Supporting Information:

Pattern recognition of toxic metal ions using a single-probe thiocoumarin array

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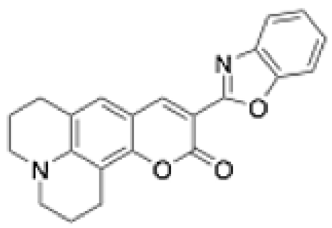
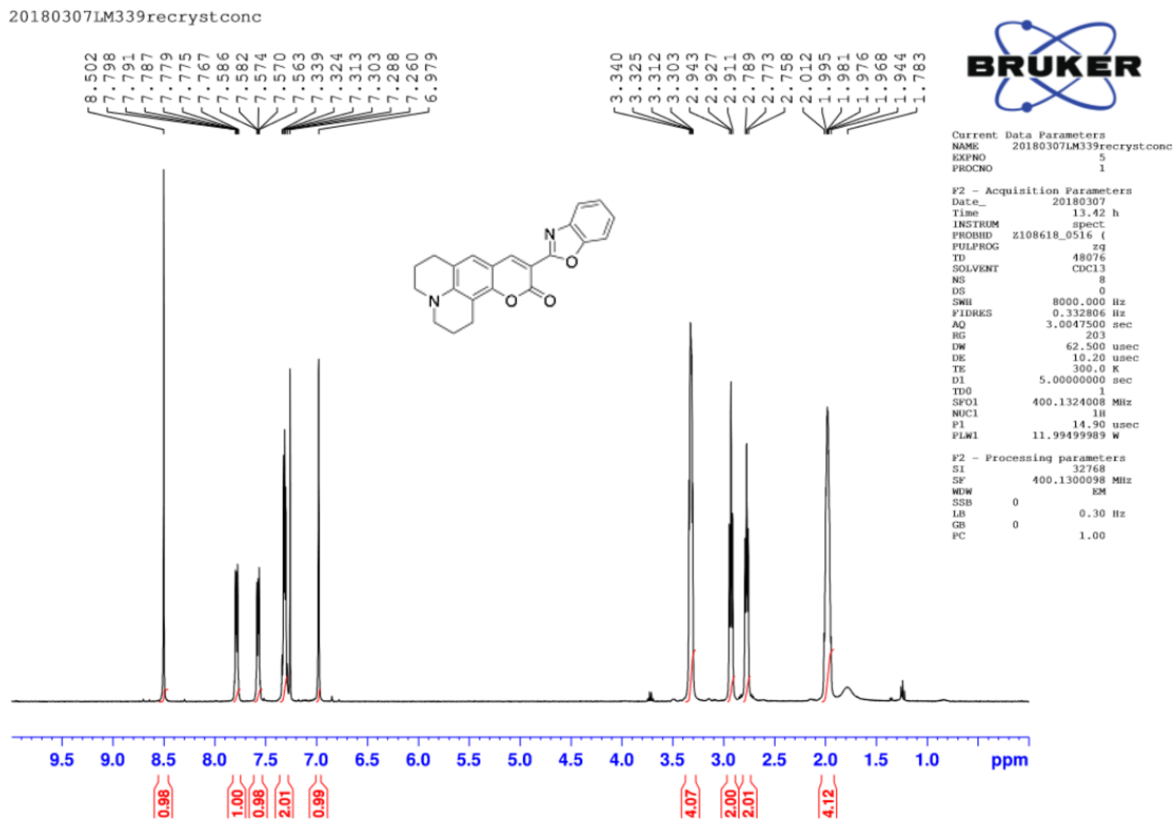
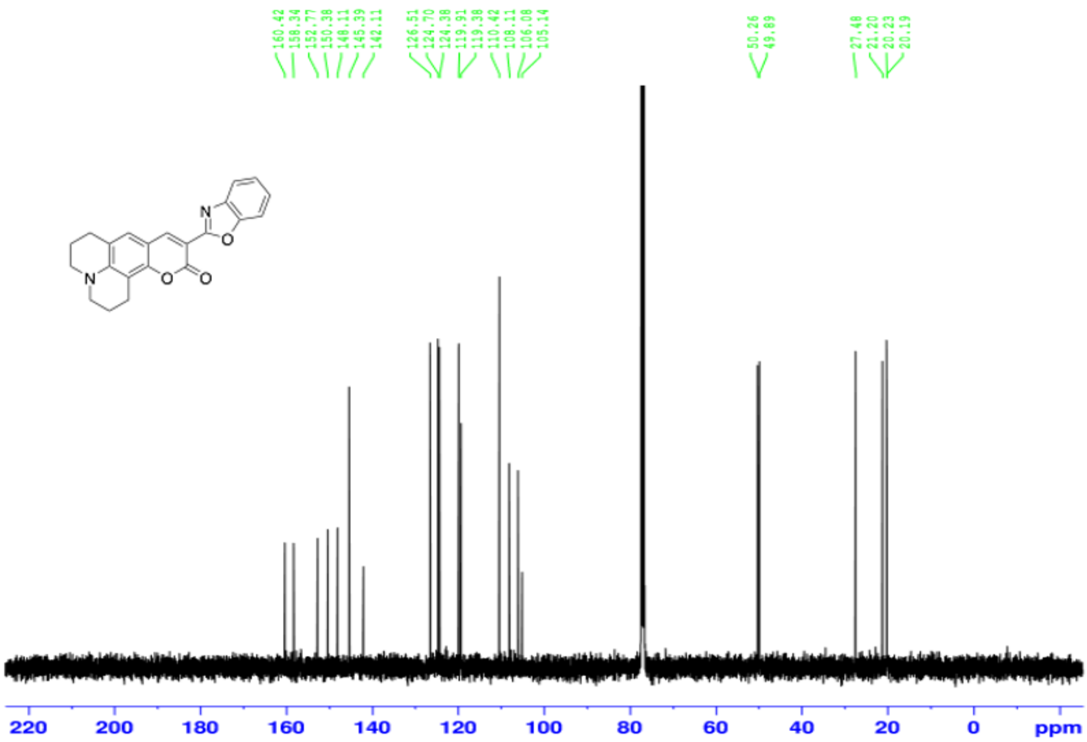
Coumarin 525



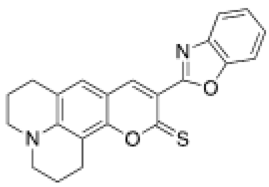
8-Hydroxyjulolidine- 9-aldehyde (200 mg, 0.92 mmol), ethyl 2-cyanoate (200 µL, 1.9 mmol), 2-aminophenol (76 µL, 0.92 mmol), benzoic acid (80 mg, 0.66 mmol) were dissolved in ethanol (10 mL) and the solution was gently refluxed under N_2 atmosphere overnight. The solution was left to cool and the precipitate, was collected by vacuum filtration. The product was purified by column chromatography (silica, DCM:MeOH 97:3), to yield an orange solid (170 mg, 52 %).

^{1}H NMR ($CDCl_3$, 400 MHz), δ(ppm): 8.50 (s, 1H), 7.80-7.77 (m, 1H), 7.59-7.56 (m, 1H), 7.34-7.29 (m, 2H), 6.98 (s, 1H), 3.34-3.30 (m, 4H), 2.93 (t, *J* = 6.4 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.01-1.94 (m, 4H). ^{13C} NMR ($CDCl_3$, 100 MHz), δ(ppm): 160.4, 158.3, 152.8, 150.4, 148.1, 145.4, 142.1, 126.5, 124.7, 124.4, 119.9, 119.4, 110.4, 108.1, 106.1, 105.1, 50.3, 49.9, 27.5, 21.2, 20.2, 20.2. ESMS (m/z): $[M+Na]^+$ calcd for $C_{22}H_{18}N_2O_{3}Na$, 380.1; found 380.1.

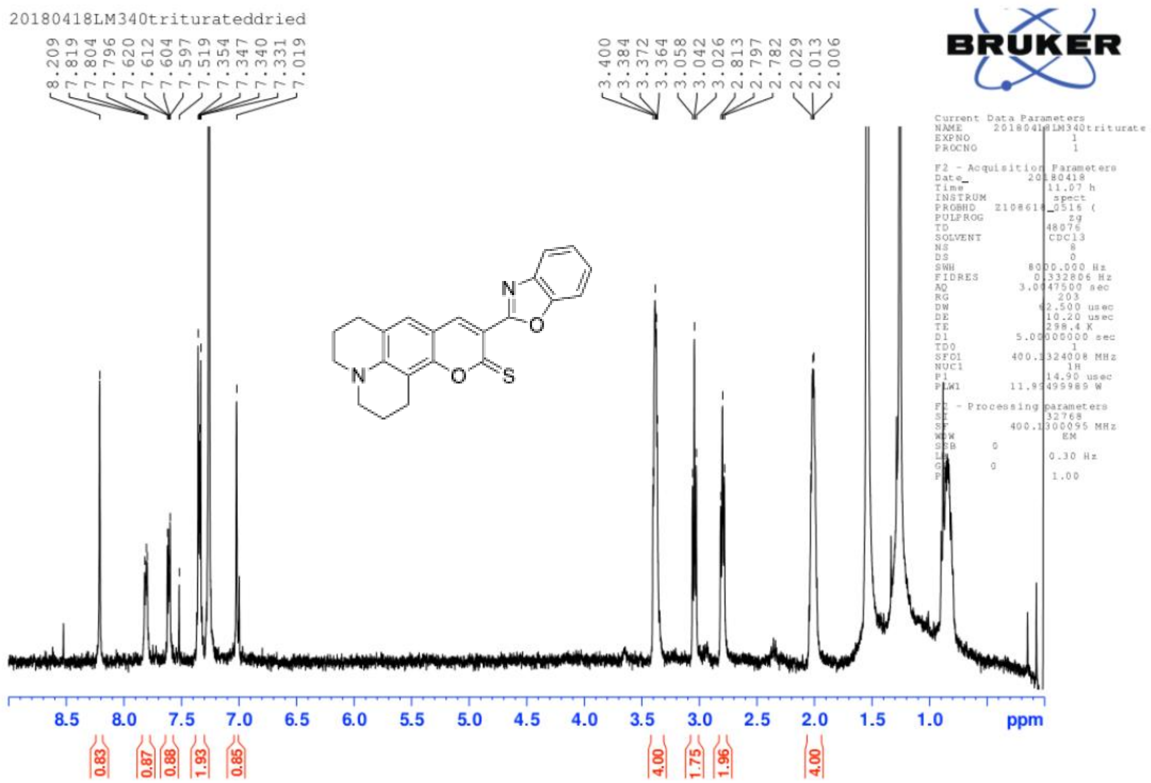




Thiocoumarin 525



Lawesson's Reagent (1.69 g, 4.19 mmol) was added to a solution of coumarin-545 oxazole (100 mg, 0.28 mmol) in degassed toluene (50 mL). The solution was left to reflux, in a nitrogen atmosphere for 3 days. The reaction was concentrated and purify by flash column chromatography (MeOH:DCM 2:98, silica gel), and further purified using preparatory thin layer chromatography (MeOH:DCM 3:97, silica gel), to yield a red powder, (30 mg, 29 %). ^1H NMR ($CDCl_3$, 400 MHz), δ(ppm): 8.21 (s, 1H), 7.82-7.80 (m, 1H), 7.62-7.60 (m, 1H), 7.35-7.33 (m, 2H), 7.02 (s, 1H), 3.40-3.36 (m, 4H), 3.04 (t, *J* = 6.4 Hz, 2H), 2.80 (t, *J* = 6.2 Hz, 2H), 2.03-2.01 (m, 4H). ESMS (m/z): $[M+H]^+$ calcd for $C_{22}H_{19}N_2O_2S$, 375.1; found 375.2. $[M+Na]^+$ calcd for $C_{22}H_{18}N_2O_2SNa$, 397.1; found 397.2.



20180418LM340trituratedried
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7.804
7.796
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7.612
7.604
7.597
7.519
7.354
7.347
7.340
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7.019
3.400
3.384
3.372
3.364
3.058
3.042
3.026
2.813
2.797
2.782
2.029
2.013
2.006
N
O
N
O
S
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EXPNO 1
PROCNO 1
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Time 11.07 h
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PULPROG zg
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SOLVENT CDCl3
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DS 0
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FIDRES 0.332806 Hz
RG 203
DE 10.20 usec
TE 298.4 K
TD0 1
NUC1 1H
P1 14.90 usec
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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
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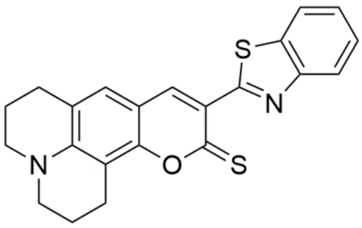
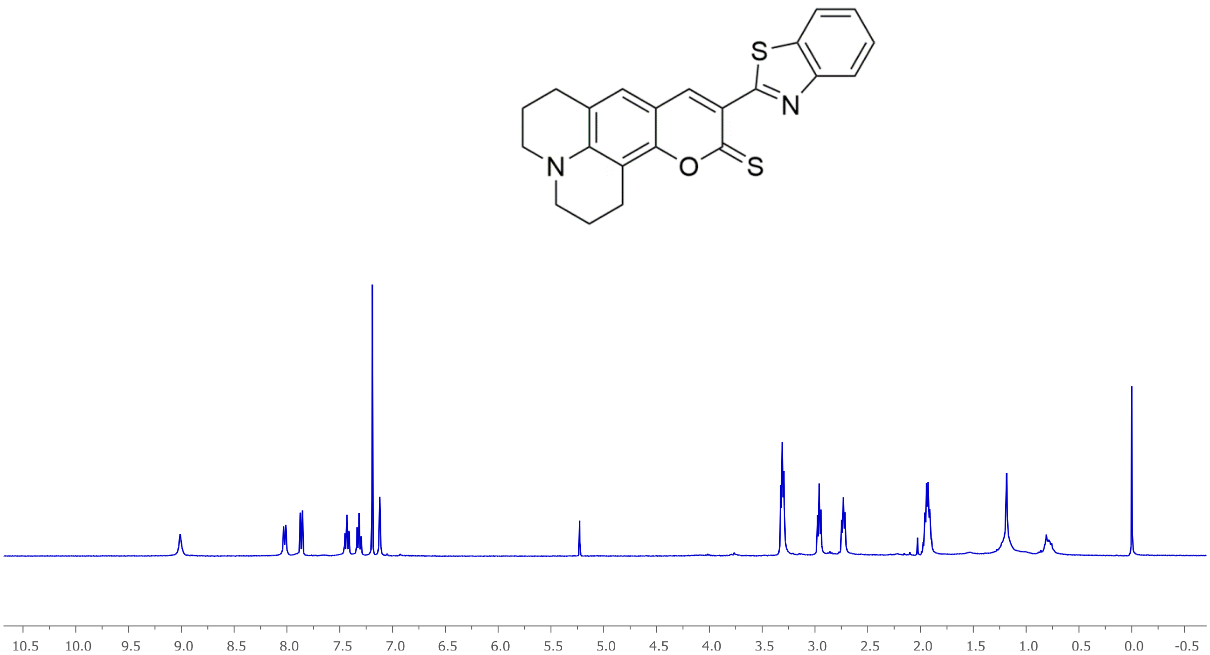
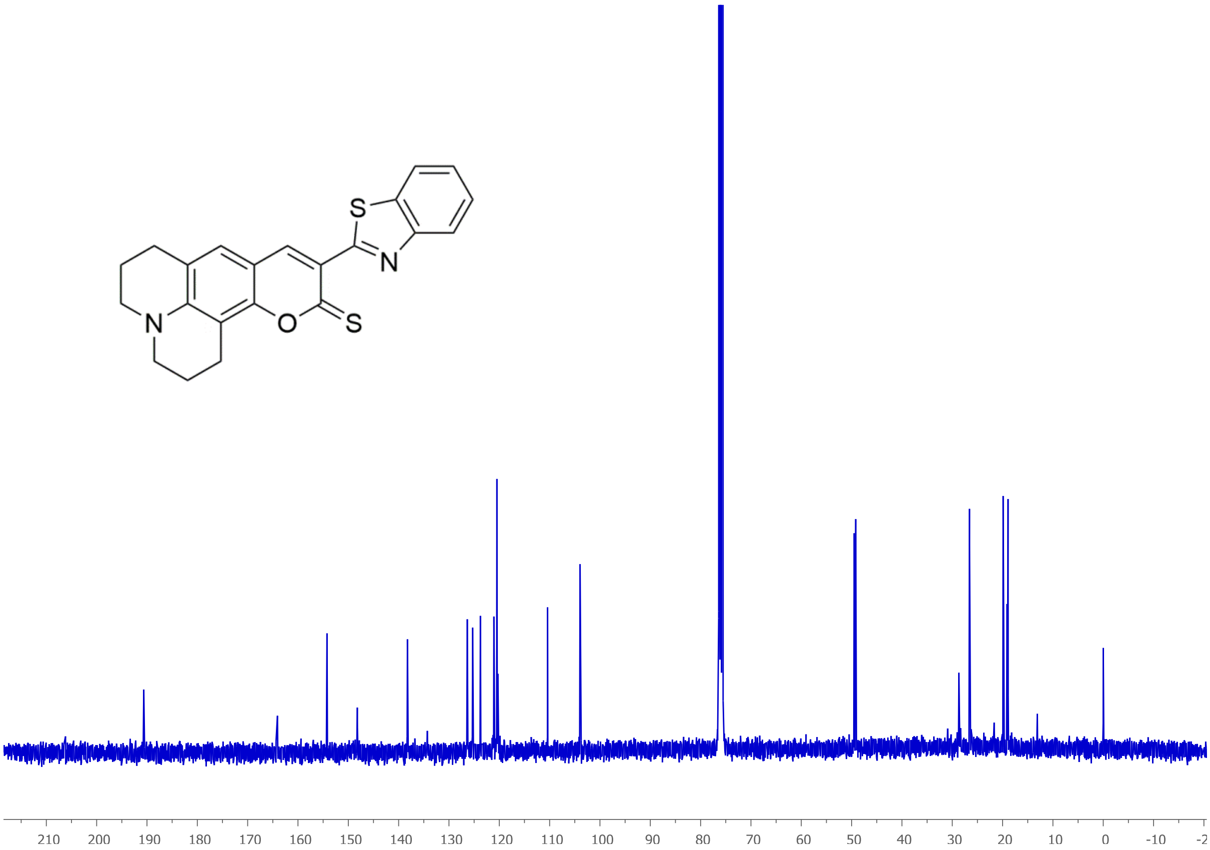
0.88
1.93
0.85
4.00
1.75
1.96
4.00

Thiocoumarin-545



Coumarin-545 (100 mg, 0.27 mmol) was dissolved in toluene and Lawesson's reagent added (325 mg, 0.80 mmol). The mixture was refluxed for 48 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (silica, DCM → 5% MeOH) as a red solid. ^{1}H NMR ($CDCl_3$, 400 MHz), δ(ppm): 9.08 (s, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.39 (t, *J* = 7.0 Hz, 1H), 7.19 (s, 1H), 3.36-3.40 (m, 4H), 3.03 (t, *J* = 6.4 Hz, 4H), 2.82 (t, *J* = 6.1 Hz, 4H), 1.97-2.04 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ(ppm): 191.8, 165.2, 155.4, 149.4, 139.4, 135.5, 127.5, 126.5, 124.9, 122.2, 121.6, 121.4, 121.4, 111.6, 105.1, 50.7, 50.3, 27.7, 21.1, 20.3, 20.1. HRMS–ESMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{19}N_2OS_2$, 391.0939; found 391.0942




S
N
N
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S
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -2

General array procedure. A stock solution of thiocoumarin was prepared as either a 1 mM or 0.1 mM solution in DMSO. Metal ion solutions ($PtCl_4$, $Cu(NO_3)_2$, $AgNO_3$, $CdCl_2$, $Hg(NO_3)_2$, $Pb(NO_3)_2$, $HAuCl_4$) were prepared as 10 mM stock solutions in water. The array experiments were performed in 96-well black polypropylene plates. For the preparation of each replicate, typically 60 µL of the stock solution of the appropriate metal ion was added to a well containing 240 µL of a solution of thiocoumarin 545 in the appropriate solvent. This solution had itself typically been prepared by mixing 30 µL of the stock probe in DMSO with 15 mL of the appropriate solvent. Measurements were recorded on a Perkin-Elmer Enspire plate-reader. An excitation wavelength of 485 nm and emission wavelength of 525 nm was used. Statistical analysis was performed using SPPS Statistics version 24.

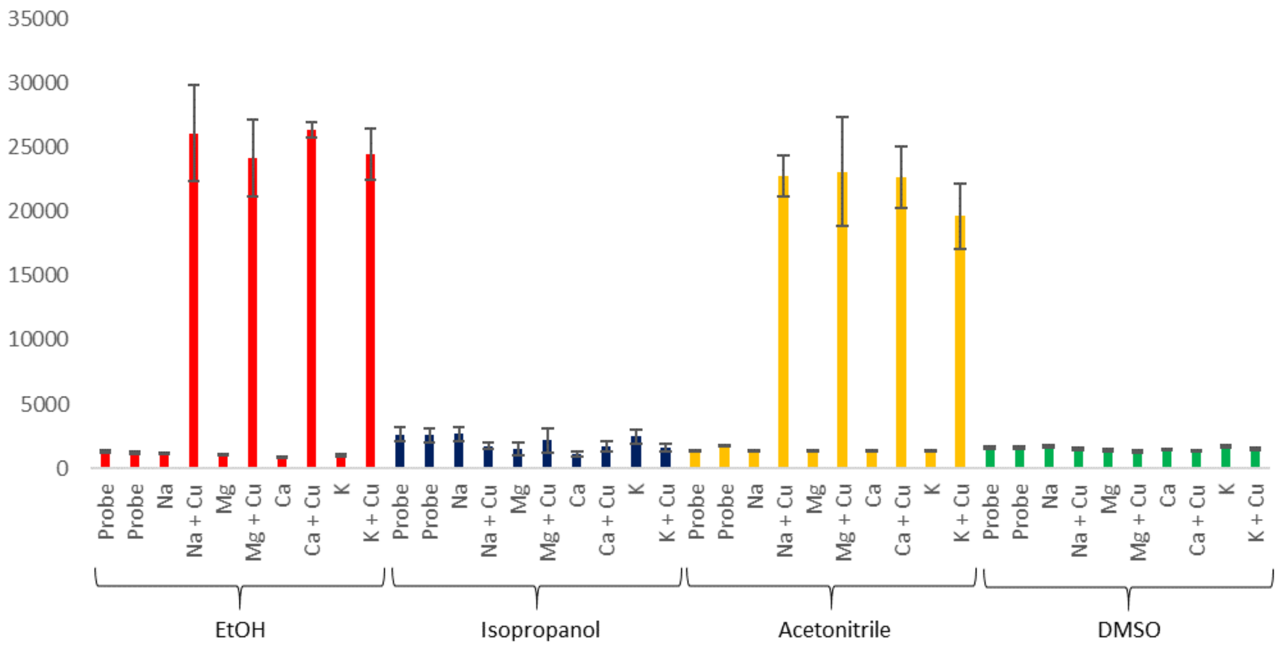

Figure S1 Effect of the addition of common metal ions on the fluorescence of thiocoumarin 525 (1.6 µM). For each solvent: EtOH (red), isopropanol (blue), acetonitrile (yellow) and DMSO (green), a common metal ion (labelled Na, Mg, Ca or K) 100 µM, was added and the fluorescence intensity recorded after 30 min. At this point 100 µM of Cu was added and after a further 30 min the fluorescnce intensity recorded. The first two bars of each set, labelled 'probe' are a control in which no metal ion was added and data collected at the same time points. Bars are an average of three replicates with the SD indicated by the error bars.

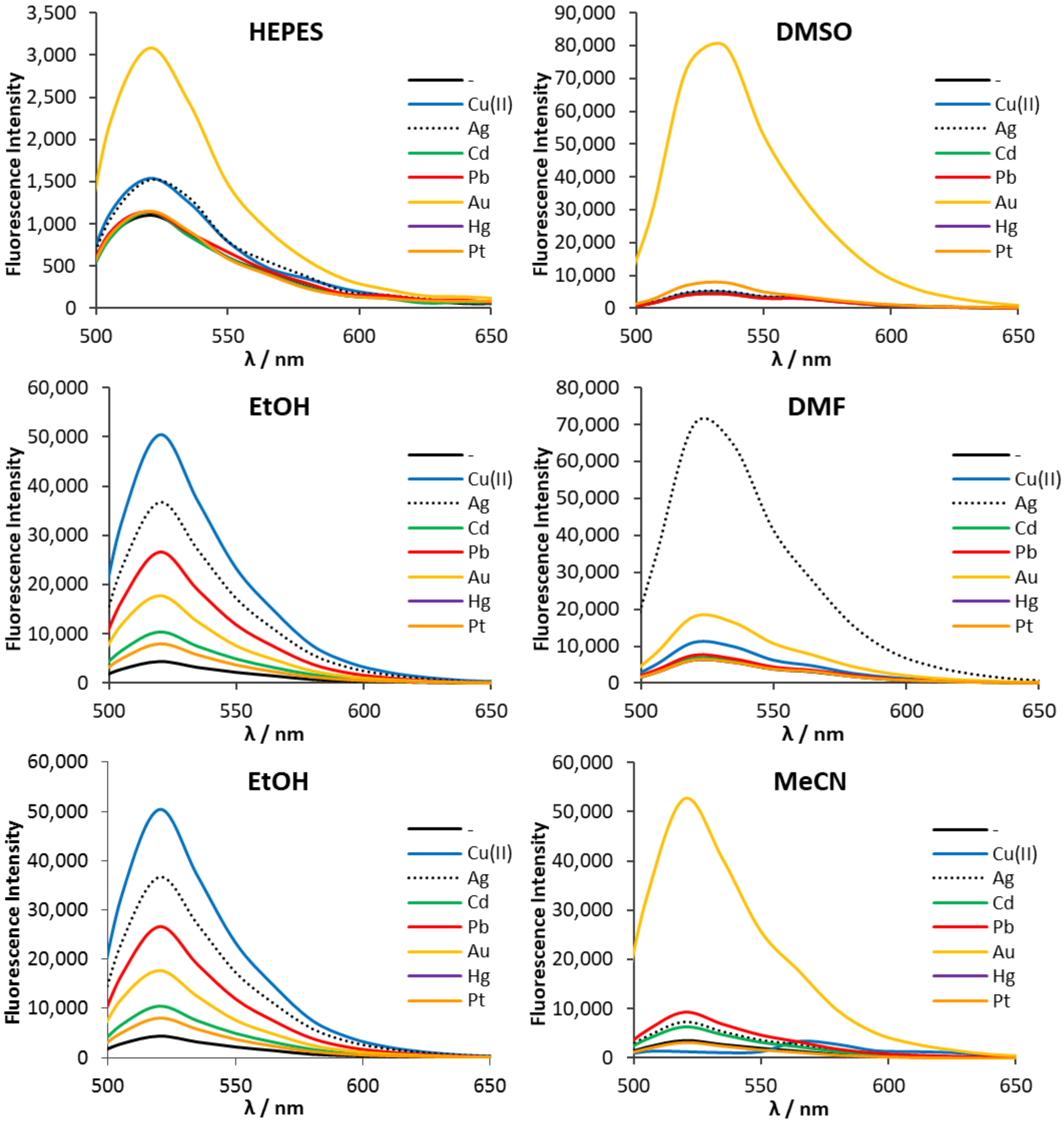

Figure S2: Emission spectra of thiocoumarin 545 (2 µM) in the specified solvent upon addition of stated metal ion (100 µM). Excitation wavelength = 480 nm.

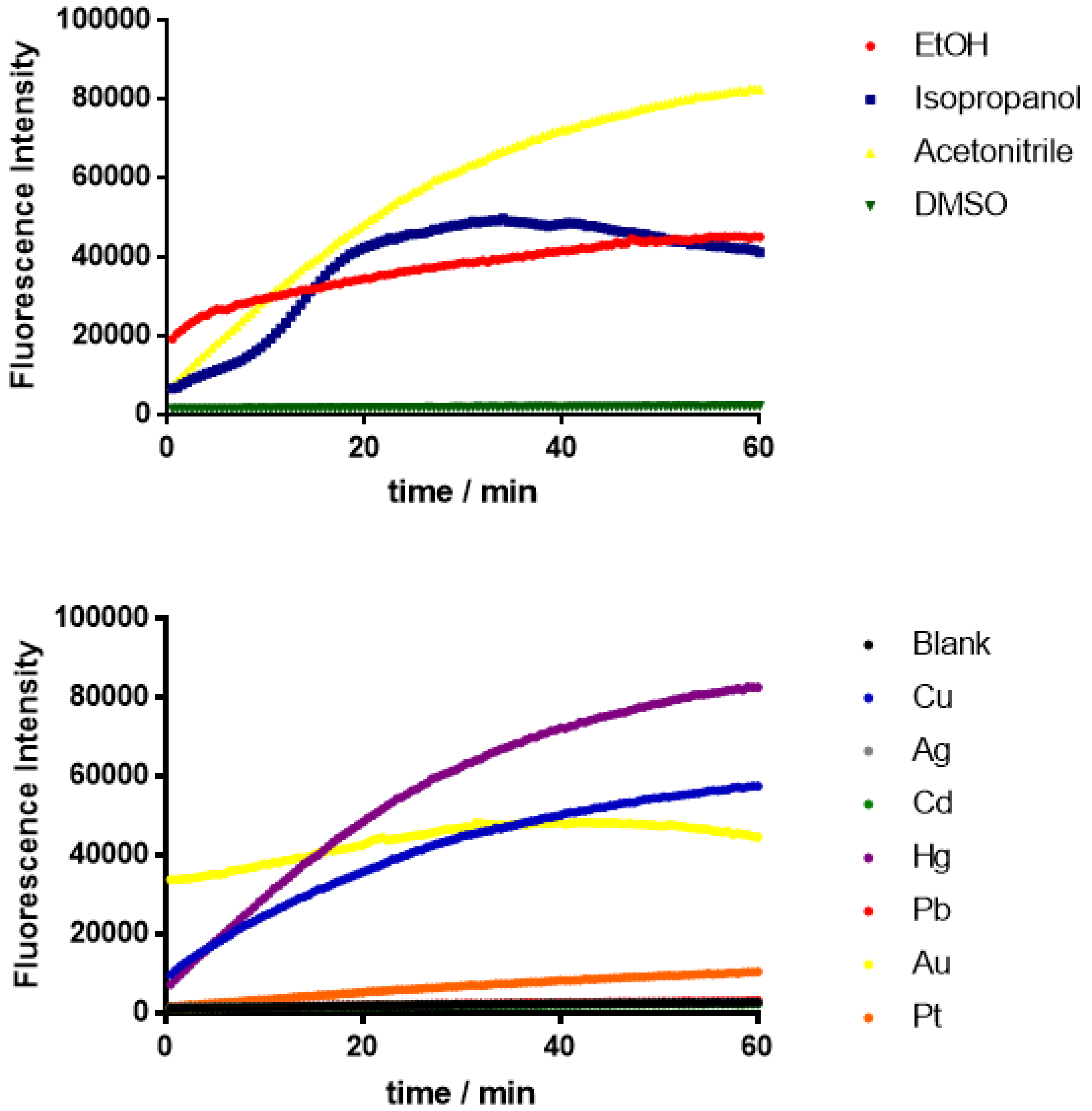

Figure S3 (a) Increase in fluorescnce intensity over time for thiocoumarin 545 (1.6 µM) in the stated solvents upon the addition of 100 µM mercury nitrate. (b) Increase in fluorescence intensity over time for thiocoumarin 545 (1.6 µM) in acetonitrile, upon the addition of the stated metal ion (100 µM).

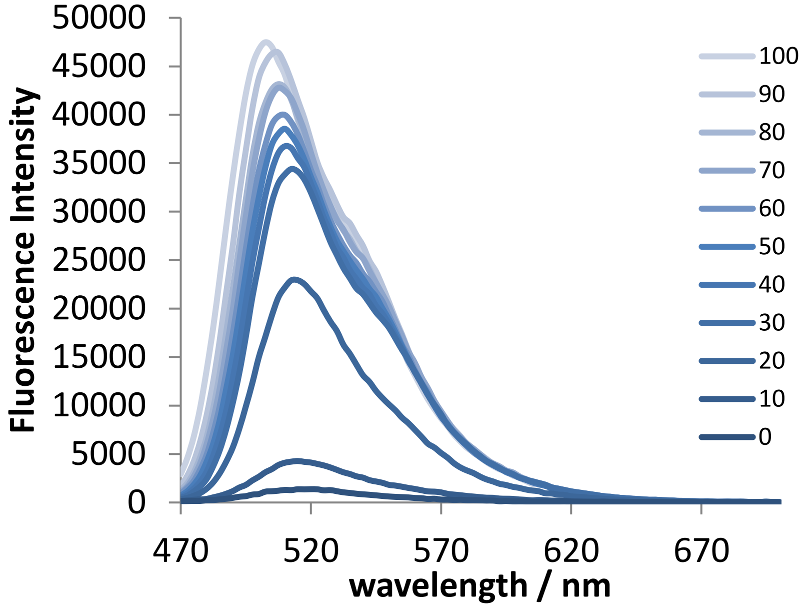

Figure S4 Fluoresence emission of thiocoumarin 545 in varying binary mixtures of acetonitrile and water (legend indicates % of water in acetonitrile).

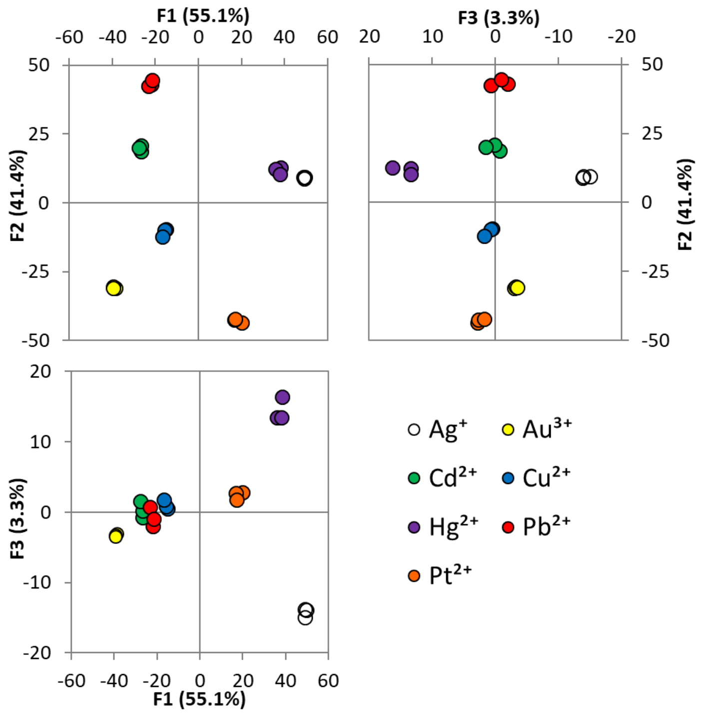

Figure S5 LDA score plot for the analysis of seven metal ions (confidence ellipsoids at 95% probability) performed performed in quintuplicate (1 mM metal ion, pH 7.4, 20 mM HEPES) describing the response to thiocoumarin 525 in eight solvents. Each pairing of the three main factors is plotted in a separate 2D plot.

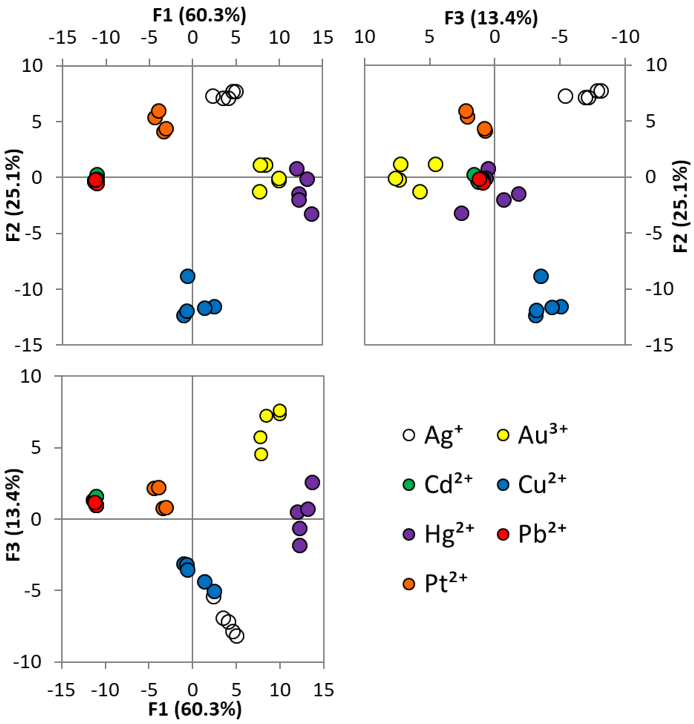

Figure S6 LDA score plot for the analysis of seven metal ions performed in quintuplicate (100 µM metal ion, pH 7.4, 20 mM HEPES) describing the response to thiocoumarin 545 in ethanol, isopropanol, acetonitrile and DMSO. Each pairing of the three main factors is plotted in a separate 2D plot.

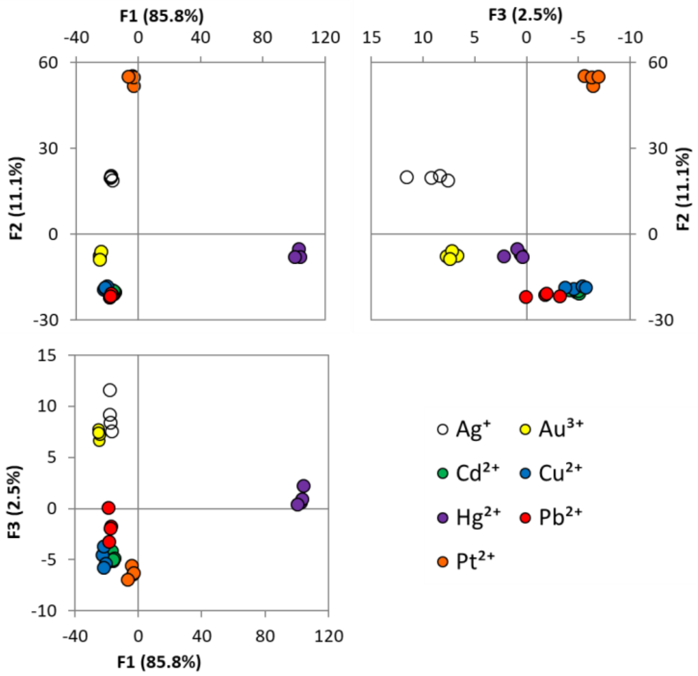

Figure S7a LDA score plot for the analysis of seven metal ions in lake-water samples (confidence ellipsoids at 95% probability) performed in quintuplicate (1 mM metal ion, pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545. Each pairing of the three main factors is plotted in a separate 2D plot.

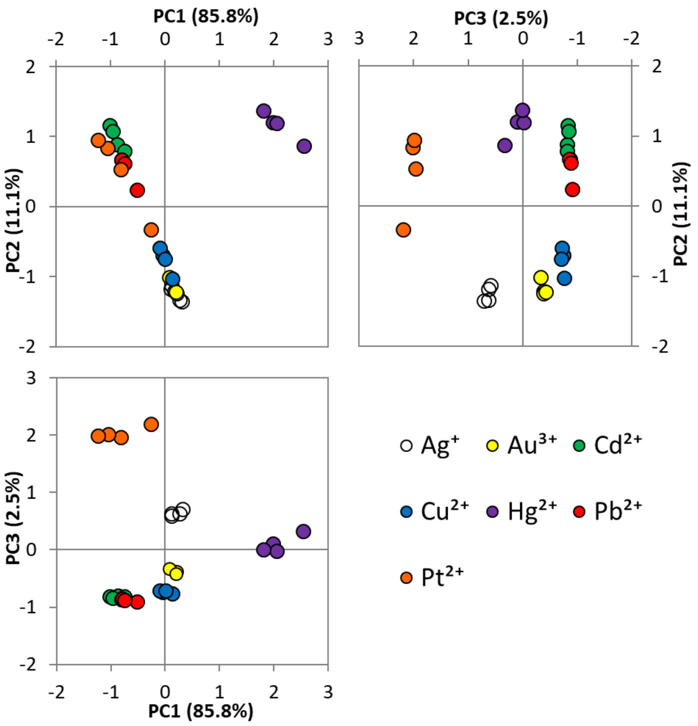

Figure S7b PCA score plot for the analysis of seven metal ions in lake-water samples (confidence ellipsoids at 95% probability) performed in quintuplicate (1 mM metal ion, pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545. Each pairing of the three main factors is plotted in a separate 2D plot.

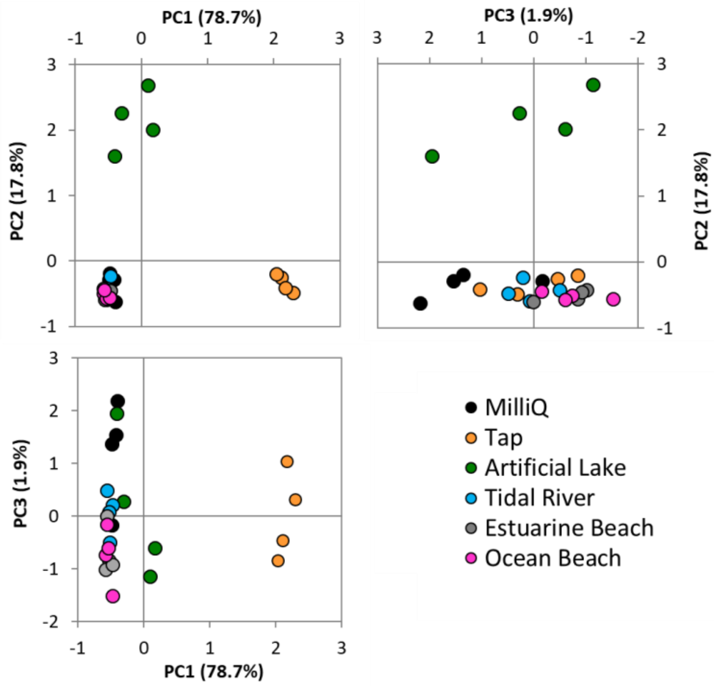

Figure S8 PCA score plot for the analysis of environmental water samples (confidence ellipsoids at 95% probability) performed in quadruplicate (pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545. Each pairing of the three main factors is plotted in a separate 2D plot.

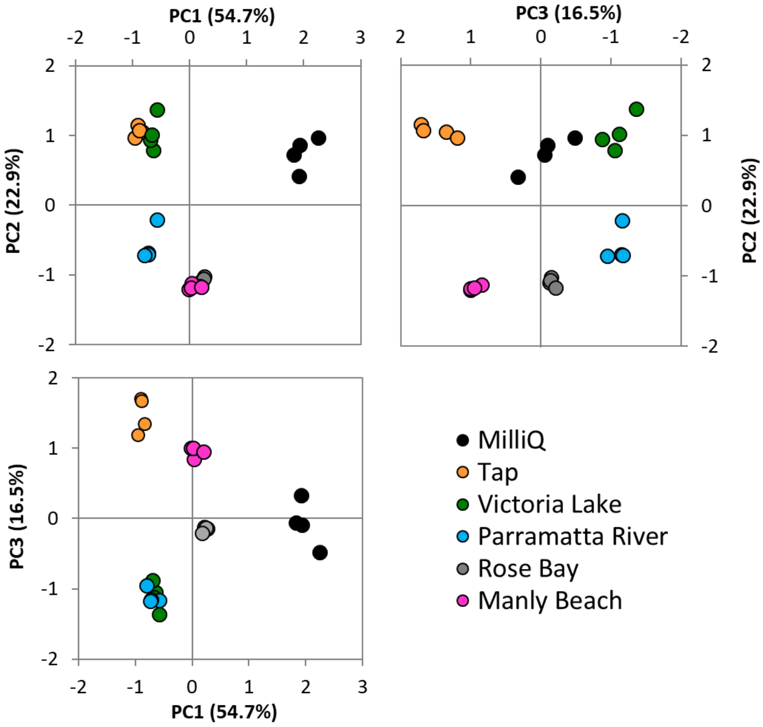

Figure S9 PCA score plot for the analysis of Cu^{2+} (100 µM) doped environmental water samples samples (confidence ellipsoids at 95% probability) performed in quadruplicate (pH 7.4, 20 mM HEPES). Each pairing of the three main factors is plotted in a separate 2D plot.

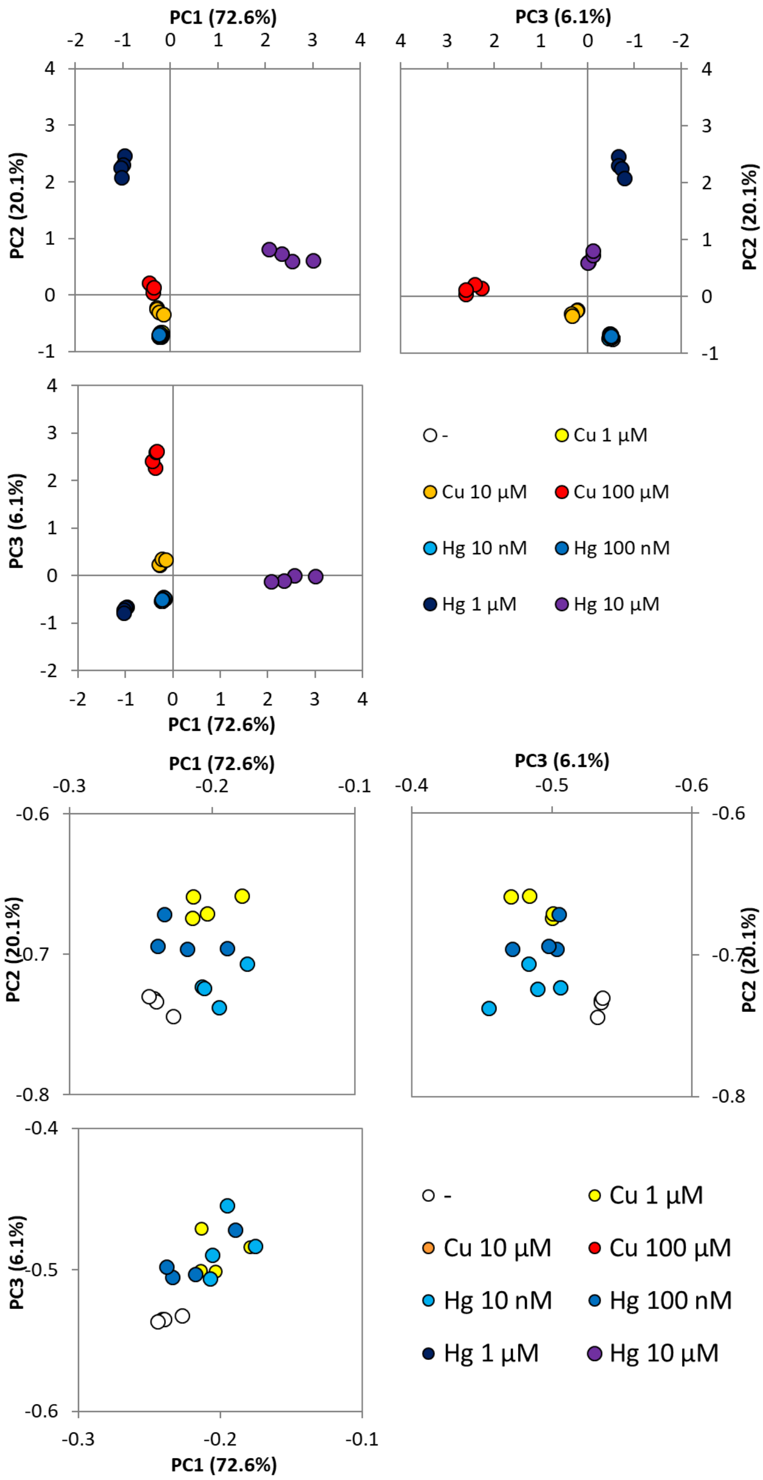

Figure S10 PCA score plots for the analysis of Hg^{2+} (10 nM to 10 µM) and Cu^{2+} (1 µM to 100 µM) solutions (confidence ellipsoids at 95% probability) performed in quadruplicate (pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545. Each pairing of the three main factors is plotted in a separate 2D plot. The lower set of three plots are an expansion of the lower concentration region.

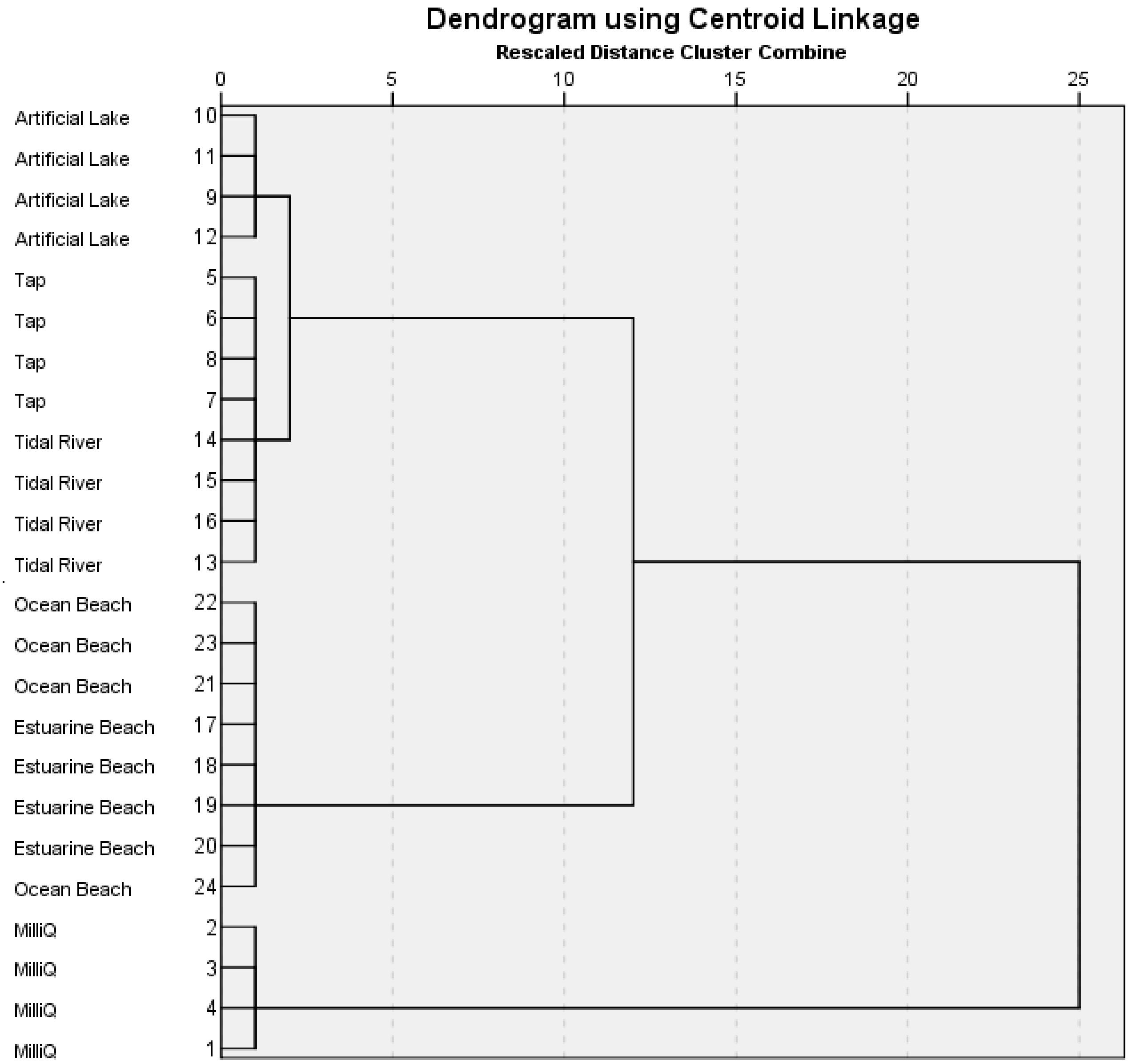

Figure 11 Hierarchical cluster analysis of Cu^{2+} (100 µM) doped environmental water samples samples.

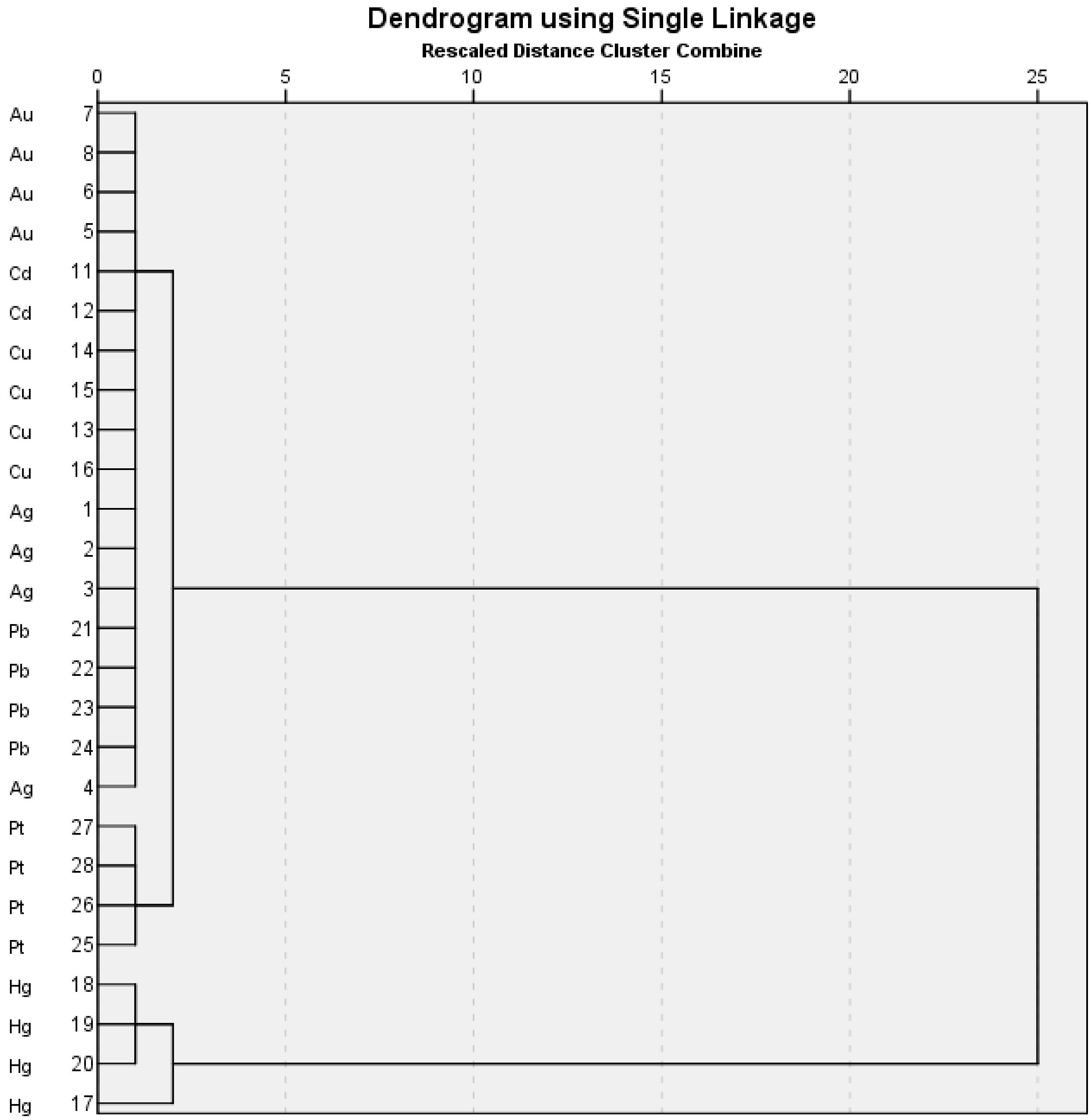

Figure 12 Hierarchical cluster analysis of analysis of seven metal ions (100 μM metal ion, pH 7.4, 20 mM HEPES)

Table S1 Rates of increase in fluorescence intensity from 0.5-5 minutes after addition of the stated metal ion. Values are the average of two repeats.

	Blank	Cu	Ag	Cd	Hg	Pb	Au	Pt
EtOH	101	165	515	113	2602	183	363	181
IPA	115	35	599	52	1106	81	346	100
MeCN	53	1984	60	37	2788	51	1144	251
DMSO	148	67	102	118	68	89	717	668

Table S2 Fluorescence intensity values for the analysis of seven metal ions performed in quintuplicate (1 mM metal ion, pH 7.4, 20 mM HEPES) describing the response to thiocoumarin 525 in eight solvents.

	HEPES	MeOH	EtOH	PrOH	MeCN	DMF	DMSO
Ag^+	197	20142	2875	643	470	2213	8020
Ag^+	178	20159	2473	588	523	2115	7818
Ag^+	161	19500	3253	1255	420	2327	6950
Ag^+	165	19799	3920	597	503	2060	7858
Ag^+	191	19902	3444	684	394	1827	7667
Au^{3+}	314	5812	5429	9780	13294	4311	9021
Au^{3+}	304	5800	4949	8277	13159	3829	8659
Au^{3+}	292	5636	3806	7244	13052	3902	8295
Au^{3+}	348	5920	4168	7109	12626	3827	8061
Au^{3+}	254	5872	3376	7178	12364	3827	7879
Cd^{2+}	70	652	448	235	383	169	272
Cd^{2+}	108	691	402	295	373	171	228
Cd^{2+}	56	645	363	199	310	125	174
Cd^{2+}	88	690	386	223	336	126	247
Cd^{2+}	129	664	378	200	323	120	207
Cu^{2+}	579	10798	19484	1242	5429	579	2038
Cu^{2+}	658	9045	18578	1179	5632	586	2126
Cu^{2+}	624	11609	18525	1107	5903	518	2641
Cu^{2+}	568	11945	18518	1266	5456	662	3577
Cu^{2+}	541	10277	19590	1221	4538	667	3780
Hg^{2+}	4015	21902	18697	14665	19513	6631	13880
Hg^{2+}	4189	21046	17943	14052	17478	6074	12609
Hg^{2+}	4552	21202	16990	13226	18806	5836	12523
Hg^{2+}	4317	21415	17392	13275	18657	5754	12422
Hg^{2+}	4669	20643	16715	14182	18361	5581	11047
Pb^{2+}	149	2031	882	256	356	144	322
Pb^{2+}	108	2162	828	248	397	179	290
Pb^{2+}	39	2063	834	216	293	82	314
Pb^{2+}	131	2330	777	233	339	189	320
Pb^{2+}	102	2236	1253	204	367	145	301
Pt^{2+}	3365	3784	9033	5798	4809	378	8731
Pt^{2+}	3292	4270	9094	6112	4877	397	8686
Pt^{2+}	3271	4192	9163	5939	4488	410	8598
Pt^{2+}	3123	3840	8653	5615	4568	381	8411

Table S3 Fluorescence intensity values of three metal salts performed in triplicate (100 µM metal ion, pH 7.4, 20 mM HEPES) describing the response to thiocoumarin 545 in ethanol, isopropanol, acetonitrile and DMSO. This data was used as a test data set, where Table Sxx had been used as a training data set, to examine the effect of the counteranion upon fluorescence output.

	EtOH	Isopropanol	Acetonitrile	DMSO
Copper chloride	12547	1832	28175	966
Copper chloride	11126	1803	27239	1028
Copper chloride	12383	1262	27564	1113
Mercury chloride	37268	18513	27796	27456
Mercury chloride	30191	20870	23107	25797
Mercury chloride	31992	23502	24524	29147
Mercury acetate	30875	21034	36663	14636
Mercury acetate	29595	23245	36151	14100
Mercury acetate	27410	22310	37728	14772

Table S4 Fluorescence intensity values for a range of metal (100 µM metal ion, pH 7.4, 20 mM HEPES) describing the response to thiocoumarin 545 in ethanol, isopropanol, acetonitrile and DMSO. Also shown is (a) the predicted group membership (98% correct classification); (b) the discriminant scores following LDA (F1-F3); and (c) the probability of group membership for each category – it can be seen that there is a greater spread and far less certainty for Ni^{2+}, Zn^{2+} and Fe^{2+}.

					(a)		(b)		(c) Predicted group membership										
	EtOH	IPA	MeCN	DMSO		F1	F2	F3	Ag	Au	Cd	Cu	Hg	Pb	Pt	Zn	Ru	Ni	Fe
Ag	29786	28054	1425	3980	Ag	13.68916	-9.08419	-8.65987	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ag	30220	30476	1394	3934	Ag	14.19673	-9.01476	-9.01443	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ag	27264	31661	1540	5216	Ag	12.89512	-8.51312	-7.83634	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ag	27126	27343	1650	4676	Ag	12.07429	-8.51102	-7.59646	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ag	24026	23984	1456	6619	Ag	10.70695	-8.85216	-5.75117	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au	12519	21978	39354	43089	Au	18.41315	0.21605	9.40706	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au	13675	24795	44476	45223	Au	20.0362	2.16101	9.46614	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au	14816	26842	34126	36358	Au	17.26142	-0.08288	6.13947	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au	16162	12544	42939	37194	Au	16.19947	3.19657	7.17316	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Au	14334	19990	45424	45374	Au	20.04209	2.05597	9.73669	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cd	1208	1062	1368	2303	Cd	-10.47038	-1.74728	1.45809	0.00	0.00	0.39	0.00	0.00	0.34	0.00	0.07	0.01	0.19	0.00
Cd	1228	1070	1392	2362	Cd	-10.42347	-1.76591	1.47091	0.00	0.00	0.39	0.00	0.00	0.35	0.00	0.06	0.01	0.18	0.00
Cd	1377	1048	1380	2222	Cd	-10.39871	-1.75576	1.38317	0.00	0.00	0.38	0.00	0.00	0.35	0.00	0.07	0.01	0.19	0.00
Cd	1350	1062	1707	2247	Cd	-10.44929	-1.56242	1.3938	0.00	0.00	0.35	0.00	0.00	0.33	0.00	0.09	0.02	0.22	0.00
Cd	1221	911	1266	3372	Cd	-9.82346	-2.32234	1.82478	0.00	0.00	0.51	0.00	0.00	0.39	0.00	0.02	0.00	0.08	0.00
Cu	20716	4308	38900	2018	Cu	-1.57412	15.06612	-5.07667	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cu	21481	3421	38826	2270	Cu	-0.93615	14.59487	-5.13558	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cu	28069	5176	41900	1846	Cu	3.31368	14.7406	-7.38378	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cu	25913	3219	40968	1905	Cu	1.74202	14.65976	-6.55355	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cu	22482	1571	33149	1973	Cu	0.26773	10.87703	-5.28728	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	25827	29873	37852	31867	Hg	22.32539	1.08529	1.13287	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	26347	32789	41458	33901	Hg	23.65364	2.37515	1.35439	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	29971	29285	40215	26005	Hg	21.4567	3.89784	-1.99995	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	28058	29439	42384	28708	Hg	21.3762	4.53992	-0.58714	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	25110	28554	51384	38005	Hg	23.39204	6.43001	3.3229	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Pb	2152	1423	1288	1858	Pb	-10.00545	-1.84963	1.00522	0.00	0.00	0.33	0.00	0.00	0.39	0.00	0.08	0.01	0.19	0.00
Pb	2090	1284	1417	1903	Pb	-10.05464	-1.78582	1.04876	0.00	0.00	0.33	0.00	0.00	0.38	0.00	0.09	0.01	0.19	0.00
Pb	2129	1179	1748	1696	Pb	-10.20645	-1.51234	0.97347	0.00	0.00	0.28	0.00	0.00	0.34	0.00	0.12	0.02	0.24	0.00
Pb	2136	1280	1951	1718	Pb	-10.20853	-1.39502	0.96719	0.00	0.00	0.26	0.00	0.00	0.32	0.00	0.14	0.03	0.25	0.00
Pb	1621	991	1305	2118	Cd	-10.2775	-1.83227	1.28375	0.00	0.00	0.38	0.00	0.00	0.37	0.00	0.07	0.01	0.18	0.00
Pt	7264	7994	3620	14639	Pt	1.56447	-7.43901	3.16526	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Pt	7904	7330	3657	15380	Pt	2.40641	-8.01444	3.28426	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Pt	10505	8943	6312	12361	Pt	2.2614	-5.69178	1.34745	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Pt	10687	9381	6364	12927	Pt	2.76194	-5.94407	1.44369	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Zn	2559	1299	3371	980	Zn	-10.54298	-0.33171	0.5785	0.00	0.00	0.06	0.00	0.00	0.10	0.00	0.31	0.20	0.30	0.03
Zn	3229	1451	3642	986	Zn	-10.0779	-0.36683	0.36909	0.00	0.00	0.06	0.00	0.00	0.12	0.00	0.36	0.16	0.28	0.01
Zn	3560	1591	3463	1055	Zn	-9.75803	-0.59658	0.28599	0.00	0.00	0.08	0.00	0.00	0.17	0.00	0.37	0.10	0.28	0.01
Zn	3261	1775	4017	1046	Zn	-10.04415	-0.15363	0.34627	0.00	0.00	0.04	0.00	0.00	0.08	0.00	0.38	0.21	0.25	0.03
Zn	3146	2238	4292	1018	Zn	-10.14289	0.09766	0.32587	0.00	0.00	0.02	0.00	0.00	0.05	0.00	0.36	0.31	0.21	0.05
Ru	2469	1961	4865	678	Ru	-10.94443	0.7846	0.42611	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.14	0.46	0.08	0.31
Ru	2469	1612	4747	705	Ru	-10.94235	0.67259	0.46744	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.17	0.45	0.10	0.26
Ru	2670	1635	4684	712	Ru	-10.78097	0.57149	0.41023	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.21	0.44	0.13	0.20
Ru	3006	1697	4547	705	Ru	-10.51567	0.39422	0.30671	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.29	0.40	0.16	0.12
Ru	3154	1529	4534	699	Ru	-10.4247	0.32962	0.27676	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.32	0.36	0.18	0.09
Ni	2189	1374	2456	1028	Ni	-10.64499	-0.78056	0.70724	0.00	0.00	0.12	0.00	0.00	0.17	0.00	0.24	0.12	0.33	0.01
Ni	2261	1385	2476	1091	Ni	-10.55707	-0.81925	0.70608	0.00	0.00	0.13	0.00	0.00	0.18	0.00	0.24	0.11	0.33	0.01
Ni	2283	1353	2407	1075	Ni	-10.5435	-0.86249	0.69809	0.00	0.00	0.14	0.00	0.00	0.19	0.00	0.24	0.10	0.33	0.01
Ni	2304	1363	2454	1089	Ni	-10.52579	-0.84657	0.69516	0.00	0.00	0.13	0.00	0.00	0.19	0.00	0.24	0.10	0.33	0.01
Ni	2598	1430	2410	1067	Ni	-10.31289	-0.94788	0.59718	0.00	0.00	0.14	0.00	0.00	0.21	0.00	0.25	0.08	0.32	0.01
Fe	1915	820	6180	1162	Fe	-11.34968	1.42203	0.83142	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.31	0.03	0.61
Fe	1847	769	6336	1221	Fe	-11.39098	1.5046	0.87325	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.29	0.02	0.65
Fe	1906	813	6873	1260	Fe	-11.39828	1.79289	0.85818	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.24	0.01	0.73

Table S5 Fluorescence intensity values for the analysis of seven metal ions performed in quintuplicate (100 µM metal ion, pH 7.4, 20 mM HEPES) describing the response to thiocoumarin 545 in ethanol, isopropanol, acetonitrile and DMSO.

	EtOH	IPA	MeCN	DMSO
Ag^+	29786	28054	1425	3980
Ag^+	30220	30476	1394	3934
Ag^+	27264	31661	1540	5216
Ag^+	27126	27343	1650	4676
Ag^+	24026	23984	1456	6619
Au^{3+}	12519	21978	39354	43089
Au^{3+}	13675	24795	44476	45223
Au^{3+}	14816	26842	34126	36358
Au^{3+}	16162	12544	42939	37194
Au^{3+}	14334	19990	45424	45374
Cd^{2+}	1208	1062	1368	2303
Cd^{2+}	1228	1070	1392	2362
Cd^{2+}	1377	1048	1380	2222
Cd^{2+}	1350	1062	1707	2247
Cd^{2+}	1221	911	1266	3372
Cu^{2+}	20716	4308	38900	2018
Cu^{2+}	21481	3421	38826	2270
Cu^{2+}	28069	5176	41900	1846
Cu^{2+}	25913	3219	40968	1905
Cu^{2+}	22482	1571	33149	1973
Hg^{2+}	25827	29873	37852	31867
Hg^{2+}	26347	32789	41458	33901
Hg^{2+}	29971	29285	40215	26005
Hg^{2+}	28058	29439	42384	28708
Hg^{2+}	25110	28554	51384	38005
Pb^{2+}	2152	1423	1288	1858
Pb^{2+}	2090	1284	1417	1903
Pb^{2+}	2129	1179	1748	1696
Pb^{2+}	2136	1280	1951	1718
Pb^{2+}	1621	991	1305	2118
Pt^{2+}	7264	7994	3620	14639
Pt^{2+}	7904	7330	3657	15380
Pt^{2+}	10505	8943	6312	12361
Pt^{2+}	10687	9381	6364	12927
Pt^{2+}	9872	13798	6621	20612

Table S6 Fluorescence intensity values for the analysis of environmental water samples performed in quadruplicate (pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545.

	HEPES	MeOH	EtOH	PrOH	MeCN	DMF	DMSO
Ag^{+}	195	955	557	759	1252	398	147
Ag^{+}	202	839	507	726	1209	357	143
Ag^{+}	233	732	483	815	1170	336	150
Ag^{+}	402	487	424	919	1433	288	101
Au^{3+}	40	2144	2830	2641	2135	1837	2770
Au^{3+}	29	2209	2306	1911	2667	1655	2687
Au^{3+}	48	2226	2319	1726	2656	1420	2585
Au^{3+}	33	2238	2249	1704	2652	1373	2647
Cd^{2+}	0	929	880	771	1338	754	1026
Cd^{2+}	0	895	854	811	1336	815	1028
Cd^{2+}	38	1020	897	809	1337	840	1108
Cd^{2+}	4	838	834	788	1303	804	971
Cu^{2+}	7	834	1046	484	1124	299	353
Cu^{2+}	69	895	983	478	1028	409	376
Cu^{2+}	85	935	1110	496	1073	392	415
Cu^{2+}	61	1008	1230	615	902	453	403
Hg^{2+}	883	10987	12262	18072	16826	5712	3222
Hg^{2+}	853	10297	10331	14722	18262	4505	2943
Hg^{2+}	872	10396	10094	13730	18440	4808	2787
Hg^{2+}	884	9685	9914	13641	17554	4584	2766
Pb^{2+}	230	813	389	500	1139	389	156
Pb^{2+}	297	730	381	467	1049	306	190
Pb^{2+}	297	843	395	388	929	356	199
Pb^{2+}	282	1079	449	407	896	307	125
Pt^{2+}	207	4810	6120	6476	2934	2130	4872
Pt^{2+}	311	4971	5213	5619	3096	1594	4463
Pt^{2+}	363	5020	4864	4294	3167	1726	4547
Pt^{2+}	351	4907	4474	3930	2751	1402	4455

Table S7 Fluorescence intensity values for the analysis of environmental water samples performed in quadruplicate (pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545. Each pairing of the three main factors is plotted in a separate 2D plot.

	HEPES	MeOH	EtOH	PrOH	MeCN	DMF	DMSO
MilliQ	265	11489	15370	690	6493	566	1988
MilliQ	324	12575	13986	471	6094	572	1928
MilliQ	273	12340	13620	568	5625	627	1765
MilliQ	305	11266	14187	625	5881	581	1834
Tap	416	3735	3313	239	1627	909	347
Tap	391	3717	3199	296	1643	823	314
Tap	339	3772	3176	243	1133	860	301
Tap	430	3714	3180	255	1514	854	384
Artificial Lake	176	1154	1607	544	888	439	529
Artificial Lake	179	1280	1448	519	889	525	551
Artificial Lake	146	1223	1521	556	907	531	509
Artificial Lake	121	1259	1646	647	965	596	518
Tidal River	62	4506	3475	334	989	398	320
Tidal River	40	4249	2870	215	1004	318	256
Tidal River	34	4088	2708	198	1018	320	298
Tidal River	68	4037	2345	185	947	318	281
Estuarine Beach	197	10793	6261	221	2625	296	809
Estuarine Beach	185	10505	6531	172	2431	310	845
Estuarine Beach	206	10483	7051	228	2340	271	762
Estuarine Beach	191	9780	6734	190	2367	246	761
Ocean Beach	393	10320	6685	275	2277	180	362
Ocean Beach	405	10364	6435	219	2129	190	445
Ocean Beach	411	10465	6405	238	2389	182	423
Ocean Beach	407	11047	6968	279	3101	171	354

Table S8 Fluorescence intensity values for the analysis of Cu^{2+} (100 µM) doped environmental water samples performed in quadruplicate (pH 7.4, 20 mM HEPES). Each pairing of the three main factors is plotted in a separate 2D plot.

	HEPES	MeOH	EtOH	PrOH	MeCN	DMF	DMSO
MilliQ	103	584	405	402	587	216	236
MilliQ	139	466	409	313	567	226	244
MilliQ	117	461	377	251	528	165	256
MilliQ	102	421	331	284	538	213	180
Tap	303	1647	2580	312	1280	307	317
Tap	274	1648	3058	286	1276	327	338
Tap	263	1605	3165	347	1237	359	355
Tap	283	1669	2570	334	1217	320	299
Artificial Lake	268	927	706	926	1533	614	300
Artificial Lake	216	1009	671	861	1136	534	324
Artificial Lake	370	1240	683	934	1304	480	298
Artificial Lake	315	1444	792	1126	1668	592	295
Tidal River	123	419	405	347	523	199	196
Tidal River	86	386	267	242	464	192	191
Tidal River	83	399	258	228	468	197	161
Tidal River	64	391	283	216	465	169	174
Estuarine Beach	66	365	287	202	407	173	143
Estuarine Beach	79	338	228	234	426	183	140
Estuarine Beach	105	362	258	232	436	162	150
Estuarine Beach	80	306	282	199	422	171	170
Ocean Beach	88	237	308	238	422	158	147
Ocean Beach	79	318	296	222	405	153	151
Ocean Beach	61	345	289	167	442	174	122
Ocean Beach	75	367	327	256	445	194	168

Table S9 Fluorescence intensity values for the analysis of Hg^{2+} (10 nM to 10 μM) and Cu^{2+} (1 μM to 100 μM) solutions performed in quadruplicate (pH 7.4, 20 mM HEPES) describing the response of thiocoumarin 545 (100 nM).

	HEPES	MeOH	EtOH	PrOH	MeCN	DMF	DMSO
-	0	23	0	0	28	0	0
-	0	14	0	0	32	0	0
-	0	5	0	0	2	0	0
-	0	0	0	0	90	0	0
Cu 1 μM	83	464	210	106	309	14	44
Cu 1 μM	75	510	258	93	387	37	35
Cu 1 μM	95	511	303	94	331	59	89
Cu 1 μM	101	551	310	148	489	89	71
Cu 10 μM	463	3142	6223	273	976	130	531
Cu 10 μM	456	2951	6141	272	850	135	558
Cu 10 μM	532	3094	5890	291	1174	148	760
Cu 10 μM	537	3164	5748	354	1593	166	727
Cu 100 μM	585	11057	11146	653	2441	976	2876
Cu 100 μM	767	11447	11117	689	2291	910	3139
Cu 100 μM	856	10374	11009	665	2269	972	3477
Cu 100 μM	886	11562	11788	748	2848	830	3332
Hg 10 nM	75	330	220	156	391	56	70
Hg 10 nM	59	261	170	135	254	10	104
Hg 10 nM	33	221	162	108	205	8	27
Hg 10 nM	32	204	163	86	214	37	55
Hg 100 nM	0	690	367	143	415	70	36
Hg 100 nM	16	534	272	147	233	0	6
Hg 100 nM	53	487	294	98	203	0	19
Hg 100 nM	15	510	175	57	189	0	25
Hg 1 μM	1710	10811	7152	1775	323	1697	168
Hg 1 μM	1566	10314	6337	1316	234	1617	164
Hg 1 μM	1638	9944	5384	1019	242	1480	175
Hg 1 μM	1511	10106	4227	1037	236	1188	99
Hg 10 μM	2288	10202	11176	12359	11618	3098	667
Hg 10 μM	2298	10085	10089	9422	11956	2596	608
Hg 10 μM	2360	10073	9751	9031	11296	2377	509
Hg 10 μM	2149	10339	9070	8591	10138	2411	547

Table S10 Jackknifie classification matrices for the LDA data:

Jackknife classification matrix for the data in figure 2b:

	Ag	Au	Cd	Cu	Hg	Pb	Pt	% correct
Ag	5	0	0	0	0	0	0	100
Au	0	5	0	0	0	0	0	100
Cd	0	0	5	0	0	0	0	100
Cu	0	0	0	5	0	0	0	100
Hg	0	0	0	0	5	0	0	100
Pb	0	0	0	0	0	5	0	100
Pt	0	0	0	0	0	0	4	100
Total								100

Jackknife classification matrix for the data in figure S2:

	Ag	Au	Cd	Cu	Hg	Pb	Pt	% correct
Ag	3	0	0	0	0	0	0	100
Au	0	3	0	0	0	0	0	100
Cd	0	0	3	0	0	0	0	100
Cu	0	0	0	3	0	0	0	100
Hg	0	0	0	0	3	0	0	100
Pb	0	0	0	0	0	3	0	100
Pt	0	0	0	0	0	0	3	100
Total								100

Jackknife classification matrix for the data in figure 3:

	Ag	Au	Cd	Cu	Hg	Pb	Pt	% correct
Ag	5	0	0	0	0	0	0	100
Au	0	5	0	0	0	0	0	100
Cd	0	0	4	0	0	0	0	80
Cu	0	0	0	5	0	0	0	100
Hg	0	0	0	0	5	0	0	100
Pb	0	0	1	0	0	5	0	100
Pt	0	0	0	0	0	0	4	100
Total								98

Jackknife classification matrix for the data in figure S4a:

	Ag	Au	Cd	Cu	Hg	Pb	Pt	% correct
Ag	4	0	0	0	0	0	0	100
Au	0	4	0	0	0	0	0	100
Cd	0	0	4	0	0	0	0	100
Cu	0	0	0	4	0	0	0	100
Hg	0	0	0	0	4	0	0	100
Pb	0	0	1	0	0	4	0	100
Pt	0	0	0	0	0	0	4	100
Total								100

Jackknife classification matrix for the data in figure 5:

	MilliQ	Tap	Lake	Tidal river	Estuarine Beach	Ocean beach	% correct
MilliQ	3	0	0	1	0	0	75
Tap	0	4	0	0	0	0	100
Lake	0	0	4	0	0	0	100
Tidal river	0	0	0	2	2	0	50
Estuarine beach	0	0	0	1	3	0	75
Ocean beach	0	0	1	1	1	2	50
Total							75

Jackknife classification matrix for the data in figure 7:

	No metal	Cu 1 µM	Cu 10 µM	Cu 100 µM	Hg 10 nM	Hg 100 nM	Hg 1 µM	Hg 10 µM	% correct
No metal	4	0	0	0	0	0	0	0	100
Cu 1 µM	0	4	0	0	0	0	0	0	100
Cu 10 µM	0	0	4	0	0	0	0	0	100
Cu 100 µM	0	0	0	4	0	0	0	0	100
Hg 10 nM	0	0	0	0	4	0	0	0	100
Hg 100 nM	0	0	0	0	0	4	0	0	100
Hg 1 µM	0	0	0	0	0	0	4	0	100
Hg 10 µM	0	0	0	0	0	0	0	4	100
Total									100