

Supplementary data

Microbial levan and pullulan as potential protective agents for reducing adverse effects of copper on *Daphnia magna* and *Vibrio fischeri*

Branka Lončarević¹, Marija Lješević¹, Marijana Marković¹, Ivan Anđelković^{2,3}, Gordana Gojgić-Cvijović¹, Dragica Jakovljević¹, Vladimir Beškoski⁴

¹ Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia.

² Innovation Center, Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade 11000, Serbia

³School of Agriculture, Food and Wine, University of Adelaide, Urrbrae SA 5064, Australia

⁴ Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade 11000, Serbia.

Table S1. Specific catalase (CAT) activity in *D. magna* after the acute toxicity test

Time, min	CL		Cu-CL		Cu-L		Cu-P	
	Specific CAT activity, U/mg	Standard deviation						
1	3.159	0.15	2.565	0.25	4.136	0.35	4.234	0.11
2	3.109	0.11	2.418	0.11	3.901	0.15	4.039	0.06
3	3.008	0.09	2.296	0.11	3.682	0.05	3.800	0.04
4	2.908	0.09	2.235	0.08	3.454	0.04	3.616	0.05
5	2.818	0.09	2.184	0.07	3.299	0	3.479	0.04
6	2.632	0.09	2.027	0.08	3.039	0.03	3.214	0.02
7	2.514	0.07	1.958	0.07	2.867	0.02	3.052	0.03
8	2.400	0.07	1.887	0.07	2.714	0.03	2.915	0.03
9	2.306	0.06	1.824	0.06	2.574	0.03	2.772	0.03
10	2.211	0.05	1.759	0.06	2.444	0.03	2.645	0.02

Table S2. Specific catalase (CAT) activity in *D. magna* after the prolonged acute toxicity test

Time, min	CL		Cu-CL		Cu-L		Cu-P	
	Specific CAT activity, U/mg	Standard deviation	Specific CAT activity, U/mg	Standard deviation	Specific CAT activity, U/mg	Standard deviation	Specific CAT activity, U/mg	Standard deviation
1	3.190	0.10	3.877	0.09	3.265	0.02	2.752	0.04
2	2.718	0.05	3.243	0.05	2.892	1.54×10^{-15}	2.357	0.03
3	2.304	0.03	2.769	0.04	2.519	7.69×10^{-16}	2.006	0.03
4	1.951	0.02	2.398	0.03	2.188	6.09×10^{-3}	1.710	0.03
5	1.669	0.01	2.087	0.03	1.914	9.75×10^{-3}	1.469	0.02
6	1.439	0.90×10^{-2}	1.829	0.02	1.675	0.01	1.270	0.02
7	1.259	5.14×10^{-3}	1.620	0.01	1.484	0.01	1.115	0.01
8	1.115	3.90×10^{-3}	1.447	0.01	1.326	0.01	0.990	0.01
9	0.995	0.40×10^{-2}	1.305	0.01	1.193	0.01	0.887	0.01
10	0.899	0.36×10^{-2}	1.183	0.91×10^{-2}	1.085	0.01	0.802	0.01

Table S3. Specific superoxide dismutase (SOD) activity in *D. magna* after the acute toxicity test

Time, min	CL		Cu-CL		Cu-L		Cu-P	
	Specific SOD activity, U/mg	Standard deviation	Specific SOD activity, U/mg	Standard deviation	Specific SOD activity, U/mg	Standard deviation	Specific SOD activity, U/mg	Standard deviation
1	9.03x10 ⁻³	0.0040396	0.060	0.02	0.042	0.01	0.051	0.02
2	9.26x10 ⁻³	0.0020107	0.051	0.01	0.035	8.27x10 ⁻³	0.041	0.01
3	8.23x10 ⁻³	0.0012137	0.043	0.01	0.029	6.68x10 ⁻³	0.033	0.01
4	7.66x10 ⁻³	0.0011005	0.037	8.18x10 ⁻³	0.025	6.04x10 ⁻³	0.027	0.01
5	7.0x10 ⁻³	0.0009983	0.032	6.15x10 ⁻³	0.022	5.74x10 ⁻³	0.021	0.01
6	6.57x10 ⁻³	0.0009371	0.028	4.67x10 ⁻³	0.019	5.58x10 ⁻³	0.017	0.01
7	6.14x10 ⁻³	0.0009445	0.024	3.89x10 ⁻³	0.017	5.58x10 ⁻³	0.014	0.01
8	5.84x10 ⁻³	0.0009507	0.021	3.24x10 ⁻³	0.015	5.49x10 ⁻³	0.011	9.15x10 ⁻³

Table S4. Effect of 50 µg/L Cu(II) and mixture of 50 µg/L Cu(II) and 100 mg/L of glucose, levan and pullulan, respectively on cumulative: (a) O₂ consumption, (b) CO₂ production in 20 *D. magna*.

Interval	Channel	Sample	Time (h)	Respiration exchange ratio, RER	Temperature (°C)	O ₂ (%)	O ₂ rate (µL/min)	Cumulative O ₂ (µL)	CO ₂ (%)	CO ₂ rate (µL/min)	Cumulative CO ₂ (µL)
0	1	CL	0.42	0	18.2	20.919	0	0	0.043	0	0
1	1	CL	2.87	0.537	19.0	20.913	-0.447	-65.767	0.045	0.135	19.862
2	1	CL	5.33	0.025	18.5	20.907	-0.472	-135.311	0.045	0.067	29.719
3	1	CL	7.78	0.225	20.5	20.907	-0.353	-187.257	0.045	0.067	39.524
4	1	CL	10.24	0.123	20.9	20.907	-0.267	-226.558	0.044	0.059	48.157
5	1	CL	12.69	0.032	18.6	20.897	-0.950	-366.413	0.044	0.006	49.062
6	1	CL	15.15	0.038	21.0	20.912	0.664	-268.608	0.044	-0.009	47.666
7	1	CL	17.6	0.025	19.6	20.897	-1.465	-484.387	0.044	-0.027	43.695
8	1	CL	25.72	0.214	18.3	20.915	-0.273	-505.194	0.042	-0.029	41.631
9	1	CL	28.17	0.042	19.0	20.907	-0.372	-559.932	0.043	0.054	49.570
10	1	CL	30.63	0.028	21.0	20.906	-0.074	-570.826	0.043	0.002	49.872
11	1	CL	33.08	0.101	20.5	20.909	-0.191	-598.911	0.043	0.000	49.911
12	1	CL	35.54	0.085	21.0	20.904	-0.312	-644.862	0.044	-0.009	48.573
13	1	CL	37.99	0.018	21.2	20.894	-0.585	-731.064	0.046	0.060	57.430
14	1	CL	40.45	0.005	20.5	20.890	-0.633	-824.290	0.046	-0.044	50.881
15	1	CL	42.9	0.019	20.3	20.885	-0.912	-958.627	0.047	0.071	61.408
16	1	CL	45.36	0.023	19.4	20.884	-0.437	-1023.049	0.045	0.000	61.430
0	2	Cu-CL	0.52	0	19.2	20.922	0	0	0.043	0	0
1	2	Cu-CL	2.97	0.585	18.9	20.917	-0.275	-40.514	0.045	0.165	24.316
2	2	Cu-CL	5.43	0.442	19.7	20.914	-0.152	-62.828	0.045	0.037	29.712

3	2	Cu-CL	7.88	0.082	20.8	20.910	-0.499	-136.329	0.045	0.100	44.464
4	2	Cu-CL	10.34	0.655	21.0	20.911	-0.084	-148.765	0.044	-0.004	43.922
5	2	Cu-CL	12.79	0.096	20.2	20.907	-0.375	-204.015	0.043	0.030	48.339
6	2	Cu-CL	15.25	0.069	21.0	20.920	0.664	-106.258	0.043	-0.018	45.700
7	2	Cu-CL	17.7	0.005	20.5	20.908	-0.925	-242.560	0.044	-0.057	37.281
8	2	Cu-CL	25.82	0.252	19.5	20.919	0.365	-215.164	0.042	-0.149	26.935
9	2	Cu-CL	28.27	0.230	20.4	20.914	-0.094	-228.954	0.042	0.048	33.998
10	2	Cu-CL	30.73	0.111	21.0	20.911	0.054	-220.981	0.043	-0.005	33.303
11	2	Cu-CL	33.18	0.012	20.8	20.917	0.154	-198.356	0.043	-0.038	27.725
12	2	Cu-CL	35.64	0.005	21.0	20.910	-0.347	-249.464	0.044	0.036	33.071
13	2	Cu-CL	38.09	0.075	21.2	20.899	-0.492	-321.898	0.045	0.002	33.329
14	2	Cu-CL	40.55	0.053	21.0	20.900	-0.163	-345.861	0.046	0.009	34.595
15	2	Cu-CL	43	0.012	21.0	20.894	-0.780	-460.806	0.046	-0.002	34.275
16	2	Cu-CL	45.46	0.065	19.8	20.893	-0.280	-501.977	0.045	0.038	39.908
0	3	Cu-G	0.62	0	20.7	20.921	0	0	0.043	0	0
1	3	Cu-G	3.07	0.238	20.3	20.915	-0.497	-73.182	0.045	0.113	16.620
2	3	Cu-G	5.53	0.104	20.5	20.907	-0.670	-171.873	0.045	0.060	25.508
3	3	Cu-G	7.98	0.078	21.0	20.907	-0.482	-242.873	0.045	0.092	39.090
4	3	Cu-G	10.44	0.063	21.0	20.907	-0.272	-283.006	0.043	-0.020	36.150
5	3	Cu-G	12.89	0.129	20.8	20.907	-0.191	-311.087	0.043	0.065	45.767
6	3	Cu-G	15.35	0.023	21.0	20.914	0.185	-283.851	0.044	-0.032	41.121
7	3	Cu-G	17.8	0.027	20.8	20.903	-1.017	-433.638	0.044	-0.073	30.373
8	3	Cu-G	25.92	0.534	20.5	20.918	0.098	-426.355	0.042	-0.045	27.331
9	3	Cu-G	28.37	0.188	20.8	20.913	-0.182	-453.148	0.043	0.077	38.663
10	3	Cu-G	30.83	0.055	21.0	20.907	-0.371	-507.739	0.043	0.037	44.154
11	3	Cu-G	33.28	1.412	20.8	20.913	-0.037	-513.154	0.045	0.077	55.474
12	3	Cu-G	35.74	0.123	21.1	20.903	-0.544	-593.223	0.047	0.145	76.767
13	3	Cu-G	38.19	0.150	21.1	20.896	-0.453	-659.935	0.048	0.097	91.083
14	3	Cu-G	40.65	0.134	17.9	20.890	-0.826	-781.618	0.049	0.091	104.479
15	3	Cu-G	43.1	0.035	18.2	20.886	-0.978	-925.664	0.049	0.089	117.635
16	3	Cu-G	45.56	0.612	19.8	20.888	-0.348	-976.854	0.049	0.163	141.643
0	4	Cu-L	0.72	0	18.4	20.919	0	0	0.044	0	0
1	4	Cu-L	3.17	0.154	20.8	20.908	-1.043	-153.644	0.046	0.145	21.326
2	4	Cu-L	5.63	0.273	20.8	20.906	-0.309	-199.197	0.045	0.037	26.735
3	4	Cu-L	8.08	0.091	21.0	20.906	-0.493	-271.848	0.045	0.059	35.477
4	4	Cu-L	10.54	0.121	21.1	20.904	-0.379	-327.703	0.044	0.038	41.140
5	4	Cu-L	12.99	0.185	21.0	20.902	-0.437	-392.027	0.043	-0.010	39.665
6	4	Cu-L	15.45	0.201	21.0	20.911	0.176	-366.120	0.044	0.026	43.518
7	4	Cu-L	17.9	0.021	21.0	20.900	-1.067	-523.203	0.044	-0.058	34.989
8	4	Cu-L	26.02	0.343	20.8	20.917	-0.108	-531.059	0.043	0.143	44.573
9	4	Cu-L	28.47	0.141	21.0	20.903	-0.968	-673.665	0.044	0.149	66.562
10	4	Cu-L	30.93	0.119	21.0	20.894	-0.884	-803.792	0.046	0.113	83.258
11	4	Cu-L	33.38	0.241	21.0	20.897	-0.685	-904.620	0.048	0.163	107.269

12	4	Cu-L	35.84	0.208	21.2	20.886	-0.972	-1047.789	0.050	0.187	134.862
13	4	Cu-L	38.29	0.122	18.5	20.876	-1.241	-1230.539	0.052	0.166	159.268
14	4	Cu-L	40.75	0.273	19.3	20.880	-0.526	-1307.992	0.052	0.144	180.471
15	4	Cu-L	43.2	0.102	19.7	20.880	-0.854	-1433.836	0.052	0.137	200.584
16	4	Cu-L	45.66	0.458	19.8	20.880	-0.510	-1508.929	0.051	0.135	220.442
0	5	Cu-P	0.82	0	20.1	20.918	0	0	0.044	0	0
1	5	Cu-P	3.27	0.279	18.1	20.911	-0.584	-85.961	0.046	0.129	19.012
2	5	Cu-P	5.73	0.126	21.0	20.907	-0.407	-145.937	0.045	0.045	25.654
3	5	Cu-P	8.18	0.048	21.0	20.908	-0.445	-211.506	0.045	0.039	31.348
4	5	Cu-P	10.64	0.342	18.5	20.909	-0.154	-234.142	0.044	0.061	40.293
5	5	Cu-P	13.09	0.163	21.0	20.907	-0.293	-277.258	0.043	0.020	43.196
6	5	Cu-P	15.55	0.398	21.0	20.914	0.053	-269.465	0.043	-0.049	36.023
7	5	Cu-P	18	0.073	21.0	20.907	-0.562	-352.265	0.044	-0.009	34.748
8	5	Cu-P	26.12	0.049	18.3	20.911	-1.008	-424.422	0.043	0.090	40.665
9	5	Cu-P	28.58	0.025	18.4	20.908	-0.333	-473.505	0.043	0.047	47.661
10	5	Cu-P	31.03	0.306	21.0	20.904	-0.232	-507.635	0.0449	0.08	59.474
11	5	Cu-P	33.48	0.230	21.0	20.906	-0.569	-591.445	0.0469	0.163	83.481
12	5	Cu-P	35.94	0.216	18.1	20.885	-1.632	-831.781	0.0518	0.375	138.786
13	5	Cu-P	38.39	0.294	20.0	20.874	-1.328	-1027.353	0.0563	0.418	200.4
14	5	Cu-P	40.85	0.327	20.5	20.873	-1.068	-1184.672	0.059	0.366	254.286
15	5	Cu-P	43.3	0.222	20.8	20.872	-1.09	-1345.163	0.0573	0.19	282.3
16	5	Cu-P	45.76	0.246	19.5	20.873	-0.82	-1465.882	0.0553	0.162	306.203