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Trace elements in human follicular fluid: development of a sensitive multielement ICP-MS method for use in biomonitoring studies

Supplementary Information

Table S1. Method validation data for Q-ICP-MS based on repeated analyses of archived serum RMs from EQA/PT programs.

Centre de toxicologie du Québec (CTQ), Québec Multielement EQA Scheme (QMEQAS)										
Analyte	QMEQAS 06S-06 (n = 32)					QMEQAS 07S-04 (n = 32)				
	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value
Be	18.5	1.2	20	6.2	>0.05	4.65	0.47	5.1	1.5	>0.05
Mo	1.08	0.18	1.1 ^a	0.4	>0.05	9.98	0.67	9.8 ^a	0.4	>0.05
Cd	1.44	0.24	1.5	0.1	>0.05	0.6	0.1	0.6	0.1	>0.05
Sn	2.14	0.21	2.3	0.4	>0.05	4.11	0.41	4.0	0.5	>0.05
Sb	0.23	0.077	0.2	0.03	>0.05	3.74	0.37	3.6	0.2	>0.05
Ba	1.5	0.25	1.4	0.3	>0.05	13.6	0.9	14	0.9	>0.05
Pt	0.2	0.067	<0.2	--	--	0.4	0.133	0.3	0.2	>0.05
Hg	2.3	0.23	2.0	0.4	0.0190	22.8	1.5	23	5.3	>0.05
Tl	0.77	0.129	0.8	0.04	>0.05	6.21	0.42	6.3	0.2	>0.05
Pb	8.2	0.55	8.7	0.3	0.0001	41.9	2.8	44	1.1	0.0034
U	0.3	0.1	0.3	0.01	>0.05	0.44	0.147	0.4	0.02	>0.05
Mn	3.97	0.4	4.0	0.3	>0.05	1.38	0.23	1.5	0.3	>0.05
Co	3.08	0.31	3.1	0.5	>0.05	0.8	0.134	0.7	0.1	0.0043
Cu	1890	123	1920	91	>0.05	1550	103	1610	82	0.0291
Zn	1940	128	2000	100	0.0432	1460	99	1550	87	0.0029
QMEQAS 08S-06 (n = 31)										
Analyte	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	QMEQAS 09S-04 (n = 31)				
	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value
Be	13.5	0.9	14	3.9	>0.05	3.62	0.36	3.8	1.1	>0.05
Mo	3	0.3	2.9	0.8	>0.05	4.1	0.41	4.2 ^b	0.3	>0.05
Cd	1.97	0.33	2.1	0.1	0.0459	6.13	0.41	6.3	0.2	>0.05
Sn	1.59	0.27	1.7	0.6	>0.05	6.3	0.42	6.3	0.6	>0.05
Sb	1.57	0.26	1.5	0.1	>0.05	2.61	0.26	2.5	0.1	0.0452
Ba	6.77	0.45	6.8	0.5	>0.05	2.76	0.28	2.7	0.4	>0.05
Pt	0.716	0.12	0.6	0.3	>0.05	0.43	0.143	0.3	0.2	>0.05
Hg	10.6	0.7	10	2.4	>0.05	3.55	0.36	2.9	0.5	0.0003
Tl	0.39	0.13	0.4	0.03	>0.05	2.37	0.24	2.5	0.1	0.0124
Pb	118	8	125	2.9	0.0001	198	13	200	4.8	>0.05
U	0.65	0.109	0.7	0.02	0.0166	0.11	0.037	0.1	0.01	>0.05
Mn	5	0.5	5.0	0.6	>0.05	1.75	0.29	1.8	0.3	>0.05
Co	1.7	0.28	1.7	0.3	>0.05	0.7	0.117	0.7	0.1	>0.05
Cu	2120	146	2210	110	0.0137	1180	81	1240	56	0.0079
Zn	2430	167	2600	140	0.0003	1230	82	1270	67	>0.05

^an = 31; ^bn = 30

New York State Department of Health PT Program															
Analyte	NYS PT SE03-05 (n = 29)					NYS PT SE03-03 (n = 29)					NYS PT SE03-02 (n = 29)				
	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value
Mo ^c	0.9		0.6 ^d	0.2	--	4		4.0 ^d	0.1	--	7.3		7.6	1.1	--
Cd ^c	NA ^e		--	--	--	3.4	0.16	3.4	0.2	>0.05	6.81	0.54	6.9	0.2	>0.05
Sn ^c	0.7		0.3	0.3	--	3.7		3.7	4.0	--	6.9		7.0	0.7	--
Tl ^c	NA		--	--	--	1.7	0	1.7	0.1	--	3.4	0.05	3.4	0.1	>0.05
Pb ^c	NA		--	--	--	16	0.86	16.7	0.4	0.0147	33.3	0.48	33.8	0.9	>0.05
Mn ^c	0.6		0.7	0.2	--	4.2	0.63	4.1	0.4	--	7.5	0.97	7.4	1.4	>0.05
Co ^c	0.7		0.4	0.1	--	1.4	0.32	1.1	0.1	0.0005	2.23	0.4	1.9	0.2	0.0188
Cu	1113	46.9	1120	50	0.0416	1444	52.5	1470	69	0.0081	1950	79.1	1960	82	0.0086
Zn	794.4	47.5	800	39	0.0010	1596	100.2	1600	79	0.0001	2336	169.9	2350	100	0.0008

^cData quality are dependent on characterization by small numbers of program participants (e.g., n = 2 for Mo, Sn; n = 3 for Cd); ^dn = 28;

^eNA = target value not assigned

Table S2. Method validation data for Q-ICP-MS based on analysis of SRMs and commercial QC materials.

NIST SRMs										
Analyte	NIST SRM 1598a Animal Serum (n = 31)					NIST SRM 1640 Natural Water (n = 26)				
	Certified (µg/L)	U ^a (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Certified (µg/L)	U ^a (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)
Be	NA ^b	--	--	--	--	34.94 ± 0.41	--	36	7.2	102
Rb	274 ± 19	--	270	17	100	2.00 ^c ± 0.02	--	<10	--	--
Sr	NA	--	--	--	--	124.2 ± 0.7	--	120	4.3	98
Mo	5.5 ^c ± 1.0	4.3 ^d	4.3	0.4	79	46.75 ± 0.26	--	46	1.8	99
Cd	0.048 ± 0.004	<0.08	<0.08	--	--	22.79 ± 0.96	--	23	0.8	103
Sb	1.00 ± 0.15	1.0	1.0	0.1	99	13.79 ± 0.42	--	13	0.7	92
Cs	0.64 ± 0.10	0.7	0.7	0.1	105	NA	--	--	--	--
Ba	NA	--	--	--	--	148.0 ± 2.2	--	140	8.9	96
Hg	0.32 ^c ± 0.19	0.1	0.1	0.2	34	NA	--	--	--	--
Tl	0.033 ^e	--	0.04	0.01	130	<0.1 ^e	--	0.01	0.004	--
Pb	NA	--	--	--	--	27.89 ± 0.14	--	31	1.0	110
Mg	NA	--	--	--	--	5828 ^c ± 56	--	5830 ^f	360	100
Mn	1.78 ± 0.33	2.3	2.3	0.3	132	121.5 ± 1.1	--	120	9.1	99
Co	1.24 ± 0.07	1.2	1.2	0.2	95	20.28 ± 0.31	--	20	1.6	97
Cu	1580 ± 90	1570	1570	73	100	85.2 ^c ± 1.2	--	95	5.0	112
Zn	880 ± 24	900	900	54	102	53.2 ^c ± 1.1	--	59	2.6	110

^aExpanded measurement uncertainty (k = 2, ± 95% confidence interval); ^bNA = certified value not assigned; ^cUncertified reference value; ^dn = 30; ^eInformational value; ^fn = 21

Commercial QC Materials															
Analyte	ClinChek Level 1 (n = 31)					ClinChek Level 2 (n = 31)					HPS CRM Bovine Liver Solution (n = 26)				
	Target (µg/L)	Range (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Target (µg/L)	Range (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Certified (µg/L)	U (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)
Be	0.970	0.679-1.26	0.06	0.2	7	20.5	16.4-24.6	21	6.1	101	NA	--	--	--	--
Rb	NA ^h	--	--	--	--	NA	--	--	--	--	200 ± 1	--	210	14	103
Mo	1.08	0.756-1.40	1.0 ⁱ	0.2	89	5.22	3.92-6.53	5.5 ⁱ	0.3	106	NA	--	--	--	--
Cd	0.233	0.163-0.303	0.2	0.1	104	4.42	3.32-5.53	4.1	0.2	93	3 ± 0.015	--	3.1	0.2	104
Sn	0.980	0.686-1.27	0.7	0.3	70	77.2	61.8-92.6	84	8.8	109	NA	--	--	--	--
Sb	6.18	4.94-7.42	5.9	0.3	95	9.58	7.66-11.5	9.6	0.6	100	NA	--	--	--	--
Pt	9.70	7.76-11.6	9.8	0.6	101	153	122-184	160	9.0	106	NA	--	--	--	--
Ba	NA	--	--	--	--	NA	--	--	--	--	10 ± 0.05	--	9.9	0.8	99
Hg	1.98	1.39-2.57	1.7	0.3	86	11.0	8.80-13.2	9.4	2.0	85	NA	--	--	--	--
Tl	1.32	0.990-1.65	1.5	0.1	111	4.27	3.42-5.12	4.8	0.2	111	NA	--	--	--	--
Pb	NA	--	--	--	--	NA	--	--	--	--	3 ± 0.015	--	3.8	0.6	128
Mg (mg/L)	12.5	11.3-13.8	12	1.3	98	26.1	23.5-28.7	21	3.7	79	6000 ± 30	--	5970 ^j	270	100
Mn	23.7	19.0-28.4	26	2.8	108	23.7	19.0-28.4	25	2.5	105	100 ± 0.5	--	99	8.5	99
Co	0.380	0.228-0.532	0.4	0.1	104	1.75	1.23-2.28	1.8	0.2	103	2 ± 0.01	--	2.0	0.2	99
Cu	817	654-980	820	44	101	1625	1300-1950	1620	84	99	2000 ± 10	--	2120	87	106
Zn	943	754-1132	950	53	101	1884	1601-2167	1890	100	100	1500 ± 7.5	--	1590	66	106

^hNA = target value not assigned; ⁱn = 30; ^jn = 21

Table S3. Spike recovery data for trace elements in pooled follicular fluid by Q-ICP-MS ($n = 27$).

Analyte	Low Spike (0.5 µg/L)			High Spike (10 µg/L)		
	Found (µg/L)	s (µg/L)	Recovery (%)	Found (µg/L)	s (µg/L)	Recovery (%)
Be	0.5 ^a	0.3	102	9.7	4.7	97
Mo	0.7	0.5	147	10.3 ^a	0.9	103
Cd	0.5	0.1	103	10.4	0.7	104
Sn	<0.3	--	--	9.1	4.1	91
Sb	0.5	0.1	107	10.2	0.8	102
Cs	0.3	0.1	62	10.2	0.3	102
Ba	0.4	0.4	75	9.9	0.9	99
W	0.2	0.4	48	9.9	0.6	99
Pt	0.6	0.4	116	10.2	1.0	102
Hg	0.4	0.2	79	9.4	2.1	94
Tl	0.5	0.03	99	10.2	0.5	102
Pb	0.5	0.1	99	10.4	0.5	104
U	0.5	0.03	103	10.3	0.6	103
Mn	0.3	0.4	66	9.5 ^a	1.2	95

^a $n = 19$

Table S4. Method validation data for SF-ICP-MS based on repeated analyses of archived serum RMs from EQA/PT programs.

Centre de toxicologie du Québec (CTQ), Québec Multielement EQA Scheme (QMEQAS)										
QMEQAS 06S-06 (n = 4)						QMEQAS 08S-06 (n = 4)				
Analyte	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value
Be	18.5	1.2	17	3.1	>0.05	13.5	0.9	13	1.5	>0.05
Mo	1.08	0.18	2.4	1.0	<0.0001	3	0.3	3.3	0.4	>0.05
Cd	1.44	0.24	1.7	0.3	>0.05	1.97	0.33	2.2	0.1	>0.05
Sn	2.14	0.21	2.0	1.4	>0.05	1.59	0.27	1.3	0.9	>0.05
Sb	0.23	0.077	0.5	0.5	>0.05	1.57	0.26	1.6	0.3	>0.05
Ba	1.5	0.25	2.7	1.3	0.0084	6.77	0.45	7.0	0.5	>0.05
Pt	0.2	0.067	0.2	0.1	>0.05	0.716	0.12	0.7	0.2	>0.05
Hg	2.3	0.23	2.3	0.3	>0.05	10.6	0.7	9.8	1.8	>0.05
Tl	0.77	0.129	0.9	0.2	>0.05	0.39	0.13	0.5	0.1	>0.05
Pb	8.2	0.55	8.4 ^a	1.0	>0.05	118	8	125	9.7	>0.05
U	0.3	0.1	0.3	0.04	>0.05	0.65	0.109	0.7	0.1	>0.05
V	0.605	0.101	0.5	0.04	>0.05	1.07	0.18	1.0	0.1	>0.05
Cr	1.14	0.19	1.1	0.1	>0.05	3.3	0.33	2.9	0.3	>0.05
Mn	3.97	0.4	3.6	0.1	>0.05	5	0.5	4.7	0.2	>0.05
Co	3.08	0.31	2.7	0.2	0.0266	1.7	0.28	1.6	0.2	>0.05
Cu	1890	123	1820	70	>0.05	2120	146	2140	78	>0.05
Zn	1940	128	1960	53	>0.05	2430	167	2570	65	>0.05
As	6.8	0.46	5.6	1.2	0.0038	9.98	0.67	8.6	1.1	0.0060
Se	276	19	260	28	>0.05	332	22	350	7.4	>0.05
QMEQAS 09S-04 (n = 4)						QMEQAS 10S-05 (n = 3)				
Analyte	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value
Be	3.62	0.36	3.8	0.5	>0.05	6.84	0.46	7.0	1.0	>0.05
Mo	4.1	0.41	4.6	0.4	0.0462	8.1	0.54	9.1	0.2	0.0049
Cd	6.13	0.41	6.3	0.7	>0.05	3.38	0.34	3.9	0.3	0.0209
Sn	6.3	0.42	4.7	3.1	>0.05	4.8	0.48	4.2	0.5	>0.05
Sb	2.61	0.26	2.8	0.4	>0.05	4.98	0.5	5.9	0.2	0.0052
Ba	2.76	0.28	2.9 ^a	0.3	>0.05	4.23	0.42	4.6	0.5	>0.05
Pt	0.43	0.143	0.4	0.2	>0.05	0.6	0.1	0.5	0.2	>0.05
Hg	3.55	0.36	3.5	0.8	>0.05	6.81	0.46	5.3	1.1	0.0011
Tl	2.37	0.24	2.5	0.2	>0.05	1.49	0.25	1.6	0.1	>0.05
Pb	198	13	210	2.6	0.0499	87.2	5.8	100	4.6	0.0008
U	0.11	0.037	0.1 ^a	0.02	>0.05	0.32	0.107	0.3	0.04	>0.05
V	2	0.33	1.9	0.2	>0.05	0.715	0.119	0.6	0.02	>0.05
Cr	4.5	0.45	4.4	0.3	>0.05	2.3	0.23	1.9	0.1	0.0081
Mn	1.75	0.29	1.7	0.3	>0.05	4	0.4	3.9	0.2	>0.05
Co	0.7	0.117	0.5	0.1	0.0098	4.2	0.42	3.6	0.4	0.0275
Cu	1180	81	1240	90	>0.05	1860	125	1880	30	>0.05
Zn	1230	82	1340	170	>0.05	1920	129	2040	140	>0.05
As	2.25	0.23	1.6	0.6	0.0056	19.7	1.3	21	1.8	>0.05
Se	141	10	160	32	0.0420	197	13	210	7.4	>0.05

New York State Department of Health PT Program															
NYS PT SE03-05 (n = 4)						NYS PT SE03-03 (n = 4)					NYS PT SE03-02 (n = 4)				
Analyte	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value	Target (µg/L)	s (µg/L)	Found (µg/L)	s (µg/L)	p value
Mo ^a	0.9		1.2	0.2	--	4		4.6	0.5	>0.05	7.3		8.3	0.6	>0.05
Cd ^a	NA ^b		0.2	0.1	--	3.4	0.16	3.6	0.3	>0.05	6.81	0.54	7.3	0.6	>0.05
Sn ^a	0.7		0.3	0.2	--	3.7		2.9	1.9	>0.05	6.9		5.4	3.6	>0.05
Tl ^a	NA		--	--	--	1.7	0	1.9	0.2	--	3.4	0.05	3.6	0.3	>0.05
Pb ^a	NA		--	--	--	16	0.86	18	2.5	>0.05	33.3	0.48	36	3.4	>0.05
Mn ^a	0.6		0.5	0.04	--	4.2	0.63	3.8	0.3	>0.05	7.5	0.97	7.0	0.3	>0.05
Co ^a	0.7		0.2	0.04	--	1.4	0.32	0.9	0.1	0.0294	2.23	0.4	1.6	0.1	0.0263
Cu	1113	46.9	1060	13	>0.05	1444	52.5	1410	62	>0.05	1950	79.1	1900	56	>0.05
Zn	794.4	47.5	790	33	>0.05	1596	100.2	1580	26	>0.05	2336	169.9	2320	21	>0.05
Se	130.7	8.5	140	5	0.0422	172.9	5.7	180	5	>0.05	220.1	8.6	230	12	0.0434

^aData quality are dependent on characterization by small numbers of program participants (e.g., n = 2 for Mo, Sn; n = 3 for Cd); ^bNA = target value not assigned

Table S5. Method validation data for SF-ICP-MS based on analysis of CRMs and commercial QC materials over a four-day period.

Analyte	NIST SRM 1598a Animal Serum				NIST SRM 1640 Natural Water				HPS CRM Bovine Liver Solution							
	Certified (µg/L)	U ^a (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Certified (µg/L)	U ^a (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Certified (µg/L)	U (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	
Be	NA ^b	--	--	--	--	34.94	± 0.41	36	3.5	103	NA	--	--	--	--	
Rb	274 ± 19	260	19	95	2.00 ^c	± 0.02	<6	--	--	--	200 ± 1	190	15	97		
Sr	NA	--	--	--	124.2	± 0.7	130	23	105	--	NA	--	--	--		
Mo	5.5 ± 1.0	5.5	0.7	101	46.75	± 0.26	40	2.1	86	--	NA	--	--	--		
Cd	0.048 ± 0.004	0.1	0.05	243 ^e	22.79	± 0.96	24	0.7	106	3	± 0.015	3.3	0.2	110		
Sb	1.00 ± 0.15	1.3	0.2	127	13.79	± 0.42	13	1.5	94	NA	--	--	--	--		
Cs	0.64 ± 0.10	0.7	0.1	117	NA	--	--	--	--	--	NA	--	--	--		
Ba	NA	--	--	--	148.0	± 2.2	170	21	117	10	± 0.05	15	6.1	151		
Hg	0.32 ^c	± 0.19	0.4	0.3	126 ^e	NA	--	--	--	--	NA	--	--	--		
Tl	0.033 ^d	--	0.05	0.004	139	<0.1 ^d	--	0.01	0.002	--	NA	--	--	--		
Pb	NA	--	--	--	27.89	± 0.14	35	3.3	124	3	± 0.015	3.7	0.8	125		
Mg	NA	--	--	--	5828 ^c	± 56	5740	480	98	6000	± 30	6000	360	100		
V	1.88 ± 0.11	2.0	0.1	104	12.99	± 0.37	11	0.8	86	0.5	± 0.0025	0.5	0.1	109		
Cr	0.33 ^c	± 0.08	0.3	0.5	98	38.6	± 1.6	34	1.7	89	1	± 0.005	1.0	0.1	97	
Mn	1.78 ± 0.33	1.7	0.1	98	121.5	± 1.1	120	3.6	95	100	± 0.5	96	2.5	96		
Co	1.24 ± 0.07	0.8	0.1	65	20.28	± 0.31	19	0.3	92	2	± 0.01	1.9	0.1	96		
Cu	1580 ± 90	1510	29	96	85.2 ^c	± 1.2	87	4.1	103	2000	± 10	2030	42	102		
Zn	880 ± 24	847	41	96	53.2 ^c	± 1.1	55 ^f	5.1	104	1500	± 7.5	1610	110	107		
As	0.3 ^d	--	1.2	1.1	392 ^e	± 0.41	23	2.0	87	0.5	± 0.0025	0.5	0.04	101		
Se	134.4 ± 5.8	110	16	85	21.96	± 0.51	19	0.7	86	10	± 0.05	9.2	0.3	92		

^aExpanded measurement uncertainty (k = 2, ± 95% confidence interval); ^bNA = certified value not assigned; ^cUncertified reference value; ^dInformational value; ^eFound values are barely detectable, and less than the limit of quantitation; ^fAnalyzed over a three-day period

Analyte	BCR CRM 637				BCR CRM 638				BCR CRM 639						
	Certified (µg/L)	U ^a (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Certified (µg/L)	U (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)	Certified (µg/L)	U (µg/L)	Found (µg/L)	s (µg/L)	Recovery (%)
Zn	1110	± 220	1040	270	94	1430	± 210	1520	110	106	2360	± 140	2390	250	101
Se	81	± 7	78	14	97	104	± 7	110	11	106	133	± 12	160	58	122

Table S6. Spike recovery data for select analytes in follicular fluid analyzed by SF-ICP-MS over a four-day period.

Analyte	Low Spike (0.5 µg/L)			Middle Spike (2 µg/L)			High Spike (10 µg/L)		
	Found (µg/L)	s (µg/L)	Recovery (%)	Found (µg/L)	s (µg/L)	Recovery (%)	Found (µg/L)	s (µg/L)	Recovery (%)
Be	0.5	0.1	101	2.0	0.2	99	10	1.2	103
Mo	<0.6	--	--	2.3	0.7	115	11	1.0	107
Cd	0.5	0.04	107	2.1	0.1	106	11	0.3	110
Sn	0.6	0.4	115	1.7	1.1	83	8.4	5.7	84
Sb	0.7	0.1	141	2.4	0.2	119	12	1.1	117
Cs	1.1	0.6	222	2.9	0.7	144	13	2.1	127
Ba	0.7	0.4	141	2.5	0.6	124	13	2.2	125
W	0.3	0.1	61	1.9	0.2	93	10	1.0	101
Pt	0.5	0.1	100	1.9	0.3	97	9.7	0.9	97
Hg	0.5	0.5	91	2.0	0.6	99	8.7	1.4	87
Tl	0.6	0.04	121	2.3	0.1	116	12	1.0	115
Pb	0.6	0.3	118	2.3	0.2	115	12	0.6	115
U	0.6	0.1	113	2.2	0.4	109	11	1.8	109
V	0.5	0.04	102	2.0	0.2	102	10	0.9	102
Cr	0.6	0.3	115	2.4	0.7	122	10	1.0	102
Mn	0.6	0.1	112	2.1	0.2	107	10	0.5	103
As	0.7 ^a	0.3	132	1.8	0.9	88	10 ^a	0.4	102

^an = 3