Removal of contaminants from wastewater using the bivalve *Corbicula fluminea* - comparative assessment of biofiltration and biosorption

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Figure S3. Bioluminescence inhibition of *A. fischeri* exposed to samples (0.5 and 1.0 $mg.L^{-1}$) before any treatment (0 h) and after 48 h of aeration (48 h – untreated; no clams) or biofiltration by *C. fluminea* (48 h – treated by clams).

Figure S4. Growth rate inhibition of *R. subcapitata* exposed to samples (0.5 and 1.0 $mg.L^{-1}$) that were previously in contact with *C. fluminea* milled shells (milled shells) or untreated (control, no shells) during 24 h.

Figure S5. Bioluminescence inhibition of *A. fischeri* exposed to samples (0.5 and 1.0 $mg.L^{-1}$) that were previously in contact with *C. fluminea* milled shells (milled shells) or untreated (control, no shells) during 24 h.

Figure S6. Comparative inhibition of the controls and the blank samples from the biofiltration experiment for the growth inhibition endpoints of *R. subcapitata*.

Section S1. Milled shells characterization

Shells were characterized concerning the point of zero charge (PZC) and the specific surface area. The point of zero charge (PZC) corresponds to the pH at which the overall electrical charge on the surface of the material equals zero, and was determined following Sousa, Otero [1]. Briefly, 0.1 M NaCl solutions (20 mL) with initial pH values (pH_i) of 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 were used and added to 2 g of milled shells. The tubes were shaken in an overhead shaker (Heidolph, Reax 2; 80 rpm) for 24 h at room temperature. After this period, the tubes were centrifuged for 5 min at 4000 rpm, and the final pH values were measured (pH_f). The differences between the initial and final pH were calculated ($\Delta pH = pH_f - pH_i$) and plotted versus pH_i. The PZC was determined based on the obtained curve and corresponds to the pH_i for which the curve intercepts the x-axis, or in other terms when $pH_i = pH_f$. The adsorbent surface is mainly positively charged at pH values below the PZC, whereas the adsorbent will be mainly negatively charged at pH values above the PZC.

The specific surface area was determined using a Micromeritics Instrument (Gemini VII 2380), performing the degassing of the samples at 120 ˚C. Nitrogen adsorption-desorption experiments were carried out through liquid nitrogen at -196 ˚C. The total pore volume (V_p) was estimated at a relative pressure of 0.99. The calculation of the specific surface area (S_{BET}) was performed using the Brunauer-Emmet-Teller equation, proposed by Brunauer, Emmett [2], within the relative pressure range of 0.001 to 0.1. Microporosity (W_0) was evaluated through the Dubinin-Astakhov equation [3], focusing on the lower relative pressure region of the nitrogen adsorption isotherm. The Stoeckli–Ballerini equation [4] was applied to determine the average micropore width (L). The average pore diameter parameter (D) was calculated following the equation $D = 2x\ V_p/S_{BET}$.

Section S2. Chemical quantification

Quantification of contaminants' concentrations in all samples was made by Reverse-phase (C18 column) High Performance Liquid Chromatography (HPLC) with UV-Vis or fluorescence detection, except for MET which was quantified by Capillary Zone Electrophoresis (CZE) with UV-Vis detection. Three instrumental replicates were run per experimental replicate. Before any quantifications, calibration curves were established. For that, solutions of each tested chemical were prepared in dechlorinated tap water. Tested chemical solutions were prepared using calibrated instruments and following the best laboratory practices. No carrier solvents were used, and full dissolution in stocks used for spiking test solutions was ensured by applying internally optimized protocols using ultrasound baths. The experimental apparatus was carefully washed and decontaminated before and after use.

For the quantification using HPLC, a Shimadzu LC 20AT Prominence system (model DGU-20A5) was utilized, featuring a DGU-20As prominence degasser, a high-pressure LC-20AD prominence pump, and a CTO-10ASVP column oven. The HPLC setup was connected to an ESA Inc. model 542 autosampler. For separation, an ACE C18-PFP column (5 µm, 150 mm × 4.6 mm) was employed. The column was conditioned daily, both before and after batch analyses, using a mobile phase of 100% acetonitrile. All mobile phases used were filtered prior to their use through a 0.2 µm polyamide membrane (Whatman). Detection was carried out using an SPD-20A prominence UV/Vis detector, while an RF-20A XS prominence fluorescence detector was specifically used for the analysis of FXT. A detailed description of each condition can be found in Table 1.

For the quantification of MET, CZE was applied using a Beckman P/ACE MDQ (Fullerton, CA, USA) system equipped with a UV/Vis detector and controlled by the 32 Karat software. The capillary was conditioned before its first use by flushing it with 1.0 M NaOH for 10 min, followed by water for 5 min, and finally with the electrolyte solution (15 mM sodium tetraborate) for 2 min.

The separation conditions for MET quantification were as follows: the capillary was rinsed with 0.1 M NaOH (1.5 min at 20 psi), followed by ultrapure water (1 min at 20 psi), and then with 15 mM sodium tetraborate (1.5 minutes at 20 psi). The sample was injected by hydrodynamic injection for 4 sec at 0.5 psi, and the capillary was rinsed with ultrapure water for 30 sec at 0.5 psi. Separation was performed with 15 mM sodium tetraborate for 2 min at 15 kV. Quantification was conducted at a wavelength of 200 nm.

Table 1. Chromatographic conditions used for each compound: caffeine (CAF), carbamazepine (CBZ), sulfamethoxazole (SMX), paracetamol (PCT), fluoxetine (FXT), ibuprofen (IBU), naproxen (NPX), and diclofenac (DIC).

	Chromatographic conditions			
	Mobile phase composition	Injection volume (µL)	Flow ($mL.min^{-1}$)	Detection (nm)
CAF	acetonitrile/water (20/80 *v*/*v*)	20	0.8	UV: 275
CBZ	acetonitrile/water (40/60 *v*/*v*)	40	0.8	UV: 215
SMX	formic acid 1%/acetonitrile (70/30 *v*/*v*)	20	0.8	UV: 254
PCT	acetonitrile/acetic acid 0.1% (10/90 *v*/*v*)	20	0.8	UV: 248
FXT	formic acid 1%/acetonitrile (65/45 *v*/*v*)	40	0.7	Fluorescence: λexc 232 λems 292
IBU NPX DIC	acetonitrile/ultrapure water (pH 2.2 adjusted with phosphoric acid) (65/35 *v*/*v*)	20	1.0	UV: 222

Validation parameters of the used chromatographic and electrophoretic methodologies were evaluated, namely, adequacy of the calibration dynamic range; limit of detection (LoD); limit of quantification (LoQ); correlation coefficient; repeatability, and intermediate repeatability analysis. Also, before each analysis, a solution containing methanol (100%) followed by ultrapure water was injected to ensure the absence of possible interferents in the HPLC column.

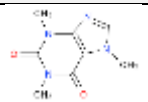
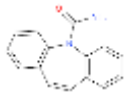
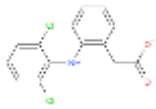
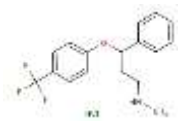
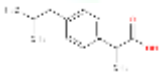
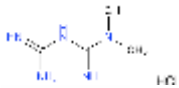
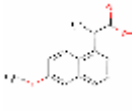
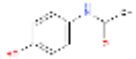
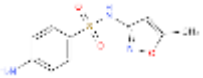
For CAF, CBZ, DIC, FXT, IBU, NAP, PCT and SMX, chromatographic peak areas were calculated from the chromatograms using integration tools as provided by the manufacturer. The previously determined calibration curves (obtained using external standards) were used to calculate the concentration in each sample. In the case of metformin, the same procedure was applied but peak areas were determined from the electropherograms.

The LoD and LoQ obtained for each compound are depicted in Table 2.

Table 2: Limit of Detection (LoD) and Limit of Quantification (LoQ) for caffeine (CAF), carbamazepine (CBZ), diclofenac (DIC), fluoxetine (FXT), ibuprofen (IBU), metformin (MET), naproxen (NPX), paracetamol (PCT), and sulfamethoxazole (SMX).

	LoD ($mg.L^{-1}$)	LoQ ($mg.L^{-1}$)
CAF	0.022	0.074
CBZ	0.013	0.043
DIC	0.028	0.094
FXT	0.026	0.086
IBU	0.051	0.17
MET	0.072	0.24
NPX	0.020	0.067
PCT	0.013	0.045
SMX	0.0086	0.029

Table S1. Chemical structure and main physicochemical properties of the tested compounds. Log Dow: log of the n-octanol/water distribution coefficient; pKa: acid dissociation constant.

Compound	Abbreviation	Chemical structure	Speciation at the studied pH	Molecular formula	Molecular mass ($g.mol^{-1}$)	Log Dow	pKa	Nr of H bond donors ([13])
caffeine	CAF		neutral ([1;10])	$C_8H_{10}N_4O_2$	194.19	-0.55 at pH 7 ([6])	0.61; 10.4 ([9])	0
carbamazepine	CBZ		neutral ([1])	$C_{15}H_{12}N_2O$	236.27	2.25 at pH 8 ([3])	2.3; 13.9 ([7])	2
diclofenac	DIC		anionic ([10])	$C_{14}H_{10}Cl_2NNaO_2$	318.13	0.9 at pH 8 ([5])	4.15 ([5])	2
fluoxetine	FXT		cationic ([11])	$C_{17}H_{19}ClF_3NO$	345.8	2.38 at pH 8 ([5])	9.80 ([5])	2
ibuprofen	IBU		anionic ([10])	$C_{13}H_{18}O_2$	206.28	0.58 at pH 8 ([5])	4.45 ([5])	1
metformin	MET		cationic ([12])	$C_4H_{12}ClN_5$	165.62	-4.31 at pH 7 ([2])	2.8; 11.6 ([8])	4
naproxen	NPX		anionic ([1])	$C_{14}H_{14}O_3$	230.26	-0.36 at pH 8 ([5])	4.15 ([1])	1
paracetamol	PCT		neutral ([1;4;10])	$C_8H_9NO_2$	151.16	0.34 at pH 7 ([2])	9.5-9.7 ([4])	2
sulfamethoxazole	SMX		anionic ([1])	$C_{10}H_{11}N_3O_3S$	253.28	-1.54 at pH 8 ([3])	1.8; 5.7 ([7])	3

Note: It was intended to have log Dow values for all compounds at pH 8 (due to being closer to the pH of the dechlorinated tap water, used in the experiments) but, due to lack of information for some compounds, the corresponding values at pH 7 were used, instead.

([1]): [5] ([2]): [6]; ([3]): [7]; ([4]): [8]; ([5]): [9]; ([6]): [10]; ([7]): [11]; ([8]): [12]; ([9]): [13]; ([10]): [14]; ([11]): [15]; ([12]): [16]; ([13]): [17]; for Met, DIC and FXT data was obtained from https://pubchem.ncbi.nlm.nih.gov/. The references are presented at the end of the document.

Table S2: Summary of literature records on the main properties of milled bivalve shells. S_{BET}: Specific Surface Area. PZC: Point of Zero Charge.

Material	Pretreatment	S_{BET} ($m^2.g^{-1}$)	Pore volume (V_p, $cm^3.g^{-1}$)	PZC	Reference
Marine bivalve shells from beaches (India)	Shells were washed with double distilled water, dried at 383 K (about 110 °C) for 24 h, and then crushed and grounded using a ball mill, and sieved.	3.6	0.0065	-	[18]
Marine bivalve *Mytella falcata* shells (Brazil)	Shells were washed and dried in an oven at 60 °C for 8 h, and then crushed in an industrial blender.	65	0.0676	-	[19]
Mussel shells (63–150 µm) (New Zealand)	Shells were scrubbed with a stiff brush with cold tap water and then air-dried for 4–5 days at room temperature with minimal sun exposure. Whole shells were crushed by a ring mill.	1.29	0.0064	-	[20]
Oyster shells (63–150 µm) (New Zealand)		4.61	0.0190	-	
Scallop shells (63–150 µm) (New Zealand)		2.23	0.0085	-	
Mussel shells (710–1180 µm) (New Zealand)		0.19	0.0009	-	
Oyster shells (710–1180 µm) (New Zealand)		1.72	0.0069	-	
Scallop shells (710–1180 µm) (New Zealand)		0.46	0.0019	-	
Marine bivalve *Anadara inaequivalvis* shells (250 µm) (Turkey)	Shells were washed with tap water, brushed, rewashed with de-ionized water, and then dried at 100 °C for 24 h. Then, they were crushed into smaller pieces, ground, and sieved to 250 µm.	1.82	Median pore width: 120.7 nm	-	[21]
Marine bivalve *Anomalocardia brasiliana* shells (Brazil)	Shells were washed in running water, dried in a heater for 3 h at 60 ˚C, triturated in a roll mill, and then in a ball mill.	4.44	0.00103	13.0	[22]
Marine bivalve *Meretrix lyrata* shells (Vietnam)	Shells were washed with tap water and then left to dry in the sun. Then, they were ground with a stone and sieved.	0.31	0.00127	-	[23]
Cockle shells (marine species)	Cockle shells were ground into powder using a blender and then sieved in the range of 80–120 mesh size. The sieved powder was oven-dried overnight at 105 ˚C.	3.4	0.017	The pH of the powdered shell was 9.62.	[24]
Scallop shells from a shellfish processing company (Ireland)	Scallop shells were mechanically cleaned and air-dried. Then, they were crushed using a hammer, and pulverized using a blender. The powder was sieved into different sizes (1.4 mm – 45 µm).	1.6207	0.007	9.4	[25]

Whelk shells from a shellfish processing company (Ireland)	Whelk shells were immersed in 3 % sodium hypochlorite solution for 1 h, rinsed thoroughly in water and oven dried at 105 ◦C for 2 h. Then, they were crushed using a hammer, and pulverized using a blender. The powder was sieved into different sizes (1.4 mm – 45 µm).	3.1837	0.0261	9.4	[25]
Shells from *Corbula trigona*, collected from a coastal lagoon (Republic of Côte d'Ivoire)	Shells were immersed in hydrogen peroxide (15 wt %) for 24 hours. After filtration, shells were washed several times with distilled water before being dried in an oven at 105 ˚C and further cooled in a desiccator. Shells were then crushed and ground in a porcelain mortar. The powder was sieved to collect the particles of sizes ranging between 100 and 250 mm.	--	--	8.2	[26]

Table S3. Removal rate (mean ± standard deviation, expressed as µg.ind^{-1}.h^{-1}) of the tested compounds by *C. fluminea* after exposure to a solution containing the compounds at an initial concentration of 0.5 mg.L^{-1} and 1.0 mg.L^{-1} during the periods 0-6 h, 6-24 h, and 24-48 h. The removal rate *per* clam was calculated as: $\text{Removal rate} = \frac{C_j - C_i}{n \times (t_j - t_i)} \times V \times 1000$, where C_i and C_j represent the average concentration of the chemical in each replicate after biofiltration at times i and j (mg.L^{-1}), respectively; n is the number of clams *per* vial; t_i and t_j are the exposure times (h) and V is the volume of medium (0.50 L).

Initial concentration (mg.L^{-1})	**Time period (h)**	**removal rate (µg.ind^{-1}.h^{-1})**				
		CAF	CBZ	DIC	FXT	IBU
0.5	0-6	0.41 ± 0.06	0.45 ± 0.05	0.6 ± 0.4	3.0 ± 0.1	0.3 ± 0.3
0.5	6-24	-- $^{\lambda}$	-- $^{\lambda}$	-- $^{\lambda}$	0.04 ± 0.01	-- $^{\lambda}$
0.5	24-48	0.20 ± 0.08	-- $^{\lambda}$	-- $^{\lambda}$	0.01 ± 0.01	0.09 ± 0.06
1.0	0-6	1 ± 1	0.73 ± 0.07	0.4 ± 0.4	6.2 ± 0.2	0.7 ± 0.2
1.0	6-24	0.3 ± 0.3	-- $^{\lambda}$	-- $^{\lambda}$	0.19 ± 0.02	0.1 ± 0.1
1.0	24-48	-- $^{\lambda}$	-- $^{\lambda}$	-- $^{\lambda}$	0.05 ± 0.03	-- $^{\lambda}$

Initial concentration (mg.L^{-1})	**Time period (h)**	**removal rate (µg.ind^{-1}.h^{-1})**			
		MET	NPX	PCT	SMX
0.5	0-6	0.6 ± 0.1	0.3 ± 0.3	0.8 ± 0.4	0.3 ± 0.1
0.5	6-24	-- $^{\lambda}$	-- $^{\lambda}$	0.10 ± 0.05	-- $^{\lambda}$
0.5	24-48	0.06 ± 0.03	--- $^{\lambda}$	0.20 ± 0.08	0.04 ± 0.04
1.0	0-6	1.7 ± 0.4	2 ± 1	1.6 ± 0.8	-- $^{\lambda}$
1.0	6-24	0.1 ± 0.1	-- $^{\lambda}$	-- $^{\lambda}$	-- $^{\lambda}$
1.0	24-48	0.5 ± 0.2	-- $^{\lambda}$	0.38 ± 0.05	-- $^{\lambda}$

$^{\lambda}$ The contaminant concentration in the treated water did not differ from the concentration in the control, and thus no removal was observed.

Table S4. Summary of the statistical analysis regarding the effect of the initial concentration (0.5 and 1.0 $mg.L^{-1}$) and the exposure time (6 h, 24 h, and 48 h) on the removal percentage by *C. fluminea*. *p*-values lower than 0.05 are highlighted in bold.

Compound	Statistical test	*p*-value
CAF	ANOVA on ranks	H(5) = 40.798; $\mathbf{p \leq 0.001}$
CBZ	2-way ANOVA	Time: $F_{(2, 66)} = 2.892$; $p = 0.062$ Initial concentration: $F_{(1, 66)} = 35.515$; $\mathbf{p < 0.001}$ Interaction: $F_{(2, 66)} = 1.012$; $p = 0.369$
DIC	2-way ANOVA	Time: $F_{(2, 65)} = 4.007$; $\mathbf{p = 0.023}$ Initial concentration: $F_{(1, 65)} = 8.569$; $\mathbf{p = 0.005}$ Interaction: $F_{(2, 65)} = 4.457$; $\mathbf{p = 0.015}$
FXT	ANOVA on ranks	H(5) = 59.655; $\mathbf{p \leq 0.001}$
IBU	2-way ANOVA	Time: $F_{(2, 65)} = 35.090$; $\mathbf{p < 0.001}$ Initial concentration: $F_{(2, 65)} = 4.614$; $\mathbf{p = 0.035}$ Interaction: $F_{(2, 65)} = 6.437$; $\mathbf{p = 0.003}$
MET	2-way ANOVA	Time: $F_{(2, 65)} = 100.583$; $\mathbf{p < 0.001}$ Initial concentration: $F_{(2, 65)} = 56.085$; $\mathbf{p < 0.001}$ Interaction: $F_{(2, 65)} = 22.892$; $\mathbf{p < 0.001}$
NPX	ANOVA on ranks	H(5) = 37.721; $\mathbf{p \leq 0.001}$
PCT	2-way ANOVA	Time: $F_{(2, 66)} = 89.009$; $\mathbf{p < 0.001}$ Initial concentration: $F_{(2, 66} = 9.686$; $\mathbf{p = 0.003}$ Interaction: $F_{(2, 66)} = 2.602$; $p = 0.082$
SMX	2-way ANOVA	Time: $F_{(2, 66)} = 11.520$; $\mathbf{p < 0.001}$ Initial concentration: $F_{(2, 66)} = 11.695$; $\mathbf{p < 0.001}$ Interaction: $F_{(2, 66)} = 9.174$; $\mathbf{p < 0.001}$

Table S5. Pearson correlation between the physicochemical properties of the tested compounds (log Dow and molar mass) and the removal endpoints, regarding the biofiltration experiments. A) for the 9 tested compounds; b) excluding compounds with molecular mass above 300 $g.mol^{-1}$ (diclofenac and fluoxetine). *p*-values below 0.05 are highlighted in bold.

a) **N = 9**	**Removal %** **0.5 $mg.L^{-1}$ 6 h**	**Removal %** **0.5 $mg.L^{-1}$ 48 h**	**Removal %** **1.0 $mg.L^{-1}$ 6 h**	**Removal %** **1.0 $mg.L^{-1}$ 48 h**
Log Dow	ρ = 0.443 *p* = 0.233	ρ = 0.355 *p* = 0.349	ρ = 0.345 *p* = 0.363	ρ = 0.158 *p* = 0.684
Molar mass	ρ = 0.587 *p* = 0.0967	ρ = 0.161 *p* = 0.680	ρ = 0.438 *p* = 0.238	ρ = 0.179 *p* = 0.645
b) **N = 7**	**Removal %** **0.5 $mg.L^{-1}$ 6 h**	**Removal %** **0.5 $mg.L^{-1}$ 48 h**	**Removal %** **1.0 $mg.L^{-1}$ 6 h**	**Removal %** **1.0 $mg.L^{-1}$ 48 h**
Log Dow	ρ = -0.0602 *p* = 0.898	ρ = 0.0653 *p* = 0.889	ρ = -0.161 *p* = 0.730	ρ = -0.381 *p* = 0.399
Molar mass	ρ = -0.813 **$p = 0.0262$**	ρ = -0.877 **$p = 9.54 \times 10^{-3}$**	ρ = -0.658 *p* = 0.108	**ρ = -0.982** **$p = 8.06 \times 10^{-5}$**

Table S6. Summary of the statistical analysis regarding the effect of the initial concentration on the removal percentage of each compound by the milled *C. fluminea* shells. *p*-values lower than 0.05 are highlighted in bold.

Compound	Statistical test	*p*-value
CAF	*t*-test	$t(16) = 7.863$; $p =$ **6.93×10^{-7}**
CBZ	*t*-test	$t(16) = 2.615$; $p =$ **0.0188**
DIC	*t*-test	U = 22.000; $p = 0.112$
FXT	*t*-test	U = 27.000; $p = 0.251$
IBU	*t*-test	$t(16) = -1.398$; $p = 0.181$
MET	*t*-test	U = 16.000; $p =$ **0.034**
NPX	*t*-test	U = 27.000; $p = 0.251$
PCT	*t*-test	$t(16) = 0.553$; $p = 0.588$
SMX	*t*-test	U = 31.000; $p = 0.427$

Table S7. Summary of the statistical analysis regarding the effect of the initial concentration on the adsorption capacity of each compound by the milled *C. fluminea* shells. *p*-values lower than 0.05 are highlighted in bold.

Compound	Statistical test	*p*-value
CAF	*t*-test	$t(4) = -11.804$; $p =$ **2.95×10^{-4}**
CBZ	*t*-test	$t(4) = 0.130$; $p = 0.903$
DIC	*t*-test	$t(4) = -0.185$; $p = 0.862$
FXT	*t*-test	$t(4) = -2.676$; $p = 0.0555$
IBU	*t*-test	$t(4) = -2.052$; $p = 0.109$
MET	*t*-test	$t(4) = 2.360$; $p = 0.0777$
NPX	*t*-test	$t(4) = -0.818$; $p = 0.459$
PCT	*t*-test	$t(4) = 0.762$; $p = 0.488$
SMX	*t*-test	$t(4) = -2.065$; $p = 0.108$

Table S8. Pearson correlation between the physicochemical properties of the tested compounds and the removal endpoints, regarding the biosorption experiments. *p*-values lower than 0.05 are highlighted in bold.

N = 9	**Removal % 0.5 mg.L^{-1} 24 h**	**Removal % 1.0 mg.L^{-1} 24 h**	**Adsorption capacity 0.5 mg.L^{-1} 24 h (nmol.g^{-1}) ***	**Adsorption capacity 1.0 mg.L^{-1} 24 h (nmol.g^{-1}) ***
Log Dow	$\rho = 0.164$; $p = 0.674$	$\rho = 0.229$; $p = 0.554$	$\rho = -0.0454$; $p = 0.908$	$\rho = 0.131$; $p = 0.737$
Molecular mass (g.mol^{-1})	$\rho = 0.175$; $p = 0.653$	$\rho = 0.266$; $p = 0.488$	$\rho = -0.0277$; $p = 0.944$	$\rho = 0.103$; $p = 0.791$
Nr of H bond donors	$\rho = -0.639$; $p = 0.0638$	$\rho = -0.609$; $p = 0.0815$	$\rho = -0.618$; $p = 0.0760$	$\rho = -0.711$; **$p = 0.0317$**

* For this analysis, adsorption capacity values were converted from μg.g^{-1} to nmol.g^{-1}, considering that the analyzed correlations should be evaluated at molecular and not mass level.

Table S9. Summary of EC50 values (when available, 95% Confidence Interval, CI, or standard deviation (SD)/error (SE), are provided within parenthesis) collected from the literature for *Raphidocelis subcapitata* exposed to each tested compound. The references are presented at the end of the document. GR: Growth Rate.

Compound	Endpoint	EC50 ($mg.L^{-1}$)	Reference
CAF	GR inhibition (72 h)	870.3 (± 3.25 SD)	[27]
CBZ	GR inhibition (72 h)	> 100	[28]
	GR inhibition (72 h)	> 100	[29]
	GR inhibition (72 h)	> 100	[30]
DIC	GR inhibition (72 h)	19.05 (CI: 15.58–23.30)	[31]
	GR inhibition (72 h)	21.30 (CI: 18.43–26.32)	[30]
	Growth inhibition (72 h)	8.03 (± 1.5 SD)	[32]
FXT	GR inhibition (72 h)	0.20 (CI: 0.18–0.21)	[30]
	Growth inhibition (96 h)	0.04499 (± 0.00176 SE)	[33]
IBU	GR inhibition (72 h)	93.26 (CI: 77.78–122.07)	[28]
	Growth inhibition (72 h)	20.05 (± 5.14 SD)	[32]
MET	GR inhibition (72 h)	> 258.3	[34]
NPX	GR inhibition (72 h)	27.10 (CI: 24.82 – 29.71)	[28]
	GR inhibition (72 h)	44.40 (CI: 40.49–45.95)*	[30]
PCT	GR inhibition (72 h)	> 100	[30]
	Growth inhibition (72 h)	317.4 (CI: 292.6-341.4)	[35]
SMX	GR inhibition (72 h)	1.12 (CI: 0.84–2.84)	[30]
	GR inhibition (72 h)	4.36 (CI: 3.46 – 5.52)	[28]
	Growth inhibition (96 h)	0.49	[36]

*Na-NPX

Table S10. Summary of EC50 values (when available, 95% Confidence Interval, CI, or standard deviation, SD, are provided within parenthesis) collected from the literature for *Aliivibrio fischeri* exposed to each tested compound, all concerning bioluminescence inhibition, at different exposure periods (Time). The references are presented at the end of the document.

Compound	Time	EC50 ($mg.L^{-1}$)	Reference
CAF	5 min	317	[37]
	15 min	632.0 (CI: 569.1–694.7)	[38]
	30 min	1244.3 (± 71.8 SD)	[39]
CBZ	15 min	52.22 (CI: 45.84–59.47)	[40]
	15 min	94.0 (CI: 82.03–105.91)	[38]
	30 min	> 81	[41]
	30 min	> 100	[42]
DIC	15 min	15.9 (CI: 14.59–17.26)	[38]
	15 min	16.31 (± 0.72 SD)	[43]
	15 min	25.36	[44]
	30 min	11.62	[42]
	30 min	11.454	[41]
FXT	15 min	35.44	[45]
IBU	15 min	39.93 (± 2.2 SD)	[43]
	15 min	18.3 (CI: 15.96–20.58)	[38]
	30 min	14.97	[42]
MET	15 min	755.4 (CI: 129.7–4400)	[40]
	30 min	> 870.79	[46]
NPX	15 min	47.07 (± 1.45 SD)	[43]
	30 min	25.17	[42]
PCT	15 min	301.41	[44]
	15 min	697 (± 48 SD)	[47]
	15 min	390.2 (CI: 241.5–630.7)	[40]
SMX	15 min	49.49	[44]
	15 min	50.51 (±2.28 SD)	[48]
	15 min	> 84	[41]
	30 min	51.77	[42]

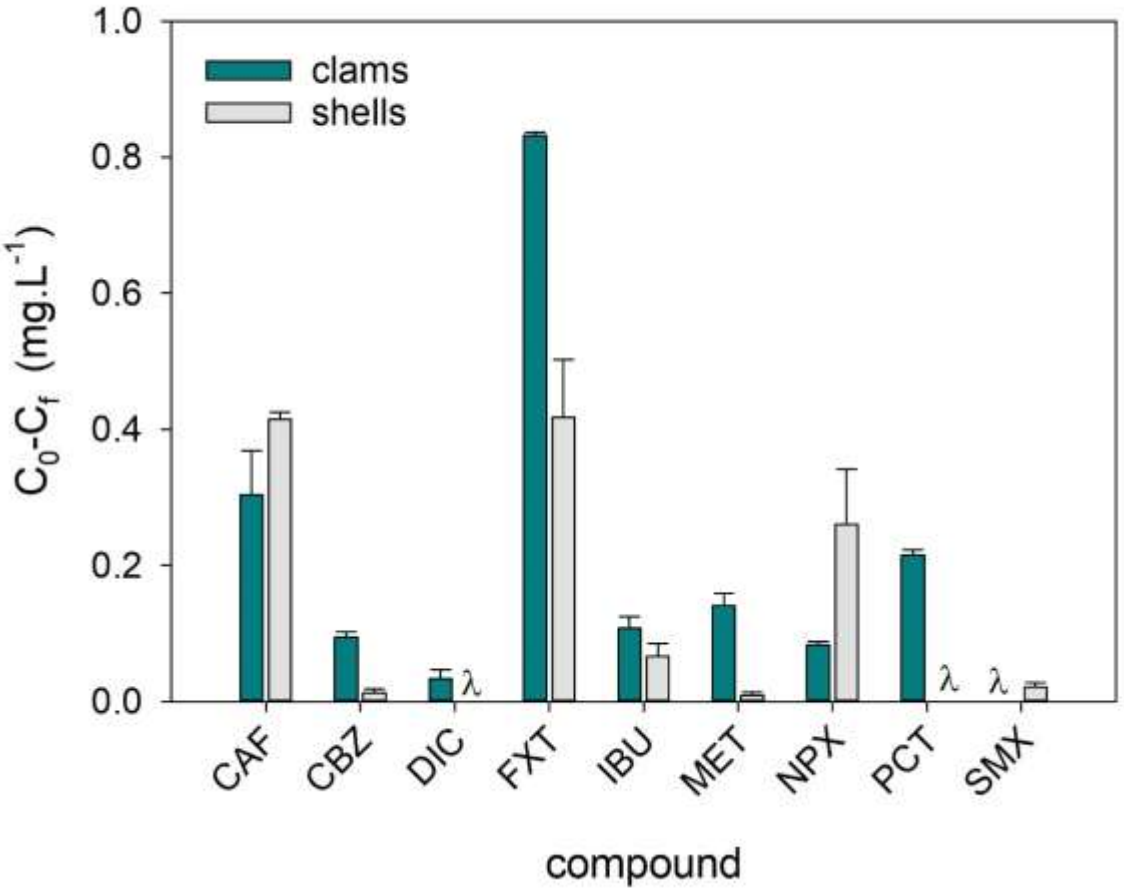


Figure S1. Decrease on the compound concentration, expressed as the difference between the contaminant concentration in the control (C_0) and the concentration in the treated sample (C_f), after treatment by *C. fluminea* (biofiltration) and by *C. fluminea* milled shells (biosorption), during 24 h, considering a solution with an initial concentration of 1.0 mg.L^{-1}. Bars represent the mean and error bars represent the standard error. The asterisks represent statistical differences between both approaches for each compound (t-test; $p < 0.05$). λ: The contaminant concentration in the treated water did not differ from the concentration in the control, and thus no removal was observed.

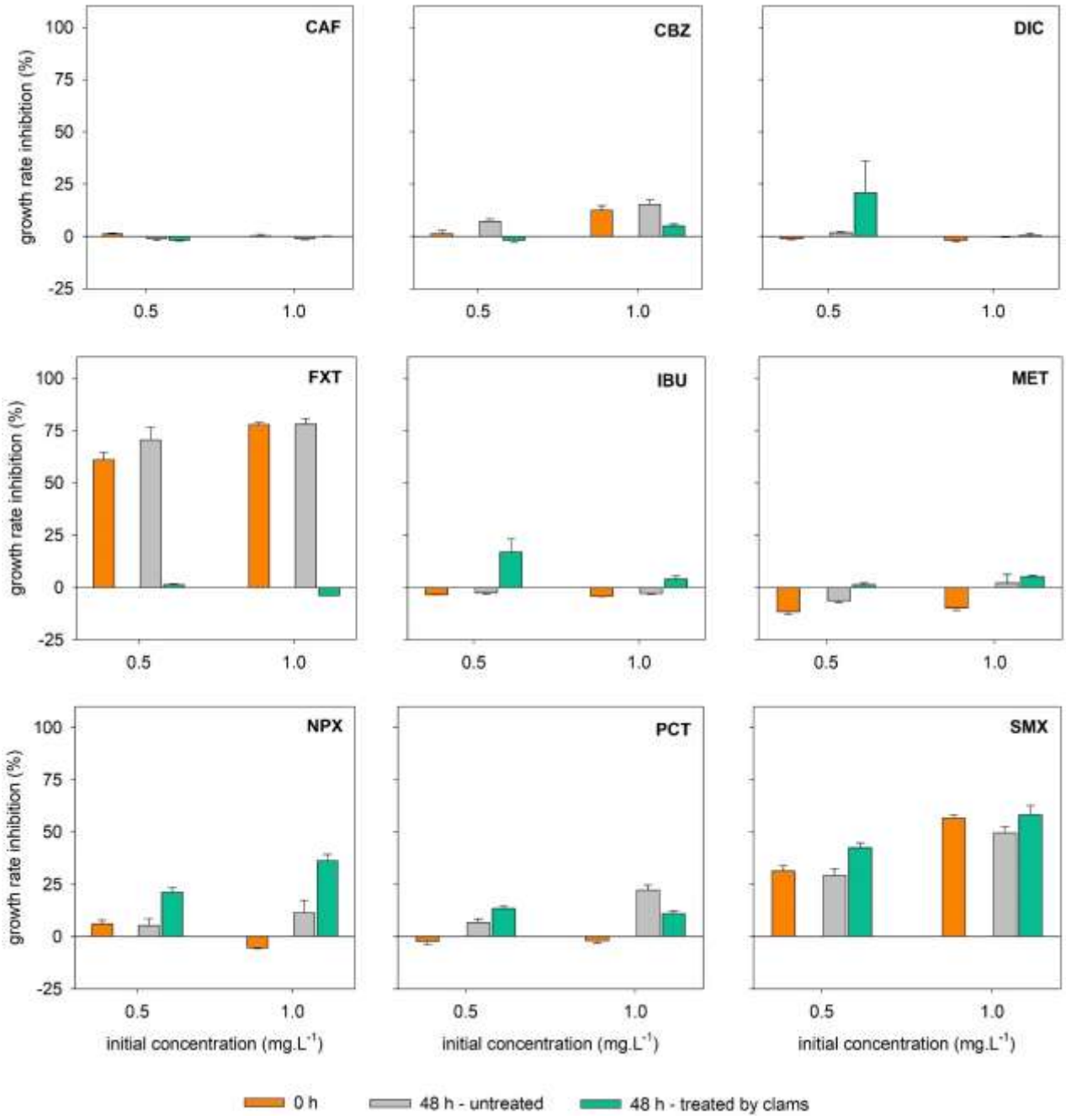


Figure S2. Growth rate inhibition of *R. subcapitata* exposed to samples (0.5 and 1.0 mg.L^{-1}) before any treatment (0 h) and after 48 h of aeration (48 h – untreated; no clams) or biofiltration by *C. fluminea* (48 h – treated by clams). Bars represent the mean and error bars represent the standard error.

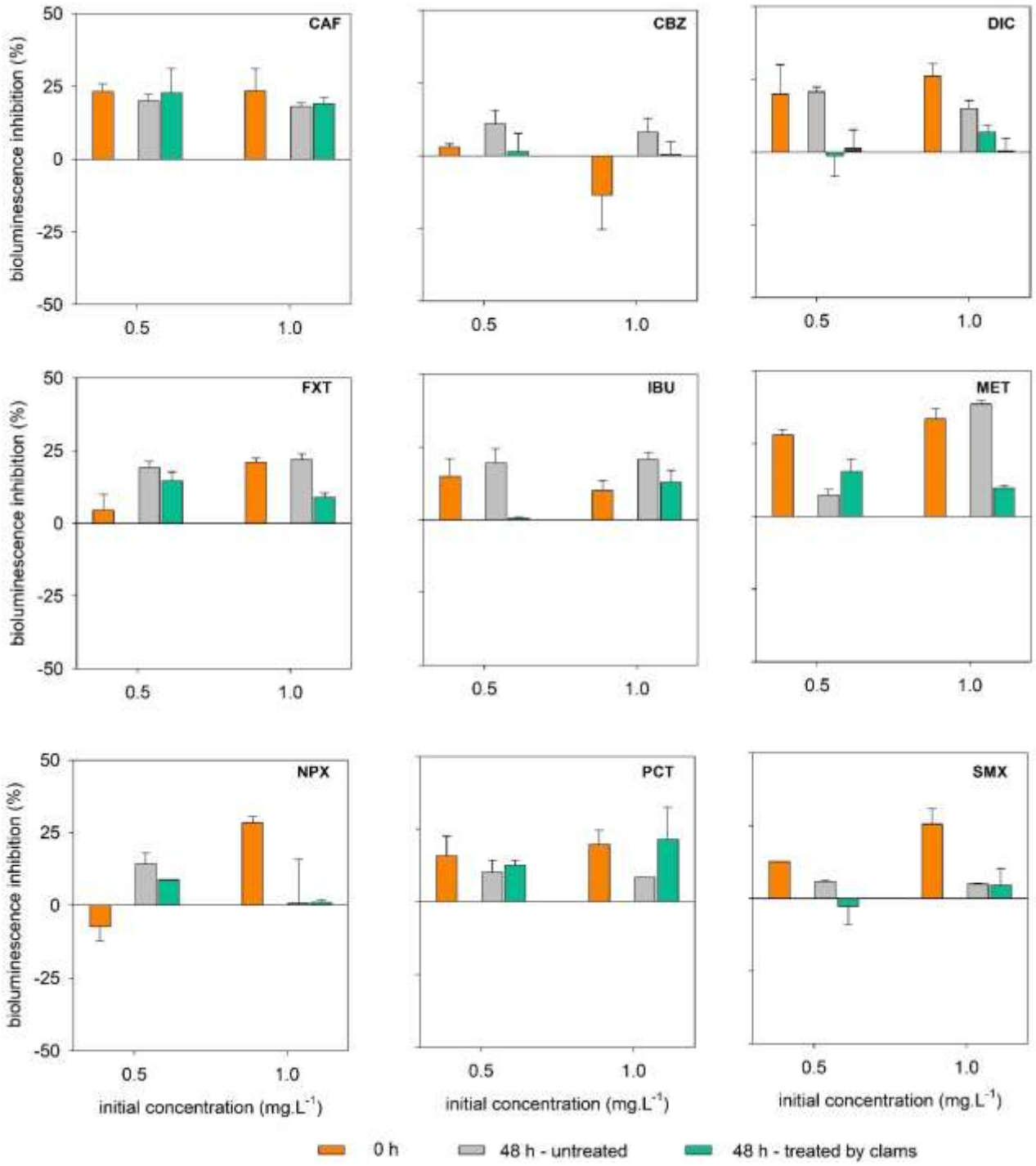


Figure S3. Bioluminescence inhibition of *A. fischeri* exposed to samples (0.5 and 1.0 $mg.L^{-1}$) before any treatment (0 h) and after 48 h of aeration (48 h – untreated; no clams) or biofiltration by *C. fluminea* (48 h – treated by clams). Bars represent the mean and error bars represent the standard error.

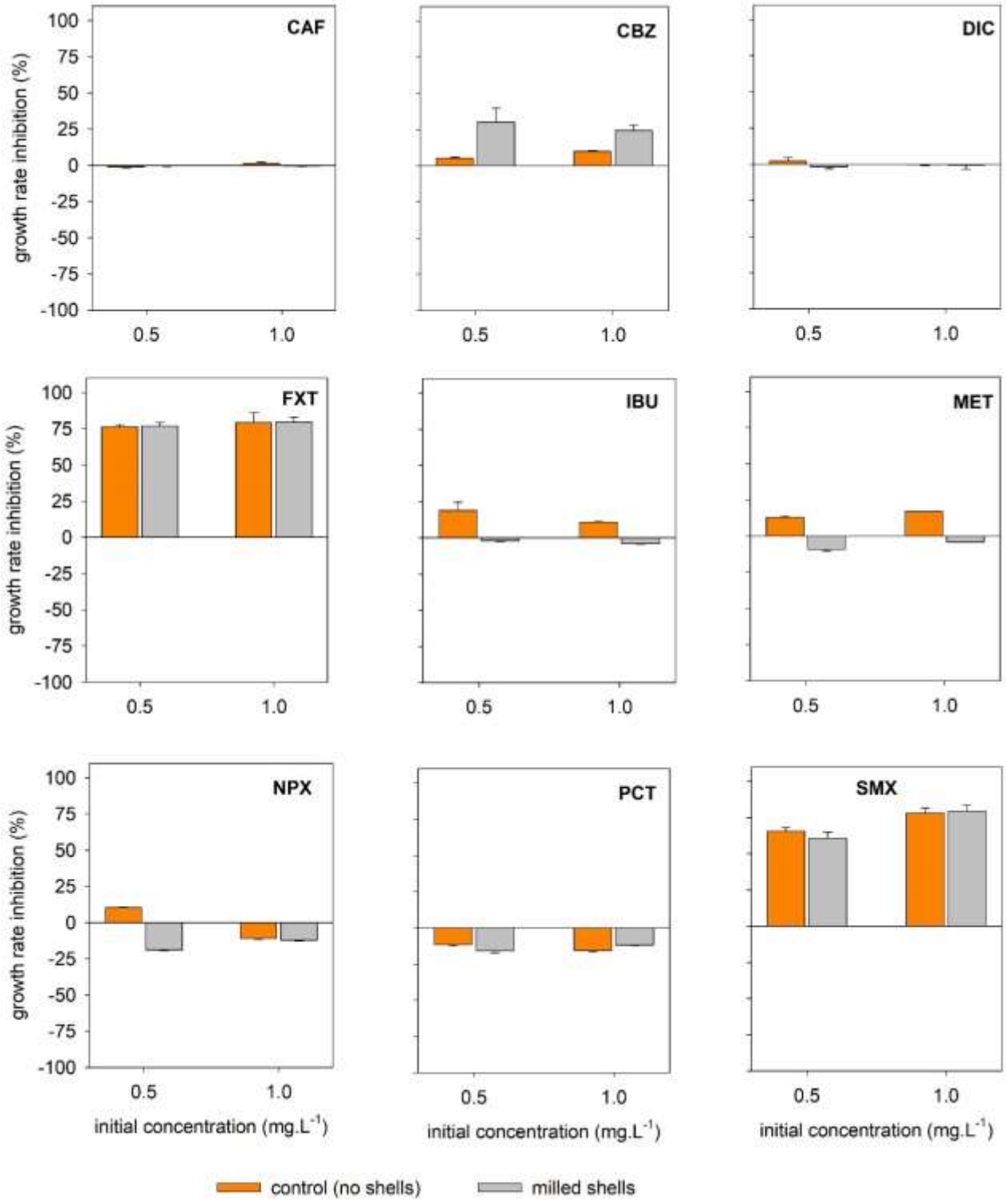


Figure S4. Growth rate inhibition of *R. subcapitata* exposed to samples (0.5 and 1.0 mg.L^{-1}) that were previously in contact with *C. fluminea* milled shells (milled shells) or untreated (control, no shells) during 24 h. Bars represent the mean and error bars represent the standard error.

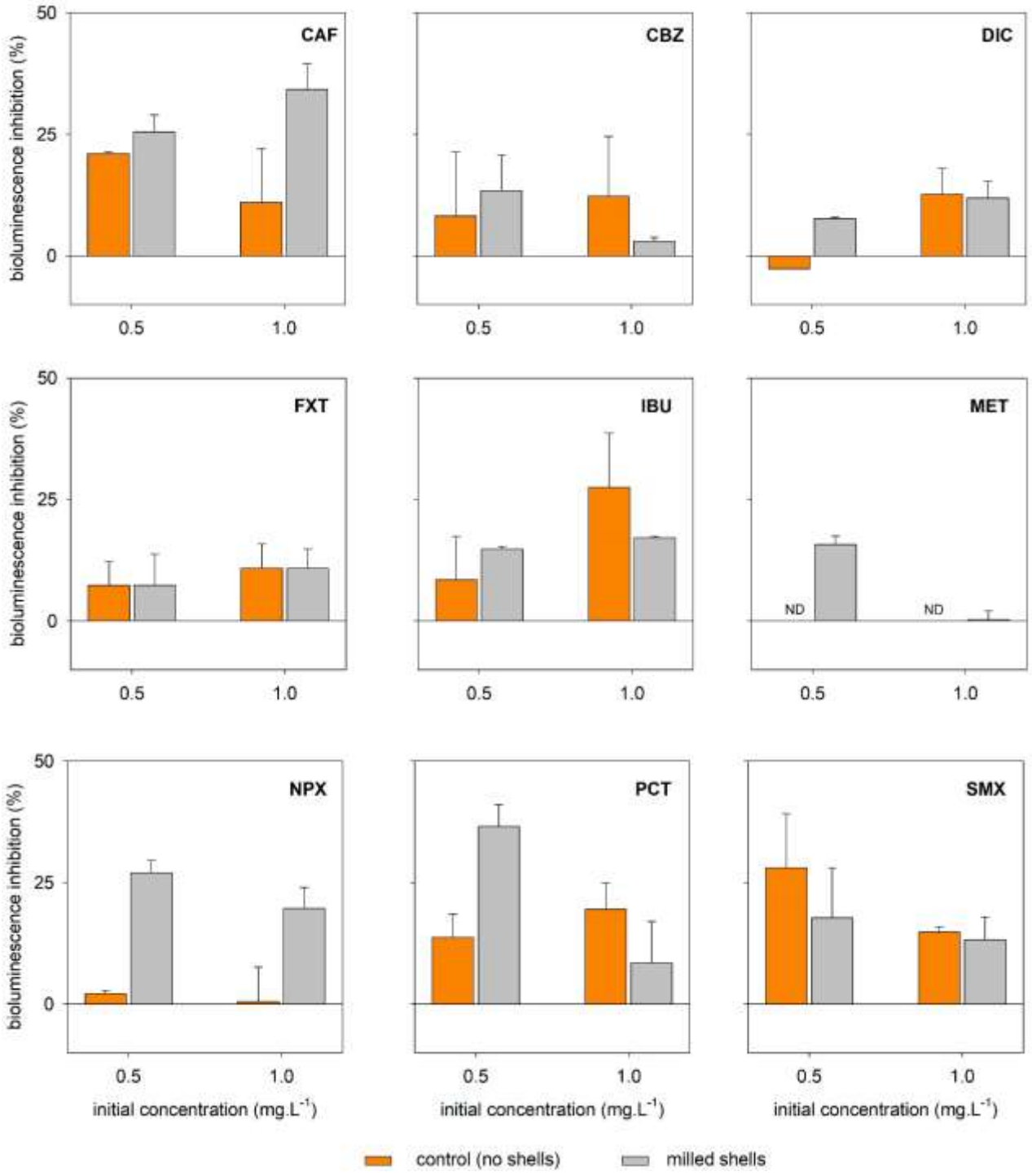


Figure S5. Bioluminescence inhibition of *A. fischeri* exposed to samples (0.5 and 1.0 mg.L^{-1}) that were previously in contact with *C. fluminea* milled shells (milled shells) or untreated (control, no shells) during 24 h. Bars represent the mean and error bars represent the standard error. ND: not determined.

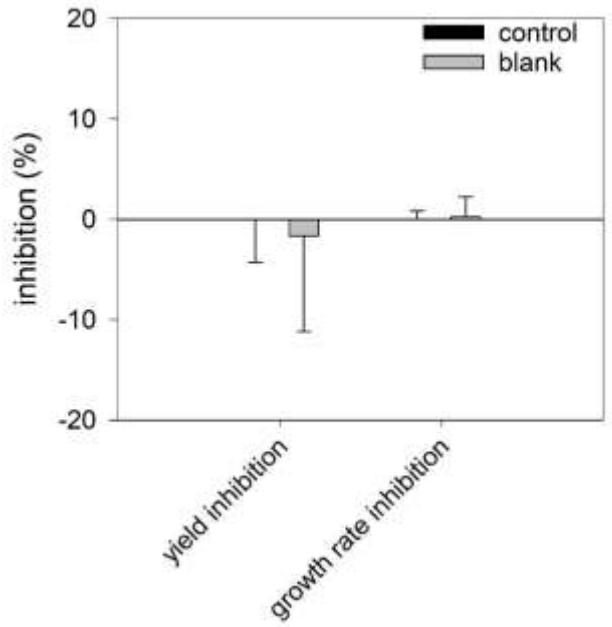


Figure S6. Comparative inhibition of the controls and the blank samples from the biofiltration experiment for the growth inhibition endpoints of *R. subcapitata*. No statistically significant differences were found for both treatments for each endpoint (*t*-test, $p \geq 0.05$).

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