Supplementary data for article:

Čukanović, J.; Tešević, V.; Jadranin, M.; Ljubojević, M.; Mladenović, E.; Kostić, S. Horse Chestnut (Aesculus Hippocastanum L.) Seed Fatty Acids, Flavonoids and Heavy Metals Plasticity to Different Urban Environments. *Biochemical Systematics and Ecology* **2020**, *89*. https://doi.org/10.1016/j.bse.2019.103980

SUPPLEMENTARY MATERIAL

HORSE CHESTNUT (*Aesculus hippocastanum* L.) SEED FATTY ACIDS, FLAVONOIDS AND HEAVY METALS PLASTICITY TO DIFFERENT URBAN ENVIRONMENTS

Supplementary Table legend:

Supplementary Table S1. Content and ratio of saturated, unsaturated and polyunsaturated fatty acids in chestnut samples (%).

Supplementary Table S2. Contents of phenolics (μg/g) in horse chestnut samples.

Supplementary Table S3. Aescin content in horse chestnut samples.

Supplementary Table S4. Principal Component Analysis (PCA) table of the Flavonoids and Aescin, showing correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis of data from 11 genotypes.

Supplementary Table S5. Seven heavy metals concentration (μ/g) in seed content of 11 genotypes.

Supplementary Table S6. Principal Component Analysis (PCA) table of the seven heavy metals, showing correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis of data from 11 genotypes.

Supplementary Figure legend:

Supplementary Figure S1. Obtained chromatograms for the flavonoids extracts of horse chesnut seed.

Supplementary Figure S2. Obtained chromatograms for the flavonoids extracts of horse chesnut seed

Supplementary Figure S3. Obtained chromatograms for the flavonoids extracts of horse chesnut seed

Supplementary Table S1. Content and ratio of saturated, unsaturated and polyunsaturated fatty acids in chestnut samples (%).

Genotype	Saturated fatty acids (SAFAs)	Unsaturated fatty acids (MUFAs)	Polyunsaturated fatty acids (PUFAs)	SAFAs / MUFAs*	PUFAs / MUFAs*
BP_2	16.60	56.40	26.40	3.39	1.59
BP_4	17.10	62.40	20.30	3.65	1.18
BP_{27}	16.70	52.10	30.80	3.12	1.84
BDS_1	28.50	63.50	1.80	2.23	0.06
BDS_2	29.20	62.60	0.70	2.14	0.02
P_1	20.60	62.10	13.70	3.01	0.66
P_4	20.70	65.10	11.90	3.14	0.57
P_5	17.50	62.30	20.10	3.56	1.14
SK_1	16.40	54.10	25.70	3.30	1.56
SK_3	16.70	56.50	26.50	3.38	1.58
SK_9	17.70	55.10	27.10	3.11	1.53
$\bar{x}_{All}$	19.01	58.44	20.64	3.16	1.19

Supplementary Table S2. Contents of phenolics (μg/g) in horse chestnut samples.

Genotype	Quercetin	Kaempferol	Rutin
BP_2	/^{a*}	0.155^{bc}	/^{e}
BP_4	0.363^{de}	0.129^{abc}	5.569^{cd}
BP_{27}	0.413^{ef}	0.119^{abc}	4.279^{b}
BDS_1	0.084^{ab}	0.060^{ab}	9.961^{g}
BDS_2	0.098^{ab}	/^{a}	1.155^{a}
P_1	0.241^{cd}	/^{a}	4.567^{b}
P_4	0.538^{f}	/^{a}	5.162^{c}
P_5	0.283^{cde}	0.429^{d}	25.784^{h}
SK_1	0.806^{g}	0.227c	5.878^{d}
SK_3	0.170^{bc}	0.019^{a}	0.982^{a}
SK_9	/^{a}	/^{a}	3.553^{f}
$\bar{x}_{\mathbf{All}}$	0.271	0.103	6.071
Cv_{All} (%)	91.61	95.66	93.15

*Means followed by different letters differ significantly, based on Duncan's test at $p<0.05$.

Legend: /-absent

Supplementary Table S3. Aescin content in horse chestnut samples.

Genotype	Seed mass (g)	Ethanol extract mass (g)	Ethanol extract in seed (%)	Aescin in ethanol extract (%)	Aescin content in seed (%)
BP_2	11.09	2.09	18.93	21.32	4.04^{c*}
BP_4	6.97	1.04	15.01	15.29	2.29^{b}
BP_{27}	7.81	1.41	18.10	9.25	1.67^{ab}
BDS_1	9.52	1.46	14.99	12.88	1.93^{ab}
BDS_2	7.52	1.37	18.22	11.97	2.18^{b}
P_1	11.70	1.51	12.94	7.16	0.93^{a}
P_4	8.11	1.91	23.55	7.85	1.85^{ab}
P_5	8.80	1.78	20.24	15.28	4.16^{c}
SK_1	6.11	144	23.70	7.60	1.80^{ab}
SK_3	21.68	0.78	3.60	13.14	1.26^{a}
SK_9	5.00	0.77	15.56	5.24	0.82^{a}
$\bar{x}_{All}$	9.48	1.41	16.80	11.54	2.07
Cv_{All} (%)	47.52	29.97	33.15	40.77	52.03

*Means followed by different letters differ significantly, based on Duncan's test at $p<0.05$.

Supplementary Table S4. Principal Component Analysis (PCA) table of the Flavonoids and Aescin, showing correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis of data from 11 genotypes.

Variable	PC1	PC2	PC3
Quercetin	-0.24	0.94*	-0.20
Kaempferol	-0.96*	0.07	-0.05
Rutin	-0.85*	0.03	0.51
Aescin	-0.79*	-0.42	-0.42
Eigenvalue	2.35	0.71	0.48
Var. (%)	58.74	26.92	12.09
Cum. (%)	58.74	85.66	100.00

41

42 Supplementary Table S5. Seven heavy metals concentration (μ/g) in seed content of 11 genotypes.

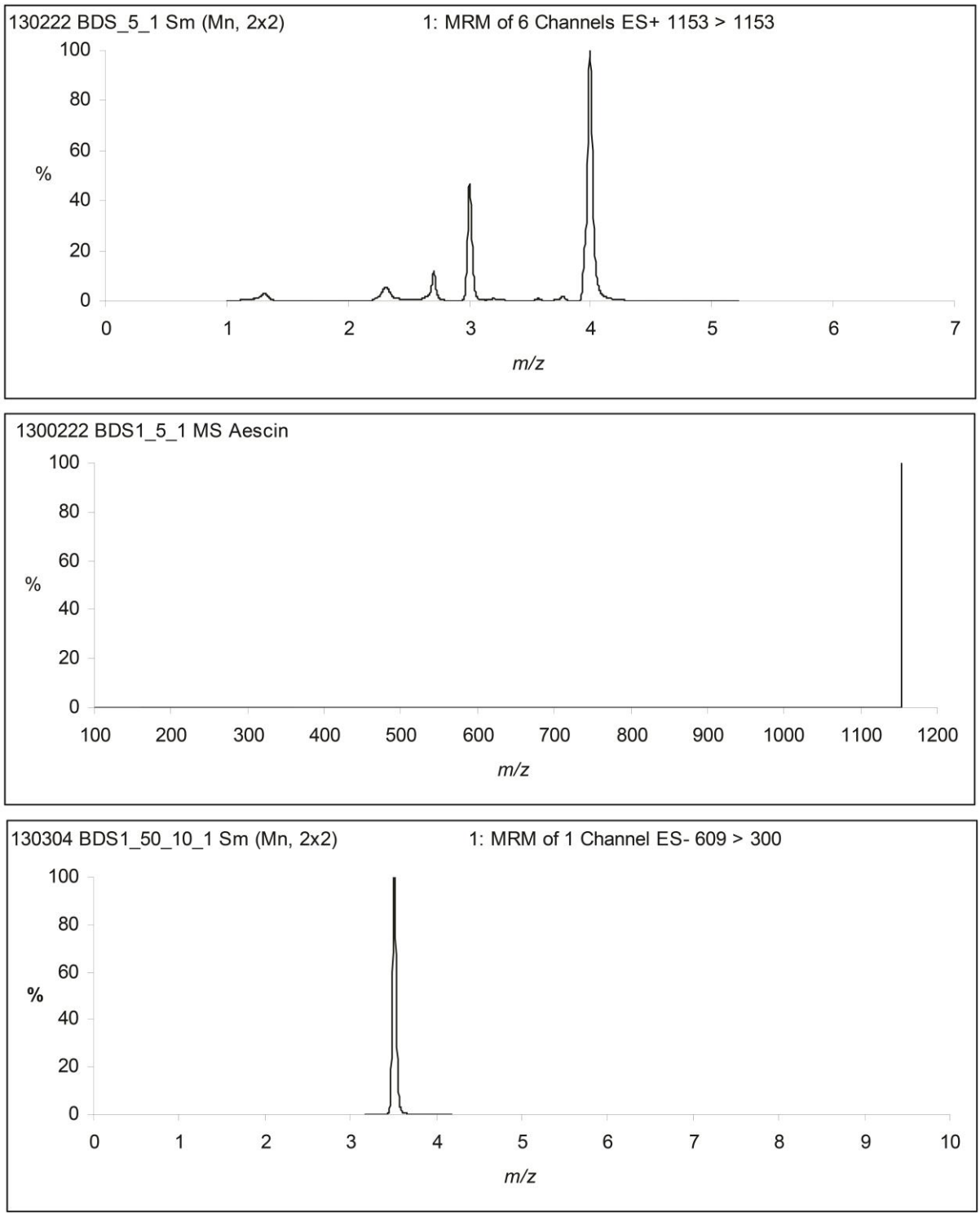
Genotype	Al	Cr	Cu	Fe	Mn	Ni	Zn	Total
BP_2	1.77^{abcd}*	0.05^{a}	8.60^{abc}	7.60^{ab}	1.80^{a}	0.50^{a}	9.00^{abcd}	29.32
BP_4	0.59^{ab}	0.05^{a}	6.60^{cd}	6.50_{a}	2.30^{a}	0.50^{a}	7.40^{a}	23.94
BP_{27}	0.50^{a}	0.05^{a}	10.0b	7.80^{abc}	3.10^{a}	0.50^{a}	8.50^{abc}	30.45
BDS_1	0.70^{ab}	0.05^{a}	7.00^{cde}	6.90^{ab}	2.20^{a}	0.50^{a}	9.80^{bcd}	27.15
BDS_2	1.40^{abc}	0.05^{a}	5.50^{c}	6.20^{a}	2.00^{a}	0.50^{a}	8.00^{ab}	23.65
P_1	1.02^{ab}	0.20^{b}	13.00^{f}	13.20^{f}	3.20^{a}	0.80^{bc}	18.10^{f}	49.52
P_4	2.54^{cd}	0.10^{a}	8.00^{ade}	7.20^{ab}	2.50^{a}	0.50^{a}	7.90^{a}	28.74
P_5	2.88^{d}	0.10^{a}	11.70^{f}	9.50^{cd}	2.50^{a}	0.60^{ab}	10.80^{d}	38.08
SK_1	0.50^{a}	0.05^{a}	9.80^{ab}	8.40^{bc}	2.10^{a}	0.60^{ab}	10.0^{cd}	31.45
SK_3	0.50^{a}	0.05^{a}	9.00^{ab}	11.30^{e}	1.80^{a}	0.60^{ab}	8.10^{ab}	31.35
SK_9	1.9^{bcd}	0.10^{a}	12.4^{f}	10.40^{de}	3.10^{a}	0.85^{c}	13.60^{e}	42.35
$\bar{x}_{All}$	1.30	0.07	9.23	8.63	2.33	0.59	10.11	32.26
Cv_{All} (%)	78.04	73.01	27.00	26.65	40.2	26.73	31.55	

43 *Means followed by the same letters within a variable are not significantly different (p<0.05).

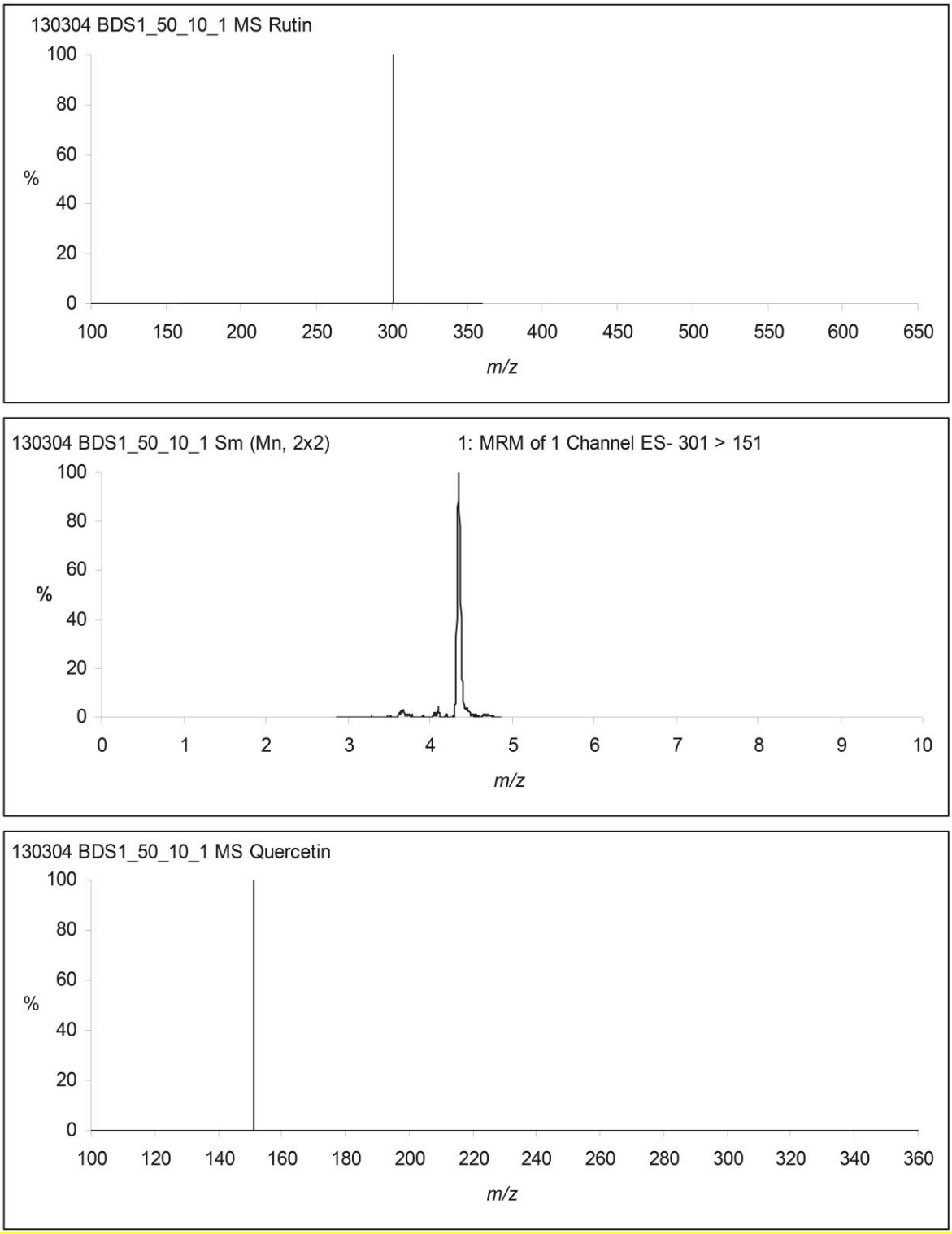
Supplementary Table S6. Principal Component Analysis (PCA) table of the seven heavy metals, showing correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis of data from 11 genotypes.

Variable	PC1	PC2	PC3
Al	-0.21	-0.96*	0.15
Cr	-0.89*	-0.18	-0.05
Cu	-0.91*	-0.02	0.06
Fe	-0.87*	0.23	0.35
Mn	-0.74*	-0.04	-0.64
Ni	-0.91*	0.12	0.14
Zn	-0.94*	0.09	/
Eigenvalue	4.68	1.04	0.59
Var. (%)	66.96	14.96	8.50
Cum. (%)	66.96	81.92	90.43

Supplementary Figure S1. Obtained chromatograms for the flavonoids extracts of horse chesnut seed



Supplementary Figure S2. Obtained chromatograms for the flavonoids extracts of horse chesnut seed



Supplementary Figure S3. Obtained chromatograms for the flavonoids extracts of horse chesnut seed