Supplementary Material

Differentiation of metallicolous and non-metallicolous *Salix caprea* populations based on phenotypic characteristics and nuclear microsatellite (SSR) markers

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Supplementary Table 1: Summary of the soil characteristics for each isolate and their growth and accumulation behaviour in perlite-based soil-less cultures exposed to elevated levels of Cd (0.5 mg L^{-1}) and Zn (5 mg L^{-1}).

Soil sample	Zn total mg/kg	Cd total mg/kg	pH	Zn labile mg/kg	Cd labile mg/kg	Plant sample Perlite	Zn Perlite mg/kg		Cd Perlite mg/kg		Biomass (g)	
Prag						Nr	mean	SE	mean	SE	mean	SE
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	1	888	87.2	119	16.93	3.52	0.23
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	2	787	22.5	82.0	3.77	5.20	0.25
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	3	773	17.8	68.8	6.39	4.10	0.44
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	4	874	32.2	97.8	7.34	2.44	0.54
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	5	797	37.9	121	22.4	3.10	0.90
P6 S	67.9	0.26	6.06	0.37	0.008	6	638	10.0	72.6	2.57	2.99	0.44
P7-8 S a	72.0	0.28	7.62	0.12	0.008	7	600	10.1	58.5	4.08	5.04	0.24
P7-8 S b	72.0	0.28	7.62	0.12	0.005	8	577	8.8	46.5	1.76	5.29	1.15
P9 S	67.5	0.29	6.57	0.20	0.006	9	680	35.1	68.0	5.28	2.24	0.43
P10-11 S	67.5	0.26	6.48	0.17	0.005	10	1187	206	147	35.0	0.89	0.48
P10-11 S	67.5	0.26	6.48	0.17	0.005	11	922	51.3	91.6	1.54	6.06	0.40
P12-13 - S	59.9	0.19	5.43	0.65	0.016	12	799	65.8	90.8	9.42	4.20	1.10
P12-13 - S	59.9	0.19	5.43	0.65	0.016	13	777	41.0	85.3	7.11	5.78	1.02
P14-16+18S	64.6	0.22	5.43	0.80	0.019	14	678	21.6	71.5	7.22	4.35	0.51
P14-16+18S	64.6	0.22	5.43	0.80	0.019	15	835	94.5	94.0	13.9	4.22	0.34
P14-16+18S	64.6	0.22	5.43	0.80	0.019	16	913	44.0	141	31.1	2.53	0.53
P17 S	60.3	0.19	5.37	0.73	0.019	17	634	16.4	61.4	1.73	4.75	0.35
P14-16+18S	64.6	0.22	5.43	0.80	0.019	18	644	58.0	86.4	17.3	3.22	0.57
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	21	752	67.7	96.6	12.8	4.22	1.20
P1-5, 21-22 S	87.4	0.41	5.83	0.85	0.016	22	815	59.4	111	10.9	3.46	0.30
Mezica												
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	1	820	19.6	119	15.4	3.24	0.28
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	3	736	23.8	122	24.7	3.61	0.49
S - M5	9534	59.4	7.2	29.4	0.63	4	845	216	146	55.6	3.36	1.02
S - M7	404	7.65	7.27	0.70	0.07	7	608	39.6	86.4	2.9	5.35	0.21
S - M9+10+11	2747	13.6	7.57	2.35	0.04	10	784	114	90.0	3.1	3.07	0.85
S - M9+10+11	2747	13.6	7.57	2.35	0.04	11	1476	235	243	50.5	1.67	0.52
S - M8, 12-14, 26	1508	15.3	6.94	1.33	0.07	13	570	23.3	76.0	3.9	4.63	0.31
S - M15	3269	28.5	7.06	7.75	0.18	15	882	52.8	145	11.2	2.61	0.35
S - M16	489	9.94	7.19	1.05	0.06	16	679	35.7	112	9.0	2.63	0.35
S - M17	131	1.93	7.45	0.40	0.02	17	541	19.5	65.4	3.0	2.69	0.12
S - M18	2267	35.3	6.64	6.99	0.20	18	686	178	86.7	38.1	1.74	1.49
S - M19+20	1867	18.3	7.28	3.60	0.10	19	719	119	133	30.2	2.78	0.40

S - M19+20	1867	18.3	7.28	3.60	0.10	20	991	50.8	116	19.3	1.16	0.35
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	21	1656	178	265	25.3	1.64	0.28
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	22	893	47.4	112	9.0	4.13	0.32
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	23	995	149	156	26.0	2.68	0.56
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	24	1228	51.4	207	16.3	1.55	0.17
S - M1-3, 21-25	1292	18.1	7.2	0.92	0.10	25	1103	120	152	12.1	2.60	0.66
S - M8, 12-14, 26	1508	15.3	6.94	1.33	0.07	26	959	64.3	140	9.4	2.89	0.29
S - M18, 27	2267	35.3	6.64	6.99	0.20	27	1154	212	138	9.2	1.87	0.82
						28	781	38.9	104	11.6	2.56	0.23
Pribram												
PR1 S	276	7.35	4.88	25.7	1.68	1	765	46.7	182	29.1	1.01	0.08
PR 2-6	202	4.70	5.5	13.1	1.22	2	743	28.4	90.8	6.51	1.74	0.26
PR 2-6	202	4.70	5.5	13.1	1.22	3	751	45.1	85.4	6.35	2.91	0.32
PR 2-6	202	4.70	5.5	13.1	1.22	4	929	63.8	111	16.8	1.74	0.65
PR 2-6	202	4.70	5.5	13.1	1.22	5	722	64.4	65.3	21.6	2.80	0.55
PR 2-6	202	4.70	5.5	13.1	1.22	6	614	33.4	52.9	2.88	3.21	0.28
PR7-9 S	4182	35.8	6.35	36.5	1.01	7	936	26.6	137	0.56	1.40	0.35
PR7-9 S	4182	35.8	6.35	36.5	1.01	8	648	47.7	67.7	10.8	3.59	0.47
PR7-9 S	4182	35.8	6.35	36.5	1.01	9	670	38.7	84.1	10.5	2.50	0.25
PR10-12 S a	4387	38.9	6.5	63.3	1.40	10	928	20.9	76.5	8.26	1.55	0.18
PR10-12 S a	4387	38.9	6.5	63.3	1.40	11	807	63.0	141	9.29	1.25	0.18
PR13 S	1139	10.3	7.35	2.01	0.13	13	715	55.5	65.2	8.44	2.74	0.33
PR14-17 S	2091	12.6	7.25	1.20	0.11	14	838	90.8	134	52.6	1.65	0.52
PR14-17 S	2091	12.6	7.25	1.20	0.11	15	859	57.3	87.6	3.84	2.00	0.12
PR14-17 S	2091	12.6	7.25	1.20	0.11	16	624	47.2	67.4	6.97	2.78	0.16
PR18-19 S	2962	30.0	5.95	221	4.69	18	879	67.8	129	14.8	1.98	0.11
PR18-19 S	2962	30.0	5.95	221	4.69	19	774	75.9	86.9	12.2	1.78	0.09
Arnoldstein												
A1-3, A21-S	2492	19.4	7.3	14.3	0.11	1	913	27.0	148	13.8	1.85	0.24
A5, A22-S	1530	11.9	6.23	57.4	0.90	5	701	22.5	118	8.61	3.00	0.38
A 6 - 8-S	1244	9.65	6.61	8.68	0.21	6	685	44.2	88.6	12.1	5.09	0.40
A 6 - 8-S	1244	9.65	6.61	8.68	0.21	8	709	68.7	138	25.7	2.13	0.40
A9-S	4465	13.5	7.89	35.1	0.17	9	590	45.9	79.0	7.97	3.84	0.22
A10-16-S	1824	3.91	7.97	7.57	0.03	13	604	67.4	100	8.38	3.51	0.30
A10-16-S	1824	3.91	7.97	7.57	0.03	11	545	23.8	78.6	1.64	3.37	0.09
A10-16-S	1824	3.91	7.97	7.57	0.03	14	733	68.0	117	15.6	3.20	0.42
A10-16-S	1824	3.91	7.97	7.57	0.03	15	900	85.9	192	25.0	2.34	0.37
A17-S	1011	3.93	7.59	0.82	0.02	16	784	25.0	129	3.55	2.87	0.61
A18-S	1898	12.0	7.44	10.0	0.15	19	903	130	153	30.5	2.82	0.51
A19,20-S	1134	6.50	7.49	2.83	0.10	20	516	57.0	65.3	16.8	4.56	0.89
A1-3, A21-S	2492	19.4	7.3	14.3	0.11	21	855	100	127	20.0	1.85	0.70
A5, A22-S	1530	11.9	6.23	57.4	0.90	22	1062	90.2	169	27.1	3.13	0.63
A19,20-S=A23	1134	6.50	7.49	2.83	0.10	23	751	36.5	122	7.68	2.47	0.09
A1-3, 21, 24 S	2492	19.4	7.3	14.3	0.11	24	1192	57.0	248	15.1	1.52	0.10
A18-S=A27	1898	12.0	7.44	10.0	0.15	27	1173	28.0	192	28.1	2.17	0.31
A9-S=A28	4465	13.5	7.89	35.1	0.17	28	910	108	179	23.6	1.16	0.18
Forchtenstein												
F2-4S	80.9	0.46	5.83	5.94	0.052	2	691	79.6	126	19.5	2.24	0.53
F2-4S	80.9	0.46	5.83	5.94	0.052	3	606	32.4	72.1	3.13	3.23	0.31
F5-9S	92.8	0.37	4.74	3.50	0.032	6	681	65.7	83.1	12.7	4.10	0.56
F5-9S	92.8	0.37	4.74	3.50	0.032	7	570	21.7	60.1	4.28	4.40	0.19
F5-9S	92.8	0.37	4.74	3.50	0.032	8	917	98.3	114	19.1	4.04	0.80
F10-18S	89.0	0.59	4.3	8.03	0.049	10	804	47.4	96.6	16.6	2.90	0.52

F10-18S	89.0	0.59	4.3	8.03	0.049	11	897	70.3	118	9.48	3.29	0.62
F10-18S	89.0	0.59	4.3	8.03	0.049	12	707	58.0	79.3	13.9	3.68	0.35
F10-18S	89.0	0.59	4.3	8.03	0.049	13	659	26.2	93.4	3.32	3.45	0.23
F10-18S	89.0	0.59	4.3	8.03	0.049	14	797	110	125	26.4	2.75	0.41
F10-18S	89.0	0.59	4.3	8.03	0.049	17	633	11.5	85.1	2.85	3.74	0.49
F19-S	519*	0.50	5.14	76.45	0.028	19	632	37.5	61.8	4.76	3.20	0.29
F20-S	228*	0.79	7.47	0.15	0.003	20	575	48.4	57.5	9.65	4.37	1.00
Kutna Hora												
KH1-2 S	8603	72.00	6.42	82.99	1.206	1	924	29.2	133	9.55	1.74	0.38
KH1-2 S	8603	72.00	6.42	82.99	1.206	2	745	74.1	76.2	6.96	2.40	0.34
KH3-5, 21 S	2740	31.50	7.91	5.36	0.124	4	741	54.3	104	15.0	2.57	0.56
KH3-5, 21 S	2740	31.50	7.91	5.36	0.124	5	650	19.9	61.0	4.88	2.37	0.49
KH6 S	8220	84.00	7.14	14.65	0.378	6	862	61.5	110	17.0	1.66	0.36
KH7 S	630	4.00	7.58	1.94	0.024	7	921	59.9	117	11.3	2.34	0.25
KH8 S	5015	54.00	7.19	9.36	0.364	8	667	27.6	82.2	2.92	1.79	0.15
KH10, 22 S	4814	61.00	7.02	18.52	0.673	10	618	33.1	47.3	5.42	3.91	0.46
KH11-12S	4010	56.00	7.08	16.19	0.353	11	955	185	85.2	9.61	1.63	0.22
KH13 S	505	4.50	8.34	1.06	0.005	13	767	152	84.5	16.0	2.27	0.26
KH14 S	515	4.00	7.07	2.92	0.141	14	617	38.9	88.6	7.13	2.38	0.14
KH15, 23 S	1801	14.00	5.12	12.74	0.272	15	931	72.2	119	26.9	1.21	0.19
KH16-17 S	715	4.00	7.5	4.55	0.180	16	807	63.0	124	14.6	2.49	0.35
KH16-17 S	715	4.00	7.5	4.55	0.180	17	699	46.4	68.2	9.00	1.54	0.30
KH11-12S	4010	56.00	7.08	16.19	0.353	12	859	70.9	105	7.32	4.10	0.28
KH18 S	109	1.50	7.67	1.685	0.045	18	768	107	123	21.8	2.81	0.33
KH19-20	105	1.50	8.46	0.88	0.005	19	745	96.2	86.1	14.5	2.67	0.39
KH19-20	105	1.50	8.46	0.88	0.005	20	667	21.8	116	7.73	2.26	0.28
KH3-5, 21 S	2740	31.50	7.91	5.36	0.124	21	1021	31.1	252	12.5	1.44	0.13
KH10, 22 S	4814	61.00	7.02	18.52	0.673	22	996	59.7	215	35.6	1.68	0.55
KH15, 23 S	1801	14.00	5.12	12.74	0.272	23	1044	62.0	178	12.1	2.79	0.26
Völkermarkt												
V1-5	63.5	0.58	6.8	0.58	0.002	1	1042	296	172	78.9	2.60	0.87
V1-5	63.5	0.58	6.8	0.58	0.002	2	927	72.8	142	23.0	2.34	0.48
V1-5	63.5	0.58	6.8	0.58	0.002	3	963	33.5	145	8.70	2.67	0.21
V1-5	63.5	0.58	6.8	0.58	0.002	4	868	170	160	27.4	2.43	0.11
V1-5	63.5	0.58	6.8	0.58	0.002	5	1116	261	190	28.6	1.14	0.47
V6-11	74.4	0.46	6.7	0.72	0.010	7	925	158	154	34.8	2.56	0.64
V6-11	74.4	0.46	6.7	0.72	0.010	9	664	84.6	103	20.7	3.05	0.41
V6-11	74.4	0.46	6.7	0.72	0.010	10	939	76.1	147	16.8	2.63	0.26
V6-11	74.4	0.46	6.7	0.72	0.010	11	888	64.7	159	6.21	0.82	0.14
V12-21	58.4	0.45	6.5	1.40	0.001	12	794	22.7	117	9.19	2.55	0.31
V12-22	58.4	0.45	6.5	1.40	0.001	13	897	107	178	36.9	2.40	0.59
V12-23	58.4	0.45	6.5	1.40	0.001	14	766	37.9	110	9.39	3.21	0.26
V12-24	58.4	0.45	6.5	1.40	0.001	15	972	189	151	30.8	3.08	0.72
V12-25	58.4	0.45	6.5	1.40	0.001	16	606	32.3	82.0	1.33	4.62	0.10
V12-26	58.4	0.45	6.5	1.40	0.001	17	942	225	160	58.2	3.19	0.73
V12-27	58.4	0.45	6.5	1.40	0.001	18	914	62.9	196	20.6	1.14	0.28
V12-28	58.4	0.45	6.5	1.40	0.001	19	1064	192	160	28.8	1.87	0.75
V12-29	58.4	0.45	6.5	1.40	0.001	20	759	59.0	106	16.0	3.95	0.41
V12-30	58.4	0.45	6.5	1.40	0.001	21	1009	150	109	12.7	2.23	0.75

***)** these two Zn soil concentrations are probably high because of a nearby rusty and leaching fence.

Supplementary Table 2 Overview of the SSR marker characteristics

Locus	Allele Size [bp]	Primer Sequence 5` - 3`	Motif in *S. caprea*	Motif in *P. trichocarpa*	Chromsomal Position
ORPM_62	170-198	F: CGGAGTCAGCTTGAGGTAGC R: CGGCAATATTGAGGAGAATGA	$[AT]_{2}$.. $[T]_{8}$	$[AT]_{4}$..$[ATTTT]_{3}$	I 16.99 Mb
ORPM_312	177-207	F: GTGGGGATCAATCCAAAAGA R: CCCATATCAAACCATTTGAAAAA	$[CTT]_{5}$	$[CCT]_{6}$	VII 9.31 Mb
ORPM_446	220-258	F: GGGCTGCAGACAAATTAAGG R: TGGGACATGCTCCATGGTAT	$[CT]_{3}$..$[CT]_{4}$	$[CT]_{3}$..$[CT]_{4}$	XIV – 3.69 Mb
				Motif in *P. nigra*	
WMPS_12	146-155	F: TTTTTCGTATTCTTATCTATCC R: CACTACTCTGACAAAACCATC	$[CA]_{4}$	$[CA]_{4}$	VI 11.36 Mb
WMPS_14	205-237	F: CAGCCGCAGCCACTGAGAAATC R: GCCTGCTGAGAAGACTGCCTTGAC	[1]$[CAG]_{21}$	[1]$[CAG]_{28}$	V 12.53 Mb
WMPS_19	152-209	F: AGCCACAGCAAATTCAGATGATGC R: CCTGCTGAGAAGACTGCCTTGACA	[2]$[CAG]_{22}$	[2]$[CAG]_{38}$	V 12.53 Mb
WMPS_21	157-184	F: TGCTGATGCAAAAGATTTAG R: TTGGAACTTCAACATTCAGAT	$[GCT]_{27}$	$[GCT]_{45}$	II 5.05 Mb
				Motif in *S. burjatica*	
SB_24	127-153	F: ACTTCAATCTCTCTGTATTCT R: CTATTTATGGGTTGGTCGATC	[3]$[TG]_{9}$	[3]$[TG]_{9}$	XI 4.70 Mb
SB_38	91-151	F: CCACTTGAGGAGTGTAAGGAT R: CTTAAATGTAAAACTGAATCT	$[TG]_{27}$	$[TG]_{27}$	IX 10.38 Mb
SB_199	95-127	F: CTATTTGGTCTCAATCACCTT R: CTTTACCTCAGAAAATCCAGA	$[TG]_{15}$	$[TG]_{11}CG[TG]_{6}$	XV 9.74 Mb
				Motif in *S. lanata*	
gSIMCT024	96-124	F: CTCCCTTCACTTGCTCCAT R: TAATACCAGCCCTTAAAGAAG	$[CT]_{14}$	$[CT]_{10}$	X 14.27 Mb

[1]Complete complex motif in *S. caprea*: $[CAG]_{4}CAT[CAG]_{12}CAT[CAG]_{3}$ and in *P. nigra*: $[CAG]_{1}CAT[CAG]_{12}CAT[CAG]_{9}CAT[CAG]_{3}$

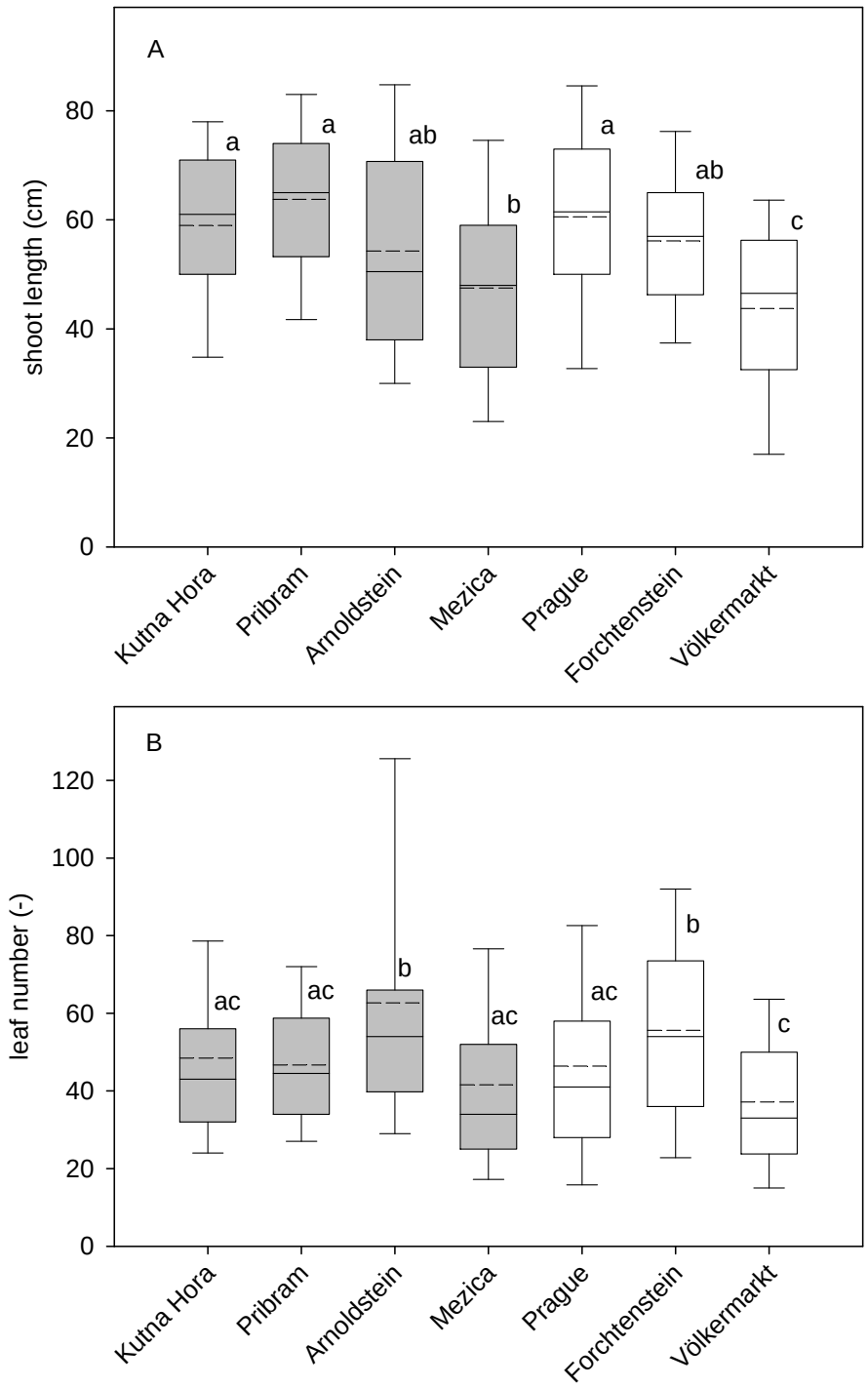
[2]Complete complex motif in *S. caprea*: $[CAG]_{2}CAT[CAG]_{8}CAT[CAG]_{10}$ and in *P. nigra*: $[CAG]_{3}CAT[CAG]_{8}CAT[CAG]_{8}CAT[CAG]_{4}CAA[CAG]_{3}$

[3]Complete complex motif in *S. caprea*: $[TG]_{9}A[TG]_{2}A[TG]_{4}$ and in *S. burjatica*: $[TG]_{9}A[TG]_{2}A[TG]_{4}..[TG]_{3}$

Supplementary Table 3 Heterozygosities, lnRH and normalized lnRH values of the 11 loci and seven populations. Loci with Norm.Θ values of larger +1.95 and smaller –1.95 have a significant reduced variability and thus might have been under selection and contribute to adaptation in the contamination area.

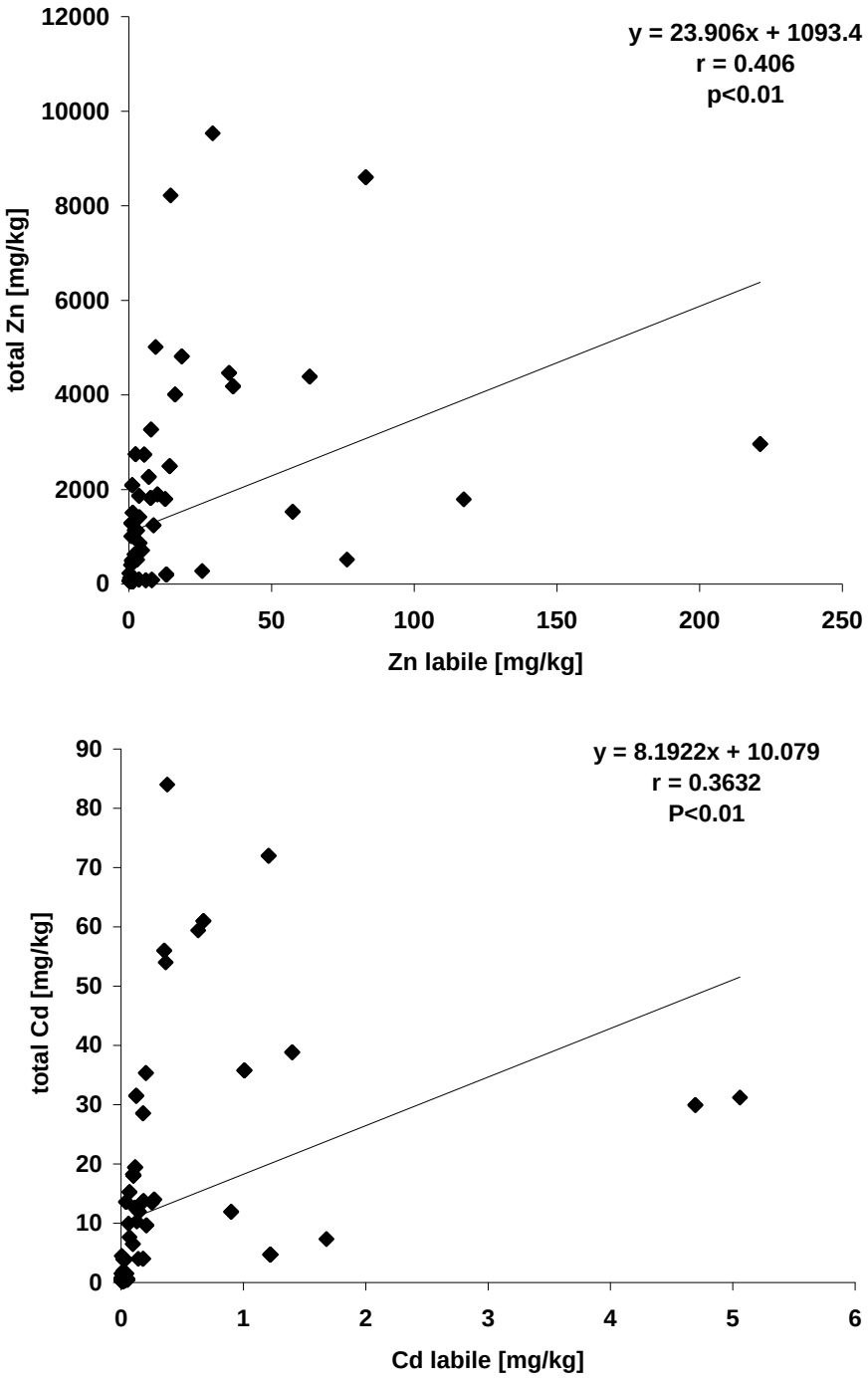
Locus	H_{obs}	H_{exp}	Populations							μ_c	μ_u	lnRH Θ	Norm.Θ
			A	M	V	F	KH	PR	P				
ORPM_62	0.49	0.74	0.79	0.55	0.67	0.70	0.75	0.78	0.71	0,72	0,69	0,18	-0,30
ORPM_312	0.81	0.82	0.85	0.82	0.84	0.73	0.88	0.77	0.74	0,83	0,77	0,63	0,79
ORPM_446	0.56	0.81	0.86	0.80	0.79	0.75	0.80	0.79	0.80	0,81	0,78	0,33	0,07
WMPS_12	0.34	0.33	0.25	0.44	0.09	0.38	0.41	0.50	0.20	0,40	0,22	0,99	1,68
WMPS_14	0.85	0.84	0.81	0.86	0.84	0.85	0.77	0.86	0.90	0,83	0,86	-0,51	-1,97*
WMPS_19	0.84	0.88	0.86	0.88	0.85	0.86	0.88	0.89	0.89	0,88	0,87	0,17	-0,32
WMPS_21	0.79	0.76	0.75	0.75	0.75	0.71	0.75	0.76	0.82	0,75	0,76	-0,07	-0,90
SB_24	0.89	0.77	0.77	0.82	0.79	0.79	0.79	0.78	0.67	0,79	0,75	0,37	0,16
SB_38	0.82	0.91	0.90	0.93	0.82	0.90	0.92	0.89	0.89	0,91	0,87	0,74	1,07
SB_199	0.61	0.76	0.80	0.79	0.79	0.81	0.78	0.65	0.65	0,76	0,75	0,04	-0,63
gSIMCT024	0.85	0.80	0.82	0.74	0.73	0.84	0.84	0.83	0.76	0,82	0,78	0,45	0,35
all loci	0.71	0.77	0.77	0.76	0.72	0.76	0.78	0.77	0.73				

* only significant without Bonferroni correction

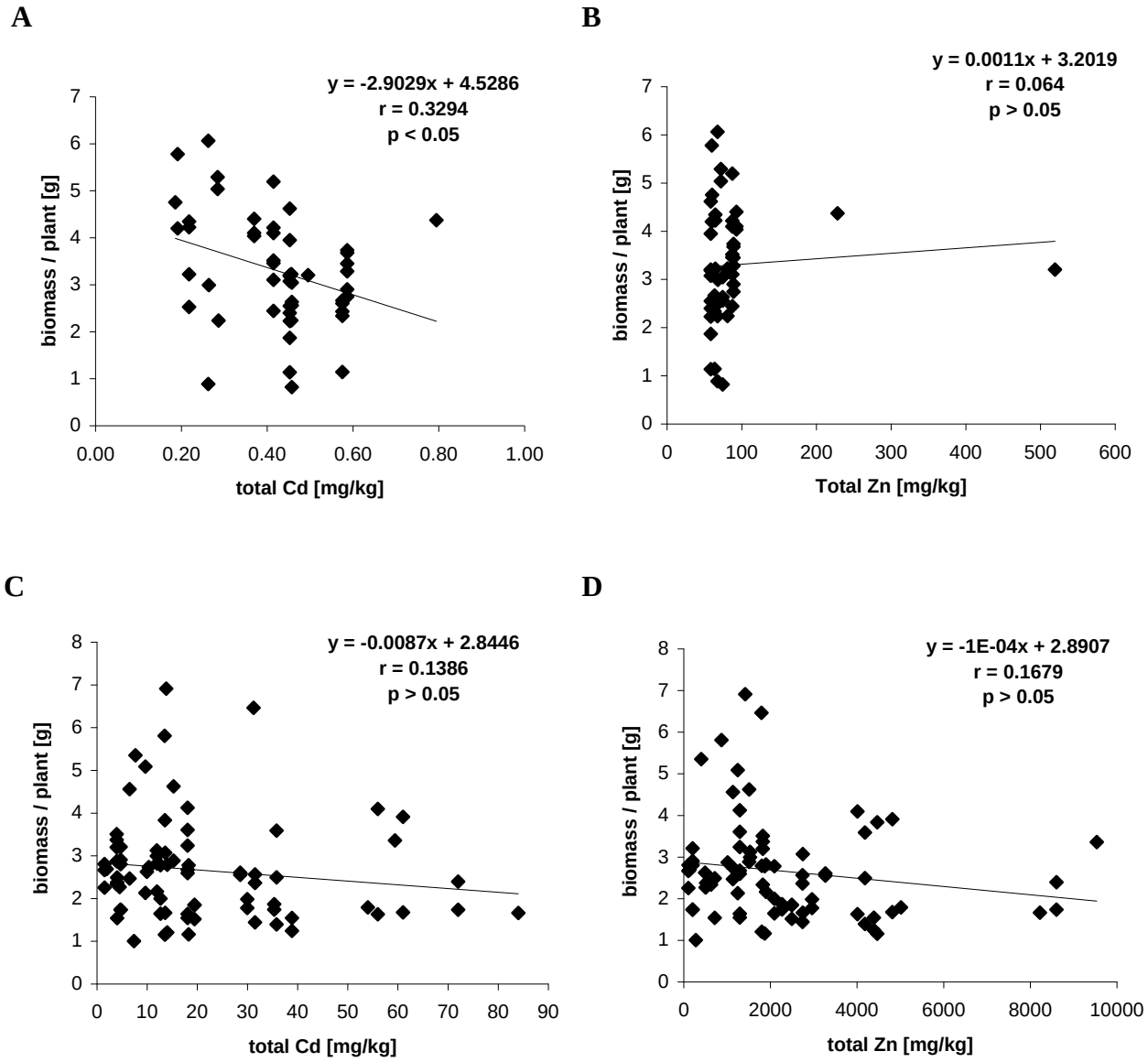


Supplementary Figure 1: Shoot length (A) and leaf number (B) of *S. caprea* isolates. Boxes represent the median (vertical solid line), the arithmetic mean (vertical dashed line), and 25 – 75 % percentile. Whiskers represent the 90^{th} and 10^{th} percentile. Significant differences were determined by a post hoc comparison of means (Scheffé test after nested analysis of variance; $p < 0.05$) and are indicated by different letters.

Supplementary Figure 2: Geographic map indicating the location of the *S. caprea* populations. South the Alps in Slovenia the contaminated population in Mežica – M, and in Austria the contaminated population Arnoldstein – (A) and the non-contaminated sites, Völkermarkt - V and Forchtenstein – F. North the Alps in the Czech Republic the two contaminated populations Příbram – PR and Kutná Hora – KH and the non-contaminated site near Prague (P).

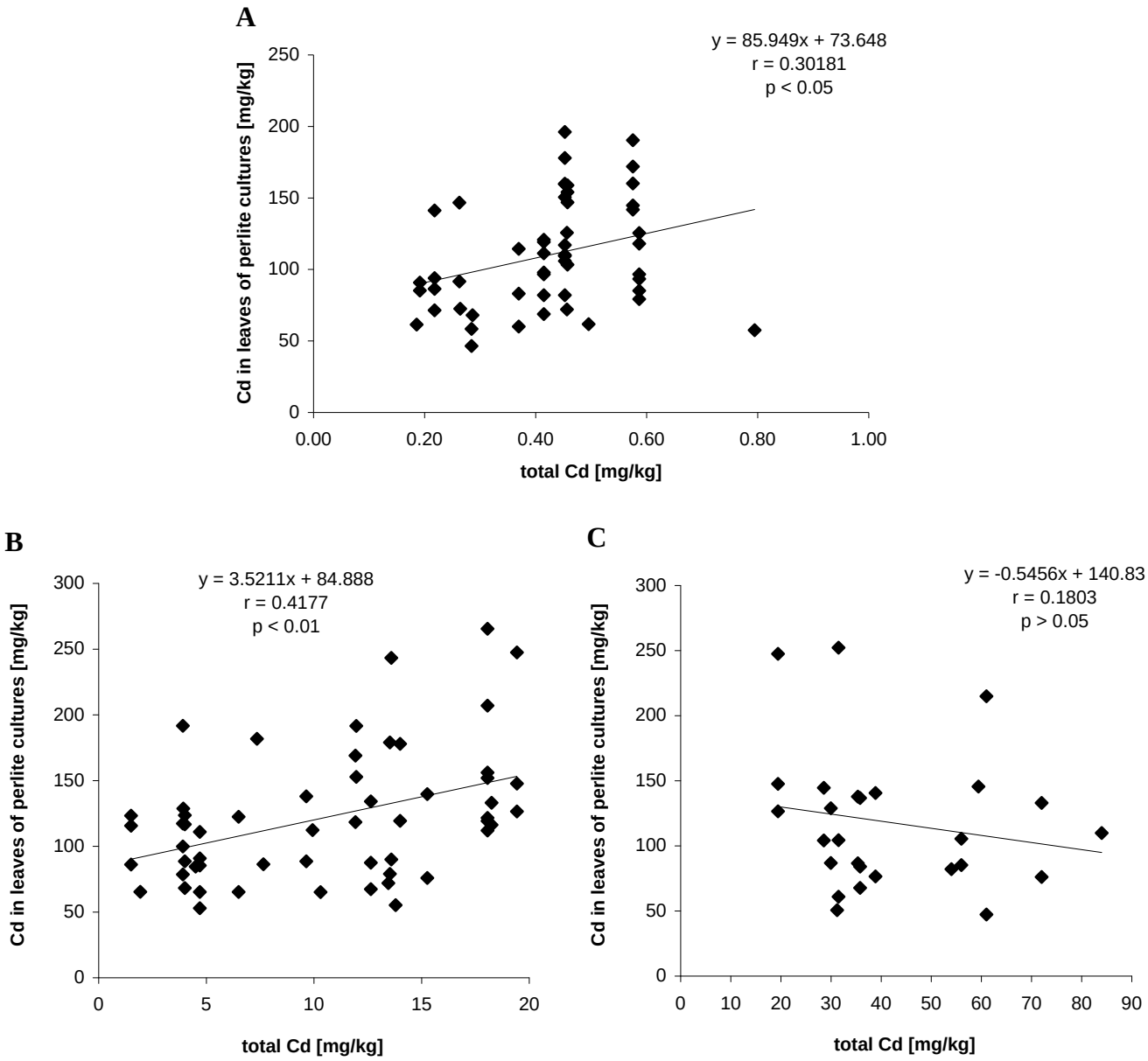


Supplementary Figure 3: The graphs show that the amount of labile Zn and Cd depends with a significance level for Pearson's correlation of $p<0.01$ on the total Zn and Cd concentration in the soil. In fact 40% of the variation of labile Zn and 36% for Cd are explained by the total Zn and Cd concentration, respectively. Other factors influencing the labile heavy metal fractions are pH, content of clay, carbonate and organic matter.



Supplementary Figure 4: Pearson's correlations between the level of contamination where the isolate originated and the biomass production in perlite cultures exposed to elevated levels of Cd and Zn. A and B are the graphs for the uncontaminated, C and D for the contaminated sites. The only significant negative correlation between Cd concentration and biomass production was seen at uncontaminated sites (A). Isolates from the contaminated sites did not show such a correlation indicating that they might have been selected to withstand higher Cd and Zn concentrations.

Note that two soil samples from the uncontaminated sites had very high Zn concentrations probably because of a nearby rusty fence that leached into the soil (B).



Supplementary Figure 5: Pearson's correlations between the level of soil contamination and Cd concentration in leaves after the exposure of the isolates in perlite to Cd and Zn. While a significant trend was found between soil contamination and accumulation capacity in perlite cultures for soil contamination below 20 mg kg^{-1} (A, B) above this soil Cd contamination level the trend diminished.