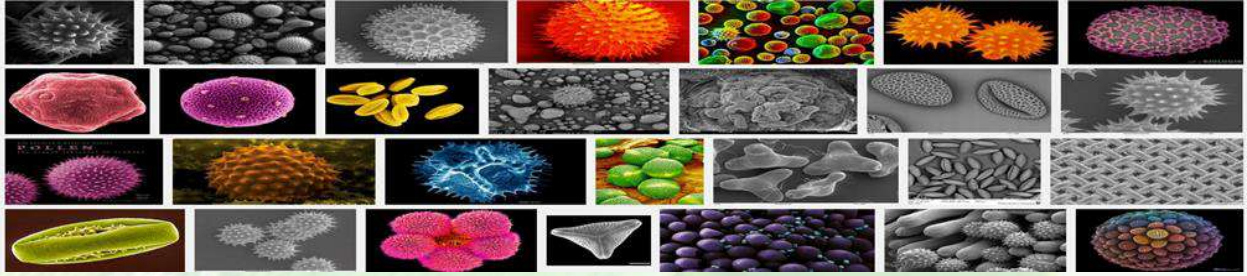
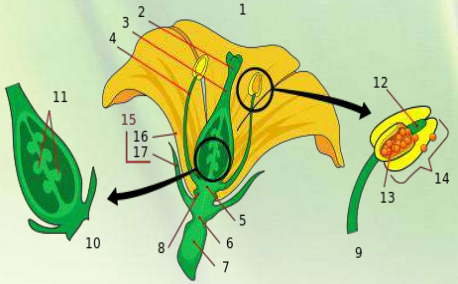
October 2018

INTRODUCTION





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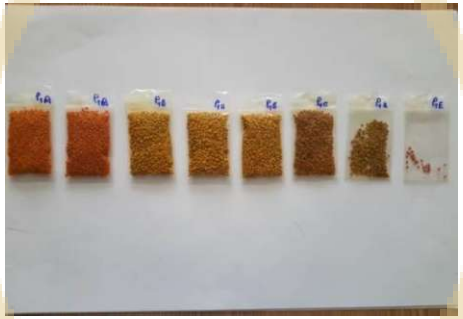
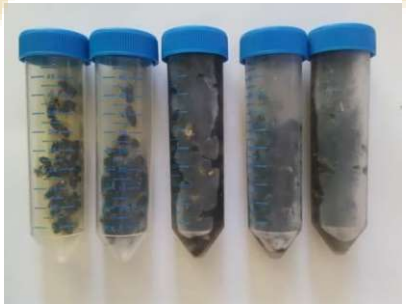
Due to the fact that the source of bee pollen is the plant, which is practically in an increasingly contaminated environment with industrial and agricultural waste, there is the problem of contamination of this product with heavy metals and other contaminants that endanger the health of bees as well as the human consumer that they ultimately reach.

MATERIALS AND METHODS





METHODS

1.Determination of botanical origin by microscopic analysis

Colour sorting

Dilution of glomeruli with 10ml H_2SO_4 5‰ in Falcon tubes

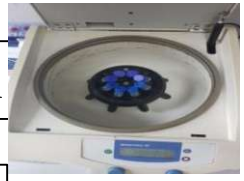
Centrifugation of Falcon tubes

Supernatant suppression

Dilution 5ml distilled water

Falcon tube shacking

Centrifugation



Supernatant suppression

Sand sediment on microscope blade

Drying

Fixing

Adding lamella

Microscopic analysis

METHODS

2. Determination of minerals and heavy metal content of pollen samples and bee samples by spectophotometry with atomic absorption.

Taking 0.3 g of each sample and place in the flask

↓

Adding 2 ml of 65% HNO_3 and 3 ml of purified water

↓

Resting 10-20 min.

↓

Closing containers

↓

Calcination kiln mineralization

Berghof microwawe digestion system MWS-2

METHODS

2. Determination of minerals and heavy metal content of pollen samples and bee samples by spectophotometry with atomic absorption**.**

Atomic Absorption Spectrometer with Aanalyst 800 Graphite Kiln, Perkin-Elmer

METHODS

3. Determination of free amino acid content of pollen samples at LC-MS

Liquid chromatograph coupled with mass spectrometer

METHODS

3. Determination of free amino acid content of pollen samples at LC-MS

0.25 g pollen was dissolved in 10 ml of ultra pure water

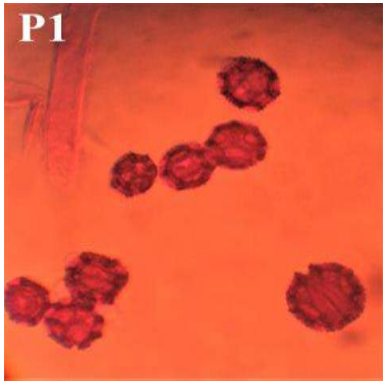
Sonicated 30 minutes

centrifuged for 10 minutes at 10000 rpm

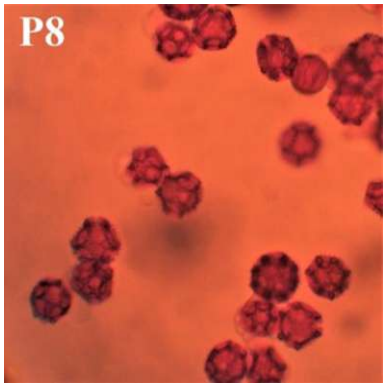
•The supernatant was taken 25 µl and the procedure described in the kit EZ:faast Phenomenex

RESULTS OF PALINOLOGICAL ANALYSIS

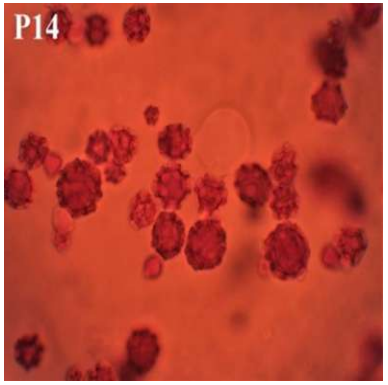
- *ASTERACEAE* FAMILIY*:*



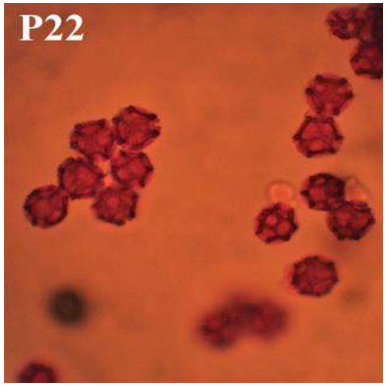
P1-Taraxacum Officinale



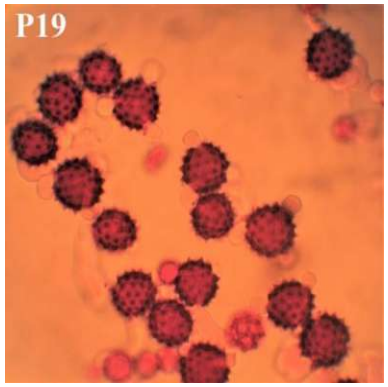
P8-Taraxacum Officinale



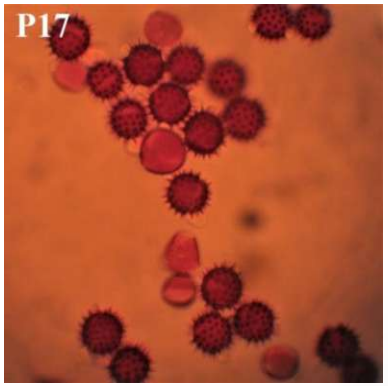
P14-Taraxacum Officinale



P22-Taraxacum Officinale



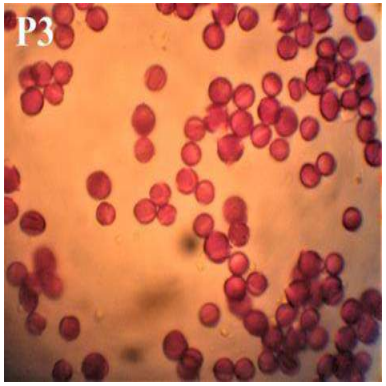
P19-Helianthus annuus



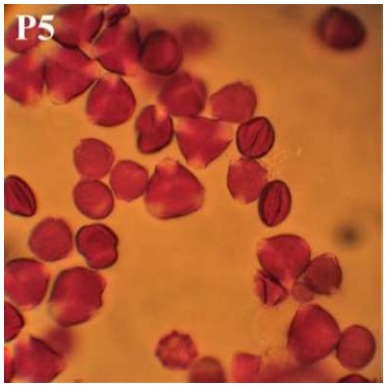
P17-Helianthus annuus

RESULTS OF PALINOLOGICAL ANALYSIS

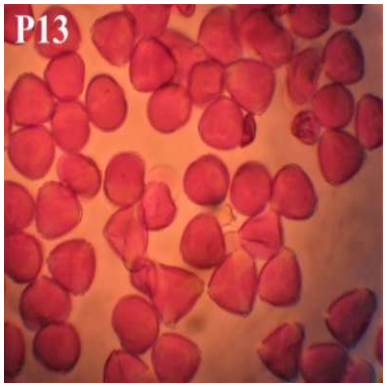
- *FABACEAE, ACERACEAE* ŞI *CUCURBITACEAE* FAMILY:



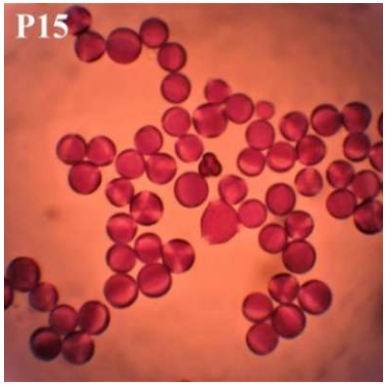
P3-Trifolium Pratense



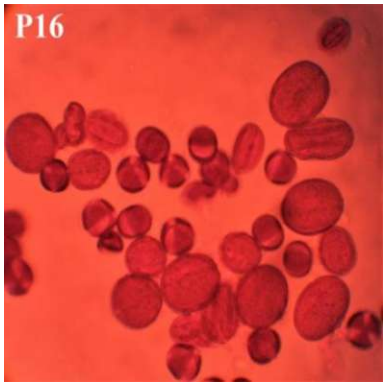
P5-Medicago sativa



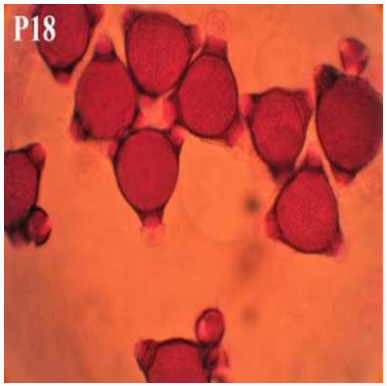
P13-Robinia pseudoacacia



P15-Acer tartaricum



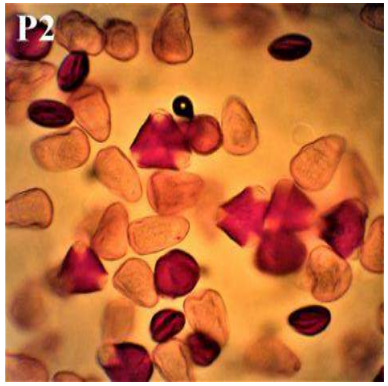
P16-Acer tartaricum



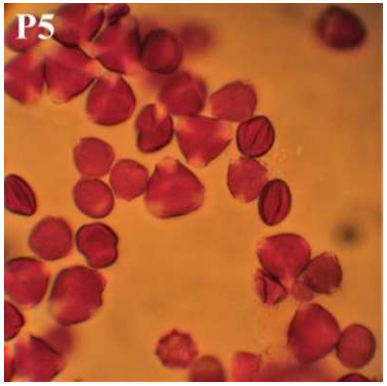
P18-Cucumis Sativus

RESULTS OF PALINOLOGICAL ANALYSIS

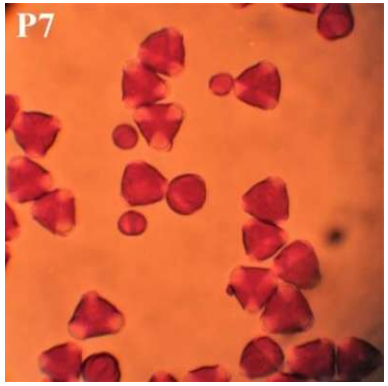
- *ROSACEAE, CIPERACEE* FAMILY:



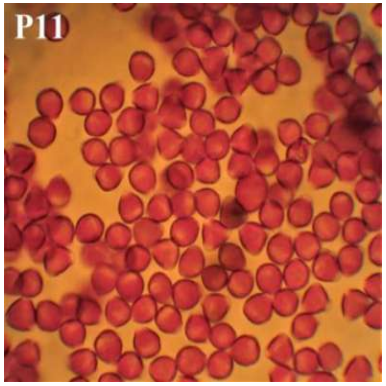
P2-Carex species



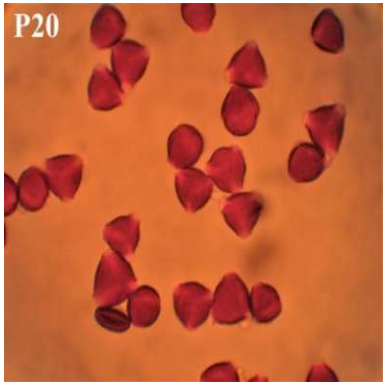
P5-Rubus idaeus



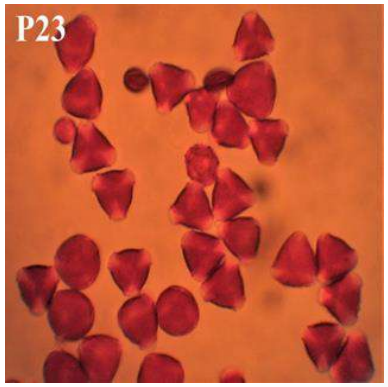
P7-Crataegus monogyna



P11-Rubus idaeus



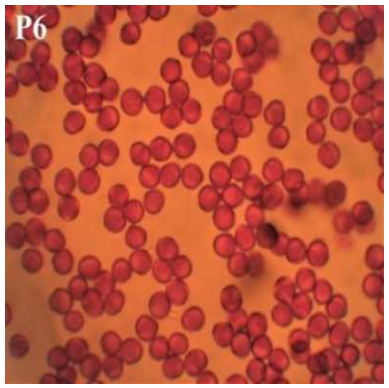
P20-Rubus idaeus



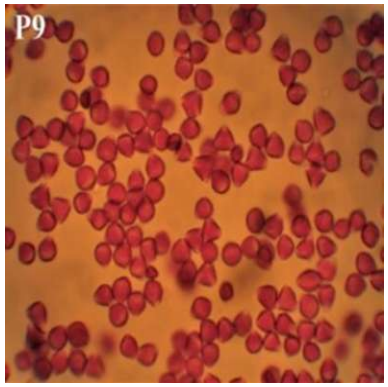
P23-Prunus avium

RESULTS OF PALINOLOGICAL ANALYSIS

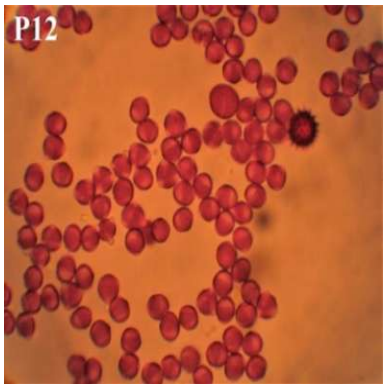
- *BRASSICACEAE ŞI ADOXACEAE* FAMILY



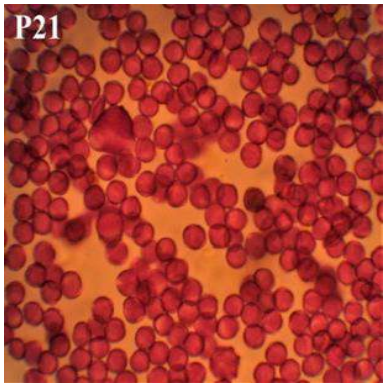
P6-*Brassica napus*



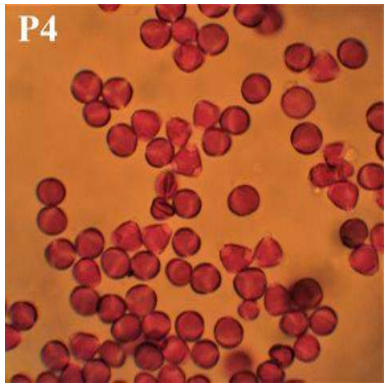
P9-*Brassica napus*



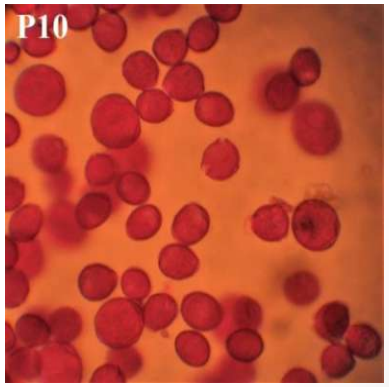
P12-*Brassica napus*



P21-*Brassica napus*

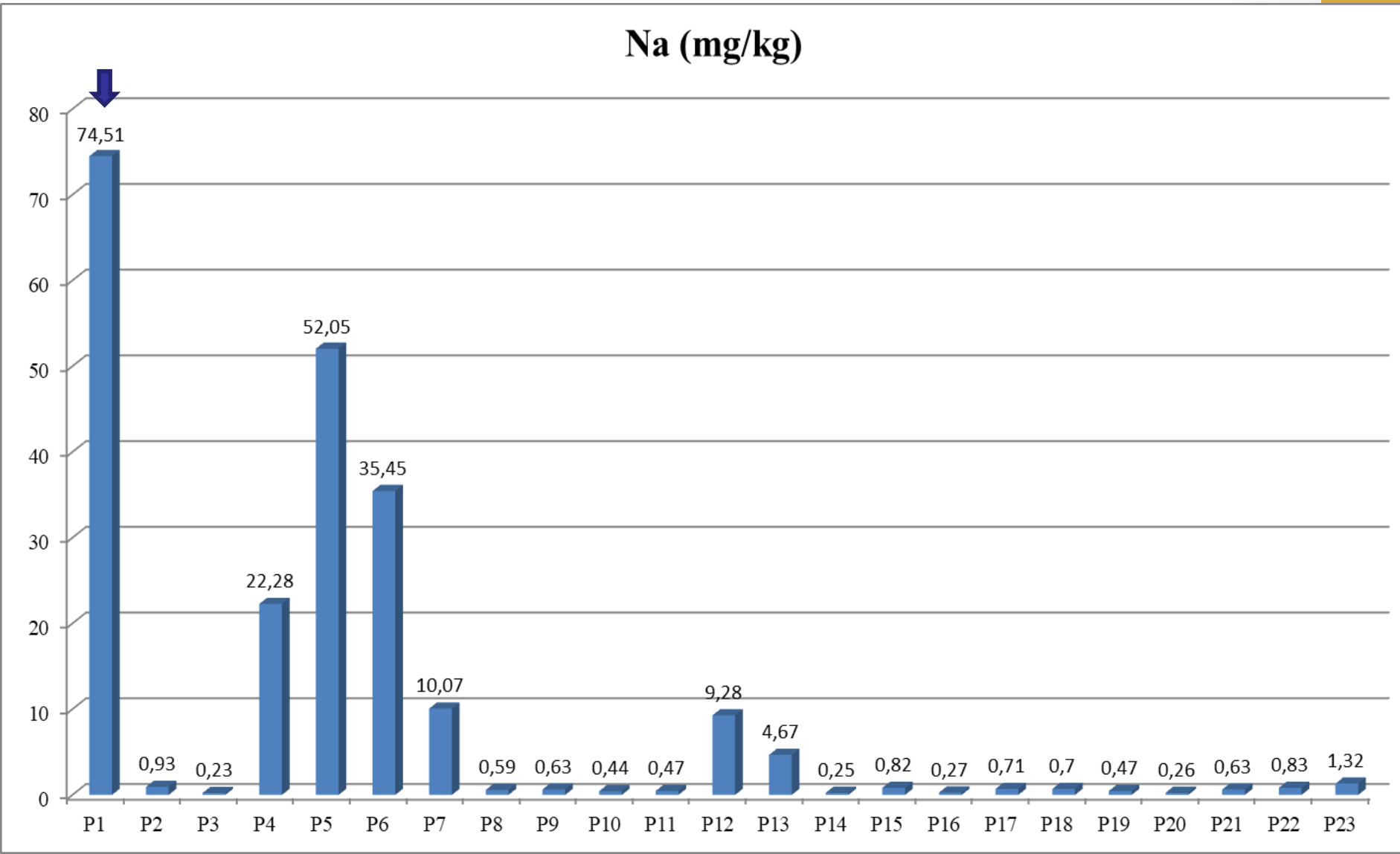


P4-*Sambucus Nigra*



P10-*Sambucus Nigra*

RESULTS ON THE CONTENT OF MACROELEMENTS OF POLEN SAMPLES



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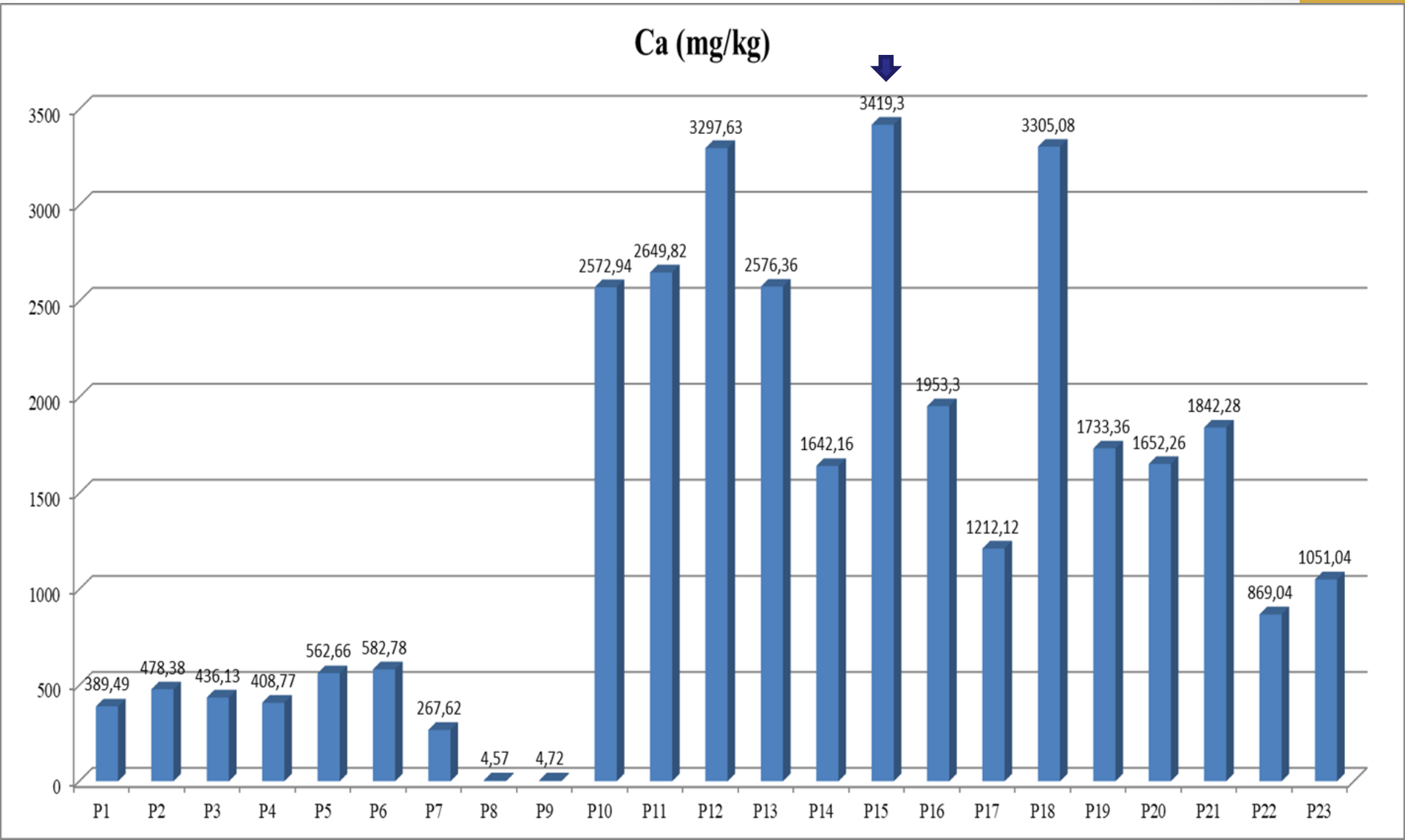

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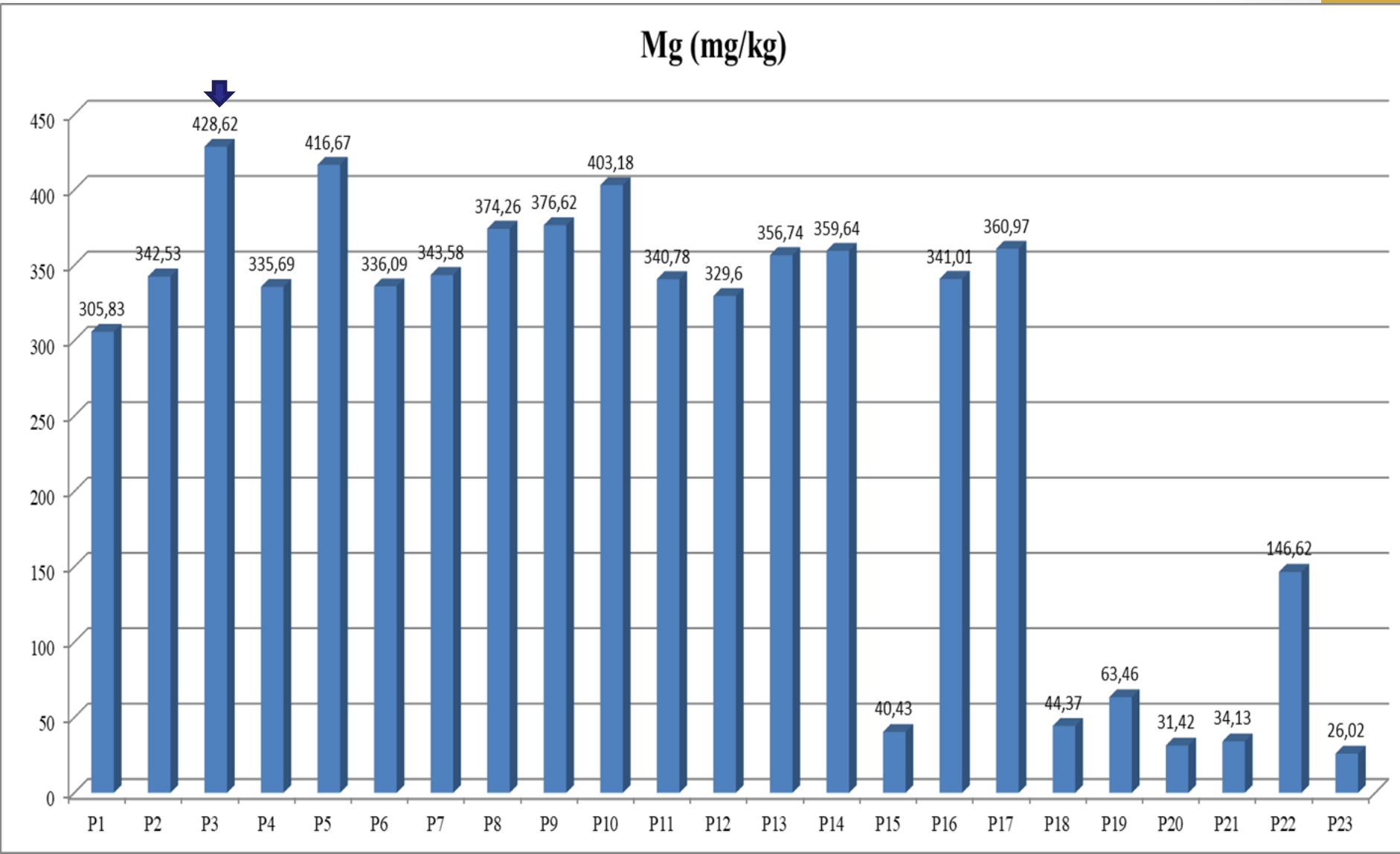

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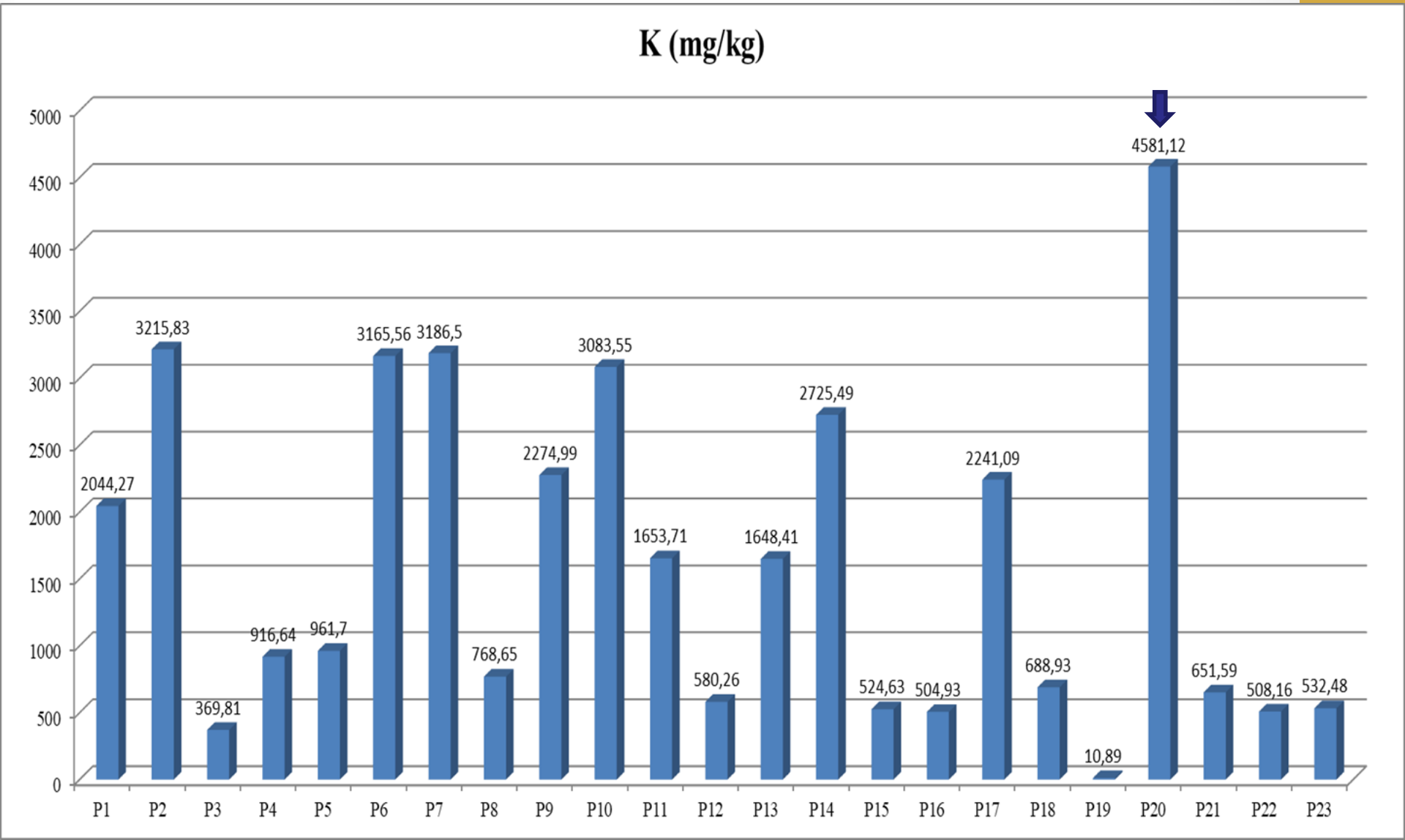
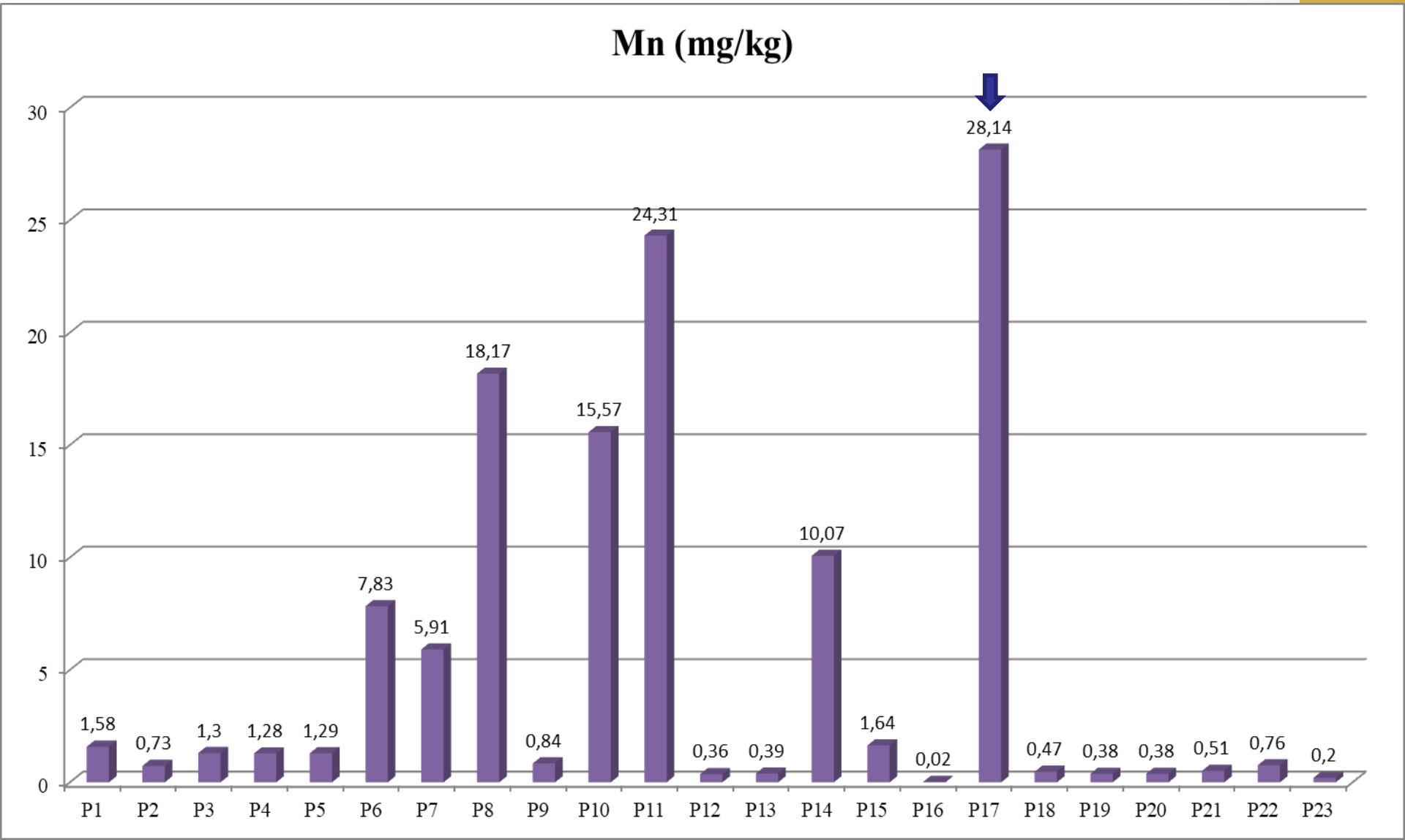
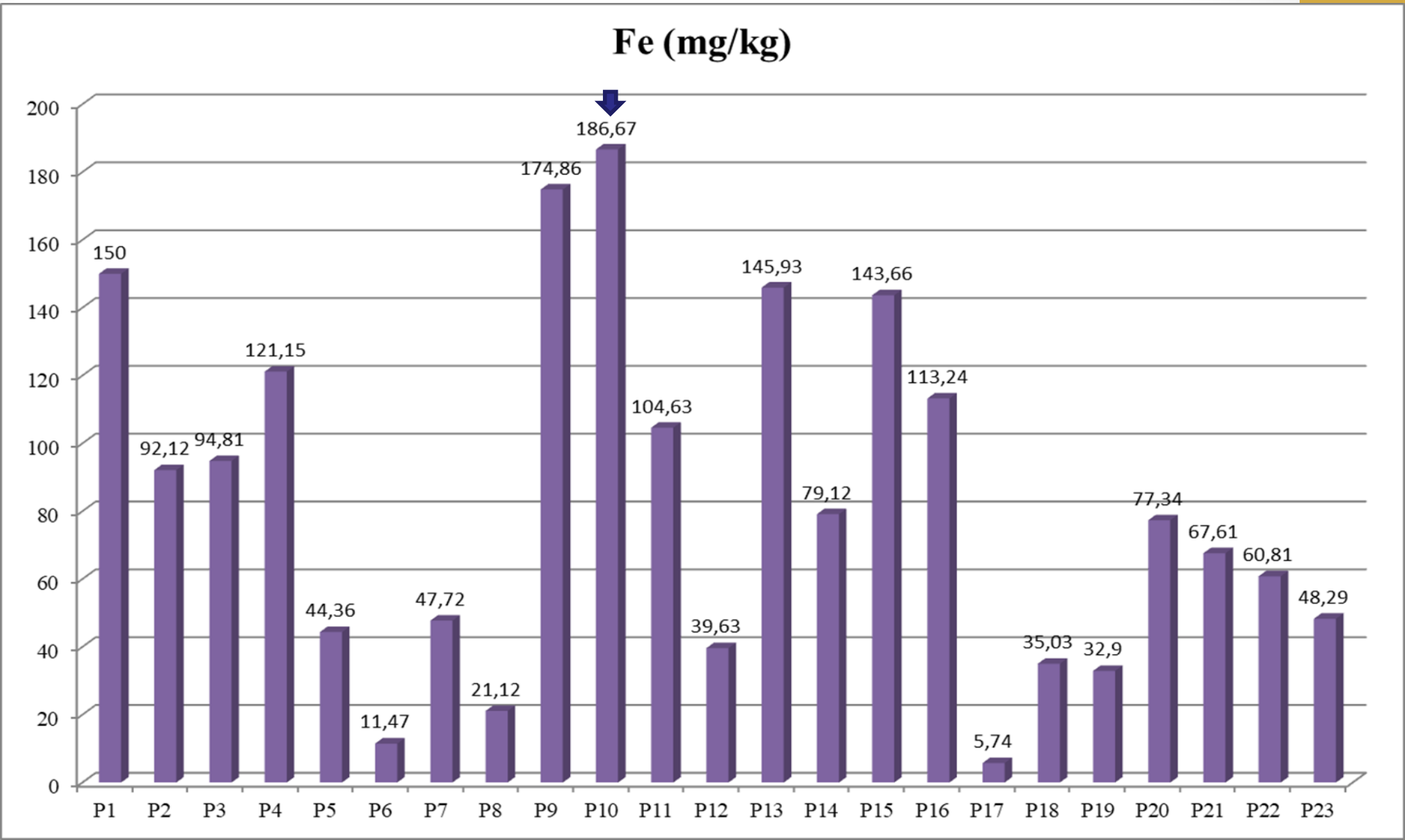


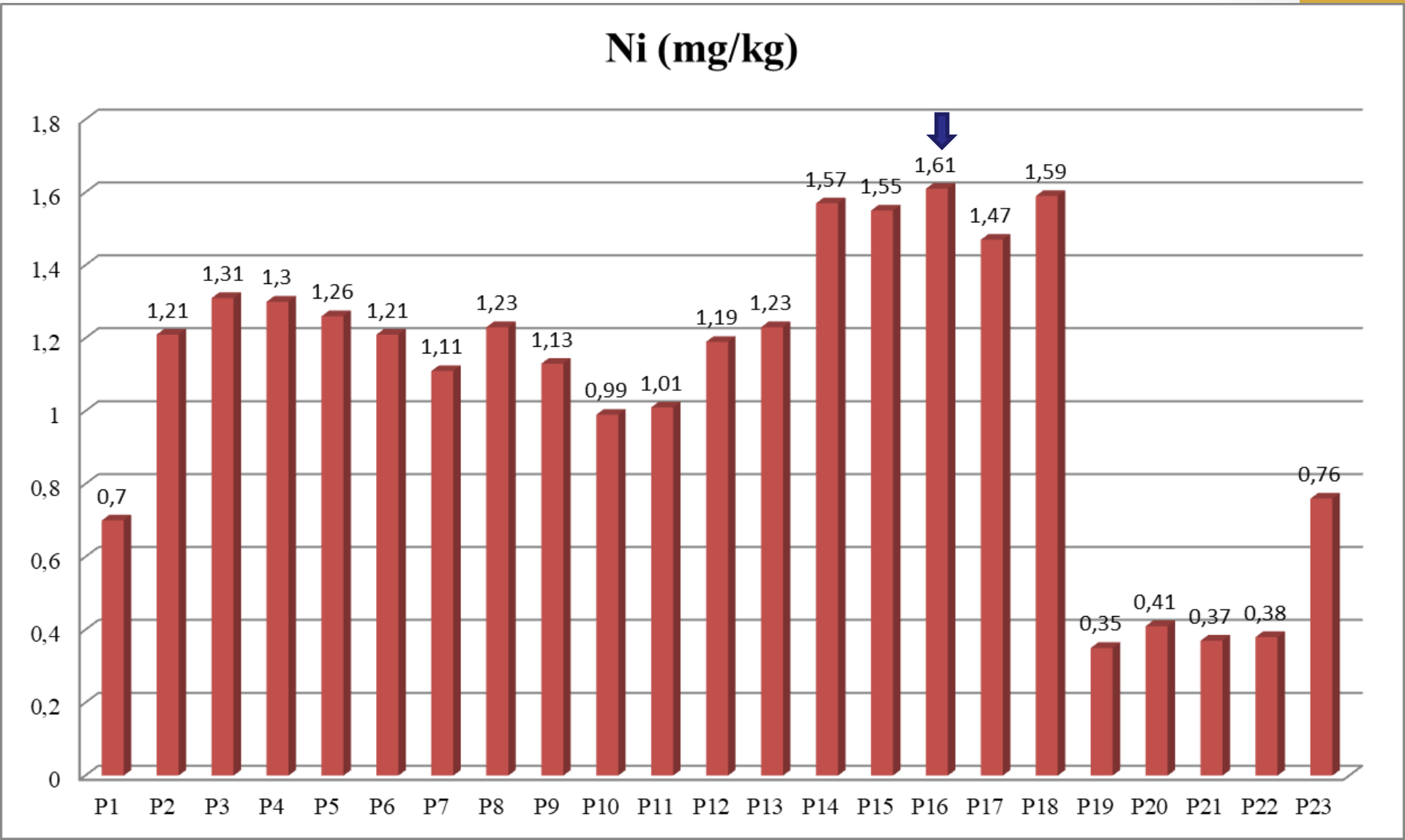
RESULTS ON THE CONTENT OF MICROELEMENTS OF POLEN SAMPLES



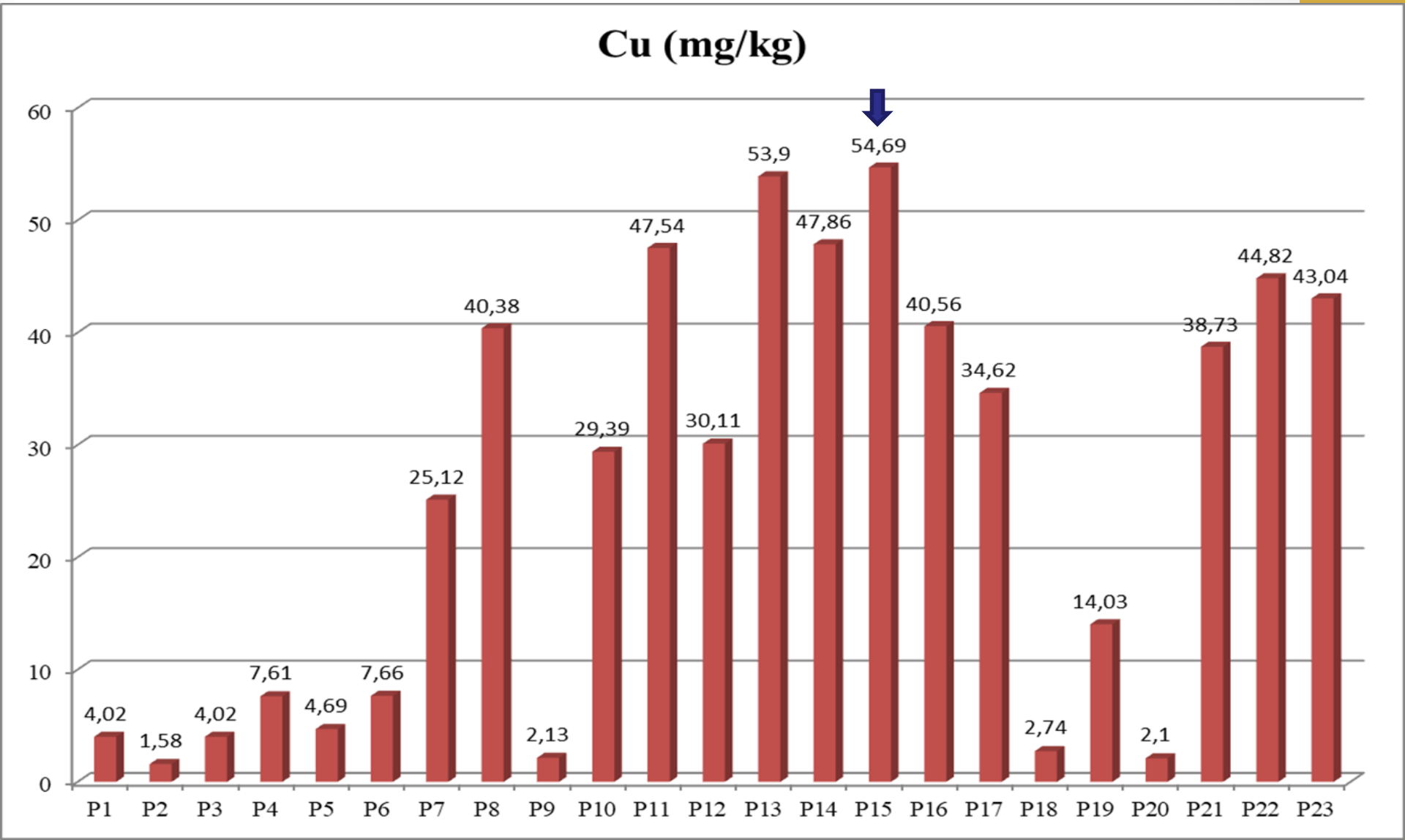
RESULTS ON THE CONTENT OF MICROELEMENTS OF POLEN SAMPLES



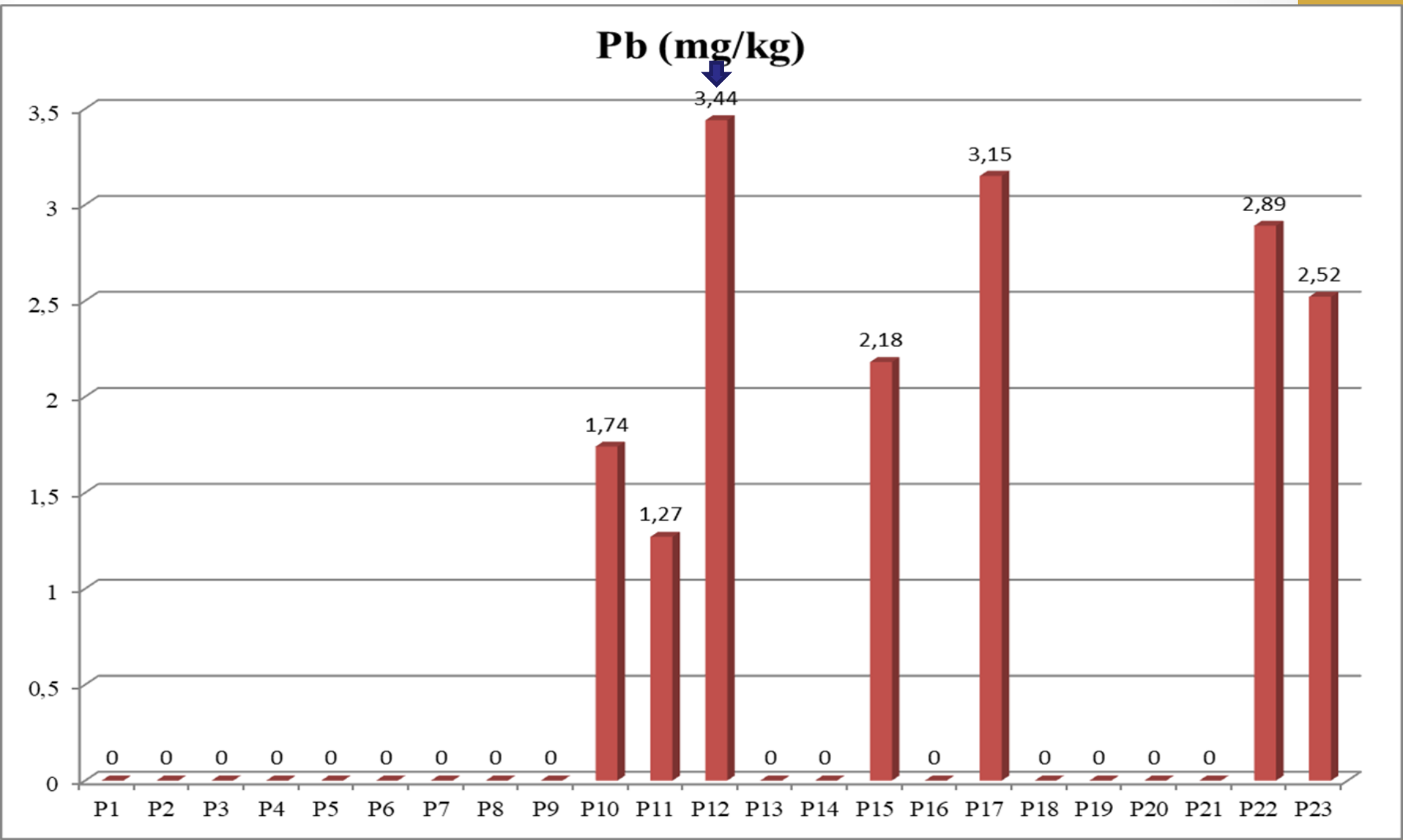
RESULTS ON THE CONTENT OF HEAVY METALS OF POLEN SAMPLES



RESULTS ON THE CONTENT OF HEAVY METALS OF POLEN SAMPLES



RESULTS ON THE CONTENT OF HEAVY METALS OF POLEN SAMPLES



RESULTS ON THE CONTENT OF HEAVY METALS OF POLEN SAMPLES

ISSN 0101-2061

DOI: http://dx.doi.org/10.1590/1678-457X.36016

Mineral element and heavy metal (ca
bee pollen i

Table 2. Mineral element and heavy metal mean values ($\mu g/g_{pollen}$) of bee pollens produced in Turkey.

No	Cr	Cu	Fe	Mn	Se	Zn	B	Ni	Si	Cd	Pb	As
1	0.536	11.157	140.223	19.456	3.912	37.813	0.946	0.004	0.261	0.113	0.176	0.015
2	1.159	14.994	340.117	201.036	5.085	39.079	0.799	0.020	0.082	0.086	0.088	0.010
4	0.337	10.616	313.532	19.713	3.242	30.387	0.693	0.007	0.132	0.040	0.055	0.009
5	0.912	7.062	60.691	30.652	1.845	27.272	34.850	1.062	7.249	0.070	0.222	0.446
6	0.730	11.622	510.235	25.087	2.127	23.873	0.841	0.005	0.110	0.061	0.304	0.009
7	0.842	11.398	725.360	18.295	2.154	27.738	0.700	0.002	0.142	0.046	0.278	0.008
8	1.170	12.430	70.406	23.067	1.836	36.040	48.524	1.184	10.380	0.090	0.293	0.435
								07	0.121	0.076	0.065	0.007
								02	0.195	0.042	0.102	0.007
								08	0.098	0.095	0.390	0.006
								03	0.245	0.047	0.479	0.010
								90	6.014	0.058	0.000	0.406
								02	0.198	0.033	0.215	0.010
								43	1.982	0.113	0.000	0.759
								36	10.500	0.051	0.117	0.983
								90	8.490	0.006	0.145	1.035
								42	2.189	0.181	0.212	0.871
								61	6.024	0.054	0.296	0.907

Research Article

Open Access

Adam Roman*, Ewa Popiela-Pleban, Paweł Migdał, Wojciech Kruszyński

As, Cr, Cd, and Pb in Bee Products from a Polish Industrialized Region

DOI: 10.1515/chem-2016-0007
received May 18, 2015; accepted January 26, 2016.

Abstract: Bee pollen and bee bread from stationary apiaries in the southwest Polish Legnica-Glogow copper district (LGOM) were analyzed for Cr, Pb, Cd and As by ICP-AES. Their concentrations in both products were As > Cr > Pb > Cd. Concentrations in bee pollen were higher than in bee bread. Average Cr, Pb, As and Cd concentrations in bee products were 0.138, 0.093, 0.325, and 0.019 mg kg^{-1}, respectively. Chromium was the most problematic element

Bees and their products have been used to monitor environmental contamination [3-9]. There are significant correlations between the level of toxic metals in soil and plants and their content in bee products [10].

The aim of this study was to determine Cr, Pb, Cd and As in bee pollen (pollen loads) and bee bread from bee colonies in industrialized southwest Poland.

2 Experimental procedure

RESULTS ON THE CONTENT OF HEAVY METALS OF BEE SAMPLES

Samples bee pollen/bees	Mn µg/g	Ni µg/g	Cu µg/g	Pb µg/g	Cd µg/g
P24 (MS)	1,45± 0,02	0,98±0,04	1,24±0,11	ND for 5µg/l	0,06±0,01
P25 (SJ)	0,31±0,05	0,99±0,01	1,27±0,09	ND for 5µg/l	0,01±0,00
P26 (CJ)	3,45±1,21	0,69±0,01	1,09±0,01	1,29±0,05	0,06±0,02
P27 (DJ)	4,25± 1,34	1,15±0,01	1,54±0,04	1,80±0,05	0,23±0,05
P28 (MM)	0,26±0,01	0,13±0,01	1,72±0,03	1,21±0,03	0,01±0,00

RESULTS ON THE CONTENT OF HEAVY METALS OF BEE SAMPLES

Heavy Metal (Hg, Cr, Cd, and Pb) Contamination in Urban Areas and Wildlife Res... as Bioindicators

Monia Perugini • Maurizio Manera • Lisa Gr... Maria Cesarina Abete • Renata Tarasco • Michele Amorena

Polish J. of Environ. Stud. Vol. 19, No. 3 (2010), 663-669

Short Communication

Levels of Copper, Selenium, Lead, and Cadmium in Forager Bees

Adam Roman*

Department of Animal Hygiene and Environment, Wroclaw University of Environmental and Life Science, Chełmońskiego 38C, 51-630 Wrocław, Poland

Received: 2 June 2009
Accepted: 30 November 2009

Abstract

The aim of this study was to determine levels of trace metals (Cu, Se, Pb and Cd) in bodies of forager bees. The study was conducted in 14 stationary apiaries situated in southeastern Opole Province (Poland) in autumn 2007 and in spring, summer and autumn 2008. Samples were mineralized using microwave method. Quantitative analysis of studied metals in bees' bodies and multiflower honey were done using the plasma

Table 1. Concentration of trace elements in bees in subsequent periods of the study [mg·kg^{-1} d.m.].

Element	Specification	Subsequent periods of the study				In total N=400
		Autumn 2007 N=100	Spring 2008 N=100	Summer 2008 N=100	Autumn 2008 N=100	
Cadmium	min.	0.29	0.33	0.38	0.09	0.09
	max.	0.93	1.82	1.58	1.24	1.82
	$\bar{x}$	**0.53** A	**0.83** B	**0.77** B	**0.46** A	**0.65**
	SD	0.17	0.36	0.30	0.26	0.32
	V [%]	31.1	43.6	38.5	56.0	49.0
r	min.	15.3	15.7	13.9	12.1	12.1
	max.	24.9	35.2	32.1	31.2	35.2
	$\bar{x}$	**20.6** Aa	**24.5** B	**23.2** b	**22.2**	**22.6**
	SD	2.6	5.4	4.7	4.6	4.6
	V [%]	12.7	22.1	20.3	20.5	20.4
	min.	0.81	0.94	0.003	0.003	0.003
	max.	4.94	6.73	3.82	3.53	6.73
	$\bar{x}$	**1.73** aA	**2.59** bAB	**2.55** B	**0.95** C	**1.95**
	SD	0.78	1.56	0.71	1.09	1.27
	V[%]	45.2	60.3	27.7	115.3	64.8
	min.	4.70	2.55	1.84	1.40	1.40
	max.	9.27	10.76	11.90	15.81	15.81
Selenium	$\bar{x}$	**6.69**	**5.81** Aa	**7.69** B	**7.95** b	**7.03**
	SD	1.28	2.32	2.26	3.85	2.70
	V [%]	19.1	39.9	29.3	48.4	38.1

A, B, C – significant statistical differences on a level of $p \leq 0.01$ between periods;

RESULTS ON THE CONTENT OF FREE AMINOACID OF POLEN SAMPLES

Free amino acids [mg/100g]	*P1*	*P2*	*P3*	*P4*	*P5*
ABA	2.2	2.1	0.8	0.8	1.4
GABA	78.2	20.4	64.2	95.9	145.6
GLU	9.2	79.3	43.9	3.6	18.1
3 MHIS	6.6	0	1	1.4	0.7
HYP	61.7	67.3	56.9	54.9	45.9
ASP	69.8	84	60.7	66.4	68.9
1 MHIS	6.9	7.8	7.6	8	6.4
ALA	55.4	88	94.5	86.2	136.1
ARG	113.5	64.6	74.6	25.7	21.4
ASN	395.1	522.7	426	125.4	221.1
PHE	33.9	22	22.2	31.6	276.5
GLY	9.4	9.4	10.3	8.6	15
GLN	130.5	105	83	87.9	79.9
HIS	173.2	112.2	152.2	242.1	140.9
ILE	14.9	6.4	6.8	7.6	7
LEU	30.7	19.3	19.9	28.4	106.8
LYS	42.1	25.3	28.6	42.9	36.8
MET	8.4	8.1	6.9	5.4	2.5
ORN	4.7	8	7.1	2.4	2.5
PRO	451.4	1299	1079.7	1168.8	1217.3
TYR	15.4	12.1	10.1	15.2	10
TRP	14.5	6.6	8	9.1	111.6
SAR	1.4	2.7	2.3	5.3	2.6
SER	84.6	74.4	67.4	59.4	85.5
THR	26.6	23	19.7	18.3	25.3
VAL	28.3	14.8	19.6	19	26.8
EAA	41.39	26.41	31.53	44.93	81.57

RESULTS ON THE CONTENT OF FREE AMINOACID OF POLEN SAMPLES

Table 3
Average content (mg/g pollen) of free and total amino acids of 32 samples analyzed and of *C. ladanifer* and *E. plantagineum* loads manually separated

Amino acid (mg/g bee-pollen)	Free amino acid			Total amino acid		
	Bee-pollen	*Cistus*	*Echium*	Bee-pollen	*Cistus*	*Echium*
Asp	0.40 ± 0.21	0.41 ± 0.02	0.57 ± 0.03	15.10 ± 3.68	13.52 ± 0.90	32.34 ± 0.69
Glu	0.25 ± 0.15	0.26 ± 0.04	0.97 ± 0.11	17.88 ± 1.98	16.09 ± 0.48	16.36 ± 0.59
α-Adip	< D.L.	< D.L.	< D.L.	< D.L.	< D.L.	< D.L.
Asn	0.52 ± 0.48	0.53 ± 0.06	2.00 ± 0.01	3.87 ± 1.03	3.43 ± 0.27	5.68 ± 0.07
Ser	0.60 ± 0.31	0.55 ± 0.36	0.30 ± 0.10	2.74 ± 0.77	3.05 ± 0.08	2.41 ± 0.12
Gln	0.60 ± 0.48	0.81 ± 0.38	0.17 ± 0.12	5.91 ± 2.53	4.98 ± 0.07	14.23 ± 0.02
His	0.74 ± 0.67	0.31 ± 0.01	0.10 ± 0.00	6.84 ± 7.15	3.78 ± 0.30	44.90 ± 2.47
Hser	0.03 ± 0.08	0.14 ± 0.01	< D.L.	–	–	–
Gly	0.21 ± 0.19	0.19 ± 0.10	0.31 ± 0.07	6.40 ± 1.09	5.69 ± 0.04	11.20 ± 0.74
Thr	0.25 ± 0.21	0.25 ± 0.03	0.83 ± 0.25	4.17 ± 0.61	4.21 ± 0.23	5.50 ± 0.10
Arg	2.48 ± 1.61	2.32 ± 1.14	1.49 ± 0.35	5.03 ± 1.49	4.26 ± 0.26	8.27 ± 0.54
Ala	0.82 ± 0.65	0.94 ± 0.12	0.97 ± 0.17	10.68 ± 1.09	9.66 ± 0.27	13.31 ± 0.11
Tau	< D.L.	< D.L.	< D.L.	< D.L.	< D.L.	< D.L.
Gaba	0.35 ± 0.33	0.70 ± 0.20	< D.L.	–	–	–
Tyr	0.32 ± 0.15	0.31 ± 0.15	0.31 ± 0.05	7.43 ± 3.13	5.71 ± 0.30	37.56 ± 1.60
Met	0.29 ± 0.30	0.24 ± 0.06	0.13 ± 0.01	4.10 ± 1.53	3.69 ± 0.12	7.23 ± 0.62
Val	0.21 ± 0.11	0.25 ± 0.05	0.11 ± 0.01	7.26 ± 1.94	5.54 ± 0.11	15.76 ± 0.52
Trp	0.09 ± 0.10	< D.L.	< D.L.	–	–	–
Phe	0.75 ± 0.40	0.73 ± 0.24	0.19 ± 0.14	9.65 ± 2.16	8.80 ± 0.09	27.16 ± 1.01
Ileu	0.51 ± 0.55	0.49 ± 0.02	0.60 ± 0.26	9.22 ± 2.01	8.57 ± 0.34	15.98 ± 0.03
Leu	0.91 ± 0.43	0.40 ± 0.21	0.65 ± 0.37	10.81 ± 1.65	8.51 ± 0.52	16.87 ± 1.53
Orn	0.08 ± 0.21	0.79 ± 0.21	< D.L.	–	–	–
Lys	0.26 ± 0.29	0.38 ± 0.24	0.24 ± 0.19	10.97 ± 1.94	9.88 ± 0.23	37.11 ± 1.01
Pro	20.27 ± 3.82	21.87 ± 0.63	12.23 ± 1.13	22.88 ± 3.53	23.91 ± 0.60	19.39 ± 0.78
Total	30.9 ± 4.49	32.5 ± 0.76	22.2 ± 2.56	161.2 ± 23.70	143.3 ± 0.62	331.3 ± 0.11

ibility (C.V.) ranged from 5.3% to 20.4%; recoveries were above 78.8%. Forty monovarietal honey samples from ilex, oak, heather and chestnut-tree were analyzed for their free amino acid profiles. α-Aminoadipic acid and homoserine are reported for the first time in honeys. Thirty-two samples of Spanish bee-pollen, made of a majority of pellets from *Cistus Ladanifer* (67.1%) and *Echium plant-*

DISCUSSIONS AND CONCLUSIONS

- Differences between means were analyzed using pearson r coefficient with the software GraphPad (GraphPad Prism7, Inc., La Jolla, CA) and p values are the following Mn: p=0.1-0.6; Ni: p=0.3-0.5; Cu: p=0.3-0.6; Pb: p=0.01-0.55; Cd: p=0.01-0.58.

- According to Polish standards, the following limits for bee pollen must not be exceeded: Cd: 0.05 mg/kg; Pb: 0.5 mg /kg (Roman, 2007; Roman et al., 2016). Campos et al., 2008 showed the following limits for Cd: 0.03 mg/kg and Pb: 0.05 mg/kg. Cu: 50*103µg/kg (Ordin nr.975/1998)

- Analyzing those five geographic areas and intake of heavy metal, it can be concluded that: Dolj county is a more polluted geographic area in comparison with other areas.

Thank you!