

Table S1. Metal content in leaves and roots of male (M) and female (F) *Salix × fragilis* specimens cultivated hydroponically in a sand-stabilized system with the addition of zinc (Zn) or copper (Cu) and under simulated drought conditions (d) subjected to isolated and combined treatments (c – control; data presented as mean  $\pm$  standard deviation, n=3; *p* – empirical level of significance for "sex  $\times$  treatment" fixed effect, identical superscripts denote no significant differences between means according to a post-hoc Tukey's HSD test following two-way ANOVA at  $\alpha=95\%$ )

Sex	Treatment	Cu [mg kg <sup>-1</sup> DW]	Zn [mg kg <sup>-1</sup> DW]	Ca [g kg <sup>-1</sup> DW]	Mg [g kg <sup>-1</sup> DW]	K [g kg <sup>-1</sup> DW]	Na [mg kg <sup>-1</sup> DW]
Roots							
M	c	10.9 <sup>d</sup> $\pm$ 2.43	44.4 <sup>de</sup> $\pm$ 2.76	3.26 <sup>cd</sup> $\pm$ 0.08	1.20 <sup>b</sup> $\pm$ 0.22	3.76 <sup>bcd</sup> $\pm$ 0.48	329 <sup>c</sup> $\pm$ 94.2
F		29.8 <sup>d</sup> $\pm$ 1.94	109 <sup>cde</sup> $\pm$ 15.3	6.71 <sup>a</sup> $\pm$ 1.06	1.89 <sup>a</sup> $\pm$ 0.49	3.09 <sup>bcd</sup> $\pm$ 1.07	700 <sup>a</sup> $\pm$ 141
M	Zn	30.7 <sup>d</sup> $\pm$ 7.34	769 <sup>a</sup> $\pm$ 163.7	4.82 <sup>b</sup> $\pm$ 1.56	0.94 <sup>bc</sup> $\pm$ 0.25	4.36 <sup>b</sup> $\pm$ 0.84	290 <sup>cd</sup> $\pm$ 45.6
F		40.4 <sup>d</sup> $\pm$ 6.15	459 <sup>b</sup> $\pm$ 85.9	2.73 <sup>d</sup> $\pm$ 0.31	1.32 <sup>b</sup> $\pm$ 0.17	2.59 <sup>cde</sup> $\pm$ 0.27	461 <sup>b</sup> $\pm$ 75.7
M	Cu	155 <sup>c</sup> $\pm$ 51.1	59.9 <sup>de</sup> $\pm$ 8.62	4.08 <sup>bc</sup> $\pm$ 0.31	0.78 <sup>bcd</sup> $\pm$ 0.02	2.99 <sup>bcd</sup> $\pm$ 1.34	462 <sup>b</sup> $\pm$ 64.5
F		135 <sup>c</sup> $\pm$ 17.6	62.4 <sup>de</sup> $\pm$ 0.91	6.80 <sup>a</sup> $\pm$ 0.89	2.16 <sup>a</sup> $\pm$ 0.73	6.04 <sup>a</sup> $\pm$ 1.95	663 <sup>a</sup> $\pm$ 56.3
M	d	14.9 <sup>d</sup> $\pm$ 1.70	44.4 <sup>de</sup> $\pm$ 1.45	4.16 <sup>bc</sup> $\pm$ 0.01	1.29 <sup>b</sup> $\pm$ 0.41	3.93 <sup>bc</sup> $\pm$ 0.32	156 <sup>ef</sup> $\pm$ 61.6
F		14.7 <sup>d</sup> $\pm$ 1.98	45.7 <sup>de</sup> $\pm$ 9.01	3.17 <sup>cd</sup> $\pm$ 1.17	1.28 <sup>b</sup> $\pm$ 0.38	2.92 <sup>bcd</sup> $\pm$ 1.22	105 <sup>ef</sup> $\pm$ 54.8
M	Zn+d	5.89 <sup>d</sup> $\pm$ 3.07	176 <sup>c</sup> $\pm$ 30.0	0.84 <sup>e</sup> $\pm$ 0.14	0.31 <sup>d</sup> $\pm$ 0.01	2.16 <sup>de</sup> $\pm$ 0.26	62 <sup>f</sup> $\pm$ 10.4
F		30.2 <sup>d</sup> $\pm$ 1.93	131 <sup>cd</sup> $\pm$ 11.9	2.16 <sup>d</sup> $\pm$ 0.70	0.37 <sup>cd</sup> $\pm$ 0.29	1.05 <sup>e</sup> $\pm$ 0.16	204 <sup>de</sup> $\pm$ 70.0
M	Cu+d	231 <sup>b</sup> $\pm$ 28.5	51.8 <sup>de</sup> $\pm$ 11.1	2.95 <sup>cd</sup> $\pm$ 0.29	0.93 <sup>bc</sup> $\pm$ 0.20	4.12 <sup>bc</sup> $\pm$ 0.60	84.7 <sup>f</sup> $\pm$ 22.3
F		478 <sup>a</sup> $\pm$ 84.5	34.9 <sup>e</sup> $\pm$ 19.1	4.17 <sup>bc</sup> $\pm$ 0.76	0.39 <sup>cd</sup> $\pm$ 0.03	2.57 <sup>cde</sup> $\pm$ 1.04	178 <sup>def</sup> $\pm$ 32.1
<i>p</i> value		0.0000	0.0001	0.0000	0.0012	0.0020	0.0009
Rod							
M	c	10.2 <sup>d</sup> $\pm$ 2.54	44.0 <sup>cd</sup> $\pm$ 13.8	4.81 <sup>cd</sup> $\pm$ 0.49	0.44 <sup>de</sup> $\pm$ 0.08	1.59 <sup>abcd</sup> $\pm$ 0.35	181 <sup>a</sup> $\pm$ 36.1
F		12.2 <sup>cd</sup> $\pm$ 1.49	56.6 <sup>bcd</sup> $\pm$ 9.9	6.97 <sup>b</sup> $\pm$ 0.51	0.48 <sup>cde</sup> $\pm$ 0.08	1.94 <sup>ab</sup> $\pm$ 0.25	17.5 <sup>fg</sup> $\pm$ 2.17
M	Zn	26.2 <sup>ab</sup> $\pm$ 8.76	75.1 <sup>ab</sup> $\pm$ 18.4	2.73 <sup>f</sup> $\pm$ 0.46	0.39 <sup>e</sup> $\pm$ 0.05	1.35 <sup>cde</sup> $\pm$ 0.56	6.38 <sup>g</sup> $\pm$ 2.82
F		12.9 <sup>cd</sup> $\pm$ 3.12	51.6 <sup>cd</sup> $\pm$ 13.2	7.24 <sup>ab</sup> $\pm$ 0.77	0.35 <sup>e</sup> $\pm$ 0.05	1.36 <sup>bcd</sup> $\pm$ 0.11	64.9 <sup>cd</sup> $\pm$ 5.56
M	Cu	19.9 <sup>bc</sup> $\pm$ 0.98	79.6 <sup>a</sup> $\pm$ 6.16	4.61 <sup>cde</sup> $\pm$ 0.08	0.89 <sup>a</sup> $\pm$ 0.05	1.90 <sup>abc</sup> $\pm$ 0.04	49.1 <sup>cde</sup> $\pm$ 2.80

F		20.0 <sup>bc</sup> ± 3.20	36.8 <sup>d</sup> ± 4.24	7.37 <sup>ab</sup> ± 1.21	0.60 <sup>cd</sup> ± 0.03	1.71 <sup>abc</sup> ± 0.11	67.9 <sup>c</sup> ± 19.3
M	d	16.8 <sup>cd</sup> ± 2.00	62.6 <sup>abc</sup> ± 6.95	4.08 <sup>de</sup> ± 0.23	0.79 <sup>ab</sup> ± 0.15	2.03 <sup>a</sup> ± 0.84	33.2 <sup>ef</sup> ± 8.00
F		19.0 <sup>bc</sup> ± 2.33	63.6 <sup>abc</sup> ± 15.3	8.38 <sup>a</sup> ± 1.46	0.45 <sup>de</sup> ± 0.19	1.06 <sup>ed</sup> ± 0.09	55.0 <sup>cde</sup> ± 14.2
M	Zn+d	13.5 <sup>cd</sup> ± 2.30	48.2 <sup>cd</sup> ± 15.0	3.49 <sup>ef</sup> ± 0.27	0.60 <sup>cd</sup> ± 0.02	1.62 <sup>abcd</sup> ± 0.20	43.2 <sup>de</sup> ± 5.45
F		32.7 <sup>a</sup> ± 9.75	76.5 <sup>ab</sup> ± 19.2	5.54 <sup>c</sup> ± 0.62	0.33 <sup>e</sup> ± 0.16	0.88 <sup>e</sup> ± 0.39	118 <sup>b</sup> ± 16.6
M	Cu+d	25.6 <sup>ab</sup> ± 6.05	63.4 <sup>abc</sup> ± 0.85	3.47 <sup>ef</sup> ± 0.09	0.65 <sup>bc</sup> ± 0.01	1.58 <sup>abcd</sup> ± 0.06	15.3 <sup>fg</sup> ± 3.31
F		31.7 <sup>a</sup> ± 5.29	42.4 <sup>cd</sup> ± 16.8	6.82 <sup>b</sup> ± 1.01	0.43 <sup>e</sup> ± 0.12	0.86 <sup>e</sup> ± 0.10	38.80 <sup>ef</sup> ± 7.18
<i>p</i> value		0.0004	0.0008	0.0264	0.0204	0.0218	0.0000

Leaves							
M	c	9.82 <sup>ef</sup> ± 1.23	28.1 <sup>f</sup> ± 19.4	2.62 <sup>fg</sup> ± 1.02	1.11 <sup>e</sup> ± 0.59	5.52 <sup>e</sup> ± 2.06	107 <sup>cd</sup> ± 17.6
F		8.34 <sup>ef</sup> ± 1.47	92.7 <sup>bcd</sup> ± 16.4	6.04 <sup>d</sup> ± 3.11	4.65 <sup>bc</sup> ± 1.12	19.0 <sup>c</sup> ± 1.81	32.5 <sup>fg</sup> ± 3.95
M	Zn	17.8 <sup>de</sup> ± 3.40	113 <sup>bcd</sup> ± 27.3	1.92 <sup>fg</sup> ± 0.37	0.74 <sup>ef</sup> ± 0.13	4.53 <sup>e</sup> ± 1.18	8.59 <sup>g</sup> ± 1.43
F		5.31 <sup>f</sup> ± 3.26	231 <sup>a</sup> ± 49.7	0.52 <sup>g</sup> ± 0.01	0.18 <sup>f</sup> ± 0.04	0.55 <sup>f</sup> ± 0.07	95.6 <sup>de</sup> ± 10.6
M	Cu	33.7 <sup>bc</sup> ± 3.55	90.1 <sup>cd</sup> ± 18.9	10.8 <sup>bc</sup> ± 1.12	5.44 <sup>ab</sup> ± 0.46	23.2 <sup>b</sup> ± 2.27	220 <sup>a</sup> ± 53.0
F		16.2 <sup>e</sup> ± 1.42	84.3 <sup>de</sup> ± 8.36	12.3 <sup>b</sup> ± 0.99	4.13 <sup>c</sup> ± 1.04	27.1 <sup>a</sup> ± 1.34	49.3 <sup>fg</sup> ± 10.7
M	d	43.3 <sup>b</sup> ± 6.90	133 <sup>b</sup> ± 31.0	15.1 <sup>a</sup> ± 1.09	6.14 <sup>a</sup> ± 0.16	26.2 <sup>ab</sup> ± 1.83	174 <sup>b</sup> ± 36.0
F		34.4 <sup>bc</sup> ± 8.75	106 <sup>bcd</sup> ± 19.7	3.60 <sup>ef</sup> ± 2.79	0.20 <sup>f</sup> ± 0.07	7.99 <sup>e</sup> ± 1.85	73.2 <sup>def</sup> ± 10.2
M	Zn+d	3.32 <sup>f</sup> ± 1.77	102 <sup>bcd</sup> ± 15.2	9.09 <sup>c</sup> ± 0.00	5.59 <sup>a</sup> ± 0.36	17.5 <sup>c</sup> ± 1.61	147 <sup>bc</sup> ± 4.30
F		27.8 <sup>cd</sup> ± 4.99	128 <sup>bc</sup> ± 24.4	0.72 <sup>g</sup> ± 0.11	0.07 <sup>f</sup> ± 0.04	0.59 <sup>f</sup> ± 0.22	168 <sup>b</sup> ± 43.6
M	Cu+d	85.2 <sup>a</sup> ± 8.95	91.4 <sup>bcd</sup> ± 13.7	5.92 <sup>de</sup> ± 0.38	2.89 <sup>d</sup> ± 0.32	13.2 <sup>d</sup> ± 5.33	147 <sup>bc</sup> ± 15.9
F		85.3 <sup>a</sup> ± 14.5	42.1 <sup>ef</sup> ± 32.8	5.46 <sup>de</sup> ± 1.05	0.56 <sup>ef</sup> ± 0.21	6.35 <sup>e</sup> ± 0.71	65.3 <sup>ef</sup> ± 9.06
<i>p</i> value		0.0001	0.0000	0.0000	0.0000	0.0000	0.0000

Table S2. Parameters of metal uptake by *Salix × fragilis* male (M) and female (F) specimens cultivated hydroponically in a sand-stabilized system with the addition of zinc (Zn) or copper (Cu) and under simulated drought conditions (d) subjected to isolated and combined treatments (c – control; data presented as mean  $\pm$  standard deviation, n=3; "-" – not calculated for variants without addition of particular metal; *p* – empirical level of significance for i) "sex  $\times$  treatment" fixed effect (identical superscript lettering indicates non-significant differences between mean values according to a post-hoc Tukey's HSD test following a two-factor ANOVA analysis significant at  $\alpha=0.05$ , ii) for non-significant ANOVAs a Dunnett's test was performed to compare the results with control for each sex separately and *p* was presented as \*\*\* (<0.001), \*\* (<0.01) or \* (<0.05))

Sex	Treatment	Cu	Zn	Ca	Mg	K	Na
Bioconcentration factor (BCF <sub>roots</sub> )							
M	c	-	-	19.2 <sup>cd</sup> $\pm$ 0.5	49.2 <sup>b</sup> $\pm$ 9.1	22.2 <sup>bcd</sup> $\pm$ 2.8	-
F		-	-	39.6 <sup>a</sup> $\pm$ 6.2	77.6 <sup>a</sup> $\pm$ 20.3	18.2 <sup>bcd</sup> $\pm$ 6.3	-
M	Zn	-	7.8 <sup>a</sup> $\pm$ 1.7	28.4 <sup>b</sup> $\pm$ 9.2	38.6 <sup>bc</sup> $\pm$ 10.1	25.7 <sup>b</sup> $\pm$ 5.0	-
F		-	4.7 <sup>b</sup> $\pm$ 0.9	16.1 <sup>d</sup> $\pm$ 1.8	54.4 <sup>b</sup> $\pm$ 6.9	15.3 <sup>cde</sup> $\pm$ 1.6	-
M	Cu	1.6 <sup>b</sup> $\pm$ 0.5	-	24.1 <sup>bc</sup> $\pm$ 1.8	32.3 <sup>bcd</sup> $\pm$ 0.8	17.7 <sup>bcd</sup> $\pm$ 7.9	-
F		1.4 <sup>b</sup> $\pm$ 0.2	-	40.1 <sup>a</sup> $\pm$ 5.3	88.9 <sup>a</sup> $\pm$ 29.9	35.6 <sup>a</sup> $\pm$ 11.5	-
M	d	-	-	24.6 <sup>bc</sup> $\pm$ 0.04	53.0 <sup>b</sup> $\pm$ 16.8	23.2 <sup>bc</sup> $\pm$ 1.9	-
F		-	-	18.7 <sup>cd</sup> $\pm$ 6.9	52.8 <sup>b</sup> $\pm$ 15.8	17.2 <sup>bcd</sup> $\pm$ 7.2	-
M	Zn+d	-	1.8 <sup>c</sup> $\pm$ 0.3	5.00 <sup>e</sup> $\pm$ 0.8	12.9 <sup>d</sup> $\pm$ 0.4	12.7 <sup>de</sup> $\pm$ 1.6	-
F		-	1.3 <sup>c</sup> $\pm$ 0.1	12.7 <sup>d</sup> $\pm$ 4.2	15.4 <sup>cd</sup> $\pm$ 11.9	6.2 <sup>e</sup> $\pm$ 0.9	-
M	Cu+d	2.4 <sup>b</sup> $\pm$ 0.3	-	17.4 <sup>cd</sup> $\pm$ 1.7	38.3 <sup>bc</sup> $\pm$ 8.4	24.3 <sup>bc</sup> $\pm$ 3.5	-
F		5.0 <sup>a</sup> $\pm$ 0.9	-	24.6 <sup>bc</sup> $\pm$ 4.5	16.2 <sup>cd</sup> $\pm$ 1.2	15.1 <sup>cde</sup> $\pm$ 6.2	-
<i>p</i> value		0.0022	0.0402	0.0000	0.0012	0.0020	-
Bioaccumulation factor (BAF <sub>rod</sub> )							
M	c	-	-	28.4 <sup>cd</sup> $\pm$ 2.9	18.1 <sup>de</sup> $\pm$ 3.3	9.4 <sup>abcd</sup> $\pm$ 2.1	-
F		-	-	41.1 <sup>b</sup> $\pm$ 3.0	19.7 <sup>cde</sup> $\pm$ 3.2	11.4 <sup>ab</sup> $\pm$ 1.5	-
M	Zn	-	0.77 $\pm$ 0.19	16.1 <sup>f</sup> $\pm$ 2.7	16.2 <sup>e</sup> $\pm$ 1.9	8.0 <sup>cde</sup> $\pm$ 3.3	-

F		-	0.53 ± 0.14	42.7 <sup>ab</sup> ± 4.5	14.3 <sup>e</sup> ± 2.0	8.0 <sup>bcd</sup> ± 0.7	-
M	Cu	0.21 ± 0.01	-	27.2 <sup>cde</sup> ± 0.5	36.5 <sup>a</sup> ± 2.0	11.2 <sup>abc</sup> ± 0.25	-
F		0.21 ± 0.03	-	43.5 <sup>ab</sup> ± 7.1	24.9 <sup>cd</sup> ± 1.4	10.1 <sup>abc</sup> ± 0.7	-
M	d	-	-	24.1 <sup>de</sup> ± 1.4	32.5 <sup>ab</sup> ± 6.1	12.0 <sup>a</sup> ± 5.0	-
F		-	-	49.4 <sup>a</sup> ± 8.6	18.6 <sup>de</sup> ± 7.8	6.2 <sup>de</sup> ± 0.6	-
M	Zn+d	-	0.49 ± 0.15	20.6 <sup>ef</sup> ± 1.6	24.7 <sup>cd</sup> ± 0.8	9.6 <sup>abcd</sup> ± 1.2	-
F		-	0.78 ± 0.20	32.7 <sup>c</sup> ± 3.7	13.7 <sup>e</sup> ± 6.7	5.2 <sup>e</sup> ± 2.3	-
M	Cu+d	0.27 ± 0.06	-	20.4 <sup>ef</sup> ± 0.5	26.6 <sup>bc</sup> ± 0.3	9.29 <sup>abcd</sup> ± 0.4	-
F		0.33 ± 0.06	-	40.3 <sup>b</sup> ± 6.0	17.6 <sup>e</sup> ± 5.1	5.09 <sup>e</sup> ± 0.6	-
<i>p</i> value		>0.05	>0.05	0.0264	0.0204	0.0219	-
Translocation factor (TF)							
M	c	0.92 ± 0.16	0.63 ± 0.42	0.81 <sup>efg</sup> ± 0.32	1.01 <sup>fg</sup> ± 0.68	1.5 <sup>def</sup> ± 0.59	0.34 <sup>de</sup> ± 0.05
F		0.28 ± 0.05	0.87 ± 0.26	0.96 <sup>defg</sup> ± 0.64	2.6 <sup>de</sup> ± 0.80	6.6 <sup>ab</sup> ± 1.8	0.05 <sup>e</sup> ± 0.01
M	Zn	0.61 ± 0.23	0.16 ± 0.07	0.44 <sup>fg</sup> ± 0.18	0.84 <sup>fg</sup> ± 0.34	1.1 <sup>def</sup> ± 0.49	0.03 <sup>e</sup> ± 0.00
F		0.13 ± 0.08	0.53 ± 0.20	0.19 <sup>g</sup> ± 0.02	0.14 <sup>g</sup> ± 0.05	0.21 <sup>f</sup> ± 0.01	0.21 <sup>e</sup> ± 0.05
M	Cu	0.23 ± 0.06	1.5 ± 0.10	2.7 <sup>bc</sup> ± 0.48	6.9 <sup>b</sup> ± 0.41	8.7 <sup>a</sup> ± 3.5	0.49 <sup>de</sup> ± 0.19
F		0.12 ± 0.03	1.4 ± 0.13	1.8 <sup>cde</sup> ± 0.37	2.1 <sup>d</sup> ± 1.0	4.8 <sup>bc</sup> ± 1.5	0.08 <sup>e</sup> ± 0.02
M	d	3.0*** ± 0.81	3.0*** ± 0.80	3.6 <sup>b</sup> ± 0.26	5.1 <sup>c</sup> ± 1.6	6.7 <sup>ab</sup> ± 0.07	1.3 <sup>bc</sup> ± 0.81
F		2.4*** ± 0.87	2.4** ± 0.61	1.0 <sup>defg</sup> ± 0.50	0.16 <sup>g</sup> ± 0.03	3.0 <sup>cd</sup> ± 0.74	0.88 <sup>cd</sup> ± 0.57
M	Zn+d	0.56 ± 0.01	0.58 ± 0.01	11.0 <sup>a</sup> ± 1.85	17.8 <sup>a</sup> ± 1.6	8.1 <sup>a</sup> ± 0.25	2.4 <sup>a</sup> ± 0.34
F		0.92 ± 0.11	0.97 ± 0.11	0.35 <sup>fg</sup> ± 0.06	0.25 <sup>g</sup> ± 0.21	0.58 <sup>ef</sup> ± 0.25	0.84 <sup>cd</sup> ± 0.10
M	Cu+d	0.38 ± 0.09	1.78* ± 0.12	2.0 <sup>cd</sup> ± 0.07	3.3 <sup>d</sup> ± 1.1	3.1 <sup>cd</sup> ± 0.85	1.9 <sup>ab</sup> ± 0.70
F		0.18 ± 0.04	1.31 ± 0.66	1.4 <sup>def</sup> ± 0.50	1.4 <sup>efg</sup> ± 0.54	2.8 <sup>cde</sup> ± 1.1	0.37 <sup>de</sup> ± 0.02
<i>p</i> value		>0.05	>0.05	0.0000	0.0000	0.0000	0.0018

Table S3. Profiling of phenolic compounds and low-molecular-weight organic acids [ $\mu\text{g g}^{-1}$  FW] in leaves and roots of male (M) and female (F) *Salix × fragilis* specimens cultivated hydroponically in a sand-stabilized system with the addition of zinc (Zn) or copper (Cu) and under simulated drought conditions subjected to isolated and combined treatments

Compound(s)	Control (c)	Zn	Cu	Drought (d)	Zn+d	Cu+d
	M/F	M/F	M/F	M/F	M/F	M/F
Caffeic acid_R	bDL/bDL	bDL/2.59	bDL/bDL	bDL/bDL	bDL/bDL	bDL/bDL
<i>p</i> -Coumaric acid_R	0.47 <sup>f</sup> /0.42 <sup>f</sup>	0.48 <sup>f</sup> /0.27 <sup>g</sup>	1.42 <sup>c</sup> /bDL	1.25 <sup>d</sup> /7.06 <sup>a</sup>	1.81 <sup>b</sup> /0.91 <sup>e</sup>	0.49 <sup>f</sup> /0.38 <sup>fg</sup>
Chlorogenic acid_R	0.60 <sup>bc</sup> /bDL	bDL/bDL	bDL/bDL	bDL/bDL	bDL/bDL	1.29 <sup>a</sup> /1.18 <sup>bc</sup>
Gallic acid_R	bDL/29 <sup>c</sup>	bDL/bDL	bDL/bDL	bDL/8.06 <sup>a</sup>	bDL/0.39 <sup>c</sup>	bDL/0.94 <sup>b</sup>
Protocatechuic acid_R	bDL/bDL	bDL/0.22 <sup>e</sup>	bDL/bDL	0.79 <sup>c</sup> /1.92 <sup>a</sup>	bDL/0.65 <sup>d</sup>	0.92 <sup>b</sup> /21 <sup>e</sup>
4-HBA_R	0.26 <sup>f</sup> /bDL	0.23 <sup>f</sup> /bDL	bDL/0.39 <sup>e</sup>	0.61 <sup>c</sup> /2.00 <sup>a</sup>	0.49 <sup>d</sup> /0.77 <sup>b</sup>	0.25 <sup>f</sup> /0.29 <sup>f</sup>
Vanillic acid_R	bDL/bDL	0.33 <sup>f</sup> /1.98 <sup>a</sup>	bDL/0.55 <sup>e</sup>	0.81 <sup>d</sup> /bDL	1.15 <sup>c</sup> /1.38 <sup>b</sup>	0.59 <sup>e</sup> /bDL
Syringic acid_R	0.35 <sup>e</sup> /bDL	0.30 <sup>e</sup> /0.25 <sup>e</sup>	bDL/0.35 <sup>e</sup>	1.00 <sup>c</sup> /7.21 <sup>a</sup>	2.16 <sup>b</sup> /bDL	0.73 <sup>d</sup> /0.69 <sup>d</sup>
Rutin_R	bDL/bDL	bDL/bDL	bDL/bDL	bDL/bDL	bDL/bDL	0.90/bDL
Catechin_R	0.14 <sup>g</sup> /bDL	0.29 <sup>d</sup> /0.26 <sup>e</sup>	0.82 <sup>a</sup> /0.30 <sup>d</sup>	0.34 <sup>c</sup> /0.44 <sup>b</sup>	bDL/0.25 <sup>e</sup>	0.17 <sup>g</sup> /0.19 <sup>f</sup>
C6-C1_R	0.61 <sup>fg</sup> /0.29 <sup>g</sup>	0.87 <sup>f</sup> /2.23 <sup>d</sup>	bDL/1.29 <sup>e</sup>	2.71 <sup>c</sup> /17.27 <sup>a</sup>	3.81 <sup>b</sup> /2.90 <sup>c</sup>	1.57 <sup>e</sup> /1.93 <sup>d</sup>
C6-C3_R	1.06 <sup>cde</sup> /0.42 <sup>de</sup>	0.48 <sup>de</sup> /2.86 <sup>b</sup>	1.42 <sup>cd</sup> /bDL	1.25 <sup>cde</sup> /7.06 <sup>a</sup>	1.81 <sup>bc</sup> /0.91 <sup>de</sup>	1.78 <sup>bc</sup> /1.56 <sup>cd</sup>
C6-C3-C6_R	0.14 <sup>h</sup> /bDL	0.29 <sup>e</sup> /0.26 <sup>f</sup>	0.82 <sup>b</sup> /0.30 <sup>e</sup>	0.34 <sup>d</sup> /0.44 <sup>c</sup>	bDL/0.25 <sup>f</sup>	1.06 <sup>a</sup> /0.19 <sup>g</sup>
Phenolics (sum)_R	1.82 <sup>de</sup> /0.71 <sup>e</sup>	1.64 <sup>de</sup> /5.55 <sup>b</sup>	2.24 <sup>d</sup> /1.60 <sup>de</sup>	5.10 <sup>bc</sup> /26.69 <sup>a</sup>	5.62 <sup>b</sup> /4.72 <sup>bc</sup>	5.33 <sup>b</sup> /3.89 <sup>c</sup>
Acetic acid_R	4.21 <sup>ef</sup> /8.08 <sup>d</sup>	5.40 <sup>e</sup> /3.23 <sup>f</sup>	bDL/bDL	16.09 <sup>b</sup> /11.85 <sup>c</sup>	42.44 <sup>a</sup> /43.82 <sup>a</sup>	9.37 <sup>d</sup> /bDL
Citric acid_R	196.76 <sup>gh</sup> /97.64 <sup>l</sup>	108.57 <sup>k</sup> /180.45 <sup>i</sup>	198.63 <sup>fg</sup> /433.77 <sup>b</sup>	255.40 <sup>e</sup> /369.71 <sup>d</sup>	586.54 <sup>a</sup> /411.58 <sup>c</sup>	144.58 <sup>j</sup> /205.01 <sup>f</sup>

Fumaric acid_R	1.26 <sup>fg</sup> /1.06 <sup>g</sup>	0.72 <sup>g</sup> /2.13 <sup>f</sup>	3.69 <sup>e</sup> /1.55 <sup>fg</sup>	13.49 <sup>b</sup> /8.25 <sup>c</sup>	48.76 <sup>a</sup> /7.16 <sup>d</sup>	6.87 <sup>d</sup> /4.31 <sup>e</sup>
Lactic acid_R	bDL/bDL	bDL/ bDL	bDL /4.64 <sup>e</sup>	19.09 <sup>b</sup> / bDL	17.41 <sup>c</sup> /25.25 <sup>a</sup>	12.44 <sup>d</sup> / bDL
LMWOAs (sum)_R	202.24 <sup>f</sup> /106.79 <sup>i</sup>	114.70 <sup>j</sup> /185.82 <sup>g</sup>	202.32 <sup>f</sup> /439.96 <sup>c</sup>	304.07 <sup>e</sup> /389.81 <sup>d</sup>	695.15 <sup>a</sup> /487.82 <sup>b</sup>	173.15 <sup>h</sup> /209.32 <sup>f</sup>
Caffeic acid_L	0.27 <sup>k</sup> /0.77 <sup>f</sup>	0.49 <sup>j</sup> /0.61 <sup>g</sup>	0.31 <sup>j</sup> /1.06 <sup>d</sup>	4.51 <sup>c</sup> /2.09 <sup>c</sup>	0.51 <sup>h</sup> /1.00 <sup>e</sup>	7.04 <sup>a</sup> /0.18 <sup>l</sup>
<i>p</i> -Coumaric acid_L	bDL/0.171 <sup>g</sup>	bDL/0.14 <sup>h</sup>	0.40 <sup>c</sup> /0.23 <sup>e</sup>	1.00 <sup>a</sup> /0.21 <sup>f</sup>	0.26 <sup>d</sup> /bDL	0.57 <sup>b</sup> /bDL
Chlorogenic acid_L	2.07 <sup>l</sup> /16.60 <sup>g</sup>	3.69 <sup>k</sup> /14.58 <sup>h</sup>	50.93 <sup>b</sup> /25.64 <sup>f</sup>	37.84 <sup>c</sup> /27.88 <sup>e</sup>	11.80 <sup>i</sup> /74.18 <sup>a</sup>	36.70 <sup>d</sup> /9.76 <sup>j</sup>
Ferulic acid_L	0.18 <sup>l</sup> /2.03 <sup>e</sup>	0.21 <sup>k</sup> /1.36 <sup>f</sup>	3.41 <sup>b</sup> /2.36 <sup>c</sup>	1.14 <sup>g</sup> /0.32 <sup>i</sup>	0.29 <sup>j</sup> /4.65 <sup>a</sup>	2.30 <sup>d</sup> /0.41 <sup>h</sup>
Sinapic acid_L	bDL/bDL	bDL/bDL	bDL/bDL	0.28/bDL	bDL/bDL	bDL/bDL
Gallic acid_L	bDL/0.13 <sup>g</sup>	bDL/0.16 <sup>f</sup>	1.02 <sup>b</sup> /0.28 <sup>e</sup>	0.73 <sup>c</sup> /bDL	bDL/0.53 <sup>d</sup>	1.29 <sup>a</sup> /bDL
Protocatechuic acid_L	bDL/0.14 <sup>b</sup>	bDL/bDL	bDL/bDL	bDL/bDL	bDL/bDL	101.0 <sup>a</sup> /bDL
4-HBA_L	bDL/0.47 <sup>f</sup>	0.10 <sup>h</sup> /bDL	1.41 <sup>e</sup> /bDL	7.03 <sup>b</sup> /3.00 <sup>c</sup>	bDL/1.88 <sup>d</sup>	8.68 <sup>a</sup> /0.15 <sup>g</sup>
Vanillic acid_L	0.25 <sup>k</sup> /0.71 <sup>f</sup>	0.36 <sup>j</sup> /0.39 <sup>i</sup>	1.77 <sup>d</sup> /0.67 <sup>g</sup>	4.39 <sup>b</sup> /2.14 <sup>c</sup>	0.54 <sup>h</sup> /0.89 <sup>e</sup>	7.22 <sup>a</sup> /0.22 <sup>l</sup>
Syringic acid_L	bDL/0.49 <sup>f</sup>	bDL/0.11 <sup>i</sup>	1.56 <sup>c</sup> /0.19 <sup>h</sup>	6.14 <sup>a</sup> /0.79 <sup>e</sup>	0.44 <sup>g</sup> /1.28 <sup>d</sup>	6.03 <sup>b</sup> /bDL
<i>t</i> -Cinnamic acid_L	bDL/bDL	bDL/1.03 <sup>d</sup>	1.29 <sup>c</sup> /1.77 <sup>b</sup>	1.76 <sup>b</sup> /3.65 <sup>a</sup>	0.14 <sup>f</sup> /0.41 <sup>e</sup>	1.29 <sup>c</sup> /0.12 <sup>f</sup>
Salicylic acid_L	2.18 <sup>f</sup> / 3.34 <sup>f</sup>	2.66 <sup>f</sup> / 13.70 <sup>d</sup>	0.25 <sup>f</sup> / 28.00 <sup>c</sup>	2.39 <sup>f</sup> / 14.80 <sup>d</sup>	0.88 <sup>f</sup> / 33.16 <sup>b</sup>	7.20 <sup>e</sup> / 105.37 <sup>a</sup>
Rutin_L	bDL/0.55 <sup>g</sup>	0.17 <sup>i</sup> /0.63 <sup>f</sup>	3.27 <sup>c</sup> /1.13 <sup>e</sup>	86.95 <sup>a</sup> /24.77 <sup>b</sup>	bDL/bDL	2.98 <sup>d</sup> /0.37 <sup>h</sup>
Quercetin_L	bDL/4.44 <sup>b</sup>	bDL/0.49 <sup>g</sup>	0.46 <sup>h</sup> /0.88 <sup>f</sup>	11.55 <sup>a</sup> /0.30 <sup>j</sup>	1.34 <sup>c</sup> /0.94 <sup>e</sup>	0.99 <sup>d</sup> /0.31 <sup>i</sup>
Catechin_L	2.81 <sup>f</sup> /12.26 <sup>c</sup>	3.49 <sup>e</sup> /0.77 <sup>h</sup>	bDL/1.33 <sup>g</sup>	21.28 <sup>a</sup> /bDL	bDL/bDL	19.16 <sup>b</sup> /7.31 <sup>d</sup>
C6-C1_L	2.43 <sup>fg</sup> /5.27 <sup>fg</sup>	3.12 <sup>gf</sup> /14.36 <sup>e</sup>	5.74 <sup>f</sup> /29.14 <sup>c</sup>	20.68 <sup>d</sup> /20.74 <sup>d</sup>	1.85 <sup>g</sup> /37.74 <sup>b</sup>	30.41 <sup>c</sup> /105.74 <sup>a</sup>
C6-C3_L	2.52 <sup>l</sup> /19.57 <sup>g</sup>	4.38 <sup>k</sup> /17.71 <sup>h</sup>	56.34 <sup>b</sup> /31.06 <sup>f</sup>	46.52 <sup>d</sup> /34.15 <sup>e</sup>	12.74 <sup>i</sup> /80.51 <sup>a</sup>	47.89 <sup>c</sup> /10.47 <sup>j</sup>

C6-C3-C6_L	2.81 <sup>i</sup> /17.26 <sup>d</sup>	3.65 <sup>h</sup> /2.20 <sup>j</sup>	4.43 <sup>f</sup> /3.84 <sup>g</sup>	123.02 <sup>a</sup> /25.48 <sup>c</sup>	4.45 <sup>f</sup> /1.24 <sup>k</sup>	93.65 <sup>b</sup> /10.22 <sup>e</sup>
Phenolics (sum)_L	7.76 <sup>j</sup> /42.10 <sup>g</sup>	11.15 <sup>j</sup> /34.26 <sup>h</sup>	66.51 <sup>f</sup> /64.04 <sup>f</sup>	190.22 <sup>a</sup> /80.38 <sup>e</sup>	19.05 <sup>i</sup> /119.49 <sup>d</sup>	171.96 <sup>b</sup> /126.43 <sup>c</sup>
Oxalic acid_L	bDL/3.05 <sup>d</sup>	bDL/bDL	0.42f/bDL	21.72a/bDL	1.25e/4.77c	6.79b/bDL
Quinic acid_L	90.68 <sup>e</sup> /bDL	98.95 <sup>d</sup> /149.61 <sup>b</sup>	bDL/392.71 <sup>a</sup>	bDL/87.39 <sup>f</sup>	bDL/bDL	bDL/121.97c
Malic acid_L	1.33 <sup>l</sup> /16.76 <sup>e</sup>	2.93 <sup>j</sup> /25.37 <sup>d</sup>	5.08 <sup>i</sup> /5.49 <sup>h</sup>	188.44 <sup>a</sup> /9.25 <sup>g</sup>	31.15 <sup>c</sup> /16.36 <sup>f</sup>	92.40 <sup>b</sup> /1.71 <sup>k</sup>
Malonic acid_L	6.06 <sup>k</sup> /26.52 <sup>g</sup>	7.16 <sup>j</sup> /18.71 <sup>i</sup>	2.74 <sup>i</sup> /48.11 <sup>e</sup>	144.65 <sup>c</sup> /231.48 <sup>b</sup>	84.15 <sup>d</sup> /28.11 <sup>f</sup>	725.15 <sup>a</sup> /19.79 <sup>h</sup>
Acetic acid_L	0.97 <sup>k</sup> /4.78 <sup>f</sup>	2.03 <sup>i</sup> /3.57 <sup>g</sup>	0.69 <sup>l</sup> /31.95 <sup>c</sup>	171.61 <sup>a</sup> /5.24 <sup>e</sup>	10.30 <sup>d</sup> /3.74 <sup>h</sup>	139.90 <sup>b</sup> /1.21 <sup>j</sup>
Citric acid_L	141.80 <sup>h</sup> /269.55 <sup>e</sup>	111.77 <sup>k</sup> /138.10 <sup>i</sup>	493.24 <sup>a</sup> /227.75 <sup>g</sup>	113.51 <sup>j</sup> /351.62 <sup>b</sup>	339.82 <sup>c</sup> /239.70 <sup>f</sup>	302.78 <sup>d</sup> /75.16 <sup>l</sup>
Fumaric acid_L	3.93 <sup>i</sup> /9.74 <sup>c</sup>	3.06 <sup>j</sup> /5.67 <sup>g</sup>	1.85 <sup>l</sup> /5.46 <sup>h</sup>	23.52 <sup>b</sup> /8.49 <sup>d</sup>	6.69 <sup>e</sup> /5.76 <sup>f</sup>	44.71 <sup>a</sup> /1.91 <sup>k</sup>
Succinic acid_L	21.66 <sup>e</sup> /bDL	40.99 <sup>c</sup> /bDL	37.66 <sup>d</sup> /10.97 <sup>f</sup>	bDL/bDL	bDL/214.73 <sup>a</sup>	95.79 <sup>b</sup> /8.37 <sup>g</sup>
Lactic acid_L	4.77 <sup>k</sup> /17.75 <sup>e</sup>	0.62 <sup>l</sup> /10.15 <sup>h</sup>	5.92 <sup>j</sup> /15.14 <sup>f</sup>	62.62 <sup>b</sup> /32.13 <sup>d</sup>	32.35 <sup>c</sup> /12.52 <sup>g</sup>	143.11 <sup>a</sup> /6.89 <sup>j</sup>
LMWOAs (sum)_L	271.21 <sup>j</sup> /348.16 <sup>i</sup>	267.52 <sup>k</sup> /351.18 <sup>h</sup>	547.62 <sup>e</sup> /737.60 <sup>b</sup>	725.98 <sup>c</sup> /725.62 <sup>d</sup>	505.71 <sup>g</sup> /525.43 <sup>f</sup>	1550.60 <sup>a</sup> /237.01 <sup>l</sup>

Data presented as mean value (n=3); 4-HBA – 4-hydroxybenzoic acid, C6-C1 – hydroxybenzoic acids, C6-C3 – phenylpropanoids, C6-C3-C6 – flavonoids, LMWOAs – low-molecular-weight organic acids, R – roots, L – leaves, bDL – below detection limit; identical superscripts denote no significant differences between means according to a post-hoc Tukey's HSD test following two-way ANOVA for fixed effect of "sex × treatment" interaction at α=95%

#### *Phenolic metabolites in roots*

The content of C6-C1 structures in roots was higher in the case of all treatments compared to the control (excluding male plants treated with Zn). For male specimens, drought simulation caused the significant accumulation of the C6-C1 compounds, while "Zn+d" treatments elevated the effect. In a contrary, "Cu+d" reduced the increased comparing to isolated drought. For females, the highest content of C6-C1 metabolites was found for simulated drought (17.3 µg g<sup>-1</sup> FW), while metal+"d" co-treatment depleted this effect to the level comparable to isolated metal treatments.

The C6-C3 content in roots of male plants did not exceed  $2 \mu\text{g g}^{-1}$  FW, with the lowest value determined for "Zn". No significant differences were found between control and treatments. The C6-C3 structures were not detected in female specimens under Cu stress, while the highest contents was found for drought simulation. Metal+"d" co-treatments reduced the content of C6-C3 metabolites compared to isolated "d" and "Zn" treatments, however an increase for "Cu+d" vs. "Cu" was noted.

The C6-C3-C6 content in roots was lower than  $1.1 \mu\text{g g}^{-1}$  FW, and the structures were not detected in female control plants and "Zn+d"-treated males. The "Cu" and "Cu+d" treatments of male specimens resulted in the highest content of the C6-C3-C6 metabolites.

The sum of the profiled phenolic compounds increased under stress conditions compared to the control (excluding male plants treated with "Zn" or "Cu"). The highest sum in roots of male specimens was noted for "d" and metal+"d" treatments ( $>5 \mu\text{g g}^{-1}$  FW), and for female specimens in the case of "d" ( $>26 \mu\text{g g}^{-1}$  FW). Moreover, for combined "Zn+d" the sum of profiled phenolics was lower than for "Zn" and "d" isolated treatments, while for "Cu+d" it was higher than for "Cu".

The phenolic composition of roots determined for male control plants consisted of p-coumaric, chlorogenic, 4-HBA and syringic acids and catechin. The content of p-coumaric acid increased under "Cu", "d" and "Zn+d" treatments, while chlorogenic acid was found for "Cu+d". The content of 4-HBA increased only for "d" and "Zn+d", while syringic acid increased under "d" and metal+"d" combined treatments. Catechin content increased under single stressors action as well as "Cu+d". As new compounds, protocatechuic acid ("d"), vanillic acid ("d" and metal+"d") and rutin ("Cu+d") were detected.

The profile of female control consisted of p-coumaric and gallic acids, and the elevation of their content was noted for "d" and metal+"d" (gallic acid) and "d" and "Zn+d" (p-coumaric acid). As new compounds, caffeic acid ("Zn"), chlorogenic ("Cu+d"), protocatechuic acid ("Zn", "d", and metal+"d"), 4-HBA ("Cu", "d" and metal+"d"), vanillic acid (isolated treatments) and syringic acid (isolated treatments and "Cu+d") were determined.

#### *Phenolic metabolites in leaves*

In leaves the synthesis of C6-C1 structures increased under stress condition reaching higher level for the female specimens. For male specimens, the C6-C1 content increased under metal and drought separate treatments compared to the control. Under "Cu+d", a significant elevation compared to the control and isolated treatments was confirmed, while for "Zn+d" the C6-C1 content was reduced compared to a single stressor. For female specimens, higher content of C6-C1 metabolites was noted for combined metal and drought treatments than for separated factors reaching  $106 \mu\text{g g}^{-1}$  FW in the case of "Cu+d"-treated females).

Compared to control, the enhanced synthesis of C6-C3 metabolites was confirmed for the majority of treatments excluding "Zn" and "Cu+d" for female plants. For male specimens, the highest content of C6-C3 was noted under "Cu", "d" and "Cu+d" action reaching  $56.3 \mu\text{g g}^{-1}$  FW. For female specimens, the C6-C3 content was higher under "Zn+d" than under isolated stressors, while under "Cu+d" it was lower vs. "Cu" and "d" treatments.



The C6-C3-C6 content increased in leaves of male plants for all treatments, with the highest value for "d" and "Cu+d" treatments (123.0 and 93.7  $\mu\text{g g}^{-1}$  FW, respectively). Metals significantly reduced the effect of drought when applied simultaneously, and the effect was more pronounced in the case of Zn. However, the action of combined stressors resulted in the elevated synthesis of C6-C3-C6 metabolites compared to isolated metals. In a contrary to male plants, the reduced C6-C3-C6 content vs. control was observed for all treatments excluding "d".

Considering the sum of profiled phenolics in *Salix* leaves, the lowest content was found in control and "Zn", and the highest for "d"-treated male specimens. Metal+"d" co-treatment of plants resulted in a higher sum of profiled phenolics than isolated metals, however not exceeding the level observed for "d". The increase was markedly noted for "Cu+d" vs. "Cu" (both sexes) and "Zn+d" vs. "Zn" (female plants).

The profile of phenolic compounds in leaves of control plants differed between sexes and was more complex for female specimens with chlorogenic and salicylic acids, and catechin being the main compounds. Moreover, caffeic, p-coumaric, ferulic, gallic, protocatechuic, 4-HBA, vanillic and syringic acids, rutin and quercetin were detected. Treatments caused the modification of leaf phenolic profiles, and the synthesis of new compounds and changes in the content of previously identified in control were noted.

The addition of Zn increased the content of the profiled phenolics (excluding salicylic acid) and induced the synthesis of new compounds (4-HBA, rutin) in male specimens. In female specimens, the drop of the majority of detected phenolics was observed compared to the control (besides salicylic and gallic acids, and rutin) and also the synthesis of trans-cinnamic acids as a new compound.

The main phenolic compounds accumulated in leaves of Cu-treated plants were chlorogenic acid (both sexes) and salicylic acid (female plants). The increase of phenolics content, lack of catechin, as well as biosynthesis of new compounds (p-coumaric, gallic, 4-HBA, syringic and trans-cinnamic acids, rutin, and quercetin) were documented in male specimens compared to control. In female specimens, the content of salicylic, caffeic, p-coumaric, chlorogenic, ferulic, gallic acids and rutin increased, while others decreased as a response to Cu. Moreover, protocatechuic acid and 4-HBA were not detected, while trans-cinnamic acid was a new compound compared to the control.

Under drought stress, the elevation of phenolic content and synthesis of new compounds were noted (p-coumaric, sinapic, gallic, 4-HBA, syringic and trans-cinnamic acids, rutin and quercetin) in male plants compared to control. Moreover, the content of the majority of phenolic compounds was higher than in the case of metal treatments. For females, the content of most phenolic compounds also increased compared to control, "Zn" and "Cu", but significant decreases were also noted. Additionally, trans-cinnamic acid was found as a new compound compared to the control.

The combined metal and drought treatments also affected the phenolic composition compared to the control and isolated stress factors. The "Zn+d" treatment resulted in an increase in phenolic compounds, with chlorogenic being the main metabolite. In leaves of male specimens, the content of phenolic compounds increased compared to control and "Zn" (with some expectations) and new compounds were detected, while the reduction of content and lack of some compounds compared to drought stress was noted (e.g. salicylic acid). For female specimens, the phenolic compounds content increased (besides p-coumaric, protocatechuic, rutin, catechin and quercetin), or new ones were synthesised (trans-cinnamic acid) compared to the control. Compared to "Zn", an increased

content of almost all compounds, and the reduction of p-coumaric and trans-cinnamic acids, rutin and catechin was observed. Compared to "d", the elevation of the content of chlorogenic, salicylic, ferulic, syringic acids, and quercetin was reported, and the synthesis of gallic as a new compound.

In male specimens under simultaneous "Cu+d" treatment, particular phenolic compounds (caffeic, gallic, protocatechuic, 4-HBA and vanillic acids) achieved the highest values, and the content of selected metabolites was higher than for "Cu" (salicylic, caffeic, p-coumaric, gallic, protocatechuic, 4-HBA, vanillic, syringic, quercetin and catechin) and/or "d" (salicylic, caffeic, ferulic, gallic, protocatechuic, 4-HBA, vanillic and syringic acids). In the case of female specimens significantly lower contents ( $<10 \mu\text{g g}^{-1}$  FW) were confirmed for nearly all profiled phenolics compared to control, with the exception of salicylic acid reaching the highest value among treatments ( $105.4 \mu\text{g g}^{-1}$  FW). Moreover, the content of the majority of phenolic compounds was suppressed compared to isolated "Cu" (excluding catechin) and "d" (excluding ferulic acid and quercetin) treatments.

#### *Low-molecular-weight organic acids in roots*

The sum of organic acids increased under stress compared to the control, except for "Zn" and "Cu+d" treatments. The highest total content of acids was found for males and females challenged with "Zn+d" ( $797$  and  $888 \mu\text{g g}^{-1}$  FW, respectively). In turn, the lowest total acid content in roots of males was found for "Zn" ( $115 \mu\text{g g}^{-1}$  FW) and in control plants for females ( $107 \mu\text{g g}^{-1}$  FW).

In roots, four organic acids (citric, fumaric and acetic) were found for both sexes. For males, in the control and Cu addition, the content of citric acid was similar ( $\sim 198 \mu\text{g g}^{-1}$  FW), while in the case of "Zn" treatment, its content was lower than in control. For "d" and "Zn+d" treatments, a significant increase in citric acid content was found compared to the control ( $256$  and  $688 \mu\text{g g}^{-1}$  FW, respectively), and for "Cu+d" a decrease in its content was noted ( $145 \mu\text{g g}^{-1}$  FW). Citric acid was also dominant in roots of females and its content increased following all applied treatments. The highest content was found for "Cu" and "Zn+d" ( $631$  and  $812 \mu\text{g g}^{-1}$  FW, respectively). Fumaric acid was determined in roots at much lower levels than citric acid. Still, similar tendencies in its content for both sexes were shown. In turn, the highest content of acetic acid was determined for "Zn+d" both in males and females ( $\sim 43 \mu\text{g g}^{-1}$  FW), and the acid was not detected in females in the case of the copper-containing treatments.

#### *Low-molecular-weight organic acids in leaves*

The sum of organic acids in leaves increased in treated plants compared to the control, except for the "Cu+d"-treated females, where a significant decrease in total acid content was found ( $237 \mu\text{g g}^{-1}$  FW). The highest total content of acids was found for "Cu+d" (males) and "Cu" (females) ( $1550$  and  $738 \mu\text{g g}^{-1}$  FW, respectively).

For males and females nine acids were determined in leaves, and citric acid was dominant as in roots. A lower content was noted for the remaining acids, and was significantly differentiated by the plant sex and applied treatment.

For male plants, the highest content of citric, quinic and succinic acids (142, 91 and 22  $\mu\text{g g}^{-1}$  FW, respectively) was determined in the control. The acids were also present in leaves of "Zn"-treated plants, however, the metal caused a decrease in citric acid with a simultaneous increase in quinic and succinic acids content (112, 99 and 41  $\mu\text{g g}^{-1}$  FW, respectively). "Cu" caused the highest content of citric and succinic acid only (493 and 38  $\mu\text{g g}^{-1}$  FW, respectively). Drought simulation led to a significant increase in oxalic, malic, fumaric and lactic acids content. At the same time "Zn+d" co-treatment, however, intensified the formation of malic, acetic, and lactic acids, and "Cu+d" also fumaric and succinic acids.

For females, the highest content of four organic acids was found for citric, malonic, lactic, and malic (279, 27, 18 and 17  $\mu\text{g g}^{-1}$  FW, respectively). As was the case for males, the same profile was determined for "Zn"-treated plants, and a decrease was noted for citric, malonic, and lactic acid (138, 19 and 10  $\mu\text{g g}^{-1}$  FW, respectively). Significantly increased content of quinic acid was also noted, while in control, the acid was not detected. Further, "Cu" treatment induced the accumulation of quinic and acetic acids.

Drought simulation and "Zn+d" treatment stimulated the formation of organic acids in *S. × fragilis* leaves. Leaves of female plants exhibited the highest contents of citric, malonic and quinic acids under "d" (352, 232 and 87  $\mu\text{g g}^{-1}$  FW). In contrast, for "Zn+d" the highest succinic acid was determined (215  $\mu\text{g g}^{-1}$  FW). In the case of "Cu+d" treatment, a significant decrease in acid formation was found compared to the control, and the most significant reduction was noted for citric acid.

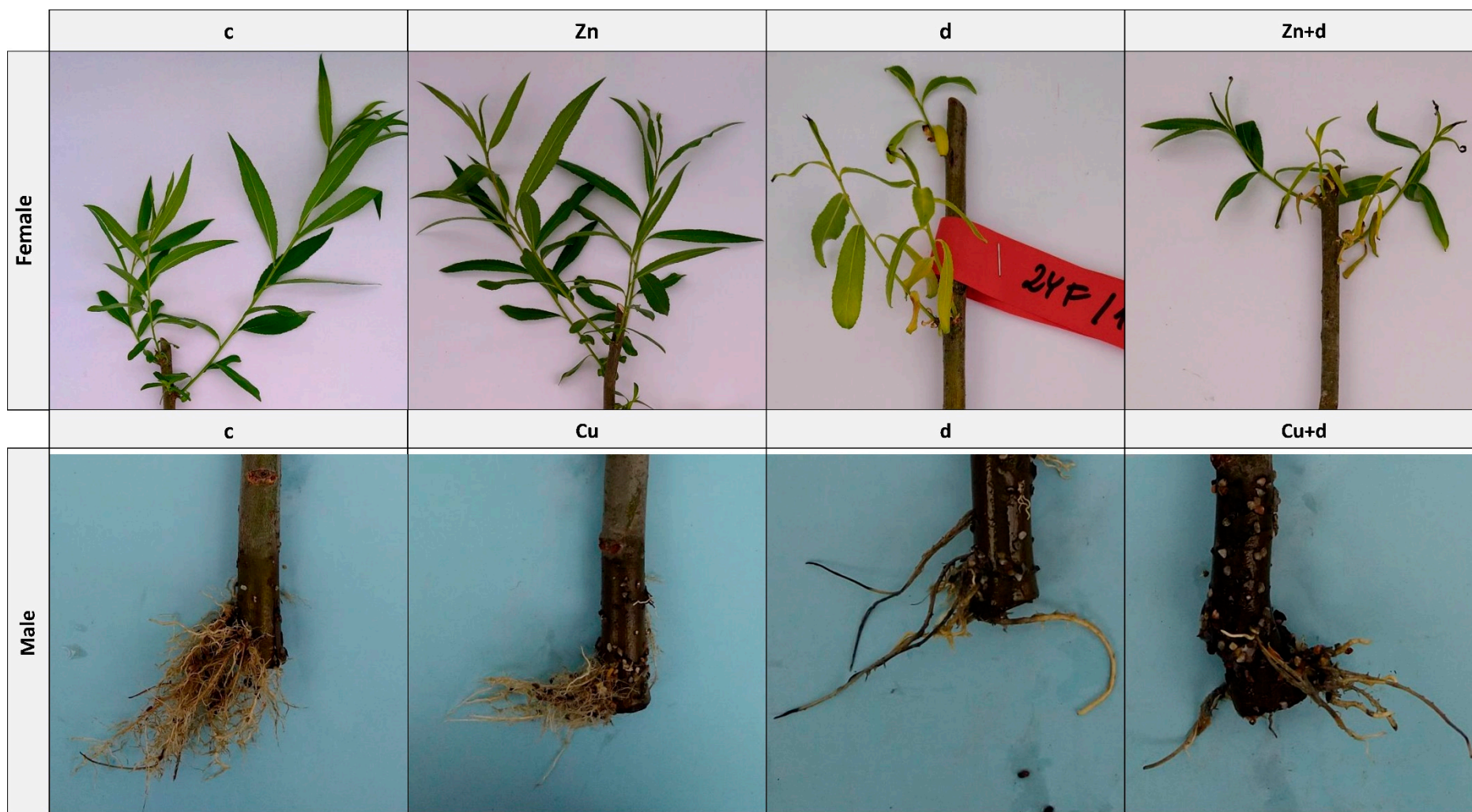


Figure S1 Exemplary images presenting toxicity symptoms of leaves and roots of *Salix × fragilis* L. treated with zinc ("Zn"), copper ("Cu"), simulated drought ("d") and under combined treatments ("Zn+d", "Cu+d") ("c" – control)