



Supplemental data

Acute Toxicity of Sodium Chloride, Nitrates, Ortho-Phosphates, Cadmium, Arsenic and Aluminum for Juveniles of the Freshwater Pearl Mussel: *Margaritifera Margaritifera* (L.1758)

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Section 1. Sodium Chloride 48-h Toxicity Test

Figure S1 corresponds to the dose-response curve obtained in the 48 hrs toxicity test to NaCl with 10-month-old juveniles of the Freshwater Pearl Mussel *Margaritifera margaritifera* (FWPM) and Table S1 presents mean values of water quality parameters (pH, conductivity, dissolved oxygen and temperature) measured at the beginning of the test.

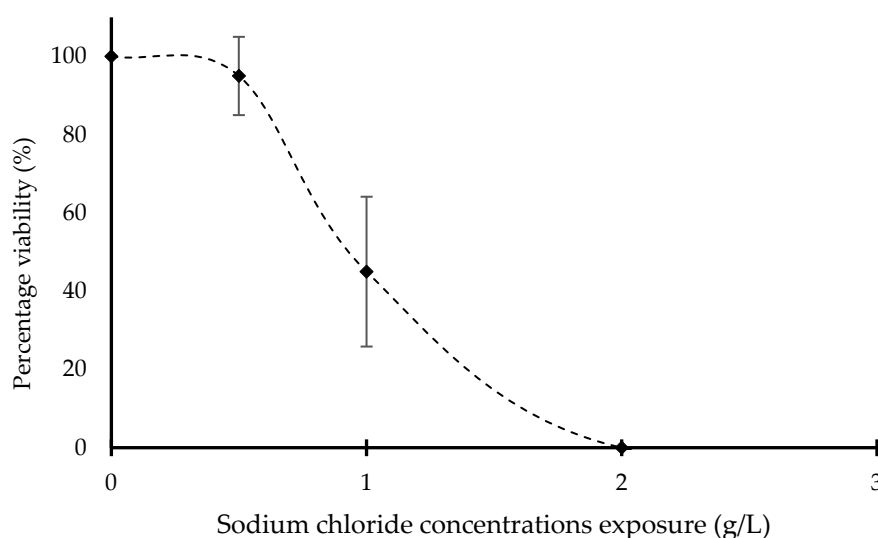


Figure S1. Viability (% $\pm$ SD) of 10-month old *M. margaritifera* juveniles exposed to sodium chloride in 48-h acute toxicity test conducted at 16 °C.

Table S1. Mean values ($\pm$ SD) measured at the beginning of the test, for water parameters (pH, conductivity, dissolved oxygen and temperature) in 48-h acute toxicity tests.

Experimental Conditions (NaCl Nominal Concentrations in g/L)	pH	Conductivity (μ S/cm)	Dissolved Oxygen (mg/L)	Temperature ($^{\circ}$ C)
<i>Control (0)</i>	7.1 ± 0.1	73 ± 6	7.7 ± 0.3	15.0 ± 0.1
<i>Low (0.5)</i>	7.1 ± 0.2	1074 ± 39	7.8 ± 0.3	14.9 ± 0
<i>Medium-low (2)</i>	6.9 ± 0.1	3707 ± 11	8.1 ± 0.3	14.8 ± 0.1
<i>High (8)</i>	6.9 ± 0.1	$13,603 \pm 101$	7.8 ± 0.2	14.7 ± 0.1

Section 2. Water Quality Parameters Values, Mean Percentage Viability and Mean Measured Concentration Values

Mean measured values of pH, temperature ($^{\circ}$ C), conductivity (μ S/cm) and dissolved oxygen (mg/L) at the beginning and at the end of each acute toxicity tests conducted in this study, in Control, low, medium and high exposure conditions are presented in the table S2. Nominal toxicant concentrations for each exposure conditions are also described in this table. Water quality parameters were measured in 3 replicates ($n = 3$) for all acute toxicity test except for phosphate exposure for which, pH was measured in one replicate at the end of the experiment. All experiments presented in this table were conducted in the presence of a substrate sand except for experiments where the absence of substrate is noticed in the Table S2.

Table S2. Mean measured values ($\pm$ SD) for pH, temperature ($^{\circ}$ C), conductivity (μ S/cm) and dissolved oxygen (mg/L) at the beginning and at the end of each acute toxicity tests conducted in this study in Control, low, medium and high exposure conditions ($n = 3$).

Pollutants (units)	Conditions	Nominal Concentrations	pH		Temperature ($^{\circ}$ C)		Conductivity (μ S/cm)		Dissolved Oxygen (mg/L)	
			Start	End	Start	End	Start	End	Start	End
NaCl without substrate (g/L) ¹	Control	0	7.02 $\pm$ 0.09	7.34 $\pm$ 0.04	16 $\pm$ 0.1	16.3 $\pm$ 0.05	59 $\pm$ 0.5	60 $\pm$ 1	7.12 $\pm$ 0.26	7.07 $\pm$ 0.09
	Low	0.2	6.71 $\pm$ 0.08	7.07 $\pm$ 0.06	15.9 $\pm$ 0	16.2 $\pm$ 0	484 $\pm$ 1	493 $\pm$ 2	7 $\pm$ 0.24	6.62 $\pm$ 0.02
	Medium	0.8	6.74 $\pm$ 0.09	7.05 $\pm$ 0.07	15.8 $\pm$ 0.05	16.2 $\pm$ 0	1707 $\pm$ 11	1816 $\pm$ 102	7.12 $\pm$ 0.27	6.92 $\pm$ 0.09
	High	1.6	6.84 $\pm$ 0.06	7.09 $\pm$ 0.07	15.7 $\pm$ 0.05	16.2 $\pm$ 0	3272 $\pm$ 10	3319 $\pm$ 16	7 $\pm$ 0.08	7 $\pm$ 0.08
NaCl with substrate (g/L) ¹	Control	0	7.12 $\pm$ 0.05	7.15 $\pm$ 0.22	16.1 $\pm$ 0.05	16.3 $\pm$ 0.06	62 $\pm$ 2	61 $\pm$ 2	7.23 $\pm$ 0.26	7.22 $\pm$ 0.45
	Low	0.2	7.02 $\pm$ 0.01	6.85 $\pm$ 0.12	16 $\pm$ 0.11	16.3 $\pm$ 0	471 $\pm$ 6	478 $\pm$ 7	7.28 $\pm$ 0.45	7.17 $\pm$ 0.12
	Medium	0.8	6.95 $\pm$ 0.02	6.96 $\pm$ 0.05	15.8 $\pm$ 0.05	16.3 $\pm$ 0	1647 $\pm$ 13	1675 $\pm$ 18	7.17 $\pm$ 0.09	6.98 $\pm$ 0.09
	High	1.6	6.92 $\pm$ 0.03	7 $\pm$ 0.09	15.7 $\pm$ 0.06	16.2 $\pm$ 0	3112 $\pm$ 73	3157 $\pm$ 64	7.17 $\pm$ 0.39	7.05 $\pm$ 0.06
NO ₃ ⁻ (mg/L)	Control	0	7.78 $\pm$ 0.19	7.62 $\pm$ 0.11	15.3 $\pm$ 0.1	15.5 $\pm$ 0.1	69 $\pm$ 2	71 $\pm$ 3	7.82 $\pm$ 0.03	7.55 $\pm$ 0.07
	Low	128	7.57 $\pm$ 0.01	7.32 $\pm$ 0.04	15.3 $\pm$ 0	15.4 $\pm$ 0	290 $\pm$ 2	295 $\pm$ 4	7.96 $\pm$ 0.01	7.54 $\pm$ 0
	Medium	512	7.32 $\pm$ 0.07	7.15 $\pm$ 0.01	15.3 $\pm$ 0	15.4 $\pm$ 0	913 $\pm$ 2	935 $\pm$ 15	8.04 $\pm$ 0.06	7.92 $\pm$ 0
	High	2048	7.10 $\pm$ 0	6.94 $\pm$ 0.02	15.3 $\pm$ 0	15.4 $\pm$ 0	3360 $\pm$ 42	3455 $\pm$ 64	7.96 $\pm$ 0.09	7.82 $\pm$ 0
PO ₄ ³⁻ (mg/L)	Control	0	7.43 $\pm$ 0.04	7.46 ²	16.05 $\pm$ 0.07	15.8 $\pm$ 0.2	68 $\pm$ 1	74 $\pm$ 5	7.69 $\pm$ 0.02	7.48 $\pm$ 0.12
	Low	0.2	7.26 $\pm$ 0.06	7.54 ²	16.05 $\pm$ 0.07	15.6 $\pm$ 0	68 $\pm$ 0	71 $\pm$ 2	7.58 $\pm$ 0.16	7.55 $\pm$ 0.04
	Medium	1.6	7.03 $\pm$ 0.03	7.48 ²	16 $\pm$ 0	15.6 $\pm$ 0	70 $\pm$ 0	74 $\pm$ 4	7.79 $\pm$ 0.03	7.49 $\pm$ 0.13
	High	6	6.9 $\pm$ 0.01	7.45 ²	16 $\pm$ 0	15.6 $\pm$ 0	74 $\pm$ 0	79 $\pm$ 5	7.58 $\pm$ 0.07	7.42 $\pm$ 0.01
Cadmium (μ g/L)	Control	0	7.08 $\pm$ 0.09	7.43 $\pm$ 0.15	15.4 $\pm$ 0	15.95 $\pm$ 0.07	70 $\pm$ 0.5	72 $\pm$ 4	7.84 $\pm$ 0.23	7.91 $\pm$ 0.29
	Low	5	7.12 $\pm$ 0.07	7.13 $\pm$ 0.14	15.3 $\pm$ 0	15.85 $\pm$ 0.07	70 $\pm$ 0	69 $\pm$ 0.5	7.61 $\pm$ 0.03	7.35 $\pm$ 0.13
	Medium	30	6.81 $\pm$ 0.15	6.78 $\pm$ 0.06	15.2 $\pm$ 0	15.85 $\pm$ 0.07	68 $\pm$ 0	68 $\pm$ 0.5	7.81 $\pm$ 0.01	7.42 $\pm$ 0.18
	High	120	6.86 $\pm$ 0.01	6.64 $\pm$ 0	15.2 $\pm$ 0	15.8 $\pm$ 0	69 $\pm$ 1	68 $\pm$ 0	7.67 $\pm$ 0.07	7.23 $\pm$ 0.05
Cadmium with substrate (μ g/L) ¹	Control	0	7.02 $\pm$ 0.05	6.79 $\pm$ 0.06	16.5 $\pm$ 0.06	15.8 $\pm$ 0.09	63 $\pm$ 0.5	64.2 $\pm$ 2	6.25 $\pm$ 0.09	6.28 $\pm$ 0.24
	Low	5	6.93 $\pm$ 0.06	6.79 $\pm$ 0.04	16.5 $\pm$ 0.05	15.6 $\pm$ 0	62 $\pm$ 0	62.9 $\pm$ 0.3	6.36 $\pm$ 0.12	6.27 $\pm$ 0.09
	Medium	30	6.88 $\pm$ 0.03	6.72 $\pm$ 0.03	16.4 $\pm$ 0	15.6 $\pm$ 0	61.5 $\pm$ 0.9	61.6 $\pm$ 0.5	6.53 $\pm$ 0.15	6.39 $\pm$ 0.04
	High	120	6.74 $\pm$ 0.04	6.76 $\pm$ 0.07	16.4 $\pm$ 0	15.5 $\pm$ 0	61.2 $\pm$ 0.8	61.3 $\pm$ 0.7	6.58 $\pm$ 0.16	6.57 $\pm$ 0.12
Cadmium without substrate (μ g/L) ₁	Control	0	7.23 $\pm$ 0.10	7.58 $\pm$ 0.13	16.4 $\pm$ 0.06	15.6 $\pm$ 0.1	59.4 $\pm$ 0.1	60.8 $\pm$ 1.3	6.38 $\pm$ 0.09	6.51 $\pm$ 0.15
	Low	5	7.01 $\pm$ 0.05	7.31 $\pm$ 0.08	16.4 $\pm$ 0	15.5 $\pm$ 0	59.2 $\pm$ 0.1	59.5 $\pm$ 0.1	6.53 $\pm$ 0.21	6.59 $\pm$ 0.11
	Medium	30	6.97 $\pm$ 0.05	7.09 $\pm$ 0.03	16.4 $\pm$ 0.05	15.5 $\pm$ 0	58.9 $\pm$ 0.2	59.5 $\pm$ 0.2	6.65 $\pm$ 0.08	6.51 $\pm$ 0.14
	High	120	6.91 $\pm$ 0.06	6.99 $\pm$ 0.05	16.3 $\pm$ 0	15.5 $\pm$ 0	58.7 $\pm$ 0.1	59.8 $\pm$ 0.5	6.53 $\pm$ 0.21	6.4 $\pm$ 0.21
Arsenic (μ g/L)	Control	0	7.38 $\pm$ 0.11	7.64 $\pm$ 0.04	16.3 $\pm$ 0	15.8 $\pm$ 0.1	70.4 $\pm$ 0.4	72.9 $\pm$ 5	7.52 $\pm$ 0.13	7.32 $\pm$ 0.18
	Low	5	7.23 $\pm$ 0.03	7.37 $\pm$ 0.12	16.2 $\pm$ 0.1	15.9 $\pm$ 0.1	69 $\pm$ 0.4	68.6 $\pm$ 0.2	7.17 $\pm$ 0.21	7.12 $\pm$ 0.04
	Medium	30	7.04 $\pm$ 0.12	7.15 $\pm$ 0.11	15.5 $\pm$ 0.1	15.6 $\pm$ 0	69.7 $\pm$ 1.1	69.4 $\pm$ 0.1	7.19 $\pm$ 0.12	7.42 $\pm$ 0.16
	High	120	6.84 $\pm$ 0.02	7.02 $\pm$ 0.13	15.3 $\pm$ 0	15.5 $\pm$ 0	68.7 $\pm$ 0.3	66.9 $\pm$ 0.6	7.1 $\pm$ 0.17	7.33 $\pm$ 0.03
Aluminium (μ g/L)	Control	0	7.59 $\pm$ 0.07	7.51 $\pm$ 0.43	15.3 $\pm$ 0.2	14.8 $\pm$ 0.1	66 $\pm$ 0.5	65.9 $\pm$ 2	7.51 $\pm$ 0.2	7.33 $\pm$ 0.5
	Low	120	7.42 $\pm$ 0.09	7.52 $\pm$ 0.11	15 $\pm$ 0	14.6 $\pm$ 0.1	66 $\pm$ 0.5	63.9 $\pm$ 0.3	7.77 $\pm$ 0.8	7.47 $\pm$ 0.2
	Medium	500	6.99 $\pm$ 0.08	7.17 $\pm$ 0.17	14.9 $\pm$ 0.2	14.6 $\pm$ 0.2	67.4 $\pm$ 0.2	69.5 $\pm$ 0.4	7.29 $\pm$ 0.2	7.65 $\pm$ 0.2
	High	1000	6.53 $\pm$ 0.13	6.34 $\pm$ 0.06	14.9 $\pm$ 0.1	14.5 $\pm$ 0.1	69.3 $\pm$ 0	80.9 $\pm$ 0.5	7.42 $\pm$ 0.1	7.69 $\pm$ 0.1

¹Acute toxicity tests conducted simultaneously for the assessment of substrate effects; ²pH values were measured only in one replicate ($n=1$)



The Table S3 presents nominal and mean measured toxicant concentrations, and mean percentage viability of juveniles at the end of each of the 96-h toxicity tests. Viability was checked on 20 juveniles/ exposure condition and was based on the immobility of the mussel.

Table S3. Nominal concentrations, mean measured concentration values $\pm$ SD ($n = 3$) and mean percentage viability $\pm$ SD ($n = 4$) at the end of the test (based on juveniles immobility) for each acute toxicity tests.

Pollutants	Nominal Concentrations	Measured Concentrations	Viability (%)
NaCl without substrate (mg/L) ¹	0	ND ²	100
	0.2	ND ²	100
	0.4	ND ²	100
	0.8	ND ²	85 $\pm$ 10
	1.2	ND ²	45 $\pm$ 10
	1.6	ND ²	30 $\pm$ 11.5
NaCl with substrate (mg/L) ¹	0	ND ²	100
	0.2	ND ²	100
	0.4	ND ²	100
	0.8	ND ²	95 $\pm$ 10
	1.2	ND ²	65 $\pm$ 19
	1.6	ND ²	25 $\pm$ 19
NO ₃ ⁻ (mg/L)	0	4 $\pm$ 0.36	100
	128	130 $\pm$ 1.39	100
	256	246 $\pm$ 8.33	100
	512	493 $\pm$ 13.01	80 $\pm$ 28
	1024	1026 $\pm$ 51.50	80 $\pm$ 16
	2048	2290 $\pm$ 85.74	0
PO ₄ ³⁻ (mg/L)	0	0.022 $\pm$ 0.009	100
	0.2	0.084 $\pm$ 0.106	100
	0.6	0.316 $\pm$ 0.215	100
	1.6	1.120 $\pm$ 0.286	100
	3	2.303 $\pm$ 0.311	100
	6	5.007 $\pm$ 0.331	100
Cadmium (µg/L)	0	0.03 $\pm$ 0.04	100
	5	3.95 $\pm$ 3.59	100
	15	11.56 $\pm$ 11.75	100
	30	23.06 $\pm$ 24.47	100
	60	51.96 $\pm$ 45.42	95 $\pm$ 10
	120	112.55 $\pm$ 80.55	80
Cadmium with substrate (µg/L) ¹	0	0.61 $\pm$ 0.44	100
	5	2.99 $\pm$ 1.82	100
	15	7.99 $\pm$ 5.03	100
	30	22.83 $\pm$ 10	100
	60	48.91 $\pm$ 15.06	100
	120	110.78 $\pm$ 31.72	100
Cadmium without substrate (µg/L) ¹	0	0.22 $\pm$ 0.32	100
	5	3.04 $\pm$ 1.09	100
	15	11.53 $\pm$ 6.65	100
	30	33.9 $\pm$ 15.96	100
	60	70.57 $\pm$ 41.41	100
	120	147.51 $\pm$ 92.28	100
Arsenic (µg/L)	0	4.89 $\pm$ 0.69	100
	5	9.84 $\pm$ 0.85	100
	15	21.65 $\pm$ 2.69	100
	30	34.64 $\pm$ 2.62	100
	60	68.46 $\pm$ 12.83	93.75

	120	127.25 ± 22.76	93.75
Aluminium (µg/L)	0	221 ± 17	100
	120	362 ± 86	100
	250	408 ± 79	100
	500	567 ± 147	100
	750	774 ± 273	100
	1000	954 ± 457	100

¹ Acute toxicity tests conducted simultaneously for the assessment of substrate effects; ² ND: not determined.



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