SUPPLEMENTARY MATERIAL TO

Quality parameters and pattern recognition methods as a tool in tracing the regional origin of multifloral honey

KRISTINA B. LAZAREVIĆ[1], JELENA Đ. TRIFKOVIĆ[2], FILIP Lj. ANDRIĆ[2],
ŽIVOSLAV Lj. TEŠIĆ[2], IVAN B. ANĐELKOVIĆ[3#], DEJAN I. RADOVIĆ[4],
NEBOJŠA M. NEDIĆ[5] and DUŠANKA M. MILOJKOVIĆ-OPSENICA[2*]

[1]*Center for Food Analysis, Zmaja od Noćaja 11, 11000 Belgrade, Serbia,* [2]*Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia,* [3]*Innovation Center, Faculty of Chemistry Ltd., Studentski trg 12–16, 11000 Belgrade, Serbia,* [4]*Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia and* [5]*Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia*

TABLE S-I. Kruskal–Wallis test

Parameter	Kruskal–Wallis test		
	Chi square	*P*	Multiple comparison
K	67.03	< 0.0001	Z(B,C,W,E,S,V), C(E)
Ca	34.15	< 0.0001	C(E,B,W,V,Z), S(E,V), E(S,V), Z(C,V)
Mg	67.29	< 0.0001	Z(B,C,W,E,S,V)
Na	8.94	0.1769	–
Zn	19.42	0.0035	B,C (Z,V,S,E,W)
Fe	26.14	0.0002	V(B,C,E,S), Z(B,C,E,S)
Cu	58.70	< 0.0001	Z(B,C,W,E,S,V), C(E), E(S)
Mn	35.54	< 0.0001	Z(V,C,E,S)
Co	12.18	0.0580	–
Cr	20.80	0.0020	E,V(C,W,S,Z)
Ni	49.96	< 0.0001	Z(B,W,V,E,C,S)
Cd	21.45	0.0015	Z(B,W,V,E,C,S)
Moisture	15.91	0.0143	Z,W(B,C,E)
EC	77.68	< 0.0001	Z(B,W,V,E,C,S)
pH	53.84	< 0.0001	Z(B,W,V,E,C,S)
FA	32.91	< 0.0001	C(S,E,V,Z), Z(C,W,S,E)
OR	57.43	< 0.0001	Z(B,C,W,E,S,V)
Trehalose	12.48	0.0521	–
Glucose	16.70	0.0104	W(Z,S,C,V)

* Corresponding author. E-mail: dusankam@chem.bg.ac.rs

TABLE S-I. Continued

Parameter	Kruskal–Wallis test		
	Chi square	P	Multiple comparison
Fructose	16.83	0.0076	C(W,E,Z), W(V,S,C)
Sucrose	19.73	0.0031	Z(B,E,S,V)
i-Maltose	20.01	0.0028	E(C,V,Z)
Melezitose	15.87	0.0144	Z,C(S,E,V)
Gentiobiose +turanose	10.72	0.0974	–
i-Maltotriose	18.78	0.0045	S,E(V,C)
Maltose	14.94	0.0207	C(B,Z,E,S,V)

LINEAR DISCRIMINANT ANALYSIS

Discrimination of samples from the Zlatibor region compared to the other regions of Serbia

TABLE S-II.I. Canonical variate analysis

Function	Eigenvalue	Incl. %	Total %	R canonical	R^2 canonical	F	d.f.1	d.f.2	P	Wilk's λ
1	1.751538	100.0	100.0	0.8815	0.7770	14.3	26	107	< 0.0001	0.223

TABLE S-II.II. Classification count; Z – Zlatibor area, RS – The rest of Serbia; reduction in classification error due to Xs = 97.0 %

Actual	Predicted		
	RS	Z	Total
RS	96	0	96
Z	2	36	38
Total	98	36	134

TABLE S-II.III. Standardized canonical coefficients

Variable	Canonical Variate 1
K	–0.750
Ca	–0.464
Mg	0.680
Na	–0.017
Zn	0.007
Fe	–0.024
Cu	–0.133
Mn	–0.181
Co	0.212
Cr	–0.216
Ni	0.482
Cd	–0.078

TABLE S-II.III. Continued

Variable	Canonical Variate 1
Moisture	0.011
EC	1.681
pH	0.094
FA	−0.497
SR	−0.192
Trehalose	−0.028
Glucose	0.186
Fructose	−0.092
Sucrose	−0.038
i-Maltose	0.025
Melezitose	−0.300
Gentiobiose+turanose	−0.046
i-Maltotriose	−0.053
Maltose	0.0054

Discrimination of samples from all regions of Serbia

TABLE S-III.I. Canonical variate analysis

Function	Eigen values	Incl. %	Total %	R canonical	R^2 canonical	F	d.f.1	d.f.2	P	Wilk's λ
1	3.78	58.6	58.6	0.8894	0.7911	3.3	156	606.4	< 0.0001	0.028
2	1.17	18.2	76.8	0.7349	0.5401	2.1	125	511.8	< 0.0001	0.135
3	0.63	9.8	86.6	0.6220	0.3869	1.6	96	414.5	0.0016	0.294
4	0.52	8.0	94.7	0.5843	0.3414	1.3	69	314.5	0.0898	0.480
5	0.22	3.5	98.1	0.4286	0.1838	0.8	44	212.0	0.7715	0.729
6	0.12	1.9	100.0	0.3277	0.1074	0.6	21	107.0	0.9011	0.893

TABLE S-III.II. Classification count; reduction in classification error due to Xs = 75.6 %

Actual	Predicted							
	Belgrade	Central	Eastern	Southern	Vojvodina	Western[a]	Zlatibor	Total
Belgrade	3	0	0	0	0	0	0	3
Central	0	11	0	3	1	0	0	15
East	0	1	14	6	3	3	0	27
South	0	1	2	25	0	0	0	28
Vojvodina	2	0	1	1	12	1	0	17
Western[a]	0	0	0	1	0	5	0	6
Zlatibor	0	0	1	0	1	0	36	38
Total	5	13	18	36	17	9	36	134

[a]Western region without Zlatibor area

TABLE S-III.III. Standardized canonical coefficients

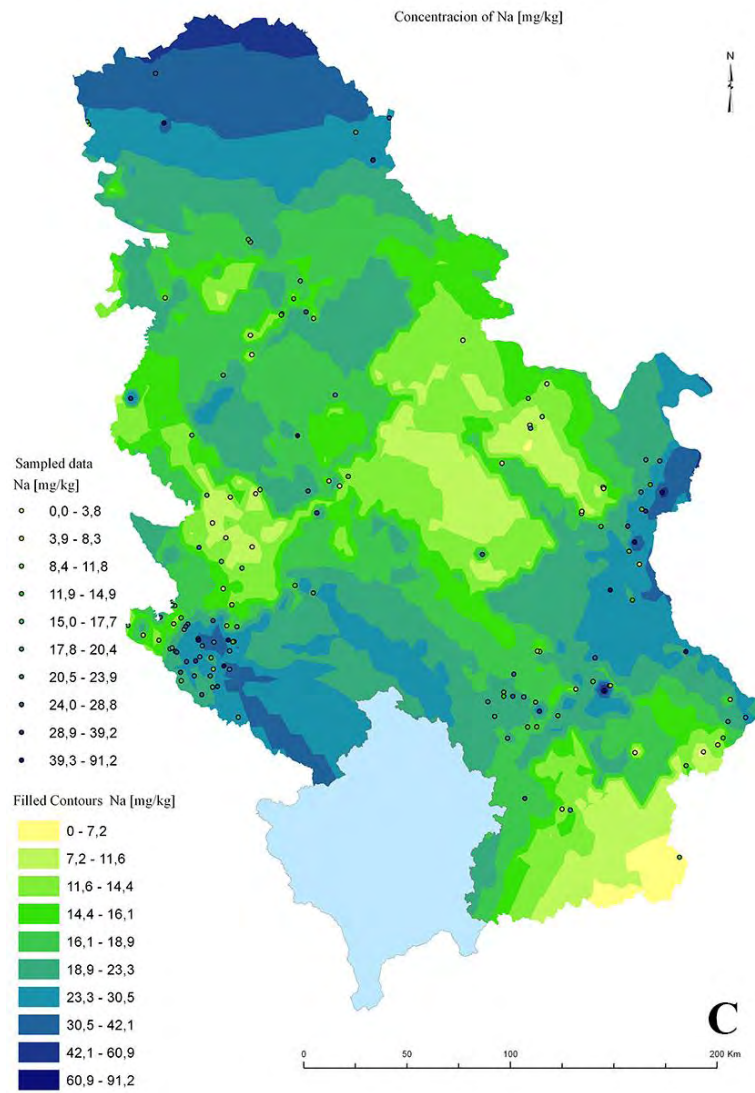| Variable	Variate 1	Variate 2	Variate 3	Variate 4	Variate 5	Variate 6
K	−0.527	0.813	−0.425	0.476	−0.384	0.160
Ca	−0.693	−0.866	0.852	0.108	0.332	0.289
Mg	0.643	−0.184	0.171	−0.435	−0.018	−0.524
Na	−0.037	0.027	−0.023	−0.055	0.036	0.574
Zn	0.033	−0.055	−0.043	0.328	−0.146	−0.181
Fe	0.004	−0.016	−0.194	−0.222	0.119	−0.446
Cu	−0.194	−0.559	−0.034	0.445	−0.417	0.015
Mn	−0.160	0.266	0.169	0.318	0.297	0.186
Co	0.284	0.162	−0.476	−0.146	0.715	0.186
Cr	−0.302	−0.590	0.070	0.058	−0.489	−0.266
Ni	0.419	−0.081	0.308	−0.372	0.269	−0.076
Cd	−0.110	0.037	0.075	−0.310	0.073	0.134
Moisture	0.045	0.186	0.142	0.237	0.273	−0.367
EC	1.602	−0.548	0.037	0.234	0.210	−0.250
pH	0.029	−0.149	−0.013	−0.614	0.045	0.215
FA	−0.405	0.137	−0.714	−0.590	−0.125	0.362
SR	−0.237	0.086	0.343	0.213	0.208	0.258
Trehalose	0.066	0.206	−0.307	−0.011	−0.125	−0.285
Glucose	0.172	−0.216	−0.271	−0.512	0.084	−0.404
Fructose	−0.112	0.102	0.098	−0.228	−0.115	0.366
Sucrose	0.015	0.203	−0.031	−0.055	−0.237	−0.270
Isomaltose	−0.047	−0.456	−0.204	−0.337	−0.134	0.165
Melezitose	−0.392	0.058	0.741	0.025	−0.265	0.137
Gentiobiose + turanose	−0.076	−0.071	0.087	−0.161	−0.450	0.092
Isomaltotriose	0.079	0.404	−0.171	0.649	0.234	−0.097
Maltose	0.035	0.326	0.293	−0.093	−0.149	−0.423

LAZAREVIĆ et al.

Concentracion of Mg [mg/kg]

Sampled data
Mg [mg/kg]
○ 5,39 - 9,52
○ 9,53 - 12,89
○ 12,90 - 16,20
○ 16,21 - 20,79
○ 20,80 - 26,80
○ 26,81 - 36,28
• 36,29 - 48,60
• 48,61 - 66,99
• 67,00 - 95,79
• 95,80 - 128,61

Filled Contours Mg [mg/kg]
    5,4 - 10,4
    10,4 - 13,7
    13,7 - 15,8
    15,8 - 19,1
    19,1 - 24,1
    24,1 - 31,8
    31,8 - 43,3
    43,3 - 61
    61 - 87,8
    87,8 - 128,6

B

0   50   100   200 Km

Concentracion of Na [mg/kg]

N

Sampled data
Na [mg/kg]

○ 0,0 - 3,8
○ 3,9 - 8,3
○ 8,4 - 11,8
○ 11,9 - 14,9
○ 15,0 - 17,7
● 17,8 - 20,4
● 20,5 - 23,9
● 24,0 - 28,8
● 28,9 - 39,2
● 39,3 - 91,2

Filled Contours Na [mg/kg]

▨ 0 - 7,2
▨ 7,2 - 11,6
▨ 11,6 - 14,4
▨ 14,4 - 16,1
▨ 16,1 - 18,9
▨ 18,9 - 23,3
▨ 23,3 - 30,5
▨ 30,5 - 42,1
▨ 42,1 - 60,9
▨ 60,9 - 91,2

C

0   50   100   200 Km

Concentracion of Ca [mg/kg]

N

Sampled data
Ca [mg/kg]

○ 17,6 - 27,1
○ 27,2 - 34,2
○ 34,3 - 39,6
○ 39,7 - 45,7
○ 45,8 - 52,7
○ 52,8 - 58,8
○ 58,9 - 71,1
● 71,2 - 85,9
● 86,0 - 103,9
● 104,0 - 133,1

Filled Contours Ca [mg/kg]

  20,6 - 30,5
  30,5 - 37,3
  37,3 - 41,9
  41,9 - 45,1
  45,1 - 49,7
  49,7 - 56,5
  56,5 - 66,4
  66,4 - 80,9
  80,9 - 102,1
  102,1 - 133,1
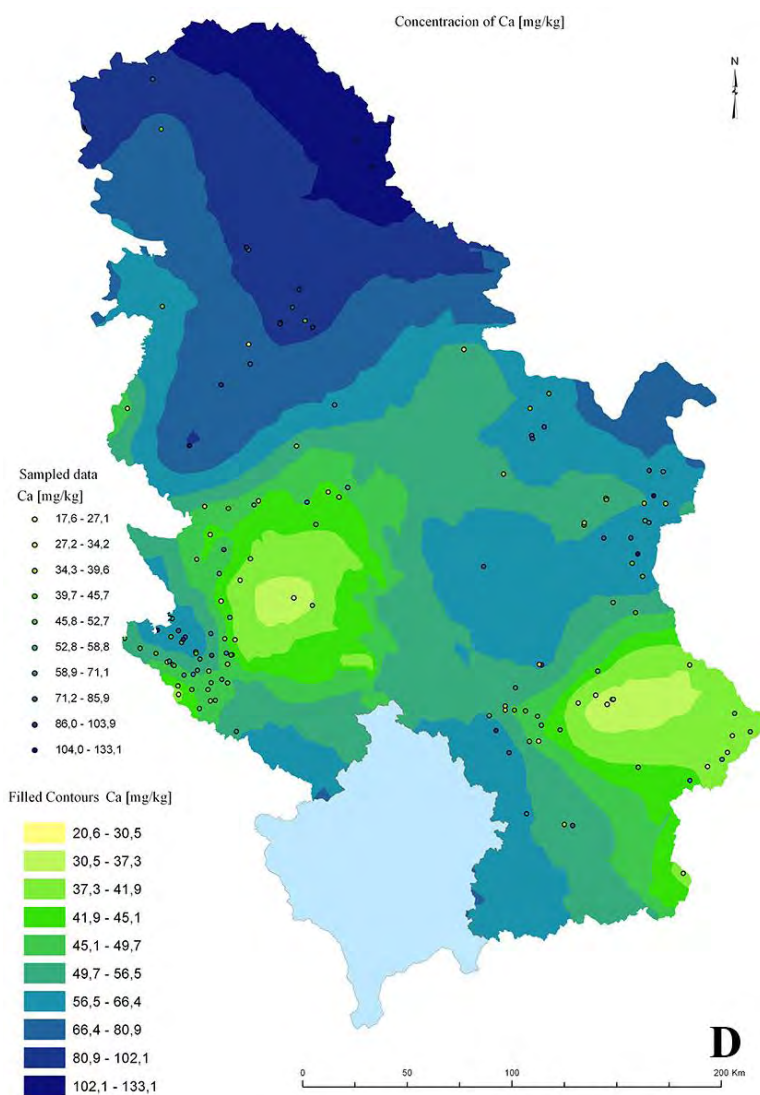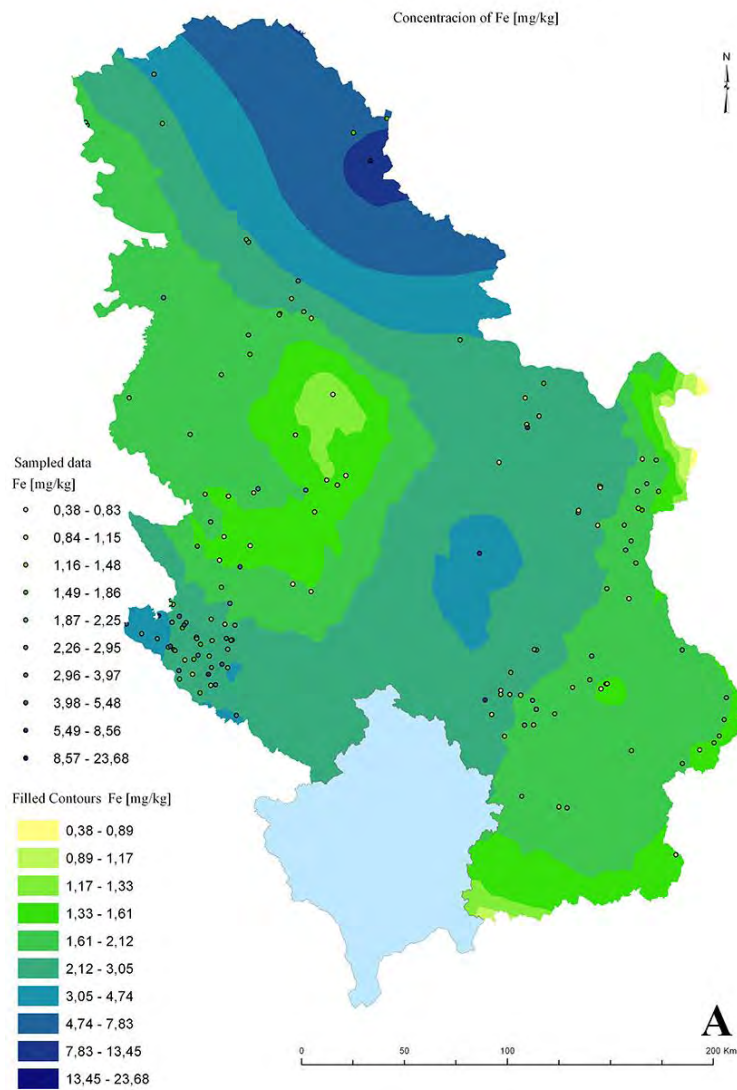
D
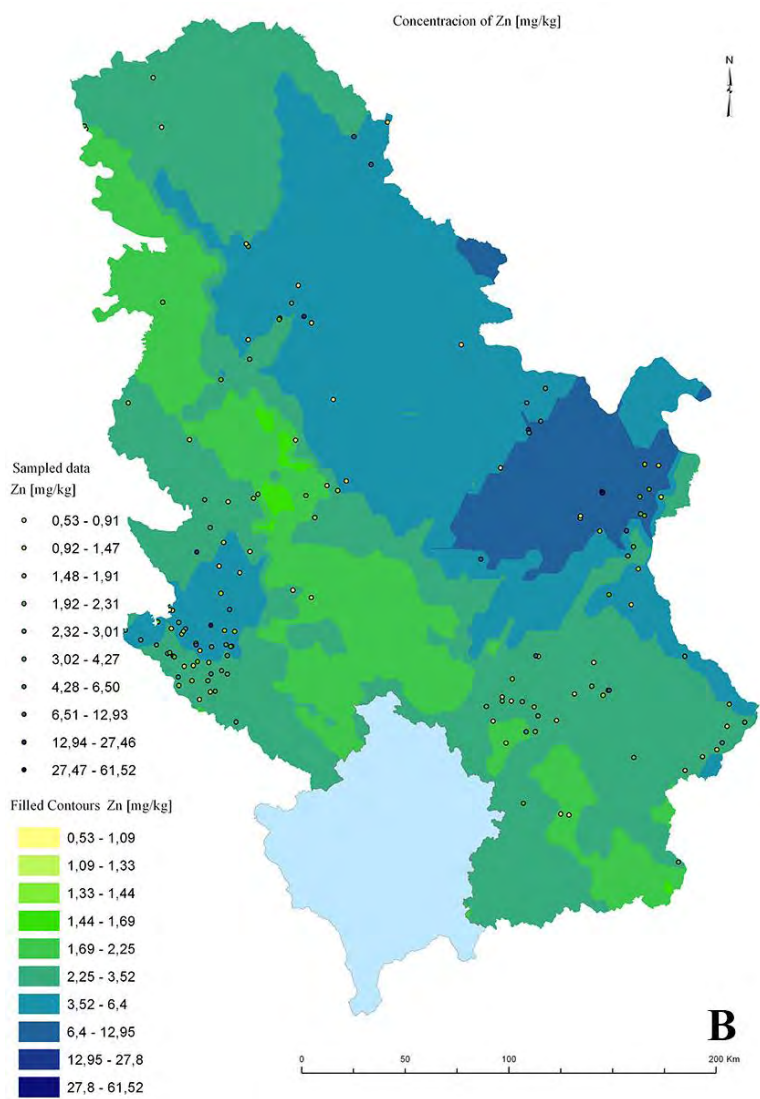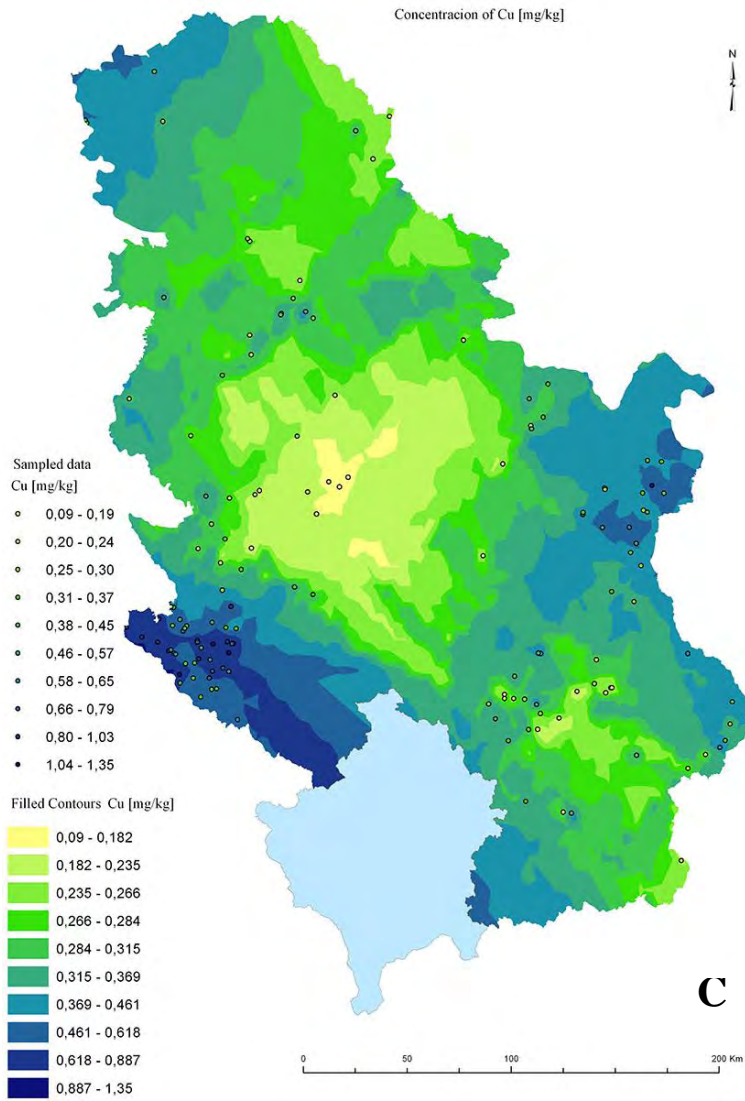
0   50   100   200 Km

Fig. S1. GIS spatial distribution of multifloral honey mineral components – macro elements.
A) K, B) Mg, C) Na and D) Ca.

Concentracion of Zn [mg/kg]

Sampled data
Zn [mg/kg]

- 0,53 - 0,91
- 0,92 - 1,47
- 1,48 - 1,91
- 1,92 - 2,31
- 2,32 - 3,01
- 3,02 - 4,27
- 4,28 - 6,50
- 6,51 - 12,93
- 12,94 - 27,46
- 27,47 - 61,52

Filled Contours Zn [mg/kg]

- 0,53 - 1,09
- 1,09 - 1,33
- 1,33 - 1,44
- 1,44 - 1,69
- 1,69 - 2,25
- 2,25 - 3,52
- 3,52 - 6,4
- 6,4 - 12,95
- 12,95 - 27,8
- 27,8 - 61,52

B

0   50   100   200 Km

LAZAREVIĆ et al.

Concentracion of Cu [mg/kg]

Sampled data
Cu [mg/kg]

- ◦ 0,09 - 0,19
- ◦ 0,20 - 0,24
- ◦ 0,25 - 0,30
- ◦ 0,31 - 0,37
- ◦ 0,38 - 0,45
- ◦ 0,46 - 0,57
- • 0,58 - 0,65
- • 0,66 - 0,79
- • 0,80 - 1,03
- • 1,04 - 1,35

Filled Contours Cu [mg/kg]

- 0,09 - 0,182
- 0,182 - 0,235
- 0,235 - 0,266
- 0,266 - 0,284
- 0,284 - 0,315
- 0,315 - 0,369
- 0,369 - 0,461
- 0,461 - 0,618
- 0,618 - 0,887
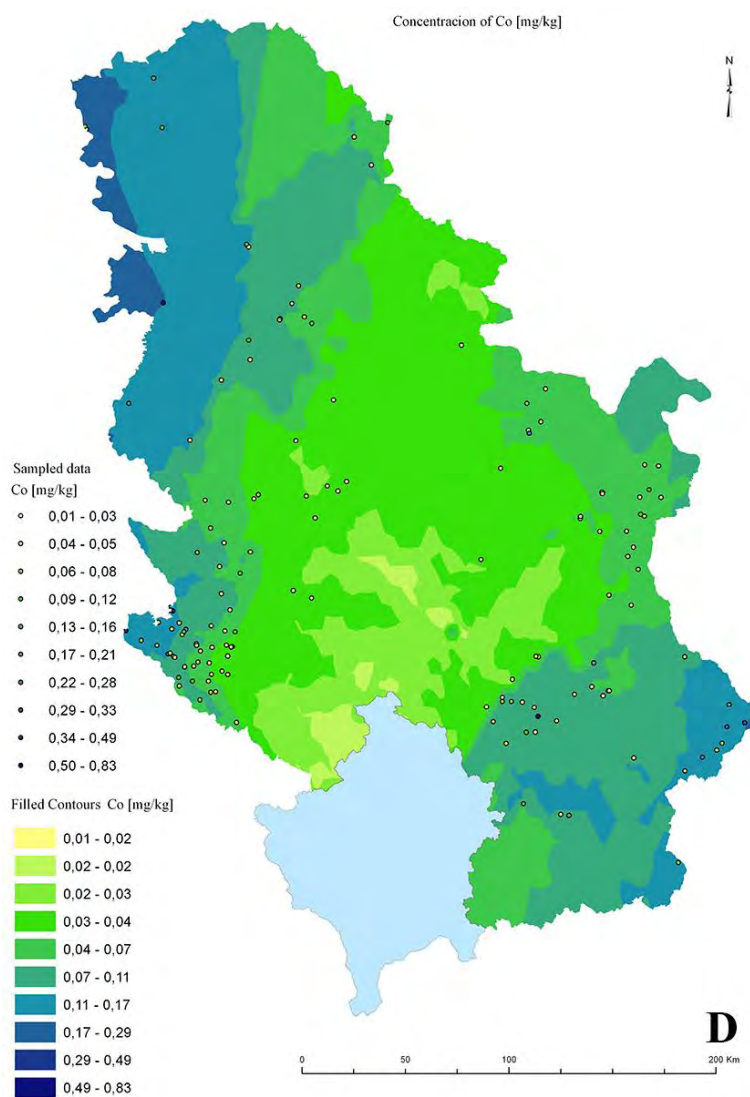- 0,887 - 1,35
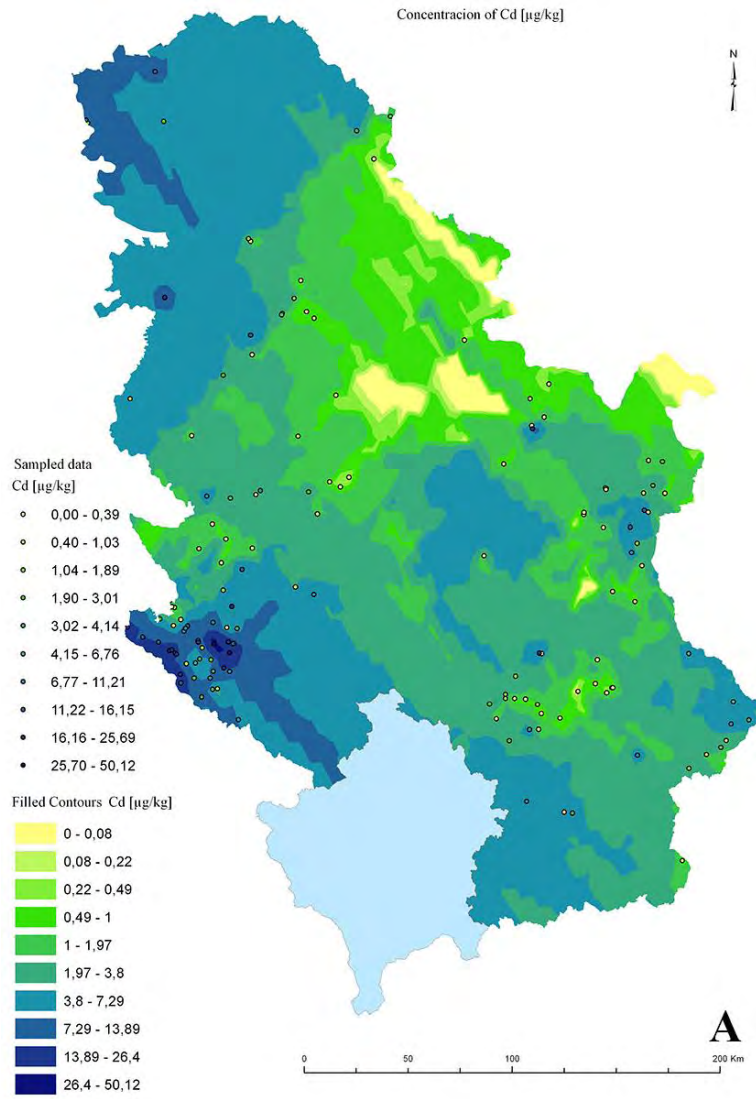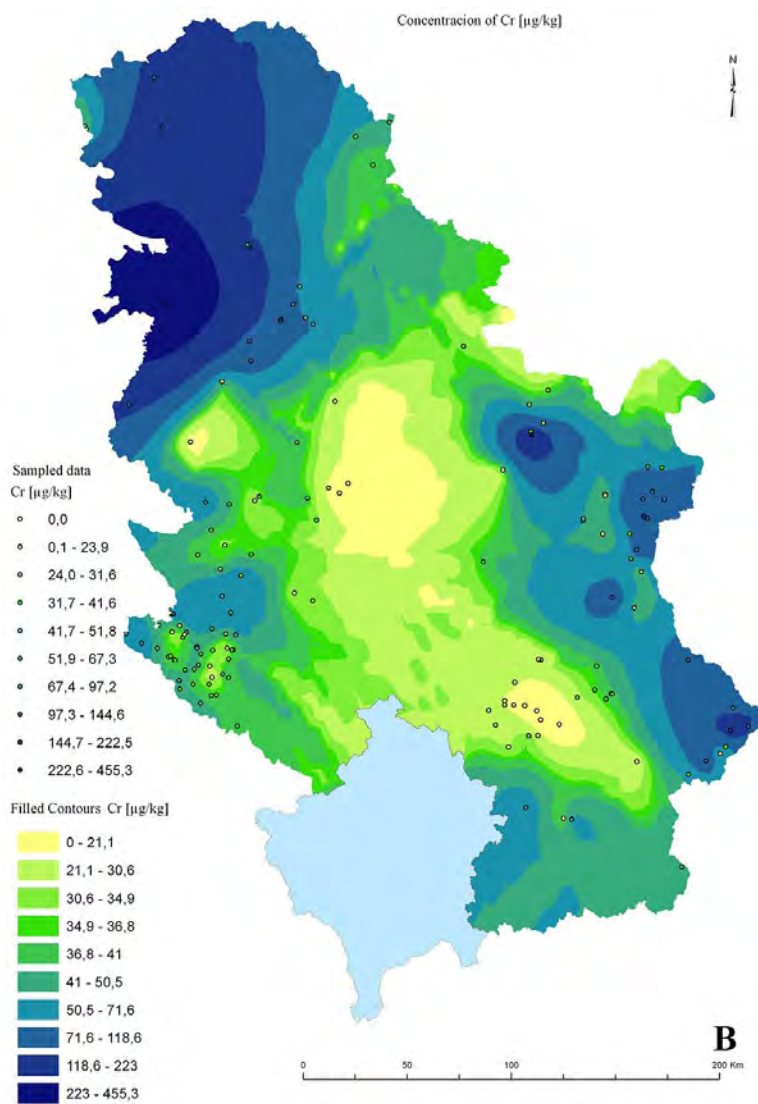
C

0   50   100   200 Km

Fig. S2. GIS spatial distribution of multifloral honey mineral components – micro elements.
A) Fe, B) Zn, C) Cu and D) Co.

LAZAREVIĆ et al.

Concentracion of Cd [μg/kg]

Sampled data
Cd [μg/kg]
- ○ 0,00 - 0,39
- ○ 0,40 - 1,03
- ○ 1,04 - 1,89
- ○ 1,90 - 3,01
- ○ 3,02 - 4,14
- ○ 4,15 - 6,76
- ○ 6,77 - 11,21
- ● 11,22 - 16,15
- ● 16,16 - 25,69
- ● 25,70 - 50,12

Filled Contours Cd [μg/kg]
- 0 - 0,08
- 0,08 - 0,22
- 0,22 - 0,49
- 0,49 - 1
- 1 - 1,97
- 1,97 - 3,8
- 3,8 - 7,29
- 7,29 - 13,89
- 13,89 - 26,4
- 26,4 - 50,12

A

0   50   100   200 Km

Concentracion of Cr [µg/kg]

Sampled data
Cr [µg/kg]

○ 0,0
○ 0,1 - 23,9
○ 24,0 - 31,6
○ 31,7 - 41,6
○ 41,7 - 51,8
○ 51,9 - 67,3
○ 67,4 - 97,2
● 97,3 - 144,6
● 144,7 - 222,5
● 222,6 - 455,3

Filled Contours Cr [µg/kg]

0 - 21,1
21,1 - 30,6
30,6 - 34,9
34,9 - 36,8
36,8 - 41
41 - 50,5
50,5 - 71,6
71,6 - 118,6
118,6 - 223
223 - 455,3

B

LAZAREVIĆ et al.

Fig. S3. GIS spatial distribution of multifloral honey mineral components – trace elements.
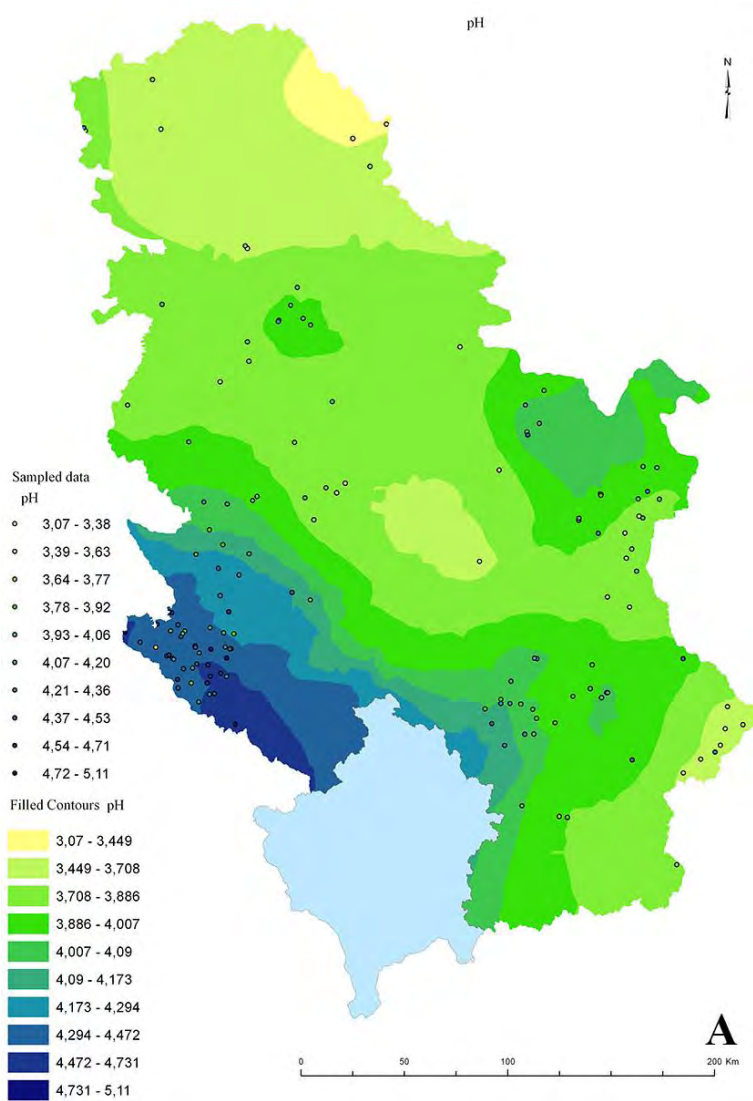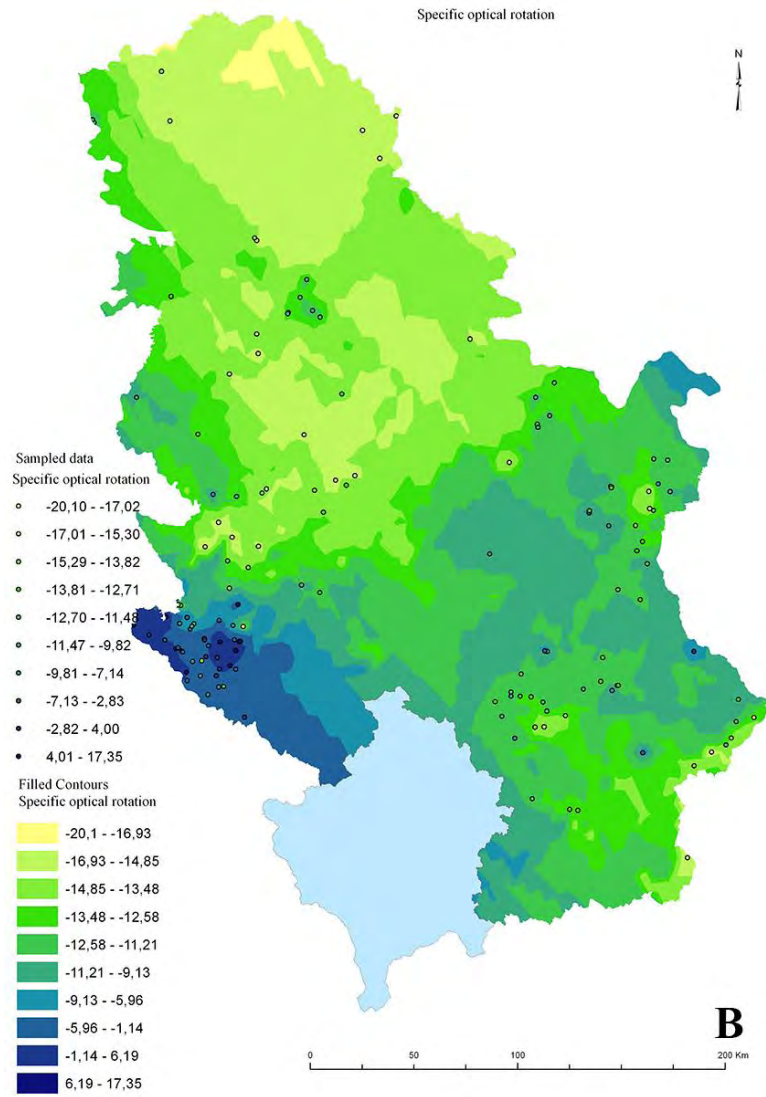A) Cd, B) Cr and C) Ni.

Specific optical rotation

Sampled data
Specific optical rotation

- ○ −20,10 − −17,02
- ○ −17,01 − −15,30
- ○ −15,29 − −13,82
- ○ −13,81 − −12,71
- ○ −12,70 − −11,48
- ○ −11,47 − −9,82
- ○ −9,81 − −7,14
- ○ −7,13 − −2,83
- · −2,82 − 4,00
- · 4,01 − 17,35

Filled Contours
Specific optical rotation

- −20,1 − −16,93
- −16,93 − −14,85
- −14,85 − −13,48
- −13,48 − −12,58
- −12,58 − −11,21
- −11,21 − −9,13
- −9,13 − −5,96
- −5,96 − −1,14
- −1,14 − 6,19
- 6,19 − 17,35

B

Free acidity

N

Sampled data
Free acidity

- ○   11,4 - 14,0
- ○   14,1 - 17,2
- ○   17,3 - 20,2
- ○   20,3 - 23,2
- ○   23,3 - 26,0
- ○   26,1 - 29,0
- ○   29,1 - 32,5
- •   32,6 - 36,0
- •   36,1 - 40,8
- •   40,9 - 51,0

Filled Contours Free acidity

- 12,3 - 17,3
- 17,3 - 21,39
- 21,39 - 24,73
- 24,73 - 27,48
- 27,48 - 29,72
- 29,72 - 32,47
- 32,47 - 35,81
- 35,81 - 39,9
- 39,9 - 44,9
- 44,9 - 51

C

0   50   100   200 Km

Electrical conductivity [S/m]

N

Sampled data
Electrical conductivity
[S/m]

- ○ 0,17 - 0,24
- ○ 0,25 - 0,30
- ○ 0,31 - 0,36
- ○ 0,37 - 0,49
- ○ 0,50 - 0,60
- ○ 0,61 - 0,69
- ● 0,70 - 0,79
- ● 0,80 - 0,94
- ● 0,95 - 1,11
- ● 1,12 - 1,39

Filled Contours
electrical conductivity [S/m]

- 0,17 - 0,21
- 0,21 - 0,24
- 0,24 - 0,28
- 0,28 - 0,33
- 0,33 - 0,41
- 0,41 - 0,51
- 0,51 - 0,64
- 0,64 - 0,82
- 0,82 - 1,06
- 1,06 - 1,39
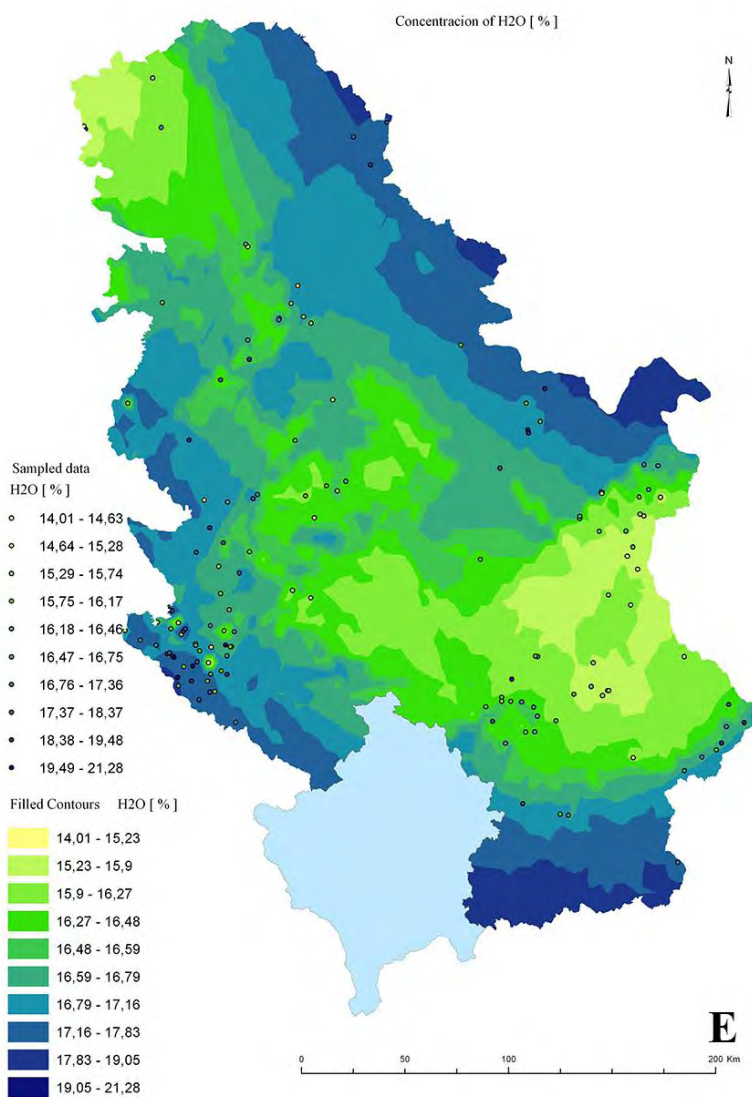
D
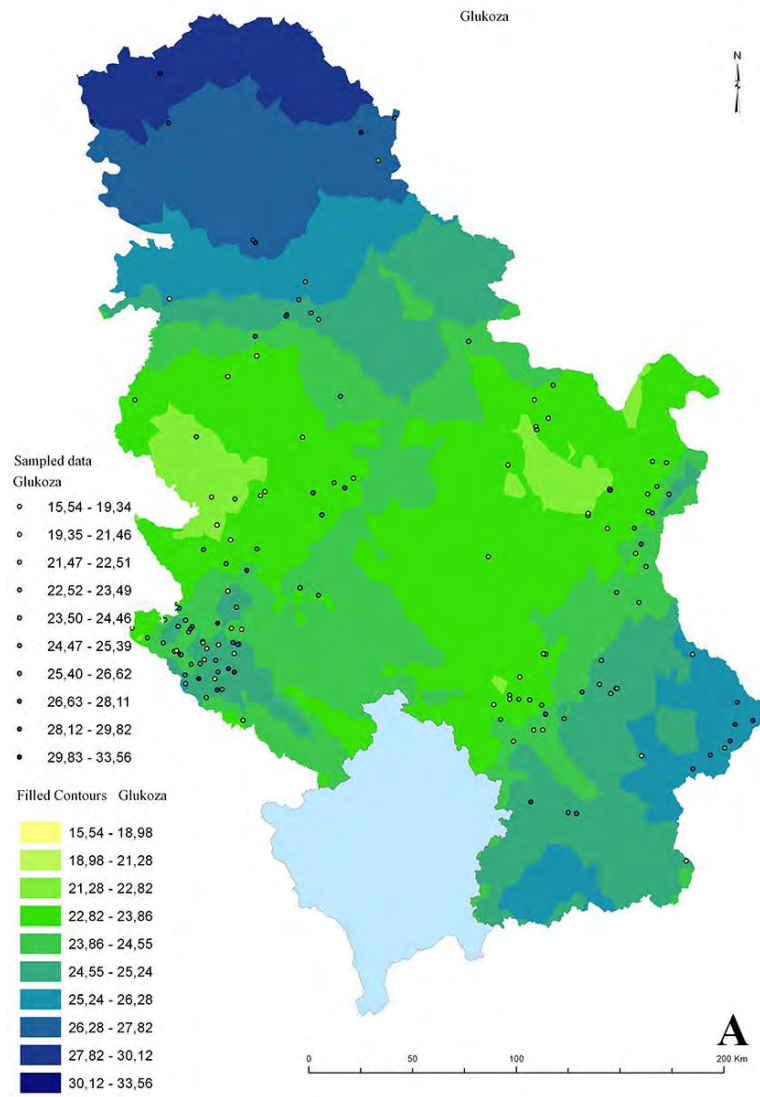
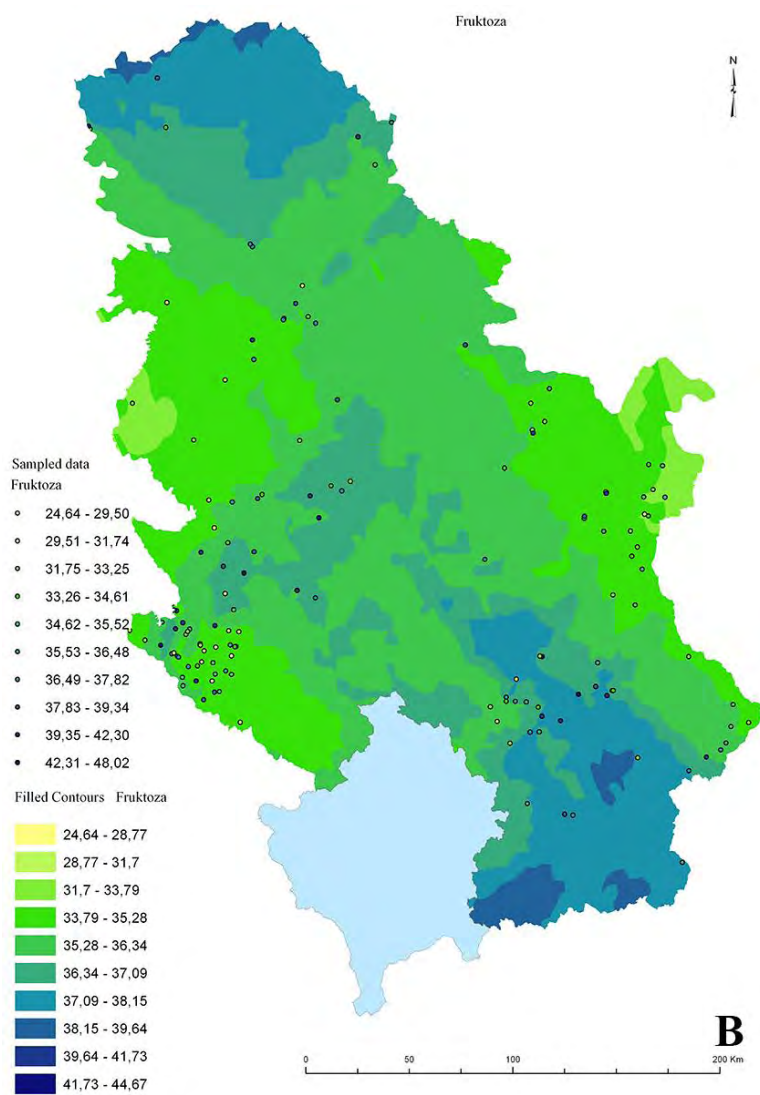0   50   100   200 Km

Fig. S4. GIS spatial distribution of multifloral honey physicochemical parameters.
A) pH, B) specific optical rotation, C) free acidity, D) electrical conductivity and E) moisture.

Fruktoza

Sampled data
Fruktoza

- ° 24,64 - 29,50
- ° 29,51 - 31,74
- ° 31,75 - 33,25
- ° 33,26 - 34,61
- ° 34,62 - 35,52
- ° 35,53 - 36,48
- ° 36,49 - 37,82
- • 37,83 - 39,34
- • 39,35 - 42,30
- • 42,31 - 48,02

Filled Contours   Fruktoza

- 24,64 - 28,77
- 28,77 - 31,7
- 31,7 - 33,79
- 33,79 - 35,28
- 35,28 - 36,34
- 36,34 - 37,09
- 37,09 - 38,15
- 38,15 - 39,64
- 39,64 - 41,73
- 41,73 - 44,67

B

0   50   100   200 Km

LAZAREVIĆ et al.

Saharoza

Sampled data
Saharoza

- ○ 0,12 - 1,30
- ○ 1,31 - 2,35
- ○ 2,36 - 3,02
- ○ 3,03 - 3,66
- ○ 3,67 - 4,54
- ○ 4,55 - 5,24
- ○ 5,25 - 6,11
- ● 6,12 - 7,53
- ● 7,54 - 9,70
- ● 9,71 - 19,50

Filled Contours Saharoza

- 0,12 - 1,69
- 1,69 - 2,68
- 2,68 - 3,3
- 3,3 - 3,69
- 3,69 - 4,32
- 4,32 - 5,31
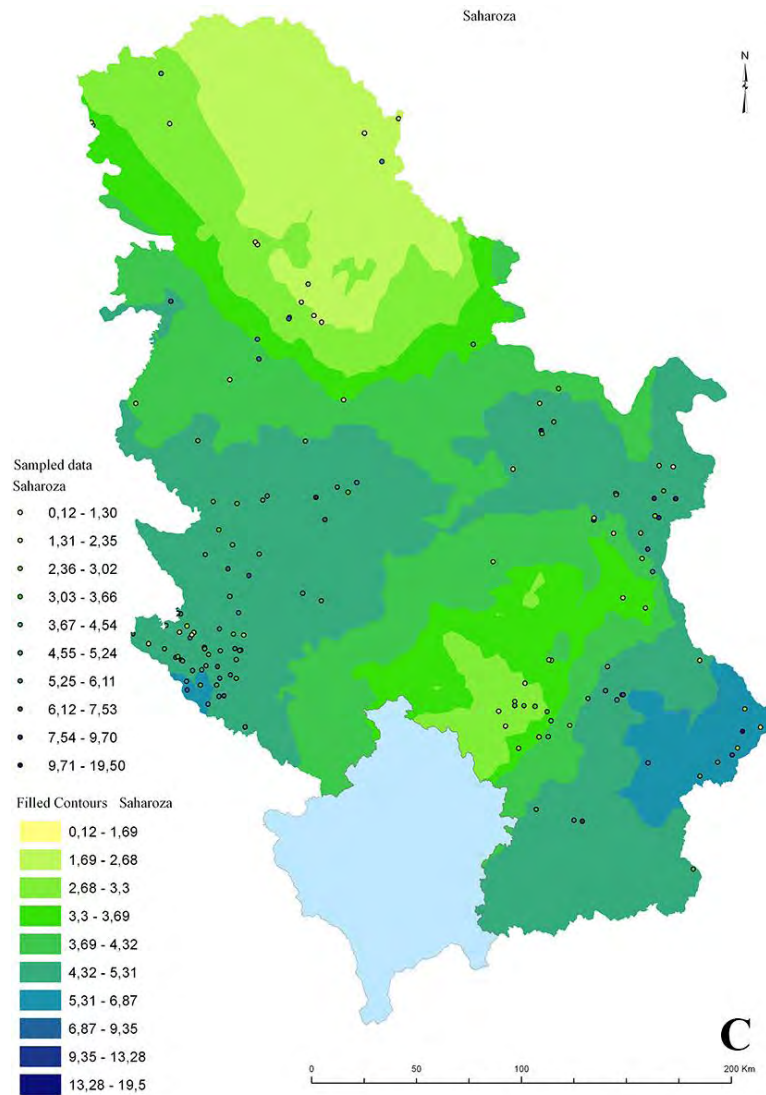- 5,31 - 6,87
- 6,87 - 9,35
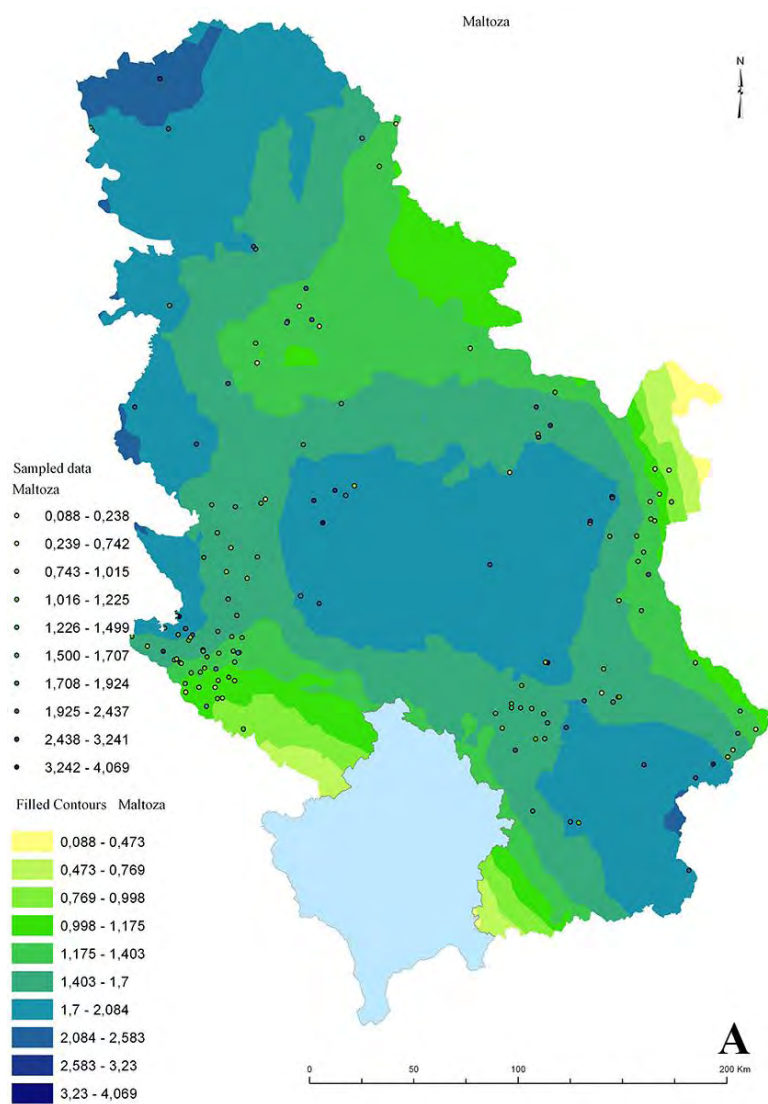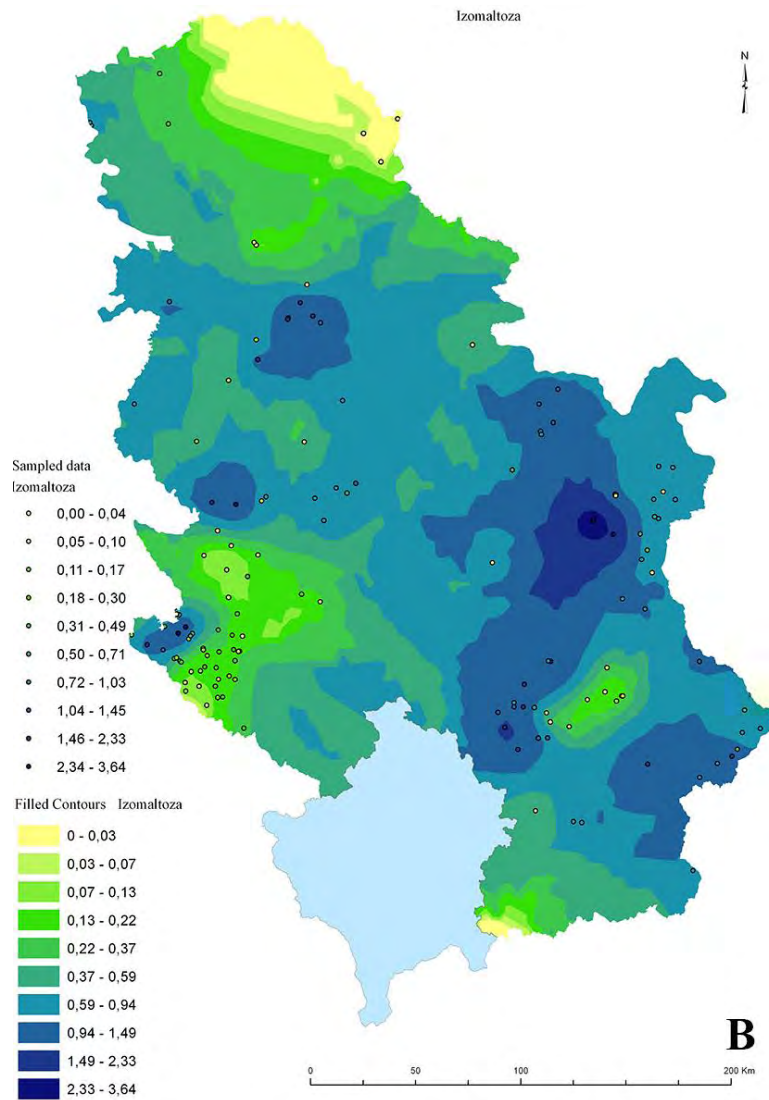- 9,35 - 13,28
- 13,28 - 19,5

C
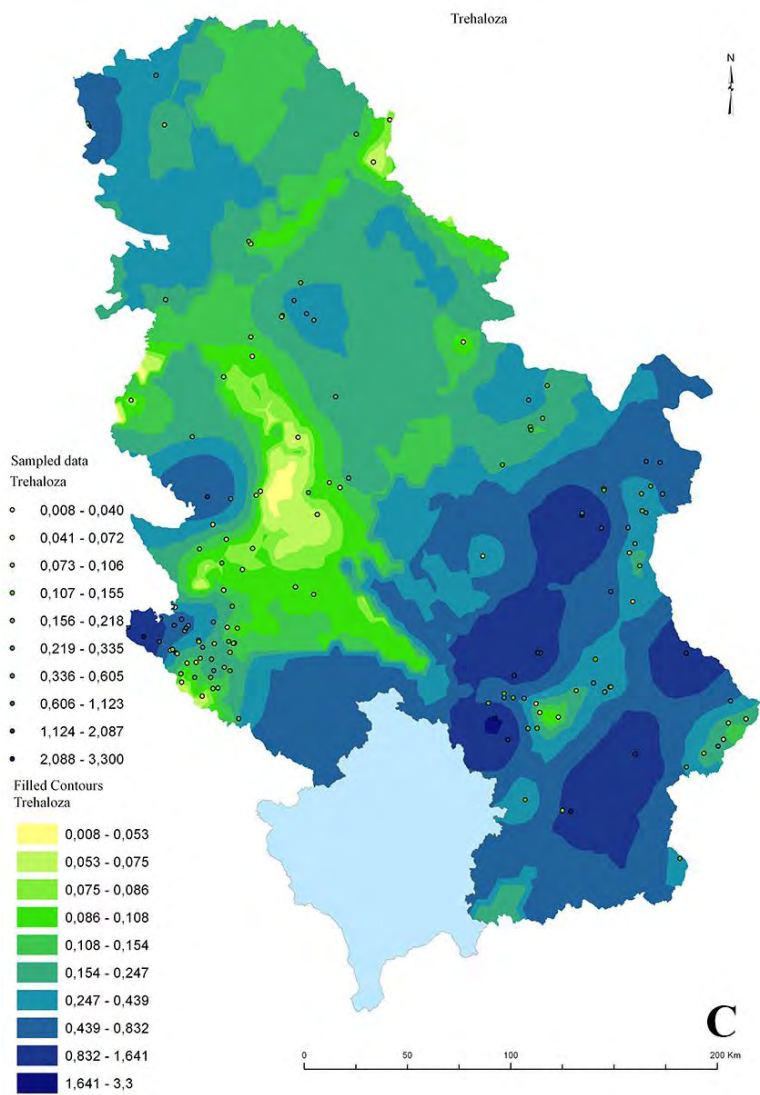
Fig. S5. GIS spatial distribution of polyfloral honey sugar content, major components:
A) glucose, B) fructose and C) sucrose.

Maltoza

Sampled data
Maltoza

○ 0,088 - 0,238
○ 0,239 - 0,742
○ 0,743 - 1,015
○ 1,016 - 1,225
○ 1,226 - 1,499
○ 1,500 - 1,707
• 1,708 - 1,924
• 1,925 - 2,437
• 2,438 - 3,241
• 3,242 - 4,069

Filled Contours Maltoza

0,088 - 0,473
0,473 - 0,769
0,769 - 0,998
0,998 - 1,175
1,175 - 1,403
1,403 - 1,7
1,7 - 2,084
2,084 - 2,583
2,583 - 3,23
3,23 - 4,069

A

Izomaltoza

Sampled data
Izomaltoza

- ◦ 0,00 - 0,04
- ◦ 0,05 - 0,10
- ◦ 0,11 - 0,17
- ◦ 0,18 - 0,30
- ◦ 0,31 - 0,49
- ◦ 0,50 - 0,71
- ◦ 0,72 - 1,03
- • 1,04 - 1,45
- • 1,46 - 2,33
- • 2,34 - 3,64

Filled Contours   Izomaltoza

- 0 - 0,03
- 0,03 - 0,07
- 0,07 - 0,13
- 0,13 - 0,22
- 0,22 - 0,37
- 0,37 - 0,59
- 0,59 - 0,94
- 0,94 - 1,49
- 1,49 - 2,33
- 2,33 - 3,64

N

B

0   50   100   200 Km

Trehaloza

N

Sampled data
Trehaloza

- ○ 0,008 - 0,040
- ○ 0,041 - 0,072
- ○ 0,073 - 0,106
- ○ 0,107 - 0,155
- ○ 0,156 - 0,218
- ○ 0,219 - 0,335
- • 0,336 - 0,605
- • 0,606 - 1,123
- • 1,124 - 2,087
- • 2,088 - 3,300

Filled Contours
Trehaloza

- 0,008 - 0,053
- 0,053 - 0,075
- 0,075 - 0,086
- 0,086 - 0,108
- 0,108 - 0,154
- 0,154 - 0,247
- 0,247 - 0,439
- 0,439 - 0,832
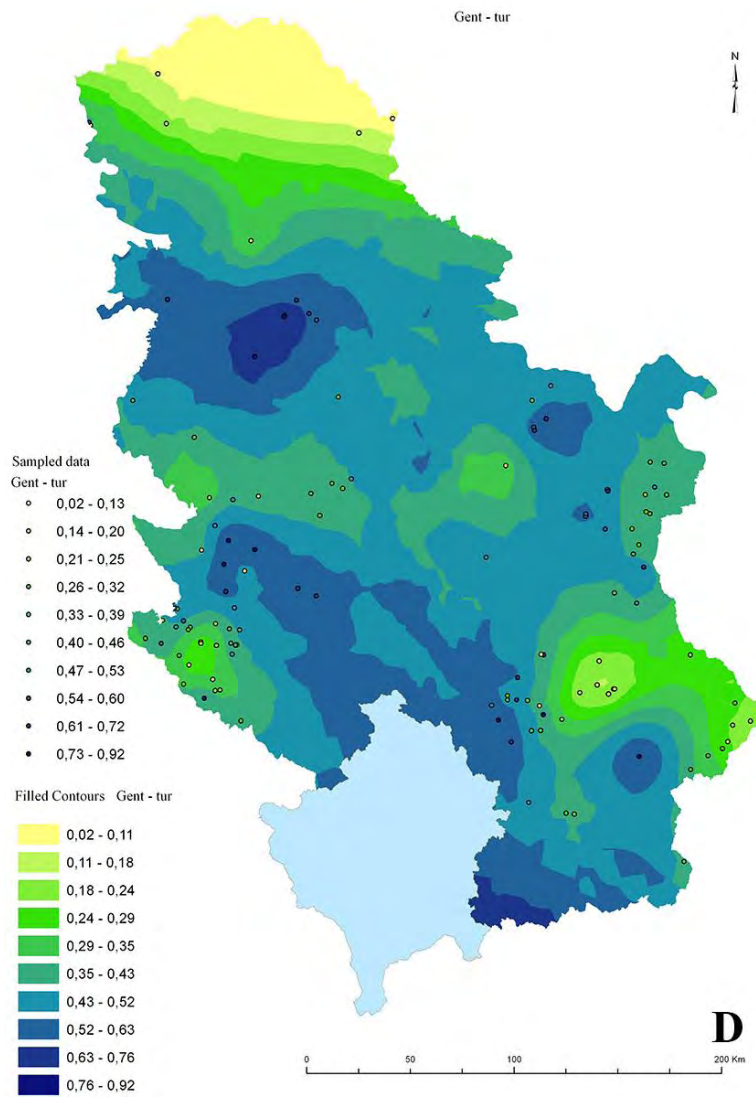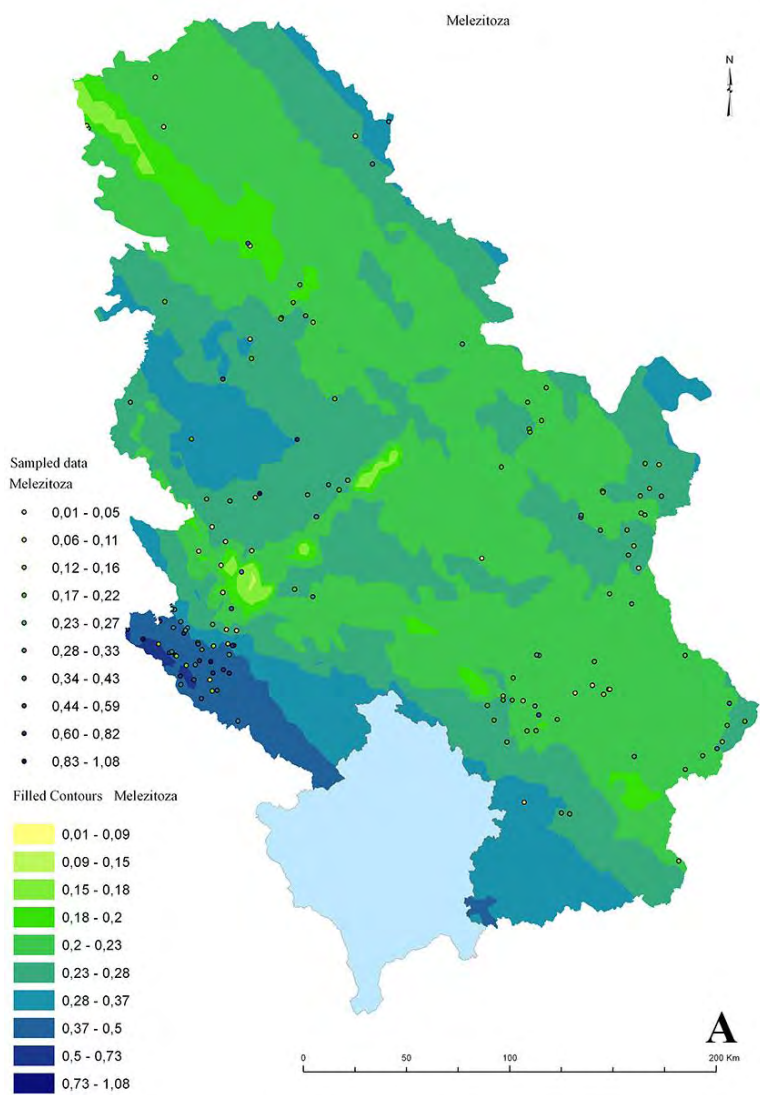- 0,832 - 1,641
- 1,641 - 3,3

C

0   50   100   200 Km

Fig. S6. GIS spatial distribution of polyfloral honey sugar content, minor components disaccharides: A) maltose, B) isomaltose, C) trehalose and D) gentiobiose + turanose).
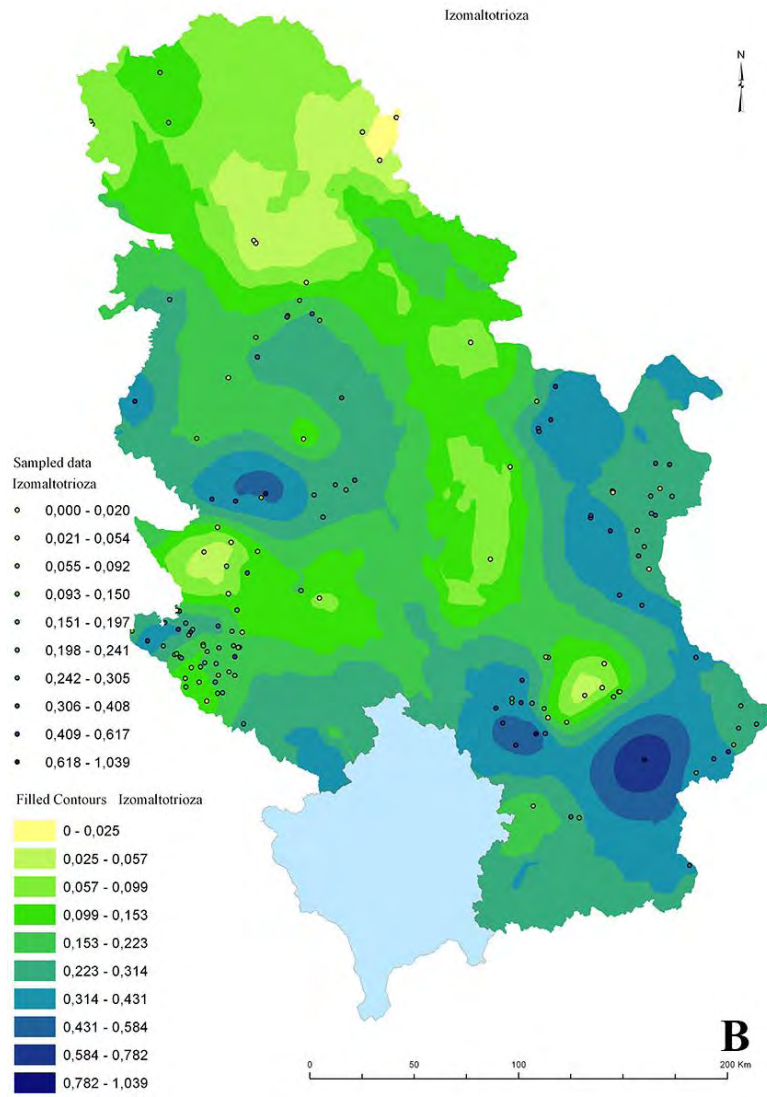
Melezitoza

Sampled data
Melezitoza

- ∘ 0,01 - 0,05
- ∘ 0,06 - 0,11
- ∘ 0,12 - 0,16
- ∘ 0,17 - 0,22
- ∘ 0,23 - 0,27
- ∘ 0,28 - 0,33
- ∘ 0,34 - 0,43
- • 0,44 - 0,59
- • 0,60 - 0,82
- • 0,83 - 1,08

Filled Contours   Melezitoza

- 0,01 - 0,09
- 0,09 - 0,15
- 0,15 - 0,18
- 0,18 - 0,2
- 0,2 - 0,23
- 0,23 - 0,28
- 0,28 - 0,37
- 0,37 - 0,5
- 0,5 - 0,73
- 0,73 - 1,08

A

0   50   100   200 Km

Fig. S7. GIS spatial distribution of polyfloral honey sugar content, minor components trisaccharides: A) melesitose and B) isomaltotriose.