

Supplementary Material

Increase of bacterial community induced-tolerance to Cr in response to soil properties and Cr level in the soil

Claudia Campillo-Cora^a, Daniel Arenas-Lago^a, Manuel Arias-Estévez^a, David Fernández-Calviño^a

^a Departamento de Biología Vexetal e Ciencia do Solo, Facultade de Ciencias, Universidade de Vigo, As Lagoas s/n, 32004 Ourense, Spain

1. Soil samples characterization

Soil texture was determined after wet sieving and the international pipette method (Day, 1965). Soil pH was measured in a ratio of 1:2.5 soil/distilled water for pH_w and soil/0.1M KCl for pH_K by a glass electrode (Crison, Spain) (Gutián and Carballas, 1976). Organic matter content was determined by loss on ignition (3 h, 550°C) (Hoogsteen et al., 2015). Total carbon (TC) and nitrogen (TN) were determined using a Thermo Finnigan EA 1112 elemental analyzer (Thermo, USA). Dissolved Organic Carbon (DOC) was measured in a 1:5 soil:water suspension using an Analyzer Multi N/C 2100 (Analytik Jena, Germany). The effective cation exchange capacity (eCEC) was calculated as the sum of exchangeable basic cations (Na, K, Ca, and Mg), extracted with 0.2 M NH_4Cl (Sumner and Miller, 1996), and of exchangeable aluminium (Al), extracted with 1 M KCl (Bertsch and Bloom, 1996). Na and K were measured by Atomic Emission Spectroscopy, and Ca, Mg and Al by Atomic Absorption Spectroscopy (AAS) using a Thermo Soolar Spectrometer (Thermo, USA).

Fe and Al associated with organic matter (Fe_{om} , Al_{om}) were extracted by using a 0.1 M Na-pyrophosphate solution (Bascomb, 1968). Inorganic non-crystalline Fe and Al oxyhydroxides and metal-hummus complexes (Fe_{ox} , Al_{ox}) were extracted with 0.2 M ammonium oxalate-oxalic acid (Blakemore, 1978). Inorganic amorphous Fe and Al (Fe_{ia} , Al_{ia}) were calculated by the difference between Fe_{ox} and Fe_{om} and between Al_{ox} and Al_{om} , respectively. Total free Fe (Fe_t), including Fe-hummus complexes, non-crystalline and crystalline Fe oxyhydroxides were determined through extraction with Na-dithionite-citrate (Holmgren, 1967). To measure total free aluminium (Al_t), a solution of 0.5 M NaOH was used as extractant. (Fe, Al) bound to crystalline structures (Fe_c , Al_c) were calculated by the difference between Fe_t and Fe_{ox} and between Al_t and Al_{ox} , respectively. All (Fe, Al)-extracts were measured by AAS using a Thermo Soolar

Spectrometer. The total amount of Cr, Cu, Ni, Pb and Zn was determined by soil digestions with HNO₃, HF and HCl (Reed and Martens, 1996) in a MarsXpress microwave oven (CEM Corporation, USA) and measuring Cr, Cu, Ni, Pb and Zn by AAs. Adapted data from Campillo-Cora et al. (2021b).

Tables

Table S1. Parent material, particle size distribution, soil pH, Dissolved Organic Carbon (DOC), organic matter, effective cation exchange capacity (eCEC), total carbon and total nitrogen (Campillo-Cora et al., 2020)

Soil	Parent Material	Sand	Silt	Clay	pH _w	pH _K	DOC	OM	Ca	Mg	Na	K	Al	eCEC	C	N
		----- % -----	%	%			g·kg ⁻¹	%	----- cmol _c ·kg ⁻¹ -----	----- % -----	%					
1	Granite	71	13	16	4.0	3.0	0.62	13.6	1.9	1.0	0.1	0.2	4.8	8.0	7.0	0.5
2	Granite	62	19	19	4.6	3.8	0.70	14.1	3.4	1.2	0.2	1.6	2.7	9.1	6.7	0.4
3	Schist	50	25	25	4.8	4.2	0.33	11.6	1.9	0.4	0.2	0.2	3.2	5.8	8.2	0.6
4	Amphibolite	46	29	20	4.9	4.5	0.14	12.0	1.7	0.2	0.1	0.2	0.9	3.1	3.7	0.3
5	Granite	68	16	16	4.9	4.2	0.30	11.2	0.4	0.1	0.1	0.1	1.2	1.9	5.0	0.4
6	Limestone	19	67	14	6.3	5.8	0.56	14.4	21.4	1.2	0.7	0.2	<0.2*	23.4	6.5	0.5
7	Limestone	20	52	28	7.5	6.9	0.53	14.8	27.8	1.1	0.1	0.1	<0.2*	29.1	5.5	0.4
8	Amphibolite	32	37	32	5.0	4.5	0.27	10.0	2.1	0.4	0.3	0.2	1.1	4.1	6.7	0.4
9	Amphibolite	45	35	19	4.7	4.3	0.25	19.6	3.3	0.4	0.2	0.3	2.6	6.8	10.3	0.8
10	Amphibolite	31	45	24	4.9	4.4	0.44	29.1	3.7	0.4	0.4	0.5	1.9	6.9	14.3	1.0

Sand %, silt % and clay % are soil texture fractions; pH_w and pH_K are pH measured in water and 0.1 KCl, respectively; DOC is Dissolved Organic Matter; OM % is organic matter; Ca, Mg, Na and K are exchangeable bases, Al is exchangeable Al; eCEC is cation exchange capacity. C % and N % are total carbon and nitrogen, respectively.

*values below the limit of detection

Table S2. (Fe, Al) compounds in the 10 studied soils and heavy metal content (adapted from Campillo-Cora et al. (2021b))

Soil	Fe _{om}	Fe _{ia}	Fe _c	Al _{om}	Al _{ia}	Al _c	Cr	Cu	Ni	Pb	Zn
	g·kg ⁻¹										
1	0.80	0.05	0.53	1.19	0.22	0.39	8	6	5	28	52
2	2.09	<0.01	0.54	2.94	<0.01*	0.40	9	6	7	48	40
3	4.34	2.12	17.59	11.44	<0.01*	5.04	87	40	65	26	118
4	4.63	2.05	24.56	8.99	1.14	12.24	373	106	196	7	124
5	0.93	0.05	0.59	4.24	<0.01*	0.94	7	4	9	54	56
6	0.91	2.55	37.40	3.32	5.27	<0.01*	103	70	185	84	203
7	1.53	3.67	32.21	1.53	2.95	<0.01*	88	40	133	57	139
8	6.57	2.16	45.89	10.72	3.51	6.50	394	69	255	11	161
9	5.56	2.76	50.23	11.03	3.79	<0.01*	286	63	79	19	117
10	4.36	1.37	47.50	16.97	7.22	<0.01*	222	64	118	23	132

Fe_{om} and Al_{om} are (Fe, Al)-humus complexes; Fe_{ia} and Al_{ia} are (Fe, Al)-inorganic amorphous oxyhydroxides; Fe_c and Al_c are (Fe, Al)-crystalline oxyhydroxides

*values below the limit of detection

Table S3 Freundlich and Langmuir adsorption constants (Campillo-Cora et al., 2020)

Soil	Freundlich		Langmuir	
	K _F	n	β	K _L
1	186.19	0.31	753.40	0.16
2	135.83	0.34	758.18	0.06
3	61.29	0.43	574.40	0.03
4	41.40	0.45	448.96	0.03
5	91.22	0.44	858.56	0.04
6	25.78	0.76	2257.96	0.01
7	3.26	1.19		
8	85.36	0.45	944.39	0.03
9	68.00	0.47	897.99	0.02
10	109.12	0.44	1041.21	0.04

K_F is the Freundlich's affinity coefficient; n is Freundlich's linearity index; β is the maximum sorption capacity of the soil; K_L is the Langmuir's constant referred to adsorption energy

Table S4

Chromium extraction from studied soils (n=10) using H₂O, CaCl₂ and DTPA, as a function of the added Cr concentrations (Campillo-Cora et al., 2021a)

Added Cr (mg·kg ⁻¹)	H ₂ O Cr (mg·kg ⁻¹)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
2000	*	*	1312.3	40.2	517.9	32.2	179.2	147.9	124.4	31.5
1000	*	*	407.2	10.7	171.7	*	32.3	21.5	19.0	11.9
500	*	*	135.5	3.2	21.1	*	15.0	8.5	6.2	7.3
250	*	*	33.2	3.4	*	*	11.4	3.1	10.2	4.7
125	*	*	8.8	6.3	*	*	6.3	3.1	11.9	6.6
62.5	*	*	1.6	3.5	*	*	6.2	6.9	13.7	5.4
31.25	*	*	*	1.5	*	*	7.1	10.2	10.4	2.7
0	*	*	*	1.2	6.6	*	*	7.6	12.1	4.2
CaCl ₂ Cr (mg·kg ⁻¹)										
2000	*	*	881.2	14.1	195.3	7.1	88.1	39.8	29.9	5.3
1000	*	*	210.2	3.1	30.5	*	4.2	3.6	3.3	1.8
500	*	*	66.6	*	2.0	*	1.5	0.2	0.3	0.6
250	*	*	15.9	*	*	*	0.9	0.4	*	*
125	*	*	1.5	*	*	*	0.5	*	*	*
62.5	*	*	*	*	*	*	0.4	0.7	*	*
31.25	*	*	*	*	*	*	0.5	0.4	*	*
0	*	*	*	*	*	*	*	0.3	*	*
DTPA Cr (mg·kg ⁻¹)										
2000	5.6	5.3	420.0	10.4	170.9	4.3	30.9	22.8	26.3	7.0
1000	4.1	3.1	154.5	4.2	51.2	0.5	1.7	5.0	4.9	2.9
500	2.8	2.9	51.9	2.4	11.3	*	0.1	1.3	1.4	1.5
250	1.1	1.6	18.1	1.1	2.3	*	*	0.6	0.2	0.5
125	0.4	0.3	4.8	*	0.2	*	*	*	*	0.1
62.5	*	*	0.5	*	*	*	*	*	*	1.0
31.25	*	*	*	*	*	*	*	*	*	0.7
0	*	*	*	*	*	*	*	*	*	0.6

*values below the limit of detection (<0.02 mg·kg⁻¹ for H₂O and CaCl₂ Cr determinations, and <0.1 mg·kg⁻¹ for DTPA Cr)

Table S5Explained variance (R^2) from log IC_{50} fitting to the logistic model

Cr (mg·kg⁻¹)	2000	1000	500	250	125	62.5	31.25	0
S1	<i>0.99</i>	<i>0.99</i>	0.99	0.99	0.99	0.99	0.99	0.99
S2	<i>0.96</i>	<i>0.95</i>	0.96	0.96	0.94	0.96	0.98	0.99
S3	*	*	0.87	0.99	0.98	0.98	0.99	0.99
S4	<i>0.99</i>	<i>0.99</i>	0.99	0.99	0.99	0.99	0.99	0.99
S5	*	<i>0.99</i>	0.99	0.99	0.99	0.99	0.99	0.99
S6	*	<i>0.98</i>	0.99	0.99	0.97	0.98	0.99	0.99
S7	*	<i>0.99</i>	0.99	0.98	0.98	0.99	0.99	0.99
S8	<i>0.98</i>	<i>0.99</i>	0.99	0.99	0.99	0.99	0.99	0.98
S9	*	*	0.98	0.96	0.95	0.99	0.99	0.99
S10	*	*	0.98	0.99	0.99	0.98	0.99	0.99

*Unadjusted data

References

- Bascomb, C.L., 1968. Distribution of pyrophosphate-extractable iron and organic carbon in soils of various groups. *Journal of Soil Science* 19, 251–268. doi:10.1111/j.1365-2389.1968.tb01538.x
- Bertsch, P.M., Bloom, P.R., 1996. Aluminum, in: Bigham, J.M. (Ed.), *Methods of Soil Analysis Part 3. Chemical Methods*, Soil Science Society of America, Madison, WI, pp. 517–550.
- Blakemore, L.C., 1978. Exchange complex dominated by amorphous material (ECDAM), in: Smith, G.D. (Ed.), *The Andisol Proposal*. Soil Bureau, Lower Hutt, New Zealand, pp. 21–22.
- Campillo-Cora, C., Conde-Cid, M., Arias-Estévez, M., Fernández-Calviño, D., Alonso-Vega, F., 2020. Specific adsorption of heavy metals in soils: Individual and competitive experiments. *Agronomy* 10, 1113. doi:10.3390/agronomy10081113
- Campillo-Cora, C., Rodríguez-González, L., Arias-Estévez, M., Fernández-Calviño, D., Soto-Gómez, D., 2021a. Influence of physicochemical properties and parent material on chromium fractionation in soils. *Processes* 9, 1073. doi:10.3390/pr9061073
- Campillo-Cora, C., Soto-Gómez, D., Arias-Estévez, M., Bååth, E., Fernández-Calviño, D., 2021b. Bacterial community tolerance to Cu in soils with geochemical baseline concentrations (GBCs) of heavy metals: Importance for pollution induced community tolerance (PICT) determinations using the leucine incorporation method. *Soil Biology and Biochemistry* 155, 108157. doi:10.1016/j.soilbio.2021.108157
- Day, P.R., 1965. Particle Fractionation and Particle-Size Analysis. *Methods of Soil Analysis, Part 1: Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling* 545–567. doi:10.2134/AGRONMONOGR9.1.C43
- Gutián, F., Carballas, T., 1976. *Técnicas de análisis de suelos*. Pico Sacro, Santiago de Compostela, Spain.
- Holmgren, G.G.S., 1967. A rapid citrate-dithionite extractable iron procedure. *Soil Science Society of America Journal* 31, 212–215.
- Hoogsteen, M.J.J., Lantinga, E.A., Bakker, E.J., Groot, J.C.J., Tittonell, P.A., 2015. Estimating soil organic carbon through loss on ignition: Effects of ignition conditions and structural water loss. *European Journal of Soil Science* 66, 320–328. doi:10.1111/ejss.12224
- Reed, S.T., Martens, D.C., 1996. Copper and Zinc, in: Bigham, J. (Ed.), *Methods of Soil Analysis Part 3. Chemical Methods*, Soil Science Society of America, pp. 703–722.
- Sumner, M.E., Miller, W.P., 1996. Cation Exchange Capacity and Exchange Coefficients, in: Bigham, J.M. (Ed.), *Methods of Soil Analysis Part 3. Chemical Methods*, Soil Science Society of America, Madison, WI, pp. 1201–1230.