Mineral element profile in African penguin (*Spheniscus Demersus*) feathers and its possible relationship with molting

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Supplementary material

Table S1 Details of samples included in the study

Penguin	Date of Sampling	PRE/POST molt or TOT	Origin
Brie 1	02/05/23	PRE	Turin
Charlotte 1	02/05/23	PRE	Turin
Chocolate 1	02/05/23	PRE	Turin
Dred 1	02/05/23	PRE	Turin
Funny 1	02/05/23	PRE	Turin
Homer 1	02/05/23	PRE	Turin
Jerry 1	02/05/23	PRE	Turin
Joker 1	02/05/23	PRE	Turin
Kyr 1	02/05/23	PRE	Turin
Pepe 1	02/05/23	PRE	Turin
Violet 1	02/05/23	PRE	Turin
Brie 2	30/08/23	POST	Turin
Charlotte 2	30/08/23	POST	Turin
Chocolate 2	30/08/23	POST	Turin
Dred 2	30/08/23	POST	Turin
Funny 2	30/08/23	POST	Turin
Jerry 2	30/08/23	POST	Turin
Joker 2	30/08/23	POST	Turin
Kyr 2	30/08/23	POST	Turin
Pepe 2	30/08/23	POST	Turin
Violet 2	30/08/23	POST	Turin
Purple 1	12/04/23	PRE	Overloon
Grey 1	12/04/23	PRE	Overloon
Pink 1	12/04/23	PRE	Overloon
Black/Blue 1	12/04/23	PRE	Overloon
Green/Pink 1	12/04/23	PRE	Overloon
Purple/Blue 1	12/04/23	PRE	Overloon
Purple 2	29/06/23	POST	Overloon
Grey 2	29/06/23	POST	Overloon

Pink 2	29/06/23	POST	Overloon
Black/Blue 2	29/06/23	POST	Overloon
Green/Pink 2	29/06/23	POST	Overloon
Purple/Blue 2	29/06/23	POST	Overloon
Erna	-	COMPOSITE	Magdeburg
Fritz	-	COMPOSITE	Magdeburg
Helmut	-	COMPOSITE	Magdeburg
Ida	-	COMPOSITE	Magdeburg
Julius	-	COMPOSITE	Magdeburg
Klaus	-	COMPOSITE	Magdeburg
Lady	-	COMPOSITE	Magdeburg
Mr. Pebody	-	COMPOSITE	Magdeburg
Petra	-	COMPOSITE	Magdeburg

Table S1 Concentration of analytes (mg kg^{-1}) in feather samples part 1

Sample ID	Origin	Molt period	Al	As	Ba	Ca	Cd	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	Pb	Sr	Zn
1_M_C	Magdeburg	COMPOSITE	16.9	0.050	1.51	2930	0.020	9,99	28.6	397	2656	4.37	0.02	243	2.01	0.150	8.71	92.7
2_M_C	Magdeburg	COMPOSITE	8.59	0.050	2.46	3760	0.020	10.5	24.3	184	197	2.30	0.03	221	3.08	0.150	9.36	114
3_M_C	Magdeburg	COMPOSITE	5.10	0.050	3.79	3650	0.020	11.5	20.2	131	175	2.06	0.03	196	2.55	0.100	9.30	103
4_M_C	Magdeburg	COMPOSITE	7.00	0.050	3.72	3230	0.010	10.8	20.3	108	119	5.13	0.02	294	2.58	0.130	7.45	103
5_M_C	Magdeburg	COMPOSITE	10.3	0.050	2.36	3670	0.020	11.5	22.1	165	151	2.12	0.02	211	3.74	0.170	8.81	104
6_M_C	Magdeburg	COMPOSITE	5.79	0.050	2.34	2830	0.020	10.3	17.2	126	118	2.60	0.04	199	3.43	0.120	6.59	107
7_M_C	Magdeburg	COMPOSITE	4.33	0.050	2.53	2820	0.020	10.3	16.0	175	150	2.30	0.03	165	2.26	0.090	5.29	101
8_M_C	Magdeburg	COMPOSITE	9.77	0.050	3.25	4040	0.020	8.66	26.5	136	138	3.62	0.02	190	2.48	0.130	9.09	106
9_M_C	Magdeburg	COMPOSITE	5.83	0.060	2.87	4170	0.020	10.8	16.8	145	175	2.54	0.02	203	2.18	0.120	8.84	112
11_O_PRE	Overloon	PRE	16.7	0.350	3.93	2630	0.080	47.1	72.2	394	145	7.47	0.11	211	8.41	0.960	9.43	159
12_O_PRE	Overloon	PRE	32.7	0.150	3.08	4360	0.050	10.84	66.7	190	272	15.4	0.07	192	7.22	0.400	15.0	160
13_O_PRE	Overloon	PRE	17.2	0.100	7.68	8960	0.090	30.7	64.3	197	523	32.0	0.07	112	10.8	0.660	36.3	161
14_O_PRE	Overloon	PRE	64.6	0.190	3.81	9740	0.100	11.3	110	362	568	36.3	0.10	201	10.7	0.630	39.9	167
15_O_PRE	Overloon	PRE	34.2	0.120	1.56	4360	0.040	9.26	39.7	105	229	14.7	0.04	120	3.34	0.250	14.7	145
10_O_MID	Overloon	MID	21.5	0.080	1.23	176	0.030	9.51	40.1	158	129	4.68	0.03	247	3.57	0.300	3.57	93.1
10_O_POST	Overloon	POST	12.4	0.080	0.710	740	0.020	7.91	29.9	124	50.6	3.37	0.04	72l3	3.81	0.220	1.77	97.9
11_O_POST	Overloon	POST	6.99	0.080	0.410	650	0.010	6.50	23.6	<LOD	44,3	3.68	0.02	<LOD	1.86	0.210	1.44	98.5
12_O_POST	Overloon	POST	9.26	0.100	0.930	723	0.020	6.93	29.0	120	60.7	3.42	0.03	72.1	4.84	0.220	1.70	114
13_O_POST	Overloon	POST	7.61	0.100	0.980	389	0.030	6.24	35.3	1,7	< LOD	2.44	0.05	4.78	4.42	0.240	<LOD	90.9
14_O_POST	Overloon	POST	7.66	0.060	0.520	365	0.020	7.82	21.9	<LOD	30.7	1.14	0.03	<LOD	2.53	0.150	0.66	94.8
15_O_POST	Overloon	POST	28.8	0.080	0.860	655	0.020	7.11	35.4	<LOD	50.5	2.59	0.03	<LOD	2.77	0.160	1.78	103

Table S2 Concentration of analytes (mg kg^{-1}) in feather samples part 2

Sample ID	Origin	Molt period	Al	As	Ba	Ca	Cd	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	Pb	Sr	Zn
16_Z_PRE	Turin	PRE	14.6	0.070	8.84	3200	0.080	18.8	0.030	134	562	7.35	30	103	35.6	0.940	12.6	156
16_Z_POST	Turin	POST	16.4	0.060	2.03	2500	0.040	8.06	0.060	297	477	5.88	0,06	191	8.79	0.250	7.72	166
17_Z_PRE	Turin	PRE	9.92	0.060	6.22	3550	0.090	10.9	0.050	565	660	5.56	0.050	186	32.5	0.540	11.8	180
17_Z_POST	Turin	POST	17.1	0.070	2.80	1930	0.100	13.2	0.050	284	405	3.20	0.050	124	47.9	0.340	6.66	116
18_Z_PRE	Turin	PRE	7.93	800	3.33	2180	0.060	22.8	16.4	261	393	3.58	0.030	126	54.8	0.430	8.81	132
18_Z_POST	Turin	POST	9.65	0.060	4.96	1620	0.040	13.3	19.4	264	423	1.65	0.050	195	65.2	0.430	5.70	112
19_Z_PRE	Turin	PRE	21.4	0.080	7.59	12200	0.090	10.6	0.040	420	1380	24.5	0.0400	359	33.9	0.460	53.4	178
19_Z_POST	Turin	POST	12.4	0.080	4.61	2800	0.060	11.5	0.130	215	664	3.14	0.130	151	14.1	0.340	9.77	153
20_Z_PRE	Turin	PRE	32.3	0.0800	7.14	4160	0.090	18.9	46.8	166	716	9.39	0.040	84.4	87.5	0.290	16.2	149
20_Z_POST	Turin	POST	4.84	0.08	3.12	2950	0.050	18.9	28.5	146	649	2.59	0.030	100	48.6	0.210	10.3	182
21_Z_PRE	Turin	PRE	12.6	0.040	1.88	2170	0.060	14.2	0.030	117	394	3.44	0.030	87.1	27.2	0.210	7.06	108
22_Z_PRE	Turin	PRE	58.4	0.070	8.25	3660	0.160	24.42	0.030	105	640	8.14	0.030	56.1	98.1	0.530	13.7	119
22_Z_POST	Turin	POST	8.30	0.1000	5,09	2230	0.100	20.0	0.060	145	536	1.72	0.060	227	39.0	0.490	8.29	142
23_Z_PRE	Turin	PRE	30.3	0.110	5.19	3150	0.110	14.3	0.070	306	706	5.08	0.070	223	39.4	0.500	12.5	143
23_Z_POST	Turin	POST	15.6	0.080	2.11	1530	0.070	10.7	0.100	263	407	<LOD	0.100	184	580	0.310	4.17	152
24_Z_PRE	Turin	PRE	62.5	0.110	18.3	1040	0.27	19.9	64.9	182	1150	14.8	0.030	98.7	86.5	0.630	42.3	17
24_Z_POST	Turin	POST	19.0	0.2000	9.79	2070	0.13	37.9	0.150	200	377	2.60	0,.150	156	20.3	0.850	5.15	153
25_Z_PRE	Turin	PRE	14.1	0.100	16.4	9320	0.13	32.6	0.040	346	969	12.8	0,04	224	82.4	0.860	43.0	140
25_Z_POST	Turin	POST	3.12	0.060	2.19	2890	0.03	100	16.6	367	526	5.00	0.060	132	14,7	0.230	8.98	153
26_Z_PRE	Turin	PRE	65.1	0.100	7.18	3680	0.12	35.2	78.2	144	599	10.1	0.030	83.9	137	1.05	11.0	132
26_Z_POST	Turin	POST	6.82	0.080	4.22	2410	0.05	14.0	19.3	255	6324	1,92	0.040	146	27.2	0.240	8.16	149

Table S3 Summary of data on elements in penguin feathers: maximum, minimum. The following groups are address: Turin and Overloon pre and post molt; all data

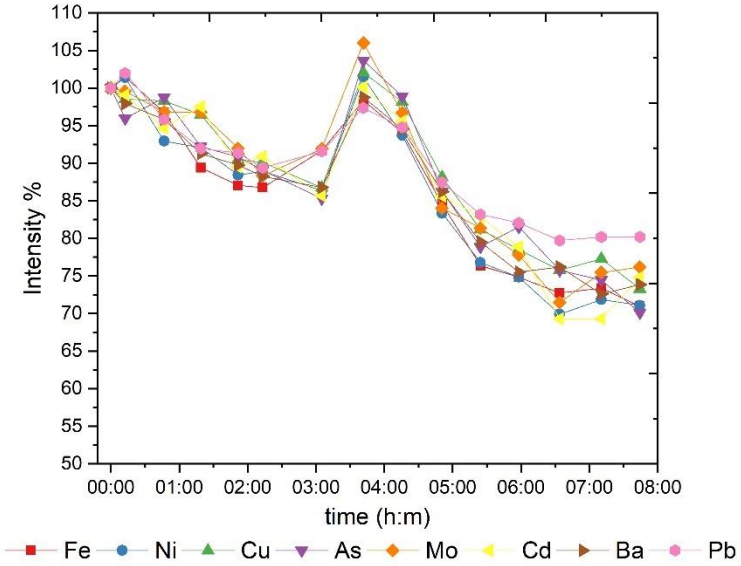
	Al	**As**	**Ba**	**Ca**	**Cd**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**Pb**	**Sr**	**Zn**
Max total	65.1	0.345	18.30	12200	0.274	47.1	110	565	1385	36.4	0.153	359	137	1.05	53.4	182
Min total	3.12	0.037	0.408	365	0.012	6.24	0.026	1.70	30.7	1.14	0.020	4.78	1.86	0.094	0.655	90,9
Turin_max pre molt	65.1	0.114	18.30	12200	0,274	35.1	78.2	565	1380	26.5	0.071	359	137	1.05	53.4	180
Turin_max post molt	19.0	0.197	9.79	2950	0.164	37.9	35.4	367	664	5.88	0.153	227	66.7	0.848	10.3	182
Turin_min premolt	7.93	0.037	1.87	2170	0.056	10.6	0.026	105	393	3.44	0.026	56.1	27.1	0.214	7.06	108
Turin_min post molt	3.12	0.056	2.02	1530	0.031	8.06	0.047	145	377	1.65	0.031	100	5,8	0.212	4.17	112
Overloon_max pre molt	64.6	0.345	7.67	9740	0.099	47.1	110	395	568	36.4	0.114	211	10.8	0.958	39.9	167
Overloon_max post molt	28.8	0.100	0.979	740	0.028	7.91	35.4	125	60.7	3.68	0.047	72.2	4.84	0.235	1.78	114
Overloon_min premolt	16.7	0.083	1.56	2630	0.045	9.26	39.7	105	145	7.47	0.036	112	3.34	0.246	9.43	117
Overloon_min post molt	6,.99	0.060	0.408	365	0.012	6.24	21.9	1.70	30.7	.,14	0.020	4.78	1.86	0.147	0.655	90.9

Table S5 Element concentrations in the quality control standard solution used for standard–sample bracketing correction during ICP-OES and ICP-MS analyses.

Element	Analytical technique	Concentration (μg l^{-1})
Al	ICP-OES	300
As	ICP-MS	1.5
Ba	ICP-MS	20
Ca	ICP-OES	10000
Cd	ICP-MS	0.5
Cu	ICP-MS	40
Fe	ICP-MS	200
K	ICP-OES	400
Mg	ICP-OES	1500
Mn	ICP-OES	50
Mo	ICP-MS	0.5
Na	ICP-OES	500
Ni	ICP-MS	100
Pb	ICP-MS	1.5
Sr	ICP-OES	50
Zn	ICP-OES	400

Table S6 Average Standard deviation, RSD% and LOD for the element analyzed. Values are expressed in mg kg^{-1}. The analytical technique and the isotope or analytical line used for the quantification are also reported.

Element	Analytical technique	Isotope (m/z) or analytical line (nm)	Standard Deviation	RSD%	LOD (µg l^{-1})
Al	ICP-OES	396.153	2.130	17	0.018
As	ICP-MS	75	0.010	12	0.002
Ba	ICP-MS	137	0.900	24	0.015
Ca	ICP-OES	317.933	377	11	0.190
Cd	ICP-MS	113	0.010	18	0.006
Cu	ICP-MS	63	1,6	13	0.050
Fe	ICP-MS	57	5.16	22	0.015
K	ICP-OES	766.457	57.2	22	1.36
Mg	ICP-OES	285.213	23.5	9	0.040
Mn	ICP-OES	257.61	0.830	15	0.002
Mo	ICP-MS	95	0.010	19	0.003
Na	ICP-OES	589.592	36.0	18	2.21
Ni	ICP-MS	61	3.62	22	0.004
Pb	ICP-MS	206	0.060	20	0.037
Sr	ICP-OES	407.771	1.45	15	0.001
Zn	ICP-OES	206.200	4.79	15	0.007



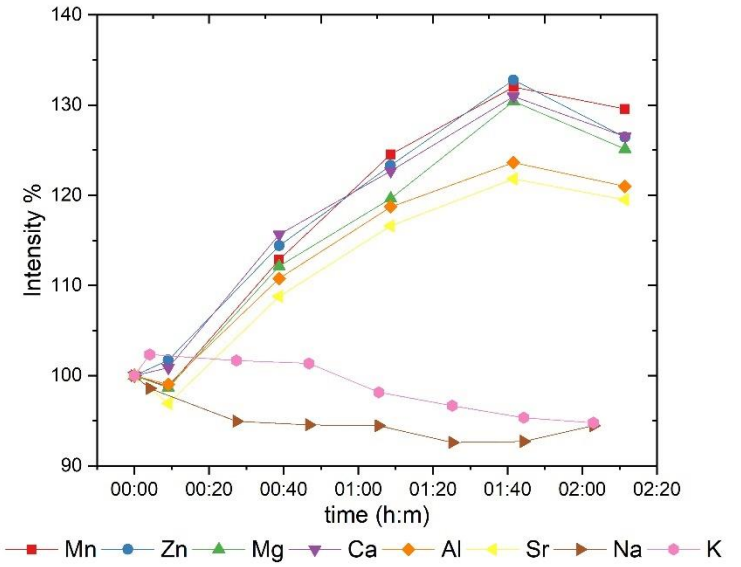
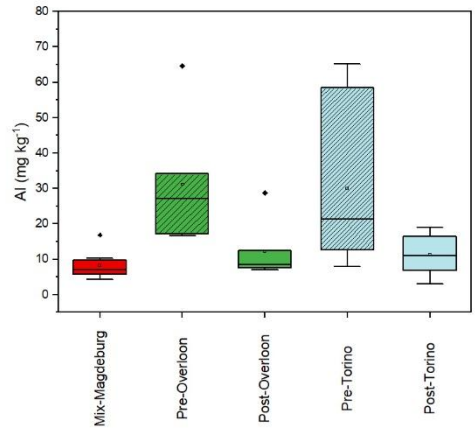
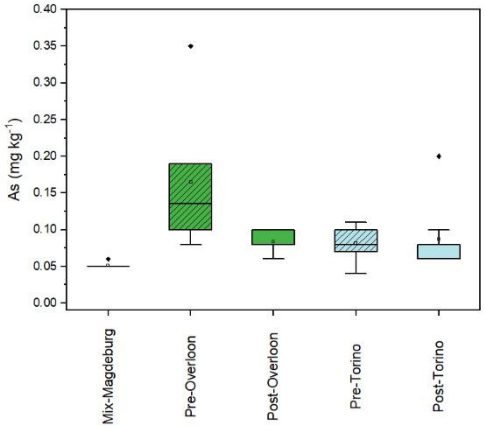
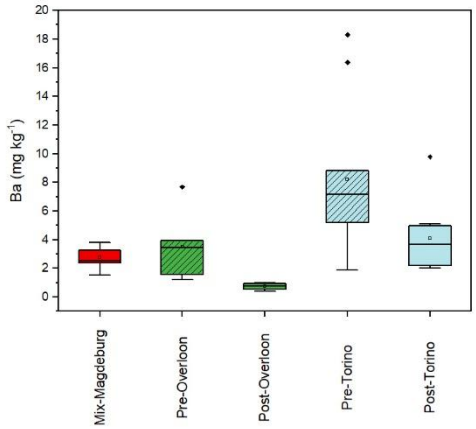
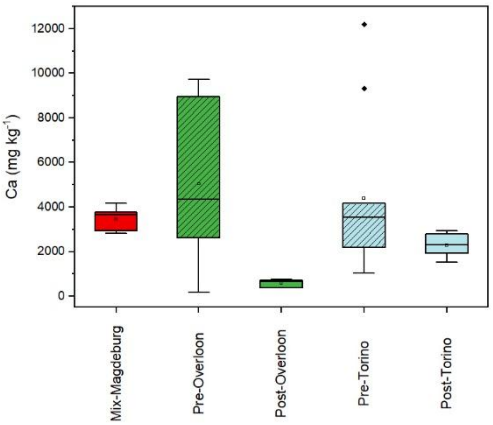


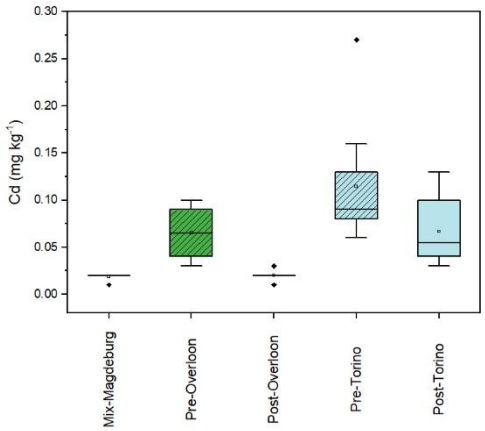
Figure S1 Relative intensity of the quality control standard solution during sample analysis by ICP-MS (top) and ICP-OES (bottom). The quantification of Na and K was performed using a different set of measurements compared to the other elements quantified by ICP-OES; therefore, the drift in signal intensity was different.

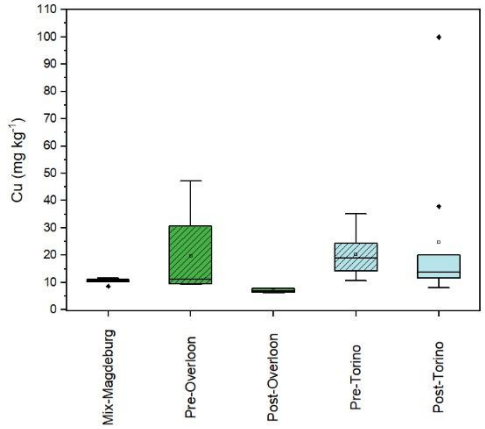

Al (mg kg-1)
0
10
20
30
40
50
60
70
80
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

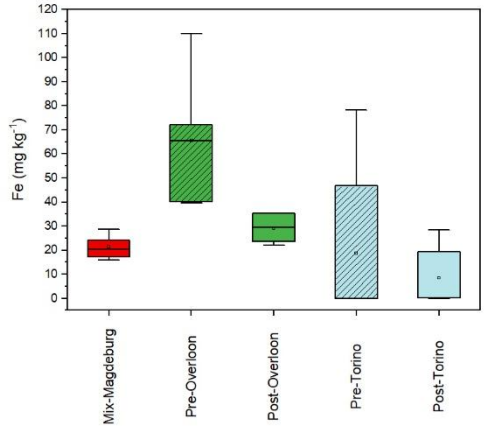

As (mg kg-1)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

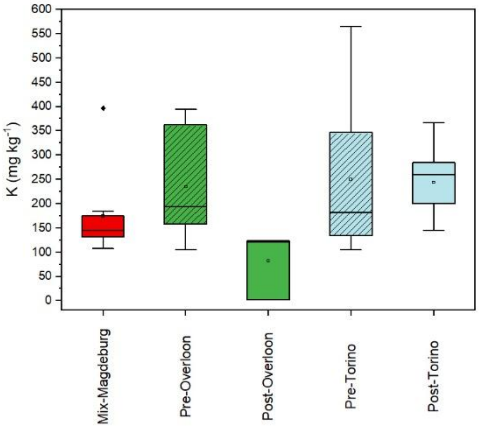

Ba (mg kg-1)
0
2
4
6
8
10
12
14
16
18
20
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

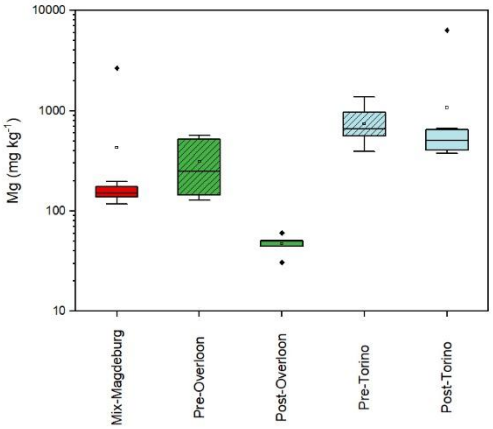

Ca (mg kg-1)
0
2000
4000
6000
8000
10000
12000
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

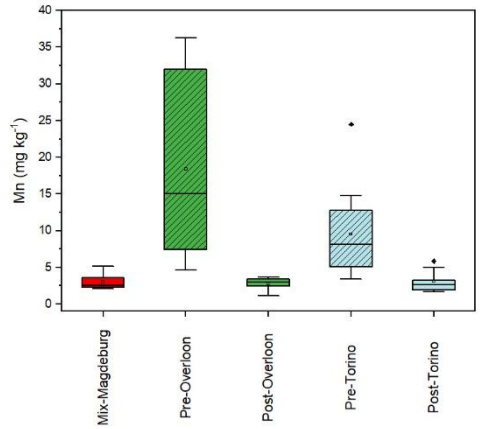

Cd (mg kg-1)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

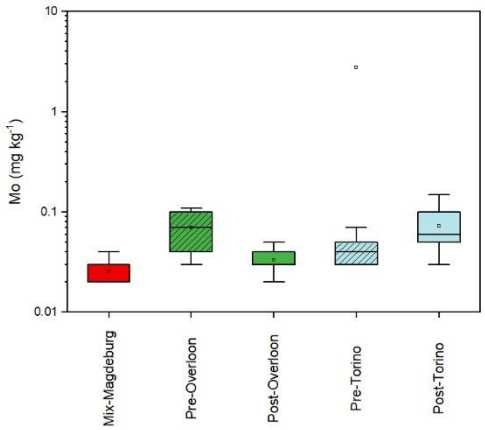

Cu (mg kg-1)
0
10
20
30
40
50
60
70
80
90
100
110
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

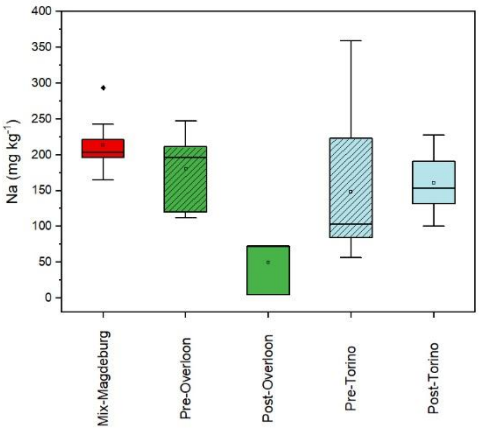

Fe (mg kg-1)
120
110
100
90
80
70
60
50
40
30
20
10
0
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino


K (mg kg-1)
600
550
500
450
400
350
300
250
200
150
100
50
0
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino


Mg (mg kg-1)
10000
1000
100
10
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino


Mn (mg kg-1)
40
35
30
25
20
15
10
5
0
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino


Mo (mg kg-1)
10
1
0.1
0.01
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino


Na (mg kg-1)
400
350
300
250
200
150
100
50
0
Mix-Magdeburg
Pre-Overloon
Post-Overloon
Pre-Torino
Post-Torino

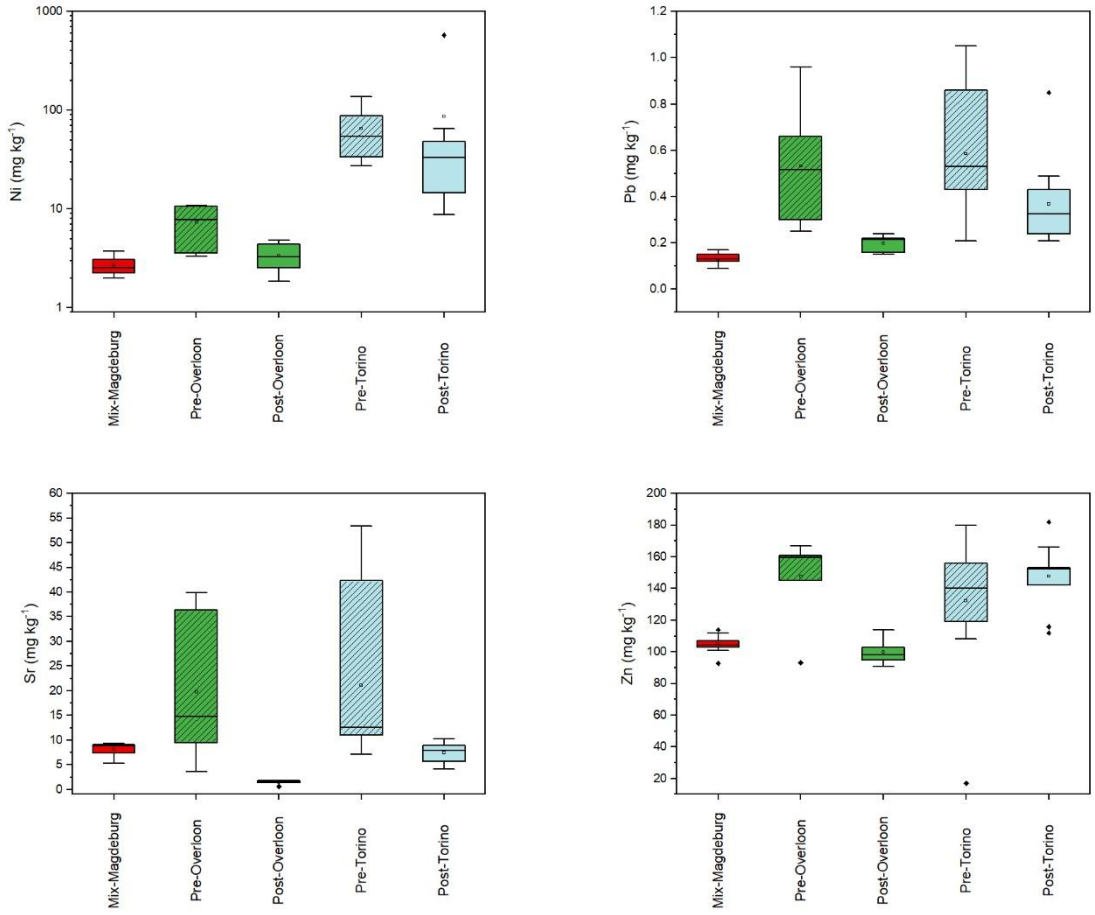


Figure S2 Boxplot of all the variables taken into account

Standard-sample bracketing correction: theoretical aspects

Instrumental signal drift during ICP-OES and ICP-MS analyses was corrected by periodic reanalysis of a selected calibration standard (S_i). For each analyte, the variation in signal intensity measured between two consecutive acquisitions of the standard solution (S_i and S_{i+k}) was used to derive a correction factor ($F_{j,}\ with\ i < j < i + k$), assuming a linear temporal drift of the instrumental response between the two standard measurements. The correction factor was calculated according to Equation (1):

$$F_j = \frac{1}{S_0} \times \left[\left(\frac{S_{i+k} - S_i}{k}\right) \times j - \left(\frac{S_{i+k} - S_i}{k}\right) \times i + S_i\right] \quad (1)$$

where i and j denote the positions in the analytical sequence of the standard measurement (with signal intensity S_i) and the sample measurement (with signal intensity X_j), respectively; S_0 represents the signal intensity of the calibration standard; k and k is the number of samples analyzed between two successive standard measurements in the analytical sequence. This value was selected on the basis of the analytical run time per sample, so as to ensure that consecutive measurements of the standard solution were carried out at time intervals not exceeding 30 min.

The correction factor F_j was subsequently applied to the signal intensity X_j of the sample measured at the j-*th* position in the analytical sequence, as expressed in Equation (2):

$$X_0 = \frac{X_j}{F_j} \quad (2)$$

The corrected intensity X_0 was finally used to determine the analyte concentration in the solution by mean of the calibration curve.