



*Emerging Pollutants: Protecting Water Quality for the Health of People and the Environment*

**Toxicity and biodegradation of the pharmaceutical diclofenac employing the green marine microalga *Tetraselmis* sp.: A preliminary study**

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## Introduction

Environmental pollution by emerging contaminants (pharmaceuticals)

<https://marcioantoniasci.wordpress.com/2022/04/08/presenca-de-medicamentos-nos-rios-e-uma-ameaca-a-saude-ambiental-e-humana/>



Toxic effects on aquatic organisms



<https://www.vecteezy.com/vector-art/88219-free-vector-cartoon-underwater-world>

### Mains goals of this research:

- ✓ Contribute to the advances on toxicology fields and biodegradation technologies;
- ✓ Contribute with data of a potential green alternative for degradation of emerging contaminants;

## Objectives

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- ✦ Evaluate the toxicity of the anti-inflammatory sodium diclofenac (DCF) on the marine microalgae *Tetraselmis* sp.;
- ✦ Evaluate the potential of marine green microalgae *Tetraselmis* sp. to biodegrade DCF.

## Methodology

### 1. *Tetraselmis* sp. culture and cell density measurement

Conway medium ( $\leq 29$  ‰, pH 8)  
21  $\pm$  3 °C and a minimum of 4500 lux.

Cell density:

UV-VIS – 680 nm

Cell counting – Neubauer chamber



### 2. Toxicity tests

ABNT NBR 16181:2021

DCF concentrations: 0.1 - 200 mg/L

Negative control

Cell density: 24 - 96 h

### 3. Biodegradation tests

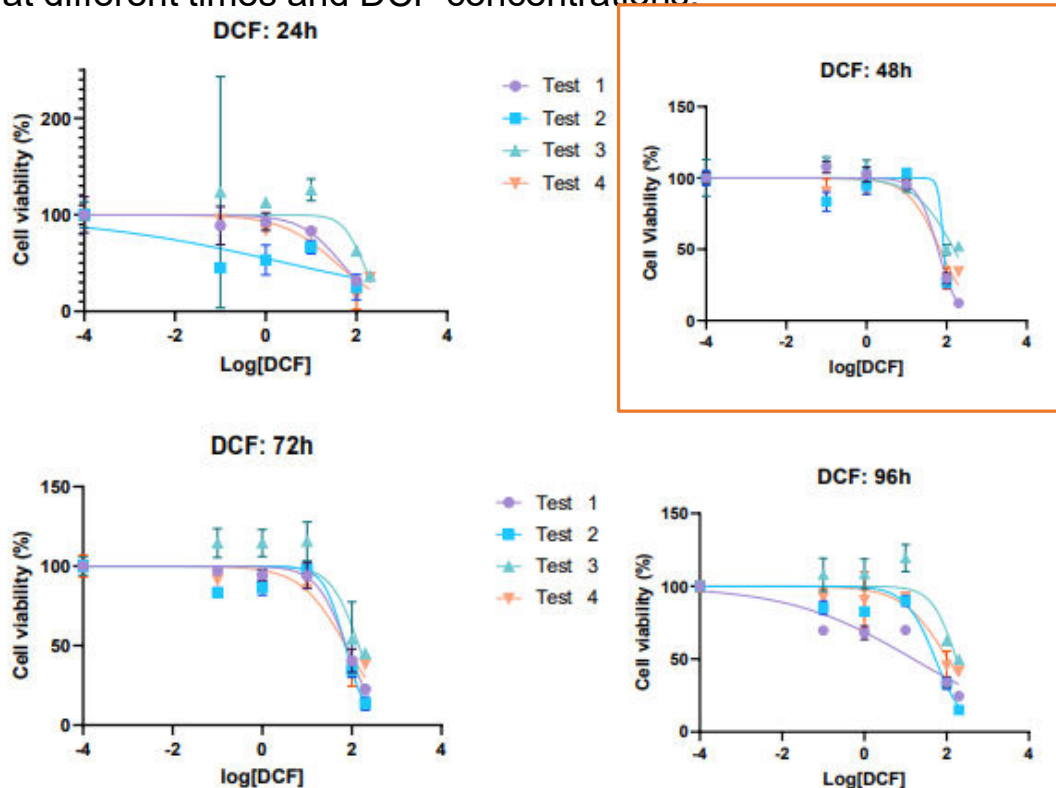
- ✓ DCF (10 and 50 mg/L) + *Tetraselmis* sp. ( $2 \times 10^5$  cell/mL)
- ✓ Negative control (*Tetraselmis* sp. at  $2 \times 10^5$  cell/mL)
- ✓ DCF control (photodegradation)
- ✓ Following times: 0 - 96 h
- ✓ DCF quantification: UV-VIS – 275 nm (DL: 1 mg/L)



# Results and discussion

## 1. Acute toxicity test: DCF

Dose-response curves of the independent tests performed at different times and DCF concentrations.



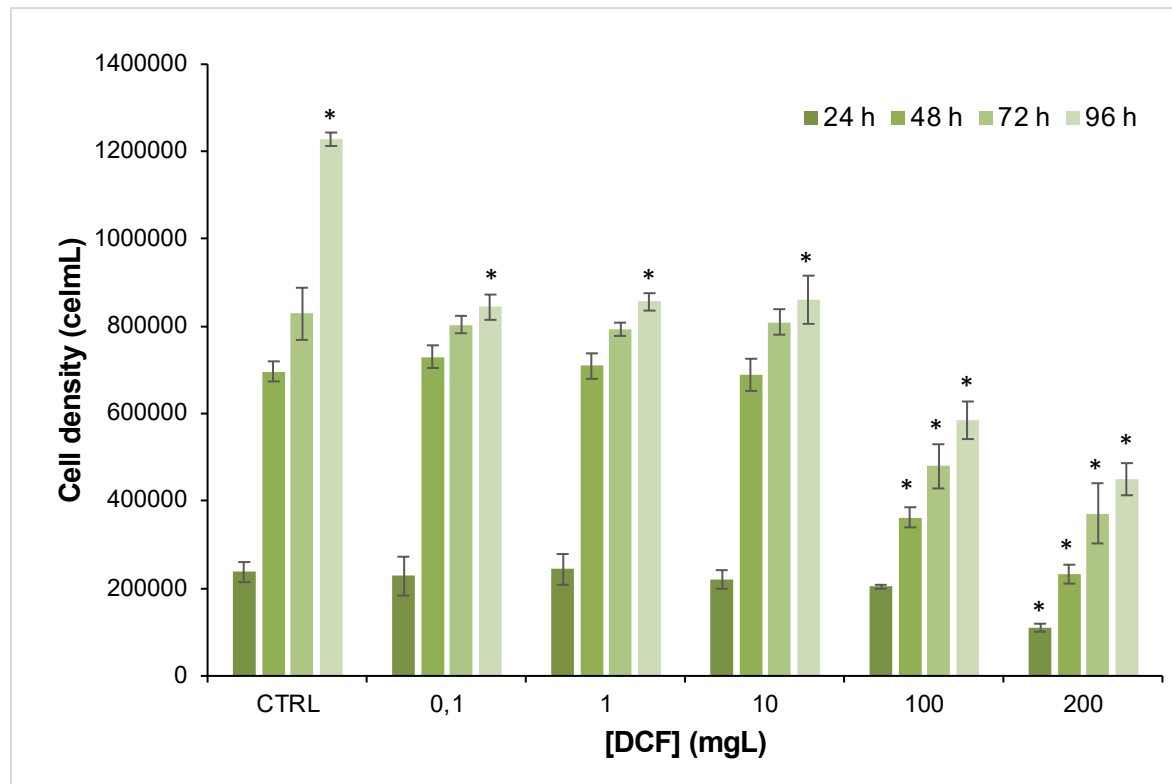
EC50 values calculated for different exposure times and respective R<sup>2</sup> (95% confidence interval) calculated in the software GraphPad.

<u>Exposure time</u>	24 h		48 h		72 h		96 h	
Test	EC50 (mg/L)	R <sup>2</sup>	EC50 (mg/L)	R <sup>2</sup>	EC50 (mg/L)	R <sup>2</sup>	EC50(mg/L)	R <sup>2</sup>
Test 1	46,53 (IC95% = 24,86 – 92,02)	0,80	60,42 (IC95% = 49,27- 74,12)	0,98	75,44 (IC95% = 62,22- 89,83)	0,97	15,54 (IC95% = 6,3- 38,75)	0,85
Test 2	2,89 (IC95% = 0,20– 109)	0,57	85,59	0,90	70,94 (IC95% = 46,12- 95,05)	0,92	53,29 (IC95% = 33,64- 82,80)	0,91
Test 3	141 (IC95% = 38,35 - )	0,31	153,4 (IC95% = 102 - 260)	0,86	143,3 (IC95% = 90,41- 265)	0,75	175,1 (IC95% = 126,1- 287,7)	0,79
Test 4	36,14 (IC95% = 19,28- 63,78)	0,87	68,73 (IC95% = 46,79- 96,78)	0,90	73,40 (IC95% = 49,82- 106)	0,90	107,2 (IC95% = 68,96- 178,6)	0,85

## Results and discussion

### 2. Chronic toxicity test

Exposure of marine microalgae *Tetraselmis* sp. to DCF



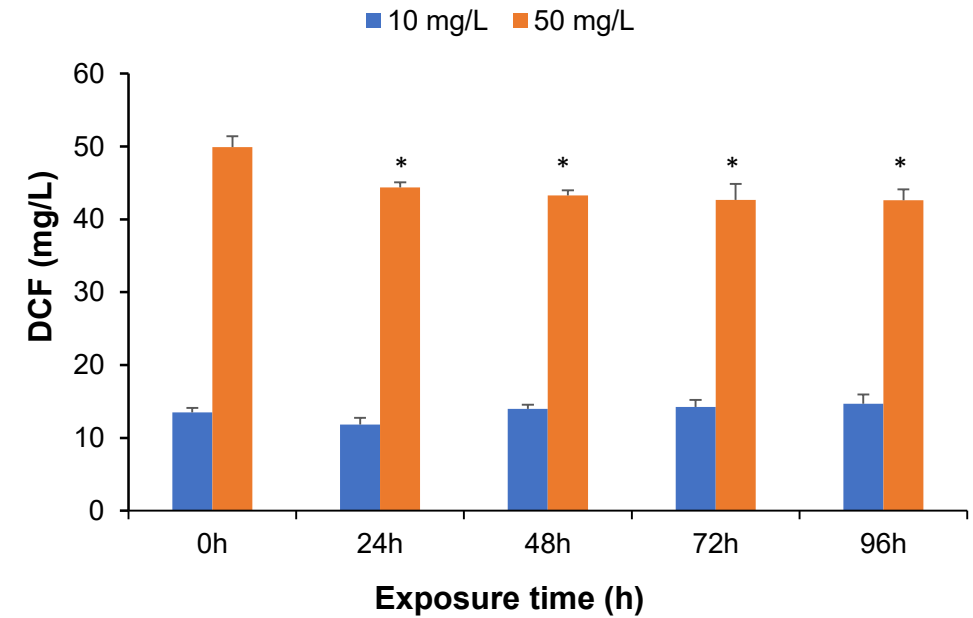
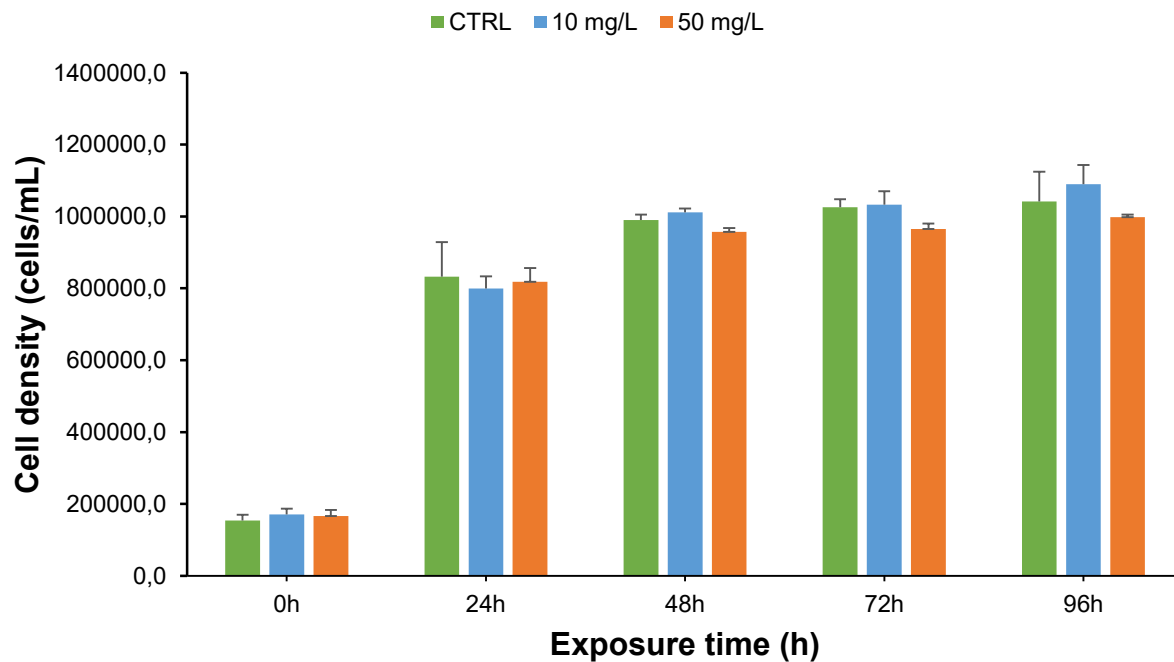
✓ Significant differences between treatment and test control ( $p < 0.05$ ) are indicated with \*

- **LOEC 48 h:** 100 mg/L
- **NOEC 48 h:** 10 mg/L

# Results and discussion

## 3. Biodegradation test

Growth of *Tetraselmis* sp. with 0 (control), 10 and 50 mg/L of DCF.



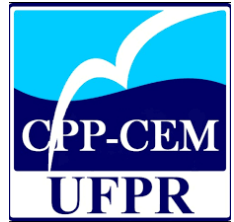
Biodegradation of DCF using the microalgae *Tetraselmis* sp. With the initial concentrations of 10 and 50 mg/L of DCF.

## Conclusions

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- ✓ Acute toxicity results with *Tetraselmis* sp. showed a  $EC_{50}$  <sub>48h</sub> to DCF of 60.42 mg/L ( $R^2 = 0.98$ );
- ✓ Chronic toxicity results indicate values to DCF of LOEC of 100 mg/L and NOEC of 10 mg/L in 48 h;
- ✓ Biodegradation tests evidenced the *Tetraselmis* sp. can be used to degrade and remove DCF from a marine medium, even though the efficiency was low.





# Thank you!

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