

SUPPLEMENTARY MATERIALS

Assessment of Advanced Oxidation Processes using Zebrafish in a Non-Forced Exposure System: A Proof of Concept

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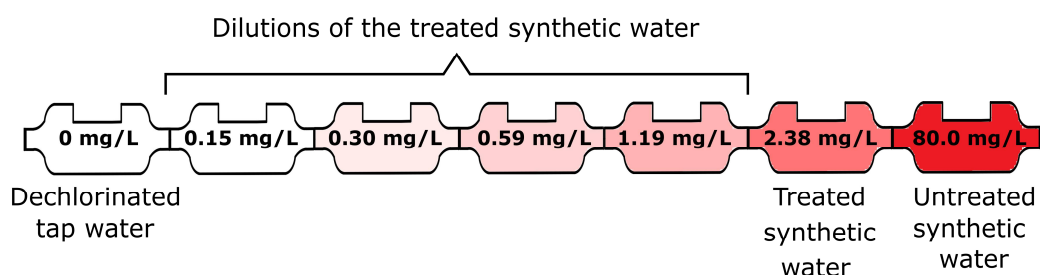
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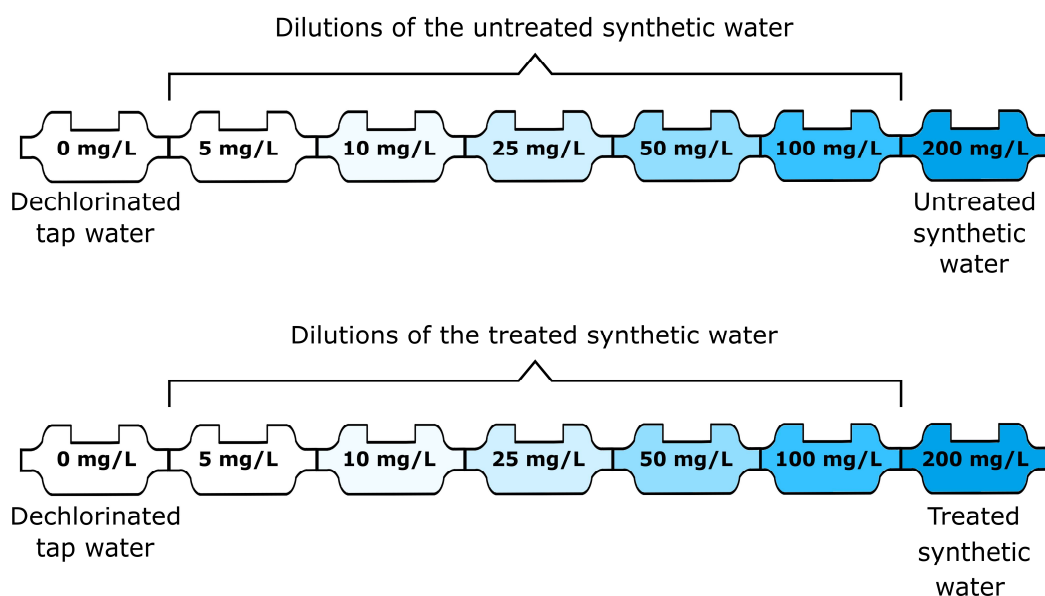
Figure S1. Linear multi-compartmented non-forced static system with seven compartments constructed with PET bottles.

Assays applied to the synthetic water from the textile industry*



* The concentration of the pollutant is expressed in mg/L of Reactive Red 120 dye.

Assays applied to the synthetic water from the cassava starch industry**



** The content of organic matter is expressed as chemical oxygen demand (COD) in mg/L.

Figure S2. Schemes showing the location of synthetic waters from the textile and cassava starch industries (and/or their dilutions) in the linear multi-compartmented non-forced static system.

Table S1. Results of the water analysis of dechlorinated tap water (water used to maintain fish and dilute synthetic waters used in assays). The measurement of the parameters was performed by the Centro de Investigación y Control Ambiental (CICAM)¹.

Parameter	Unit	Results
Total alkalinity	mg CaCO ₃ /L	39
Residual free chlorine	mg/L	< 0.2
Biochemical oxygen demand, BOD ₅	mg O ₂ /L	< 2
Chemical oxygen demand, COD	mg O ₂ /L	43
Total hardness	mg CaCO ₃ /L	47
Dissolved oxygen	mg O ₂ /L	6.6
pH	-	7.23
Total solids	mg/L	136
Total dissolved solids	mg/L	82
Turbidity	NTU	0.31

¹ CICAM is a laboratory accredited by the Ecuadorian Service of Accreditation (SAE) under ISO/IEC 17025:2006. This accreditation includes all the parameters listed in the table.



Figure S3. Untreated and treated synthetic water from the textile industry. The decolorization due to the treatment is noticeable.

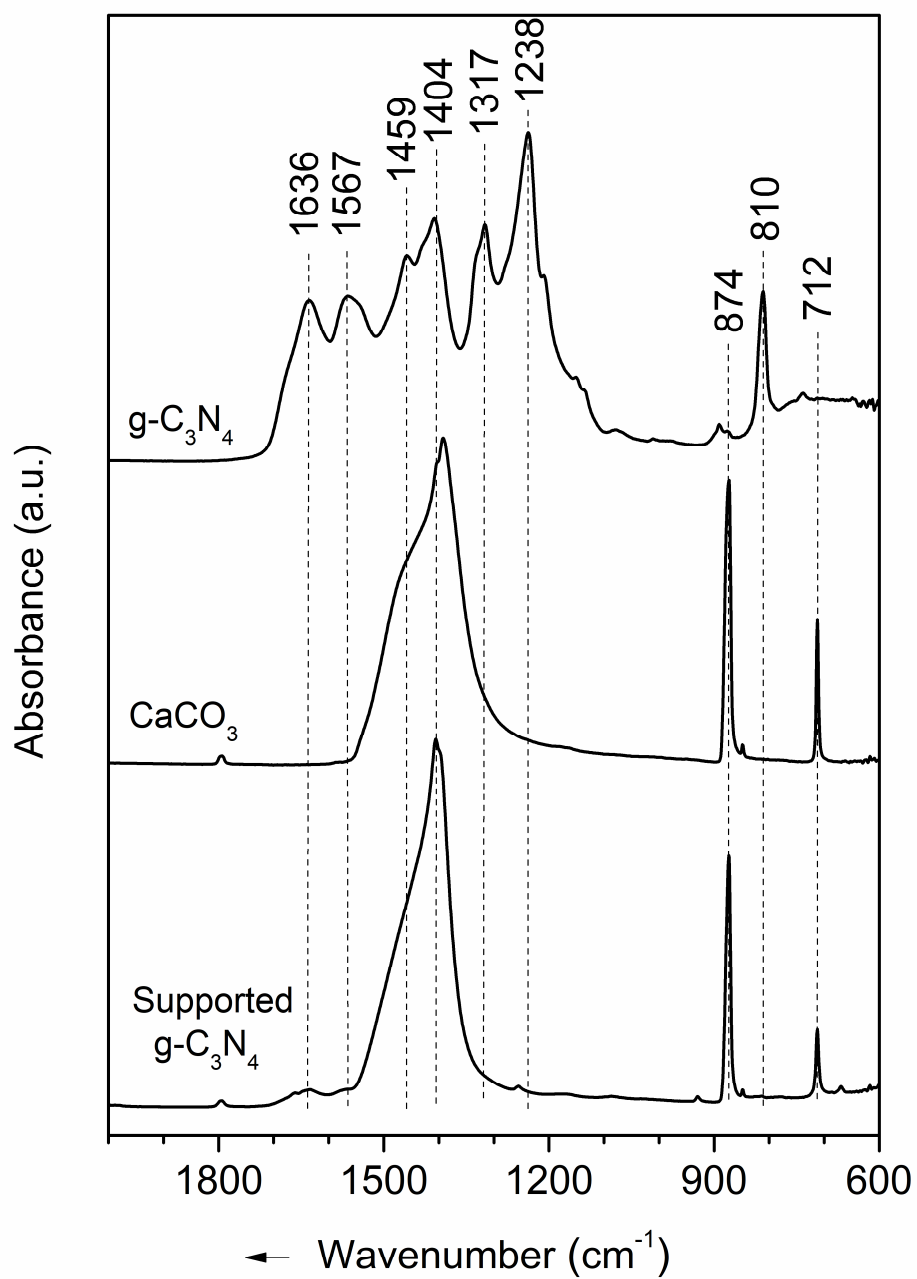


Figure S4. Infrared spectra of the supported g-C₃N₄, and pyrolyzed urea and calcium oxalate monohydrate (g-C₃N₄ and CaCO₃, respectively).

Table S2. Electrical conductivity of aqueous solutions of NaCl in the compartments for the test of heterogeneity.

ELECTRICAL CONDUCTIVITY ($\mu\text{S}/\text{cm}$)							
Compartment*	1	2	3	4	5	6	7
0 h	170.18	201.60	231.44	265.29	295.81	326.99	359.38
4 h	174.46	204.00	236.95	269.08	299.40	330.21	358.66
6 h	178.35	210.25	244.30	278.40	305.30	336.20	363.20
Average	174.33	205.28	237.56	270.92	300.17	331.13	360.42
SD	4.08	4.47	6.45	6.75	4.79	4.67	2.44
CV (%)	2.34	2.18	2.72	2.49	1.60	1.41	0.68

SD: Standard Deviation, CV: Coefficient of Variation

* Initial concentration of NaCl of solutions into the compartments in increasing order: 0, 17, 34, 50, 66, 84 and 100 mg/L.

Table S3. Distribution of organisms in the compartments filled with dechlorinated water after 4 h.

Repetition	Compartments							Total
	1	2	3	4	5	6	7	
System 1	2	4	4	2	4	2	3	21
System 2	3	3	3	4	3	2	3	21
System 3	4	2	4	2	2	3	4	21
System 4	2	4	3	3	3	4	2	21
Total	11	13	14	11	12	11	12	84

Table S4. Distribution of organisms in the compartments filled with dechlorinated water after 6 h.

Repetition	Compartments							Total
	1	2	3	4	5	6	7	
System 1	2	3	4	3	4	2	3	21
System 2	3	4	3	4	3	2	2	21
System 3	4	2	4	2	2	3	4	21
System 4	3	4	3	3	3	3	2	21
Total	12	13	14	12	12	10	11	84

Table S5. Number of organisms (zebrafish) observed after 4 h in each compartment of the non-forced exposure system for the assays with the untreated and treated synthetic waters from the textile industry.

Repetition	Concentration of RR-120 (mg/L)						
	Treated water						Untreated water
	0	0.15	0.30	0.59	1.19	2.38	80
1	7	7	0	0	5	0	2
2	8	4	7	2	0	0	0
3	1	1	6	2	5	5	1
Total organisms per compartment	16	12	13	4	10	5	3
% Organisms per compartment	25.40	19.05	20.63	6.35	15.87	7.94	4.76
Fisher test (p value) ¹	-	>0.05	>0.05	=0.05	>0.05	>0.05	<0.05

¹ Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of organisms in the control was used as reference. Values in bold represent statistically significant differences.

Table S6. Number of organisms (zebrafish) observed after 6 h in each compartment of the non-forced exposure system for the assays with the untreated and treated synthetic waters from the textile industry.

Repetition	Concentration of RR-120 (mg/L)						
	Treated water						Untreated water
	0	0.15	0.30	0.59	1.19	2.38	80
1	3	9	2	3	3	0	1
2	3	4	10	2	1	1	0
3	2	3	1	3	6	5	1
Total organisms per compartment	8	16	13	8	10	6	2
% Organisms per compartment	12.70	25.40	20.63	12.70	15.87	9.52	3.17
Fisher test (p value) ¹	-	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Fisher test (p value) ²	>0.05	-	>0.05	>0.05	>0.05	>0.05	<0.05

¹ Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of organisms in the control was used as reference. Values in bold represent statistically significant differences.

² Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of the compartment with the highest number of organisms (treated water: 0.15 mg/L) was used as reference. Values in bold represent statistically significant differences.

Table S7. Number of organisms (zebrafish) observed after 4 h in each compartment of the non-forced exposure system for the assays with the untreated synthetic water from the cassava starch industry.

Repetition	COD content (mg/L)						
	0	5	10	25	50	100	200
1	14	4	1	2	0	0	0
2	10	10	1	0	0	0	0
3	14	5	2	0	0	0	0
Total organisms per compartment	38	19	4	2	0	0	0
% Organisms per compartment	60.32	30.16	6.35	3.17	0.00	0.00	0.00
Fisher test (p value) ¹	-	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

¹ Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of organisms in the control was used as reference. Values in bold represent statistically significant differences.

Table S8. Number of organisms (zebrafish) observed after 6 h in each compartment of the non-forced exposure system for the assays with the untreated synthetic water from the cassava starch industry.

Repetition	COD content (mg/L)						
	0	5	10	25	50	100	200
1	6	13	1	1	0	0	0
2	14	4	2	1	0	0	0
3	11	7	2	1	0	0	0
Total organisms per compartment	31	24	5	3	0	0	0
% Organisms per compartment	49.21	38.10	7.94	4.76	0.00	0.00	0.00
Fisher test (p value) ¹	-	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

¹ Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of organisms in the control was used as reference. Values in bold represent statistically significant differences.

Table S9. Number of organisms (zebrafish) observed after 4 h in each compartment of the non-forced exposure system for the assays with the treated synthetic water from the cassava starch industry.

Repetition	COD content (mg/L)						
	0	5	10	25	50	100	200
1	0	2	2	1	4	9	3
2	3	5	2	0	3	3	5
3	0	0	1	2	4	7	7
Total organisms per compartment	3	7	5	3	11	19	15
% Organisms per compartment	4.76	11.11	7.94	4.76	17.46	30.16	23.81
Fisher test (p value) ¹	-	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05
Fisher test (p value) ²	<0.05	>0.05	<0.05	<0.05	>0.05	-	>0.05

¹ Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of organisms in the control was used as reference. Values in bold represent statistically significant differences.

² Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of the compartment with the highest number of organisms (treated water: 0.15 mg/L) was used as reference. Values in bold represent statistically significant differences.

Table S10. Number of organisms (zebrafish) observed after 6 h in each compartment of the non-forced exposure system for the assays with the treated synthetic water from the cassava starch industry.

Repetition	COD content (mg/L)						
	0	5	10	25	50	100	200
1	0	1	6	5	4	5	0
2	2	0	1	3	5	3	7
3	2	0	0	3	4	7	5
Total organisms per compartment	4	1	7	11	13	15	12
% Organisms per compartment	6.35	1.59	11.11	17.46	20.63	23.81	19.05
Fisher test (p value) ¹	-	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Fisher test (p value) ²	>0.05	<0.05	>0.05	>0.05	>0.05	-	>0.05

¹ Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of organisms in the control was used as reference.

² Comparisons (expected vs. observed) were performed using the total of organisms (considering the sum of the three replicates) and the distribution of the compartment with the highest number of organisms (treated water: 0.15 mg/L) was used as reference. Values in bold represent statistically significant differences.