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7.

Annex

Paper published and/or submitted

1. Evaluation and standardization of different purification procedures for fish bile and liver metallothionein quantification by spectrophotometry and SDS-PAGE analyses. Carolina Lyrio Tenório-Daussat. Marcia Carolina Martinho Resende. Roberta L. Ziolli. Rachel Ann Hauser-Davis. Dirk Schaumlöffel. Tatiana D. Saint'Pierre. **Talanta. V.120. p. 491-497. 2014. Impact factor: 3.733. Level A2 (CAPES).**

2. Peptide derivatization and complexation with Lanthanides-NHS-ester-DOTA by nano-IP-RP-HPLC-UV. Carolina Lyrio Tenório-Daussat; Rachel Ann Hauser-Davis; Tatiana D. Saint'Pierre; Dirk Schaumlöffel. **Submitted. Michrochemical Journal. Impact factor: 2.850. Level A2 (CAPES).**

Annex 1. ICP-MS detection for liver control and elemental exposition.

Unit	**µg/L**										
Element	**Pb**	**V**	**Cr**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Cd**
MW	**208**	**51**	**53**	**57**	**59**	**60**	**65**	**66**	**75**	**82**	**114**
Ctrl 1	3.10	45.9	100	1954	85.8	313	6580	609	2.73	334	2.13
Ctrl 2	2.40	36.7	44.5	2020	25.3	255	786	96.9	1.97	265	< 0.001
Ctrl 3	3.39	48.6	40.5	1997	28.8	215	1932	145	1.89	250	< 0.001
Ctrl 4	4.45	28.5	69.5	1737	25.2	237	1171	218	2.34	352	8.63
Ctrl 5	1.22	57.2	86.7	2296	50.6	319	853	267	5.90	320	< 0.001
Ctrl 6	3.38	62.0	82.0	1575	27.8	251	1851	337	5.51	357	< 0.001
Ctrl 7	2.01	36.5	58.7	2225	25.3	312	875	163	8.34	275	< 0.001
Ctrl 8	4.40	24.0	70.4	909	17.4	182	1037	113	3.91	196	0.61
Ctrl 9	2.59	29.9	75.0	1516	28.5	219	681	161	5.09	308	< 0.001
Ni 2	2.95	86.5	121	2665	71.4	67.2	2516	330	6.93	519	1.46
Ni 3	2.16	22.8	237	5070	37.0	111	2568	376	2.16	294	0.62
Ni 4	4.26	26.0	34.7	3625	44.8	101	2490	348	1.43	384	< 0.001
Ni 5	4.17	29.4	48.2	3235	29.7	255	2613	343	3.61	232	0.63
Ni 6	4.76	23.3	63.2	1552	35.2	280	1926	54.3	8.21	239	21.0
Ni 9	8.47	62.0	92.1	1818	67.1	215	2523	225	7.12	336	5.39
Ni 10	11.1	56.4	107	1426	39.2	354	2975	279	7.43	192	3.08
Ni 11	7.38	31.1	59.6	2294	38.0	231	3097	283	6.01	221	2.08
Ni 12	7.92	20.8	74.1	1582	26.5	241	1556	194	3.69	263	1.98
Cd 1	3.59	83.3	55.5	1850	37.8	205	1522	246	6.10	492	23.2
Cd 2	4.40	41.6	39.4	1542	20.7	186	652	129	7.16	250	2.14
Cd 3	4.51	28.3	63.9	2329	36.4	205	2632	202	7.32	375	10.0

Cd 4	6.80	44.8	55.7	5237	39.5	281	1215	172	6.42	321	23.0
Cd 5	3.62	58.6	28.0	2093	33.4	208	1660	99.3	5.70	365	1.69
Cd 6	5.26	16.9	66.9	4936	41.8	244	868	183	6.90	438	13.0
Cd 7	9.77	41.2	100	1425	36.2	273	932	273	7.71	436	18.6
Cd 8	5.00	30.5	85.9	1743	26.3	205	815	157	2.79	376	1.68
Cd 9	9.20	81.8	162	1671	29.6	255	1743	203	4.40	501	3.20
Zn 1	5.15	42.5	81.4	2433	62.7	248	2601	279	6.89	341	< 0.001
Zn 2	1.41	51.8	42.5	1828	42.9	183	2494	222	2.41	457	< 0.001
Zn 3	0.61	17.0	< 0.001	3274	63.3	214	4014	172	6.18	496	0.45
Zn 4	1.04	26.2	7.06	1935	41.4	220	993	234	2.41	237	0.30
Zn 5	0.69	25.4	29.3	2044	47.2	163	2574	981	2.69	222	0.66
Zn 6	0.068	20.4	57.7	3210	52.4	344	1731	358	3.98	309	0.79
Zn 7	0.78	28.7	423	2059	48.6	330	2512	131	3.28	252	< 0.001
Zn 8	9.89	33.2	67.8	4832	104	321	3217	586	3.77	273	4.29
Zn 9	0.81	31.9	37.2	1978	70.0	257	2221	176	4.06	205	2.27
Pb 1	< 0.001	31.3	48.7	2750	25.7	199	851	334	3.99	246	< 0.001
Pb 2	13.1	10.8	45.4	1720	26.6	199	813	123	5.01	374	< 0.001
Pb 3	< 0.001	28.9	67.2	2163	19.4	202	1445	221	4.84	252	< 0.001

Annex 2. ICP-MS detection for fish bile control and elemental exposition.

Unit	µg/L										
Element	**Pb**	**V**	**Cr**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Cd**
MW	**208**	**51**	**53**	**57**	**59**	**60**	**65**	**66**	**75**	**82**	**114**
Ctrl 1	< 0.001	< 0.001	< 0.001	855	< 0.001	1.20	63.8	124	< 0.001	74.9	< 0.001
Ctrl 2	< 0.001	< 0.001	< 0.001	1532	< 0.001	< 0.001	31.0	< 0.001	< 0.001	28.7	< 0.001
Ctrl 3	< 0.001	< 0.001	< 0.001	665	< 0.001	3.38	26.1	32.4	< 0.001	26.5	< 0.001
Ctrl 4	< 0.001	< 0.001	< 0.001	651	< 0.001	18.1	15.2	< 0.001	< 0.001	13.9	< 0.001
Ctrl 5	< 0.001	< 0.001	< 0.001	639	< 0.001	3.35	9.65	< 0.001	< 0.001	17.3	< 0.001
Ctrl 6	< 0.001	< 0.001	< 0.001	1897	< 0.001	20.7	43.4	132	< 0.001	75.7	< 0.001
Ctrl 8	< 0.001	< 0.001	< 0.001	690	< 0.001	< 0.001	18.3	< 0.001	< 0.001	15.5	< 0.001
Ctrl 9	< 0.001	< 0.001	64.7	777	< 0.001	3.08	38.1	16.1	< 0.001	47.8	< 0.001
Cd 1	< 0.001	< 0.001	1.28	656	< 0.001	2.78	22.3	24.0	< 0.001	35.6	< 0.001
Cd 2	0.44	< 0.001	< 0.001	700	< 0.001	3.97	14.1	4.28	< 0.001	25.2	< 0.001
Cd 3	< 0.001	< 0.001	< 0.001	695	< 0.001	6.58	30.0	< 0.001	< 0.001	37.4	< 0.001
Cd 4	< 0.001	< 0.001	< 0.001	719	< 0.001	2.35	56.0	35.0	< 0.001	57.0	< 0.001
Cd 5	< 0.001	< 0.001	< 0.001	796	< 0.001	9.52	63.0	< 0.001	< 0.001	55.1	< 0.001
Cd 7	11.6	< 0.001	< 0.001	683	< 0.001	11.4	105	76.0	< 0.001	14.5	< 0.001
Cd 8	< 0.001	< 0.001	< 0.001	716	< 0.001	1.90	25.5	< 0.001	< 0.001	34.1	< 0.001
Cd 9	< 0.001	< 0.001	< 0.001	844	< 0.001	7.87	38.9	< 0.001	< 0.001	60.6	< 0.001
Zn 1	< 0.001	< 0.001	< 0.001	735	< 0.001	14.0	15.8	< 0.001	< 0.001	47.2	< 0.001
Zn 2	4.40	< 0.001	2.11	924	< 0.001	17.2	61.0	293	< 0.001	22.6	< 0.001
Zn 3	0.32	< 0.001	< 0.001	818	< 0.001	4.04	88.2	66.0	< 0.001	46.9	< 0.001
Zn 4	< 0.001	< 0.001	< 0.001	721	0.18	5.30	79.2	< 0.001	< 0.001	61.8	< 0.001
Zn 5	< 0.001	< 0.001	< 0.001	689	< 0.001	7.79	136	147	< 0.001	31.0	< 0.001

Zn 6	3.55	< 0.001	< 0.001	758	< 0.001	5.46	124	18.8	< 0.001	38.3	< 0.001
Zn 7	< 0.001	< 0.001	< 0.001	656	< 0.001	3.79	55.8	30.0	< 0.001	28.6	< 0.001
Zn 8	< 0.001	< 0.001	< 0.001	1501	< 0.001	13.6	39.8	119	< 0.001	54.2	< 0.001
Zn 9	< 0.001	< 0.001	< 0.001	712	< 0.001	< 0.001	22.4	< 0.001	< 0.001	20.1	< 0.001
Pb 1	7.86	< 0.001	< 0.001	777	< 0.001	7.08	32.6	78.2	< 0.001	42.7	1.18
Pb 2	5.62	< 0.001	< 0.001	729	< 0.001	10.8	83.5	110	< 0.001	58.5	< 0.001
Ni 1	< 0.001	< 0.001	< 0.001	760	< 0.001	7.57	56.0	< 0.001	< 0.001	57.4	< 0.001
Ni 2	< 0.001	< 0.001	< 0.001	699	< 0.001	7.24	135	< 0.001	< 0.001	69.0	< 0.001
Ni 3	< 0.001	< 0.001	< 0.001	739	< 0.001	3.35	260	39.7	< 0.001	98.2	< 0.001
Ni 4	< 0.001	< 0.001	< 0.001	733	< 0.001	5.70	139	66.0	0.055	58.4	1.22
Ni 6	< 0.001	< 0.001	< 0.001	692	< 0.001	1.79	66.0	< 0.001	< 0.001	39.7	< 0.001
Ni 9	< 0.001	< 0.001	173	908	1.25	50.1	234	167	0.33	58.1	< 0.001
Ni 8	< 0.001	< 0.001	< 0.001	668	< 0.001	2.20	67.8	9.63	< 0.001	27.5	< 0.001
Ni 11	< 0.001	< 0.001	< 0.001	729	< 0.001	7.95	97.5	14.0	< 0.001	38.8	< 0.001

Annex 3. MT concentrations for fish bile and liver samples.

bile				liver			
Sample	μmol/L MT	Sample	μmol/L MT	Sample	μmol/L MT	Sample	μmol/L MT
Pb1	54.7	Ni1	19.5	Pb1	8.8	Ctrl9	3.2
	56.0		20.5		10.5		2.8
Pb2	65.2	Ni2	23.0	Pb2	5.7	Ni2	6.0
	77.3		22.2		6.2		7.2
Zn1	74.7	Ni3	33.0	Pb3	23.8	Ni3	17.5
	71.5		34.3		21.7		18.8
Zn2	72.0	Ni4	28.7	Zn1	9.8	Ni4	11.2
	70.0		29.0		11.2		12.0
Zn3	50.0	Ni5	29.2	Zn2	3.7	Ni5	7.2
	47.7		30.7		5.7		7.8
Zn4	39.7	Ni6	59.2	Zn3	13.0	Ni6	10.2
	41.3		63.3		12.0		11.0
Zn5	51.2	Ni7	23.5	Zn4	12.7	Ni9	10.5
	49.8		24.2		13.2		11.2
Zn6	54.0	Ni8	36.7	Zn5	29.3	Ni10	7.5
	68.2		37.3		28.3		8.8
Zn7	47.0	Cd1	64.7	Zn6	11.8	Ni11	7.8
	44.3		66.7		13.3		8.8
Zn8	91.2	Cd2	95.8	Zn7	4.5	Ni12	6.5
	88.8		97.2		4.0		7.2
Zn9	56.0	Cd3	14.0	Zn8	4.7	Cd1	2.7
	57.2		15.5		5.3		3.2
Ctrl1	5.8	Cd4	19.5	Zn9	12.3	Cd2	5.3
	7.2		20.7		12.8		6.3
Ctrl2	34.2	Cd5	22.8	Ctrl1	7.7	Cd3	4.7
	40.7		23.3		9.0		5.5
Ctrl3	52.3	Cd6	21.2	Ctrl2	7.0	Cd4	5.7
	53.5		21.0		7.2		7.2
Ctrl4	41.0	Cd7	76.5	Ctrl3	6.2	Cd5	5.0
	43.2		75.0		6.8		5.5
Ctrl5	41.0	Cd8	86.3	Ctrl4	3.7	Cd6	3.3
	72.2		87.3		4.0		3.5
Ctrl6	70.8			Ctrl5	7.2	Cd7	5.2
	74.0				7.8		5.3
Ctrl8	38.7			Ctrl6	3.3	Cd8	4.2
	40.7				5.0		5.8
Ctrl9	13.3			Ctrl7	10.8	Cd9	6.0
	14.3				12.0		7.2
				Ctrl8	1.3		
					1.7		

Annex 4. Metal concentrations for DORM-2 quantified by ICP-MS NexIon 300X - PerkinElmer.

Metal	DORM-2 mg kg^{-1}	
	Certified value	Quantified value
Cu	2.34 ± 0.16	2.18
Pb	0.065 ± 0.007	0.052
Cr	34.7 ± 5.5	33.2
Fe	142 ± 10	134
Co	0.182 ± 0.031	0.177
Ni	19.4 ± 3.1	18.2
Zn	25.6 ± 2.3	25.0
As	18.0 ± 1.1	16.3
Se	1.40 ± 0.09	1.25
Cd	0.043 ± 0.008	0.033
Hg	4.64 ± 0.26	3.99

Annex 5. Spectrophotometric results for metallothionein concentration of extraction procedures for bile and liver.

BILE					
		μmol/L MT			average value μmol/L MT
	Procedure				
β-mercaptoethanol	A	13.86	17.33	12.13	14.44
	B	19.73	13.81	15.78	16.44
	C	13.50	16.88	11.81	14.06
DTT	A	15.83	11.08	12.66	13.19
	B	13.38	16.73	11.71	13.94
	C	12.78	15.98	11.18	13.31
TCEP	A	50.10	62.63	43.84	52.19
	B	121.13	84.79	96.90	100.94
	C	36.06	45.08	31.55	37.56
LIVER					
b-mercaptoethanol	A	32.82	41.03	28.72	34.19
	B	32.70	40.88	28.61	34.06
	C	23.34	29.18	20.42	24.31
DTT	A	38.93	27.25	38.93	32.44
	B	33.06	41.33	28.93	34.44
	C	20.10	25.13	17.59	20.94
TCEP	A	51.42	64.28	44.99	53.56
	B	90.90	113.63	79.54	94.69
	C	36.06	45.08	31.55	37.56

Annex 6. Concentration of MT in bile and liver at different water bath temperature.

Temperature		BILE μmol/L MT		Average
50 °C	33.40	35.31	26.72	31.81
60 °C	38.26	40.45	30.61	36.44
70 °C	57.95	61.26	46.36	55.19
80 °C	39.83	42.11	31.87	37.94
90 °C	21.85	23.10	17.48	20.81
		LIVER μmol/L MT		
50 °C	45.19	52.98	57.65	51.94
60 °C	29.42	34.49	37.53	33.81
70 °C	59.21	69.42	75.55	68.06
80 °C	16.48	19.32	21.02	18.94
90 °C	4.40	5.16	5.62	5.06

Annex 7. Concentration of MT in bile and liver at different centrifugation times.

Procedure				
	BILE			
		μmol/L MT		Average
A''	78.30	62.64	54.81	65.25
B''	76.80	61.44	53.76	64.00
C''	74.85	59.88	52.40	62.38
	LIVER			
		μmol/L MT		
A''	69.67	73.89	67.56	70.38
B''	66.33	70.35	64.32	67.00
C''	58.04	61.56	56.28	58.63