To what extent does the concentration of Ibuprofen (0, 75, 150, 225, 300, 375 and 750µg L-1) affect the growth of Skeletonema marinoi and Nannochloropsis salina measured by spectrophotometry (OD600 absorbance)?

Dosing the Baltic Sea: what happens when algae encounters Ibuprofen?

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List of acronyms and abbreviations

EP ..... Emerging Pollutants
NSAID ..... Non-Steroidal Anti-Inflammatory Drugs
IBU ..... Ibuprofen
HELCOM ..... Baltic Marine Environment Protection Commission
EUSBSR ..... Policy Area Hazards of the European Union Strategy for the Baltic Sea Region
ATP ..... Adenosine Triphosphate
CPP ..... Cyclic Photophosphorylation
PSI ..... Photosystem One
PSII ..... Photosystem Two
NADPH ..... Nicotinamide Adenine Dinucleotide Phosphate
CAO ..... Chlorophyllide *A* Oxygenase
SWCNT ..... Single-Walled Carbon Nanotubes
MWCNT ..... Multi-Walled Carbon Nanotubes
MWNT-COOH ..... Carboxylated Multi-Walled Carbon Nanotubes
CYP ..... Human Cytochrome P450
PCR ..... Polymerase Chain Reaction
PTM ..... Post-Translational Modifications
WWTP ..... Wastewater Treatment Plant

1 - INTRODUCTION

1.1 - BACKGROUND INFORMATION

1.1.1 - IBUPROFEN AS AN EMERGING POLLUTANT

Emerging pollutants (EPs) are defined as compounds that only in recent years have been classified as potentially dangerous when in the environment (GPA-UNEP, 2020), and they have been put in the limelight as concern has grown. One such EP is the non-steroidal anti-inflammatory classed drug (NSAID) Ibuprofen (IBU). In a status report from 2017 by the Baltic Marine Environment Protection Commission (HELCOM) in cooperation with Policy Area Hazards of the European Union Strategy for the Baltic Sea Region (EUSBSR) Ibuprofen was detected in 38 out of 280 samples with concentrations reaching 159µg L^{-1}. However, looking beyond the Baltic Sea Region concentrations upwards of 1400µg L^{-1} have been detected in surface waters along Korean and Chinese coasts (Jan-Roblero & Cruz-Maya, 2023). This may be cause for major concern as the potential ecotoxicity of Ibuprofen for microscopic marine life only recently has begun to be investigated.

1.1.2 - ALGAE IN MARINE ECOSYSTEMS

As primary producers, algae are a vital part of the marine food web (Johansson et al., 2019). However, their photosynthetic abilities make them responsible for a large percentage of the atmospheric oxygen levels (The United States Environmental Protection Agency, 2024). Thus, their role in the global ecosystem can be compared to that of the rainforests (Xin et al., 2022). Diatoms alone are responsible for approximately 40% of primary production in marine biomes (Serôdio et al., 2020,). *Skeletonema marinoi* is a diatom primarily found in the Baltic Sea with a diameter of 2-10µm and a length of 4-21µm (Swedish Biodiversity Data Infrastructure & Swedish Meteorological and Hydrological Institute, n.d.). They possess a siliceous structure and are a chain forming algae. *Nannochloropsis salina* can be found along western European coastlines as well as south and east Asian coastal areas (Swedish Biodiversity Data Infrastructure & Swedish Meteorological and Hydrological Institute, n.d.). It has a spherical cell structure and ranges from 2-5µm, rather small for a green alga (Kannah et al., 2021).

In the status report from HELCOM and EUSBSR other EPs were detected such as Diclofenac and Metformin, however, Ibuprofen was selected for its widespread, increasing usage in over 100 countries (Diener et al., 2008, 225-231). Due to its low environmental degradation rate, it is accumulating in major bodies of water such as the Baltic Sea (Jan-Roblero & Cruz-Maya, 2023). Because of the overlapping in geographical distribution of *S. marinoi* and *N. salina* habitats and identified bodies of water with presence of Ibuprofen, said species were selected for this investigation to simulate what these algae currently are facing.

Figure 1. Micrograph of *N. salina* (Mohammady, 2005)

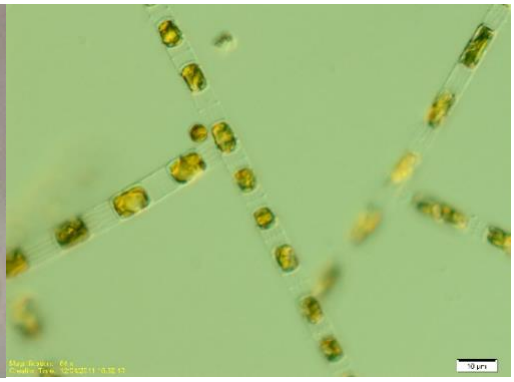

Figure 2. Micrograph of *S. marinoi* (Widdicombe, 2011)

The ecotoxicity of Ibuprofen on the diatom *Phaeodactylum tricornutum* has been previously determined. Monoclonal cultures of the algal species were grown for 4 days. After exposure to IBU at concentrations of 0-300µg L^{-1} cell counts showed a decelerated growth, which biochemical analytical approaches (fatty acid profiling, cellular energy expenditure, oxidative stress) confirmed (Silva et al., 2020). However, more relevant species for the Baltic Sea have not been researched. This investigation will try to determine following: To what extent does the concentration of Ibuprofen (0, 75, 150, 225, 300, 375 and 750µg L^{-1}) affect the growth of *Skeletonema marinoi* and *Nannochloropsis salina* measured by spectrophotometry (OD_{600} absorbance)?

1.2 - HYPOTHESES

1.2.1 - NULL HYPOTHESIS

The null hypothesis (H_0) states there is no correlation between increasing concentrations of Ibuprofen (0, 75, 150, 225, 300, 375 and 750µg L^{-1}) and growth of *Skeletonema marinoi* and *Nannochloropsis salina*.

1.2.2 - ALTERNATE HYPOTHESIS

The alternate hypothesis (H_1) states there will be a correlation between increasing concentrations of Ibuprofen (0, 75, 150, 225, 300, 375 and 750µg L^{-1}) and growth of *Skeletonema marinoi* and *Nannochloropsis salina*.

1.3 - VARIABLES

1.3.1 - INDEPENDENT AND DEPENDENT VARIABLES

In this investigation the independent variable is concentrations of the nonsteroidal anti-inflammatory drug Ibuprofen (Orifarm Generics) which will consist of 0, 75, 150, 225, 300, 375 and 750µg L^{-1} solutions. The concentrations of 0-750µg L^{-1} were determined through two steps. Initially environmentally present levels were identified through the status report from 2017 by HELCOM in cooperation with the EUSBSR. From this the range was decided upon. Increments were found through a pre-trial which allowed for 75µg L^{-1}

increments to be identified (see Appendix 1). OD_{600} is the dependent variable in this investigation. OD_{600} absorbance is a way of estimating concentration of cells (in this case algal) in a sample. A light source of 600nm is aimed at the sample which scatters and absorbs the light. A detector picks up the passing light which amounts to a light scattering value used to estimate cell concentration (Tip Biosystems, 2023). Since ecotoxic substances at elevated concentration generally inhibit algal growth, measuring concentration of algal cells in samples before and after exposure allows for the evaluation of Ibuprofen's ecotoxic properties. Optical density was also selected as a quantitative measurement method due to its ability to effectively measure a high number of samples (Beal et al., 2020).

1.3.2 - CONTROLLED VARIABLES

Following are all the controlled variables that were considered in order to maintain the preciseness of the investigation including their unit, potential influence over results and methods of managing. Algal species: Varying species of algae proliferate at different rates and must therefore be determined (Metsoviti et al., 2019, 279). Prepared, verified cultures by GUmacc were used and spatially separated when preparing solutions. Separate tools were used for both species to eliminate cross contamination. Time (Hours): Algal growth relies on time (Jong et al., 2017, 823-840). All trials were prepared in close time range and were measured after the same time. Temperature (°C): Increase in temperature has a positive correlation with algal proliferation (Singh & Singh, 2015, 431-444). All trials were kept in the same area to eliminate exposure to different temperatures. Nutrient type: Nitrogen and phosphorus are a prerequisite for algal growth (Yaakob et al., 2021, 393). A two-part verified F/2 growth medium was used to ensure presence of vital nutrients and minerals in growth solution (see Appendix 2). Nutrient quantity: A decreased concentration of nitrogen and phosphorus negatively correlates with algal proliferation (Yaakob et al., 2021, 393). F/2 medium was homogenised into the same solution used to prepare all trials in order to avoid fluctuations of nutrient quantity. Light intensity: Increasing light intensity positively correlates with algal proliferation (Metsoviti et al., 2019, 31). All trials were kept in the same area to eliminate exposure to different light intensities. Salinity (NaCl%): NaCl concentration correlates with algal proliferation (Haris et al., 2021). Same 2.6% NaCl solution was used to prepare all trials to avoid fluctuations in salinity.

2 - METHODOLOGY

2.1 - ADAPTATIONS TO PROCEDURE

Adaptation to the procedure were made based on previous trials (see appendix 1).

2.2 - PROCEDURE

The chosen experimental method is inspired by the OECD Freshwater and Cyanobacteria Growth Inhibition test (OECD, 2011) modified to account for environmentally and geographically relevant levels of Ibuprofen.

2.2.1 - MATERIALS

In order to perform this investigation different chemicals and equipment were utilised, all listed in the Table 2.

Table 2. Displaying lists of all the materials and equipment used during the investigation divided by category and area of use.

Chemicals	General Equipment	Measurement Equipment
Distilled water Sodium chloride Sodium silicate F/2 Algae Food *Skeletonema marinoi* culture from GUmacc *Nannochloropsis salina* culture from GUmacc Ibuprofen 400mg film coated tablet (Orifarm Generics)	100ml beaker 250ml beaker 500ml beaker 500ml flask with lid 2000ml flask with lid Petri dish (60mm x 15mm) Permanent marker Masking tape Plastic tray Stirring rod Magnetic stirrer Magnetic flea Parafilm wrap	Amersham Biosciences Novaspec Plus Visible spectrophotometer (+/- 0.001OD_{600}) Micropet micropipette 1-5ml (+/- 0.1ml) Finnpipette micropipette 0.5-10µl (+/- 0.1µl) Finnpipette micropipette 10-100µl (+/- 0.1µl) Mettler PC 400 laboratory scale (+/- 0.01g) Measuring cylinder 1000ml (+/- 5ml) Measuring flask 100ml (+/- 0.1ml)

2.2.2 - EXPERIMENTAL SETUP

To prepare the algal solutions to which IBU would be added a two-litre flask was filled with 1948ml of distilled water and 52g of salt (NaCl) to reach the prescribed salinity concentration of 2.6% for optimal algal growth as per GUmacc, source of the *S. marinoi* and *N. salina* cultures (Gothenburg University Marine Algae Culture Collection, n.d.). The flask was magnetically stirred until the salt was fully dissolved. Successively a two-part algal growth media (1:1 ratio of 0.132ml/L each, formulation found in Appendix 2) was added in order to reach a final concentration that of Guillard's F/2 medium.

To prepare the IBU concentration an Ibuprofen pill (Orifarm Generics) of 400mg was dissolved into 500ml of distilled water using a magnetic stirrer.

Two 500ml beakers were filled with approximately 50ml of previously prepared algal cultures of the two respective species and were diluted with previously prepared F/2 solution to reach an optimal initial OD_{600} for measuring algal growth. Subsequently it was divided into seven 100ml separate beakers to prepare the final solutions. To each beaker a relative volume of IBU solution was added via micropipette to reach the set concentrations of 0, 75, 150, 225, 300, 375 and 750µg L^{-1}. The range was selected based on environmentally accurate data (Niina Vieno et al., 2017) and increments were determined via a pre-trial were 0, 50 and 500µg L^{-1} were tested. An extreme of 750µg L^{-1} was added to account for the future possibility of IBU reaching an average occurrence in surface water in that range. From each prepared

concentration 5 petri dishes were filled with 15ml. The petri dishes had been marked with trial number, concentration and species name and then arranged into rows accordingly (see Figure 3).

Figure 3. This image displays how the petri dishes were marked and arranged during the experiment.

These trays were placed in an area with natural light cycles to emulate the algaes' natural environment. Remaining solutions were kept in their respective 100ml beakers and put on a separate tray to allow for qualitative data to be collected.

2.2.3 - DATA COLLECTION

To measure percentage change in OD_{600} 3ml from each petri dish was extracted using a 1-5 ml micropipette, added to a cuvette and then analysed using the Novaspec Plus Visible spectrophotometer (Amersham Biosciences). An initial OD_{600} for each concentration and trial was accounted for. After 144 hours in natural daylight cycles the OD_{600} was measured once again and written down.

2.2.4 - RAW DATA PROCESSING METHODS

The raw data collected (found in Appendix 3 & 4) consists of the initial and final OD_{600} for each trial (1-5) at all seven IBU concentrations including the control of 0µg L^{-1}. An OD_{600} change in percent was calculated for all trials and compiled into a mean percentage change for each increasing increment of IBU. This mean was plotted against the concentrations and a trendline was fitted for the scatter plots. As the data is continuous an R^2 could be calculated as well as a Pearson correlation coefficient. This was then used to determine statistical significance at $P < 0.05$.

2.2.5 - ENVIRONMENTAL CONSIDERATIONS

The leftover solutions containing IBU were disposed of together with biological waste to avoid it ending up in the sewage system and contributing to IBU levels entering the marine environment.

3 - RESULTS

3.1 - QUALITATIVE DATA

At the end of the incubation period there was a visual difference between the different concentrations of IBU for *S. salina*. The control of 0µg L^{-1} displayed a murky, more opaque appearance while the higher concentrations of 300, 375 and 750µg L^{-1} had a paler appearance, with the two latter being almost completely clear. A multitude of bubbles could be observed on the bottom of all petri dishes with 0µg L^{-1} IBU, subsequently decreasing with higher levels of IBU with the last two concentrations containing no bubbles at all.

In the 100ml beakers that were kept for qualitative data, the 0µg L^{-1} had a collection of *N. salina* cells suspended in the solution with a vivid, green colour. The 750µg L^{-1} contained a collection of algae as well, however, showing a yellow hue.

Figure 4. Shows two beakers of 100ml containing *Nannochloropsis salina* cultures exposed to 0µg L^{-1} and 750µg L^{-1} of Ibuprofen.

3.2 - QUANTITATIVE DATA

3.2.1 - RAW DATA

Due to the high number of trials run the raw data in its entirety for the live experiment can be found in Appendices 2 & 3 (includes both species). Raw data for the pre-trial can be found in Appendix 5 (only *S. marinoi*).

3.2.2 - CALCULATIONS

Following equation was implemented to determine the percentage change in OD_{600} for all trials:

$$\frac{(final\ OD_{600}\ -\ initial\ OD_{600})}{initial\ OD_{600}} \times\ 100 = percentage\ OD_{600}\ change$$

An example of how this formula can be used is displayed below, taken from trial 1 of the 225µg L^{-1} increment:

$$\frac{(1.446-0.230)}{0.230} \times 100 = 528.696$$

As per the formula beneath the mean of the trial datasets can be calculated. In the place of *n,* the total number of data values is inserted. The index notation *i* describes at which data value to start. The sum of the data points between selected *i* and *n* are added per the sigma notation and then multiplied by $\frac{1}{n}$ to obtain the mean:

$$\frac{1}{n}\sum_{i=1}^{n} x_i = \underline{x}$$

Below follows an example of how this formula can be employed, as for the 225µg L^{-1} increment:

$$\frac{1}{5} \times (552.632 + 545.217 + 542.035 + 544.589 + 548.430) = 546.581$$

Standard deviation was calculated using the command =STDEV in Excel. Through putting in this command in the table column for standard deviation and then selecting all the trials for one increment of IBU concentration, a standard deviation value was output. Same was done for all increments. In Tables 3, 4 & 5 all the results from above mentioned calculations are listed.

3.2.3 - PROCESSED DATA

Table 3. This table contains the calculated percentage change from all trials and concentrations (0, 75, 150, 225, 300, 375µg L^{-1}) *S. marinoi* cells were exposed to during the live experiment. A calculated average of those values including the standard deviation for the resulting number is included.

Skeletonema marinoi							
Ibuprofen concentration (+/- 0.1µl)						**Mean % change**	
*0*µg L^{-1}	585.3	575.1	580.6	573.5	579.2	578.7	4.66
*75*µg L^{-1}	557.9	562.7	557.0	553.7	549.1	556.1	5.05
*150*µg L^{-1}	552.6	545.2	542.0	544.6	548.4	546.6	4.08
*225*µg L^{-1}	528.7	540.9	540.4	539.9	535.5	537.1	5.16
*300*µg L^{-1}	527.5	527.9	524.4	531.3	514.2	525.1	6.53
*375*µg L^{-1}	494.6	505.8	471.1	518.8	494.9	497.0	17.57

Table 4. This table contains the calculated percentage change from all trials and concentrations (0, 75, 150, 225, 300, 375µg L^{-1}) *N. salina* cells were exposed to during the live experiment. A calculated average of those values including the standard deviation for the resulting number is included.

Nannochloropsis salina							
Ibuprofen concentration (+/- 0.1µl)						**Mean % change**	
*0*µg L^{-1}	369.3	375.2	361.4	328.4	331.0	353.0	21.89
*75*µg L^{-1}	380.9	371.4	355.8	355.7	352.7	363.3	12.26
*150*µg L^{-1}	384.7	399.0	397.0	403.4	372.2	391.2	12.73
*225*µg L^{-1}	341.4	333.8	336.5	338.7	334.5	337.0	3.13
*300*µg L^{-1}	356.8	354.0	351.7	361.8	362.6	357.4	4.77
*375*µg L^{-1}	347.7	359.5	354.8	361.4	355.6	355.8	5.26

Table 3 and Table 4 contain the calculated averages for all the trials giving and initial insight into the result of the study. For *S. marinoi* decreasing numbers can be seen under the mean percentage change while in the sample column for *N. salina* the numbers seem more arbitrary. Both tables include the standard deviation which for *S. marinoi* is minimal with the exception of 375µg L^{-1} with a deviation of 17.57, and for *N. salina* the deviation varied greatly between 3.13 and 21.89 at its highest.

3.2.4 - GRAPHED DATA

Below follow the graphs plotted from all the data collected in order to perform statistical analyses.

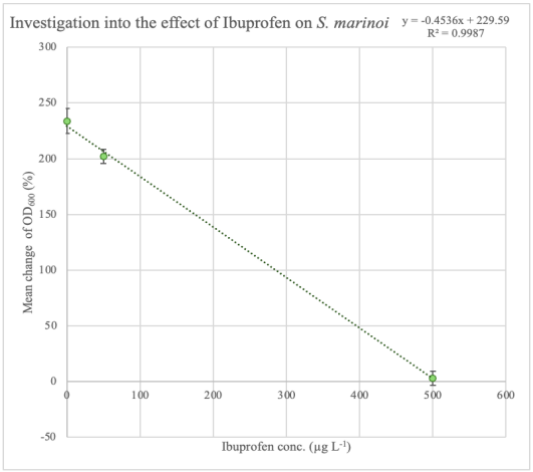

Figure 5. This scatter plot displays the change in OD_{600} of *S. marinoi* after being exposed to varying levels (0, 50 & 500g L^{-1}) of Ibuprofen for approximately 11 days.

In Figure 5 from the pre-trial a strong negative trendline can be observed with an R-squared value of 0,9978. With no IBU the growth was close to a 2.5x increase in OD_{600} while the 50µg L^{-1} approximately shows a 2x increase. However, at 500µg L^{-1} the growth is close to zero, at some points even being negative suggesting a decrease in OD_{600} after the 11 days for some samples.

From the raw data (found in Appendices 3 & 4) mean percentages were calculated for each trial. As the methodology consists of collecting continuous data the mean percentage increase of OD_{600} were plotted against the set increments of IBU. To validify the results a P-value was calculated for each and compared to the 0.05 significance level. For this investigation a P-value > 0.05 was considered not significant and a P-value < 0.05 could be considered significant.

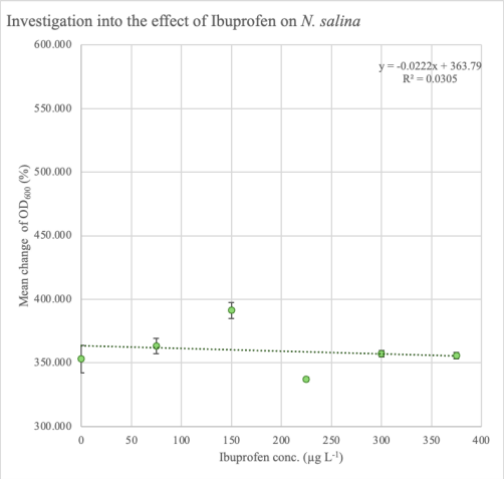

Figure 6. A scatterplot displaying the mean percentage change in OD_{600} of *N. salina* when exposed to Ibuprofen concentrations from 0 to 375µg L^{-1} with 75µg L^{-1} increments for 6 days. Error bars are adjusted with standard deviation.

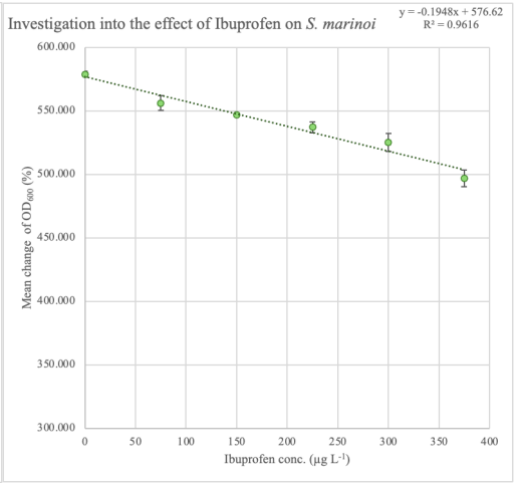

Figure 7. A scatter plot displaying the mean mean percentage change in OD_{600} of *S. marinoi* when exposed to Ibuprofen concentrations 0 to 375µg L^{-1} with 75µg L^{-1} increments for 6 days. Error bars are adjusted with standard deviation.

In Figure 6 there is no clear correlation to be identified as the data points deviate rather far from the fitted trendline. The error bars highlight the variation within the control group as well as the 75 and 150 µg L^{-1}. There is partial overlap of error bars between multiple data points.

Figure 7 illustrates a steady decrease in mean change of OD_{600} as the IBU concentrations incrementally increase. Error bars are rather short with less variation within each dataset, however the 375µg L^{-1} trial shows some more variation compared to other concentrations.

3.2.5 - STATISTICAL ANALYSES

In order to determine whether there was a significant correlation between Ibuprofen concentration and algal growth a Pearson Correlation Coefficient was computed for both species of algae. In Figure 6 for *N. salina* only a very weak negative correlation was identified, $r(5) = -.17$, $p = .74$, meaning that the results were not statistically significant at $P < 0.05$. However, in Figure 7 for *S. marinoi* a strong negative correlation was identified between the two variables, $r(5) = -.98$, $p = .0001$. This result shows statistical significance at alpha value $P < 0.05$.

Due to the above-mentioned results the alternate hypothesis can be partially accepted and null hypothesis partially rejected due to the varying values between the species (expanded upon in section 4.1.1 & 4.1.2)

4 - DISCUSSION

4.1 - EVALUATION OF RESULTS

The focus of this study was to investigate the possible effect of Ibuprofen on the growth of *S. marinoi* and *N. salina*. Through taking into account findings of IBU plotted on a map and overlapping this with algal species present in those areas the real-life relevance of the study was established. Both a diatom and a

green alga were selected to determine whether their difference in cellular structure may affect their susceptibility to IBU. Environmentally relevant levels were tested ranging from 0 to 375µg L^{-1} at 75µg L^{-1} increments. Since algal toxicity will be determined through calculating a growth percentage a spectrophotometer was used to measure optical density. It was hypothesised that Ibuprofen indeed would have an algistatic effect thus inhibiting the growth of both species of algae selected. However, due to the different results the two species will be discussed separately.

4.1.1 - SKELETONEMA MARINOI

In Figure 7 the negative trendline is clear. The control data where no IBU was added shows an increase in OD_{600} of nearly 580%. In all the following solutions the diatoms did still proliferate but with each increment there was a clear decrease in the extent of the growth. This suggests an IBU dose-dependent growth inhibition of *S. marinoi*. The R-squared value of 0.962 suggests that nearly all of variance in algal growth can be explained by the IBU dosage. The data collected had a P-value = .0001 confirming the strong statistical significance and therefore the alternate hypothesis can be accepted and the null hypothesis rejected in regard to *S. marinoi*. The algistatic effect of IBU could be attributed to its ability to interrupt multiple cellular apparatuses.

Photosystems I and II are responsible for absorbing and funnelling photon transmitted light energy into a reaction centre where a few chlorophyll molecules are photoactivated thus exciting electrons from their ground state. As these electrons move along the electron transport chain a proton gradient is built up within the thylakoid space of the chloroplast. Through chemiosmosis the protons move back into the stroma via the transmembrane protein ATP-synthase and adenosine triphosphate (ATP) is synthesised, an energy provider for crucial algal metabolic processes (Clegg et al., 2023, 440-443). IBU can possibly inhibit the energy transduction in the electron transport chain of photosystem II (PS II) as the genes coding for the integral membrane proteins of the photosynthetic electron transport chain are down-regulated (Silva et al., 2020). Due to the impairment of PS II the diatom cell would need to revert to cyclic photophosphorylation (CPP) and rely on Photosystem I (PS I). CPP can only maintain ATP production and not that of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) reducing the rate at which the light-independent reactions of the Calvin cycle can occur. Furthermore, CPP, due to only relying on PSI, does not require water and does not generate any oxygen while non-cyclic photophosphorylation does lead to oxygen formation (Clegg et al., 2023, 443). In connection to the fact that a richness of bubbles was observed in the control trials with none being present at the higher concentrations further substantiate that PSII is indeed harmed since less oxygen was released by the *S. marinoi* cells exposed to 375 and 750µg L^{-1} of IBU.

The Calvin cycle is vital to produce essential biomolecules for the algae such as lipids, amino acids and sugars. These carbon compounds support the growth and proliferation of algae and without an adequate supply the internal molecular processes slow down, impairing growth.

Furthermore, IBU could initiate the peroxidation of lipids and alter the lipid content (Silva et al., 2020) which in its case can cause a cell membrane with higher fluidity causing deterioration of the structural integrity of the cell and leading to ruptures and therefore slowing down the rate at which *S. marinoi* cultures grows. In a study of IBU and its effects on another diatom, *Phaeodactylum tricornutum,* comparable results were produced. The *P. tricornutum* cells were exposed to varying dosages of IBU and at the higher levels of 100 and 300µg L^{-1} a decelerated growth could be observed (Silva et al., 2020).

In short, IBU most likely possesses the ability to interrupt the movement of electrons through the electron transport chain in photosynthesis which reduces the rate at which ATP can be synthesised. This could cause the algal metabolism to slow down, hindering proliferation of *S. marinoi*.

4.1.2 - NANNOCHLOROPSIS SALINA

In Figure 6 there is no clear trendline. The control solution of 0µg L^{-1} IBU shows one of the lowest growths at an increase of around 355% in OD_{600}. At 150µg L^{-1} IBU the most proliferation took place as the algal density increased by approximately 390%. Furthermore, the standard deviation of the first three solutions is rather high causing an overlap between the first, second as well as fourth and sixth concentrations and thus they are statistically indistinguishable. These results suggest that at these doses of IBU there is no relevant correlation with growth of

N. salina. The R-squared value of 0.031 indicates that very little of the variance in algal growth can be explained by the IBU doses. With a P-value = .74 the correlation cannot be deemed statistically significant at $P < 0.05$. Due to these statistical evaluations the alternate hypothesis has to be rejected and the null hypothesis, stating that there is no correlation between increasing concentrations of Ibuprofen and growth of *N. salina,* has to be accepted.

These results may be due to the IBU concentrations being too low to affect the cellular systems of *N. salina*. In a similar study on the green algae *Scenedesmus obliquus,* which has spindle shaped cells similar in size to *N. salina* at 8-13µm (Chen et al., 2011) the $IC_{50\text{-}24h}$ was determined to be between 107.91 and 123.29 mg L^{-1} (Wang et al., 2020), considerably more than the maximum of 375µg L^{-1} which was tried for in this study. $IC_{50\text{-}24h}$ is defined as the half-maximal inhibitory concentration for 24 hours, i.e. the concentration of a drug required for 50% growth inhibition after 24 hours, in this case (Swinney, 2011).

In the 100ml beakers a clear visible difference was distinguishable between the 0µg L^{-1} and 750µg L^{-1} concentrations. The prior had a vivid green colour suggesting a healthy algae culture while the latter had a

yellow hue. When algae are exposed to stress factors their production of chlorophyll can be partially inhibited. The chlorophyllide *a* oxygenase gene (CAO) is part of the biosynthetic pathway of chlorophyll (Dey et al., 2023, 225-235) and possibly one of the genes that IBU could affect. One of chlorophyll's functions is the photochemical charge separation in PSI and PSII creating strong oxidative and reductive compounds allowing for electrons to travel through the electron transport chain (Sousa et al., 2012, 200-216). As previously mentioned, this is where IBU on a genetic level might impair the photosynthetic pathways of the algae cell. The yellow hue of the algae culture indicates lower levels of chlorophyll and a lower level of activity in the photosystems. However, the impairments are not acute enough to halt growth.

To summarise, the metabolism of *N. salina* did not seem to slow down significantly. On the other hand, the varying hues of yellow and green in the high and low concentrations of *N. salina* could indicate the cells' struggle to synthesise chlorophyll.

4.1.3 - LITERATURE REVIEW

The influences of other drugs classified as NSAIDs have been investigated. Ibuprofen, due to its high relative levels detected in marine environments poses the most imminent threat but the NSAID market is increasing in value year by year as new medications are emerging and existing ones are used at higher rates. Indomethacin, another NSAID, has been found to have dose-dependent negative correlation with algal growth as well, however, only in quite large doses does it have algistatic properties (Taschina et al., 2022). The NSAID Diclofenac which is one of the most prevalent substances from this group and was found in 79 out of 322 (25%) samples in the Baltic Sea and along shorelines, has been studied in relation to the green algae *Chlamydomonas reinhardtii*. It was identified as having a phytotoxicity which can deter the photolysis of the cell. A possible correlation with the impaired function of the mitochondria was underlined as well, which once again can slow down the metabolic processes required for cell division (Harshkova et al., 2020). What this study further highlighted was that it is the long-term exposure which truly becomes detrimental for the algal populations. In Figure 5 this can be seen. Albeit being a pre-trial, after the *S. marinoi* cells were exposed to a 500µg L^{-1} dose of IBU for 11 days they experienced a near zero growth, some of the petri dishes even saw a negative OD_{600} change suggesting the IBU led to some cell death. In the final experiment that ran for 6 days this was neither observed for *S. marinoi* nor *N. salina* suggesting that a longer exposure time of 11 days compared to 6 can have even more adverse consequences.

Although this study focused on eukaryotic algae of the sorts green algae and diatoms, cyanobacteria are also exposed to the same EPs. In a recent study the effect of multiple NSAIDs (diclofenac, diflunisal, ibuprofen, mefenamic acid, piroxicam) were investigated in relation to natural algal assemblages containing cyanobacteria like *Synechococcus elongatus* and eukaryotic algae like *Desmodesmus communis* (Bácsi et al., 2016). The natural assemblages aimed to mimic the ratios at which the different

species can be found in their natural environment. In the assemblages dominated by cyanobacteria the NSAIDs had a weaker effect. When the individual cyanobacteria and algae cultures were exposed to the chemical stress of the drugs it was indeed confirmed that the unicellular cyanobacteria showed stronger resistance towards the harmful effects compared to that of the eukaryotic algae. As increasing levels of multiple different NSAIDs make their way into the marine biome they may be the cause of changes in the distribution of different marine species of phytoplankton. These contaminants may contribute to the expansion and accumulation of cyanobacteria populations in water bodies and surface waters repelling the growth of eukaryotic algae (Bácsi et al., 2016). Due to the ability of cyanobacteria to produce cyanotoxins, which can cause gastroenteritis, liver and kidney damage, cancer and rashes for humans as well as be lethal for marine life, the NSAID levels should be monitored to avoid cyanobacteria to outcompete eukaryotic algae in natural systems.

The main pathway of Ibuprofen into the water is via poor treatment technologies of wastewater as well as sewage sludge, however, discarded drugs as well as by-products from medication productions may contribute to the pollution (Jan-Roblero & Cruz-Maya, 2023). Due to many NSAIDs containing aromatic groups as functional groups their biological and chemical degradation rates once released into the environment are decelerated meaning they persist longer in water bodies (Bácsi et al., 2016). Improvements are necessary when it comes to the handling of sewage and wastewater in order to prevent these substances from reaching an environment where they can provoke negative effects and expose organisms to extended chemical stress. However, on top of making sure they do not reach water bodies, efforts need to be made to investigate possible ways of aiding the biodegradation of Ibuprofen and other NSAIDs that are already present in our marine environments.

The effect of pharmaceutical EPs is not limited to algae. Studies performed on higher order animals have shown worrying results. IBU has been identified as causing general stress on clams (*Corbicula fluminea*) at as low levels as 0.1 - 1µg L^{-1} (Aguirre-Martínez et al., 2015). Furthermore, in zebrafish (*Danio rerio*), downregulation of female biased genes, induced malformations and mortality in embryos, apoptosis in larvae and an induced male biased sex ratio was observed at IBU levels exceeding 1mg L^{-1} (Bereketoglu et al., 2020) and in Sharptooth catfish (*Clarias gariepinus*) IBU was seen to induce oxidative stress in multiple organs and potentially causing kidney, liver and gill malformations (Akinola Ogunwole et al., 2021). Due to algae being a primary producer at the lowest trophic level, the species mentioned above, which are located at higher trophic levels, may on top direct interaction with IBU be threatened by the process of biomagnification (Clegg et al., 2023, 810). Due to the low chemical and biological degradation rate of many pharmaceuticals they have the potential to biomagnify, with studies identifying IBU as having biomagnification potential under certain conditions, from algae to zooplankton (Fragoso-Fuentes et al.,

2024). What this suggests is that IBU could have even more detrimental effects on species located higher up in the trophic hierarchy due to accumulating IBU levels in their tissue.

4.1.4 - LIMITATIONS

All data points collected in regard to *S. marinoi* are consistent, underlined by the rather small standard deviation within each group of trials. However, in the trials with *N. salina* variation can be observed within the increments of IBU suggesting a wider spread and larger uncertainty when it comes to the average increase in optical density.

For both algae tested there was variation in the initial optical density measured, which may be due to algae having a tendency to accumulate at the bottom of a flask and therefore not being evenly spread throughout the solution. But due to the experimental design this did not affect the final results as they were calculated individually for each petri dish. Because the concentrations of IBU investigated were miniscule, in the microgram range, the relative scale of uncertainty to measurement becomes larger, possibly leading to a loss of accuracy.

Though being a fast and effective measurement of cellular density, spectrophotometry results can be difficult to compare between different instruments unless a standardised calibration protocol is followed which can lead to figures that can be misinterpreted. Furthermore, spectrophotometry is an estimate of the cell density within a sample. For this investigation the same instrument was used for measuring both initial and final instrument to avoid any differences due to different calibrations. However, for more accurate results and a direct measure of cell count a haemocytometer and light microscope could be used to perform cell counts and calculate growth. If spectrophotometry is preferred, a standardised calibration protocol can be employed to ensure repeatability.

4.1.5 - FURTHER INVESTIGATION

In order to improve on the experimental setup, a new way of including silica in the growth solution for *S. marinoi* could be investigated in order to avoid precipitation.

This study is rather limited in the sense that only two algae strains were tested in correlation with a single pharmaceutical substance. However, per the joint report from HELCOM and EUSBSR a long list of NSAIDs as well as other pharmaceuticals such as antimicrobial and cardiovascular agents have been detected along the full Baltic coast. In order to make sure that the equilibrium between the algae and cyanobacteria stays intact the effect of all agents found in our waters need to be investigated in relation to relevant marine microbes. To further this specific investigation, one could extend the exposure time to capture the long-term effects, perhaps even in the span of multiple weeks. Different concentrations of IBU could also be investigated with *N. salina* to figure out at what levels the species could be threatened.

Another option that could aid in the determination of where our efforts should be aimed, geographically, is to investigate the IC_{50} of keystone algal species in relation to the most commonly identified pharmaceutical EPs in the status report from HELCOM and EUSBSR. This would allow for identification of which species are more susceptible to be negatively affected by e.g. IBU and therefore, in combination with recurring testing of IBU levels around the Baltic Sea, allow a directed response for removal from the ocean where it matters the most.

In order to investigate the long-term effect of slowly increasing levels of emerging pollutants (EPs) of medicinal origin, natural algal assemblages can be cultivated and exposed to varying EPs such as Ibuprofen to study how species composition changes and to observe any possible effects on species diversity as EPs might drive competitive exclusion leading to a loss of marine biodiversity.

4.2 - POSSIBLE APPROACHES TO RESOLUTION

Identifying the threat of EPs such as IBU to the world's marine biomes; algae and other higher order species, is one step in the right direction. However, different possibilities have to be investigated in order to identify an efficient, cost-effective process of removing IBU and other medicinal substances from our wastewater. This section will review the current, most promising candidates and other potential techniques of removing IBU from wastewater.

4.2.1 - ABSORPTION

Currently the most promising method investigated is absorption. These advanced absorption methods consist of different absorbents such as metal-organic frameworks, polymers and biosorbents, but the leading results have been achieved with a range of carbon-based materials (Osman et al., 2023). Carbon nanotubes are a nano-allotrope of carbon consisting of a graphene sheet rolled up forming a one tube. They can be single walled (SWCNT) or multiwalled (MWCNT) (Kausar, 2022, 23-53). A study investigating the MWCNTs, both in pristine condition and carboxylated MWCNTs (MWCNT-COOH), had promising results with almost complete removal of IBU. The MWCNTs-COOH showed the most promise and performed better than the MWCNTs in pristine condition. However, this filtration is electrochemical, meaning it requires electricity to be applied, 2-3V DC, and furthermore, in order for the electrochemical filtration to properly and effectively occur acidic conditions had to be achieved (Bakr & Rahaman, 2016). Another study identified SWCNTs as possessing a multitude of characteristics that make them suitable for removal of IBU, and other pharmaceutical agents; large specific surface area, contain high density of pores (Duarte et al., 2022). Furthermore, due to the possibility of functionalizing these nano-absorbents, one can selectively give it properties such as catalytic potential and large number of active sites in order to enhance the absorption and degradation of IBU.

Nevertheless, large scale production of CNTs is expensive with a simplified method of production still costing $3/g (Saravanan et al., 2010). On top of this, functionalization of SWCNTs is also a costly chemical process. Therefore, even though it is an effective way of removing nearly 100% of IBU from an aqueous sample, it is hard to implement on the scale of wastewater treatment plants. Furthermore, this method would be hard to include in the already established routine wastewater treatment including physical, chemical and biological stages of wastewater treatment (Trifiro & Zanirato, 2024).

Activated charcoal is one of the most common adsorbents when it comes to water treatment, and therefore its effectiveness in regard to IBU has been studied. It has been shown to absorb between 10.8 and 408mg/g from an aqueous solution (Osman et al., 2023). Another similar material, porous and rich in carbon is biochar, which is obtained through the pyrolysis of biomass. Biochar obtained from various sources such as industrial sludge, agave and pinewood showed an absorption of IBU from 22.7-309mg/g from an aqueous solution (Zhan et al., 2023). These methods often use residue from other industries and are rather ecologically sustainable, and while they are generally rather cheap and can be implemented in the mechanical stages of filtration, they are not as effective. Yeast-based carbon, produced with residue from the ethanol industry, only showed a 60% absorption, which leaves 40% of IBU residue to enter the oceans (Labuto et al., 2022).

4.2.2 - ALGAE

Algae have been studied as a way of removing IBU. A paper investigating *Chlorella vulgaris,* a single-celled green algae (Abdel-Latif et al., 2022), found the removal efficiency of *C. vulgaris* to be 67.25% in an IBU solution of 25mg L^{-1} after a 10-day long treatment. However, at a 100mg L^{-1} concentration the percentage of IBU removed was reduced to 32.77% with the study also identifying the higher level to decrease growth and photosynthetic activity (Zhou et al., 2022). Though, huge advancements have been made in the field of genetic engineering, and this may offer a possible solution. The genetic modification of algae is a method already in use, mainly for the production of biocompounds. They can synthesise metabolites such as lipids, amino acids, fatty acids, vitamins and pigments. In order to increase metabolic efficiency, thus higher production yield and economic viability, genetic engineering is emerging as an increasingly suitable option (Hanafi Ahmad Kamal et al., 2024). This being said, genetic modification of algae is not a novelty process, and could therefore theoretically be applied in the case of IBU removal from wastewater.

The metabolic pathway of IBU in the human body is complex but the primary degradation route includes the oxidative metabolism by Human cytochrome P450 (CYP) enzymes, which leaves inactive metabolites such as carboxy-ibuprofen and 2-hydroxy-ibuprofen. CYP enzymes are a family of membrane-bound haemoproteins which are crucial for the metabolism and detoxification of many different therapeutic drugs, being responsible for circa 50% of all metabolism of commonly used medicinal substances (Zhao et al., 2021). CYP2C is a cluster gene responsible for various proteins all involved in the clearance of

consumed medicines. Four members of this subfamily are responsible for around 20% of all therapeutic drug metabolism, including CYP2C8, CYP2C9, CYP2C18 & CYP2C19 (Chen & Goldstein, 2009). These are all located in a 500kb region on chromosome 10q24 (van Booven et al., 2010). Out of these CYP2C9 is the primary isoform responsible for specifically IBU metabolism. In order to isolate the gene CYP2C9 it is possible to extract the mRNA transcription of the gene, ensuring that only the protein-coding segment is selected (Alberts et al., 2002). Using reverse transcriptase one can synthesise a complementary DNA (cDNA) chain which can be amplified using the polymerase chain reaction (PCR). The production of specifically CYP2C9 cDNA-expressed enzymes has been performed in a study investigating metabolism of IBU, showing that this technique is viable (Hamman et al., 1997). There are many techniques for delivery of foreign genes into algal cells for transcription and translation, however, electroporation is likely the most effective since it has been well studied in algal species such as *Chlorella* sp. which is one of the most commonly used algae already in use for producing a host of human therapeutic proteins including antibodies and immunotoxins (Banerjee & Ward, 2022). The method includes applying an electric shock for short periods of time, generating a trans-membranal voltage difference, temporarily causing the phospholipid bilayer to open pores through which the desired CYP2C9 cDNA can enter the cell. As mentioned before, stable gene transfers to *Chlorella* sp. have previously been performed (Sreenikethanam et al., 2022). Post-translational modifications (PTMs) are an important part for the creation of a functionally active enzyme with proper bioactivity, and many algal species have shown the ability to perform effective and proper PTMs in the production of pharmaceutical proteins which suggest that they contain the right protein synthesis machinery for the production of CYP2C9 enzyme (Dehghani et al., 2022). This is further supported by another study investigating the production of therapeutic proteins in microalgae, where the protein expression systems were shown to be highly promising in effective production of human proteins (Banerjee & Ward, 2022). This suggests that the genetic modification of algal species, e.g. *Chlorella* sp. to express the CYP2C9 gene could give it the ability to metabolize IBU effectively into inactive metabolites for safe release into the ocean.

Genetic modification poses one major challenge, ethical considerations in relation to environmental risks. The general risk of genetically modified microalgae to human health and the environment has previously been determined to be low, but certain safety mechanisms can be utilised, such as through genetic modification making the algae reliant on a nonnatural nutrient, thus if the algae were to be released into a natural environment its survival would not be supported (Sebesta et al., 2022). A strong benefit of this potential method is its easy implementation into already existing wastewater treatment plants (WWTPs). Biological treatment already includes the usage of microbial organisms, often bacteria or algae, for the purification of water which suggests that implementation of genetically engineered algae would not require massive modifications of current WWTPs leading to an economically viable option (Trifiro & Zanirato, 2024).

- APPENDICES

Received upon request

- REFERENCES

Abdel-Latif, H. M. R., El-Ahsram, S., Yilmaz, S., Naiel, M. A. E., Kari, Z. A., Khalidah Abdul Hamid, N., Dawood, M. A. O., Nowosad, J., & Kucharczyk, D. (2022, June). The effectiveness of Arthrospira platensis and microalgae in relieving stressful conditions affecting finfish and shellfish species: An overview. *Agriculture Reports*, *24*. ScienceDirect. https://doi.org/10.1016/j.aqrep.2022.101135

Aguirre-Martínez, G. V., DelValls, A. T., & Martín-Díaz, M. L. (2015, October). Yes, caffeine, ibuprofen, carbamazepine, novobiocin and tamoxifen have an effect on Corbicula fluminea (Müller, 1774). *Ecotoxicology and Environmental Safety*, *120*, 142-154. ScienceDirect. https://doi.org/10.1016/j.ecoenv.2015.05.036

Akinola Ogunwole, G., Efetobor Abiya, S., Henry Amaeze, N., & ThankGod Eze, C. (2021, July). Antioxidant markers in gills, liver and muscle tissue of the African Sharptooth Catfish (Clarias gariepinus) exposed to subchronic levels of Ibuprofen and Dibutyl phthalate. *Scientific African*, *12*. ScienceDirect. https://doi.org/10.1016/j.sciaf.2021.e00816

Alberts, J. B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *Molecular Biology of the Cell* (4th ed.). Garland Sciences. https://www.ncbi.nlm.nih.gov/books/NBK26837/#:~:text=This%20is%20done%20by%20extracting,chain%20on%20an%20RNA%20template.

Bácsi, I., Béres, V. B., Kókai, Z., Gonda, S., Novák, Z., Nagy, S. A., & Vasas, G. (2016, March 9). Effects of non-steroidal anti-inflammatory drugs on cyanobacteria and algae in laboratory strains and in natural algal assemblages. *Environmental Pollution*, *212*. ScienceDirect. https://doi.org/10.1016/j.envpol.2016.02.031

Bakr, A. R., & Rahaman, S. (2016, March 29). Electrochemical efficacy of a carboxylated multiwalled carbon nanotube filter for the removal of ibuprofen from aqueous solutions under acidic conditions. *Chemosphere*, *153*, 508-520. Elsevier. https://doi.org/10.1016/j.chemosphere.2016.03.078

Banerjee, A., & Ward, V. (2022, December). Production of recombinant and therapeutic proteins in microalgae. *Current Opinion in Biotechnology*, *78*. ScienceDirect. https://doi.org/10.1016/j.copbio.2022.102784

Beal, J., Farny, N. G., Haddock-Angelli, T., Selvarajah, V., Baldwin, G. S., Buckley-Taylor, R., Gershater, M., Kiga, D., Marken, J., Sanchania, V., Sison, A., Workman, C. T., & iGEM Interlab Study Contributors. (2020, September 17). Robust Estimation of Bacterial Cell Count from Optical Density. *Communications Biology*, *3*(512). PubMed Central. https://doi.org/10.1038/s42003-020-01127-5

Bereketoglu, C., Pradhan, A., & Olsson, P.-E. (2020, June). Nonsteroidal anti-inflammatory drugs (NSAIDs) cause male-biased sex differentiation in zebrafish. *Aquatic Toxicology*, *223*. ScienceDirect. https://doi.org/10.1016/j.aquatox.2020.105476

Chen, F., Li, J., Dai, X., Sun, Y., & Chen, F. (2011, August 1). *Effect of phosphorus and temperature on chlorophyll a contents and cell sizes of Scenedesmus obliquus and Microcystis aeruginosa*. Limnology. https://www.researchgate.net/figure/Cells-of-Scenedesmus-obliquus-and-Microcystis-aeruginosa-magnification-9400_fig1_226450766

Chen, Y., & Goldstein, J. A. (2009, July). The transcriptional regulation of the human CYP2C genes. *Current Drug Metabolism*, *10*(6), 567-578. PubMed Central. https://doi.org/10.2174/138920009789375397

Clegg, C. J., Davis, A., & Talbot, C. (2023). *Biology for the IB Diploma* (3rd ed.). Hodder Education.

Dehghani, J., Movafeghi, A., Mathieu-Rivet, E., Mati-Baouche, N., Calbo, S., Lerouge, P., & Bardor, M. (2022, October 23). Microalgae as an Efficient Vehicle for the Production and Targeted Delivery of Therapeutic Glycoproteins against SARS-CoV-2 Variants. *Marine Drugs*, *20*(11), 657. PubMed Central. https://doi.org/10.3390/md20110657

Dey, D., Tanaka, R., & Ito, H. (2023, March 3). Structural Characterization of the Chlorophyllide a Oxygenase (CAO) Enzyme Through an In Silico Approach. *Journal of Molecular Evolution*, *91*(2), 225-235. PubMed Central. https://doi.org/10.1007/s00239-023-10100-9

Diener, H. C., Schneider, R., & Aicher, B. (2008, July 10). Per-capita consumption of analgesics: a nine-country survey over 20 years. *The Journal of Headache and Pain*, *9*, 225-231. Springer Nature. https://doi.org/10.1007/s10194-008-0046-6

Din Mohammady, N. G.-E. (2005). *Temporal alterations of Nannochloropsis salina (Eustigmatophyceae) grown under aqueous diesel fuel stress* (17th ed.). Journal of Applied Phycology. https://planktonnet.awi.de/index.php?contenttype=image_details&itemid=62110#content

Duarte, E. D. V., Oliveira, M. G., Spaolonzi, M. P., Costa, H. P. S., da Silva, T. L., da Silva, M. G. C., & Vieira, M. G. A. (2022, October 22). Adsorption of pharmaceutical products from aqueous solutions on functionalized carbon nanotubes by conventional and green methods: A critical review. *Journal of Cleaner Production*, *372*. ScienceDirect. https://doi.org/10.1016/j.jclepro.2022.133743

European Union Strategy for the Baltic Sea Region, United Nations Educational, Scientific and Cultural Organization, Baltic Marine Environment Protection Commission, Vieno, N., Hallgren, P., & Wallberg, P. (2017). Pharmaceuticals in the aquatic environment of the Baltic Sea region. *International Hydrological Programme*, *1*. https://helcom.fi/wp-content/uploads/2019/08/BSEP149.pdf

Fragoso-Fuentes, S., Rico-Martínez, R., & Alberto Arzate-Cárdenas, M. (2024, Aril 17). Effect of the Non-Steroidal Anti-Inflammatory Drug Ibuprofen on the Chydorid Alona guttata (Chydoridae: Aloninae) and the Rotifer Lecane papuana (Monogononta: Lecanidae) Fed on Different Algal Densities. *Polish Journal of Environmental Studies*, *33*(4). 10.15244/pjoes/177184

Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities & United Nations Environment Programme. (2020, February 20). *Emerging Pollutants in Wastewater: An Increasing Threat*. UNEP. https://www.unep.org/events/unep-event/emerging-pollutants-wastewater-increasing-threat

Gothernburg University Marine Algae Culture Collection. (n.d.). https://fmprod27.it.gu.se/fmi/webd/GUMACC?homeurl=https://fmprod27.it.gu.se/gumacc/index.html

Hamman, M. A., Thompson, G. A., & Hall, S. D. (1997, July 1). Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochemical Pharmacology*, *54*(1), 33-41. ScienceDirect. https://doi.org/10.1016/S0006-2952(97)00143-3

Hanafi Ahmad Kamal, A., Farahdevah Mohd Hamidi, N., Faiz Zakaria, M., Ahmad, A., Razif Harun, M., Chandra Segaran, T., & Jusoh, M. (2024, September 9). Genetically engineered microalgae for enhanced bioactive compounds. *Discover Applied Sciences*, *6*. SpringerNature Link. https://doi.org/10.1007/s42452-024-06116-5

Haris, N., Manan, H., Jusoh, M., Khatoon, H., Katayama, T., & Kasan, N. A. (2021, November 4). Effect of different salinity on the growth performance and proximate composition of isolated indigenous microalgae species. *Aquaculture Reports*, *22*. ScienceDirect. https://doi.org/10.1016/j.aqrep.2021.100925

Harshkova, D., Majewska, M., Pokora, W., Baścik-Remisiewicz, A., Tutodziecki, S., & Aksmann, A. (2020, November 23). Diclofenac and atrazine restrict the growth of a synchronous Chlamydomonas reinhardtii population via various mechanisms. *Aquatic Toxicology*, *230*. ScienceDirect. https://doi.org/10.1016/j.aquatox.2020.105698

Jan-Roblero, J., & Cruz-Maya, J. A. (2023, February 23). Ibuprofen: Toxicology and Biodegradation of an Emerging Contaminant (N. S. Thomaidis, Ed.). *Molecules*, *28*(5). PubMed Central. 10.3390/molecules28052097

Johansson, O. N., Töpel, M., Pinder, M. I. M., Kourtchenko, O., Blomberg, A., Godhe, A., & Clarke, A. K. (2019, April 2). Skeletonema marinoi as a new genetic model for marine chain-forming diatoms. *Scientific Reports*, *9*(5391). Nature Portfolio. https://doi.org/10.1038/s41598-019-41085-5

Jong, L. W., Fujiwara, T., Nozaki, H., & Miyagashima, S. (2017, December 11). Cell size for commitment to cell division and number of successive cell divisions in multicellular volvocine green algae Tetrabaena socialis and Gonium pectorale. *Proceedings of the Japan Academy, Series B Physicals and Biological Sciences*, *93*(10), 832-840. PubMed Central. https://doi.org/10.2183%2Fpjab.93.052

Kannah, R. Y., Kavitha, S., Karthikeyan, O. P., Rene, E. R., Kumar, G., & Banu, J. R. (2021, March 27). A review on anaerobic digestion of energy and cost effective microalgae pretreatment for biogas production. *Bioresource Technology*, *332*. ScienceDirect. https://doi.org/10.1016/j.biortech.2021.125055

Kausar, A. (2022). *Polymeric Nanocomposites with Carbonaceous Nanofillers for Aerospace Applications*. Elsevier Science. https://doi.org/10.1016/B978-0-323-99657-0.00009-0

Labuto, G., Carvalho, A. P., Mestre, A. S., dos Santos, M. S., Modesto, H. R., Martins, T. D., Lemos, S. G., da Silva, H. D. T., Vasconcelos Martins Carrilho, E. N., & Carvalho, W. A. (2022, September). Individual and competitive adsorption of ibuprofen and caffeine from primary sewage effluent by yeast-based activated carbon and magnetic carbon nanocomposite. *Sustainable Chemistry and Pharmacy*, *28*. ScienceDirect. https://doi.org/10.1016/j.scp.2022.100703

Metsoviti, M. N., Papapolymerou, G., Karapanagiotidis, I. T., & Katsoulas, N. (2019, August 10). Comparison of Growth Rate and Nutrient Content of Five Microalgae Species Cultivated in Greenhouses. *Plants*, *8*(8), 279. PubMed Central. https://doi.org/10.3390%2Fplants8080279

Osman, A. I., Ayati, A., Farghali, M., Krivoshapkin, P., Tanhaei, B., Karimi-Maleh, H., Krivoshapkina, E., Taheri, P., Tracey, C., Al-fatesh, A., Ihara, I., Rooney, D. W., & Sillanpaä, M. (2023, August 25). Advanced adsorbents for ibuprofen removal from aquatic environments: a review. *Environmental Chemistry Letters*, *22*. SpringerNature Link. https://doi.org/10.1007/s10311-023-01647-6

Saravanan, S., Babu, K., Katakam, S., & Ma, J. (2010, October). Techno-Economics of Carbon Nanotubes Produced by Open Air Arc Discharge Method. *International Journal of Engineering Science and Technology*, *2*(5). ResearchGate. http://dx.doi.org/10.4314/ijest.v2i5.60128

Sebesta, J., Xiong, W., Guarnieri, M. T., & Yu, J. (2022). Biocontainment of Genetically Engineered Algae. *Frontiers in Plant Science - Aquatic Photosynthetic Organism*, *13*. Frontiers. https://doi.org/10.3389/fpls.2022.839446

Serôdio, J., Lavaud, J., & Encyclopedia of the UN Sustainable Development Goals. (2020, August 18). Diatoms and Their Ecological Importance. *Life Below Water*. Springer Link. https://doi.org/10.1007/978-3-319-71064-8_12-1

Silva, M., Feijão, E., da Carvalho, R. d. C., Duarte, I. A., Matos, A. R., Marques, J. C., Cabrita, M. T., Barreiro, A., Lemos, M. F. L., Novais, S. C., Cqcador, I., Reis-Santos, P., Fonseca, V. F., & Duarte, B. (2020, August 5). Comfortably numb: Ecotoxicity of the non-steroidal anti-inflammatory drug ibuprofen on Phaeodactylum tricornutum. *Marine Environmental Research*, *161*. ScienceDirect. https://doi.org/10.1016/j.marenvres.2020.105109

Singh, S. P., & Singh, P. (2015, May 29). Effect of temperature and light on the growth of algae species: A review. *Renewable and Sustainable Energy Reviews*, *50*, 431-444. ScienceDirect. https://doi.org/10.1016/j.rser.2015.05.024

Sousa, F. L., Shavit-Grievink, L., Allen, J. F., & Martin, W. F. (2012, December 19). Chlorophyll Biosynthesis Gene Evolution Indicates Photosystem Gene Duplication, Not Photosystem Merger, at the Origin of Oxygenic Photosynthesis. *Genome Biology and Evolution*, *5*(1), 200-216. https://doi.org/10.1093/gbe/evs127

Sreenikethanam, A., Raj, S., Banu, R. J., Gugulothu, P., & Bajhaiya, A. K. (2022, March 23). Genetic Engineering of Microalgae for Secondary Metabolite Production: Recent Developments, Challenges, and Future Prospects. *Frontiers in Bioengineering and Biotechnology - Bioprocess Engineering*, *10*. Frontiers. https://doi.org/10.3389/fbioe.2022.836056

Swedish Biodiversity Data Infrastructure & Swedish Meteorogolical and Hydrological Institue. (n.d.). *Nordic Microalgae*. Nordic Microalgae. https://nordicmicroalgae.org/taxon/skeletonema-marinoi/

Swinney, D. C. (2011, October 14). Chapter 18 - Molecular Mechanism of Action (MMoA) in Drug Discovery. *Annual Reports in Medicinal Chemistry*, *46*, 301-317. ScienceDirect. https://doi.org/10.1016/B978-0-12-386009-5.00009-6

Taschina, M., Moisa, C., Lupitu, A., Copolovici, D. M., & Copolovici, L. (2022, June 21). Influence of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) on Photosynthetic Parameters and Secondary Metabolites of Plants from Fabaceae Family. *Applied Sciences*, *12*(13). MDPI. https://doi.org/10.3390/app12136326

Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. (2011, July 28). *OECD Guidelines for the Testing of Chemicals, Section 2*. OECD iLibrary. https://doi.org/10.1787/20745761

Tip Biosystems. (2023, December 15). *Understanding OD600 and Measuring Cell Growth*. Tip Biosystems. Retrieved October 17, 2024, from https://www.tipbiosystems.com/applications/understanding-od600-and-measuring-cell-growth/

Trifiro, F., & Zanirato, P. (2024, November 30). Water Purification: Physical, Mechanical, Chemical and Biological Treatments. *Mathews Journal of Pharmaceutical Science*, *8*(3). https://doi.org/10.30654/MJPS.10042

The United States Environmental Protection Agency. (2024, July 15). *Indicators: Sediment Diatoms | US EPA*. Environmental Protection Agency (EPA). https://www.epa.gov/national-aquatic-resource-surveys/indicators-sediment-diatoms

van Booven, D., Marsh, S., McLeod, H., Whirl Carrillo, M., Sangkuhl, K., Klein, T. E., & Altman, R. B. (2010, April). Cytochrome P450 2C9-CYP2C9. *Pharmacogenetics and Genomics*, *20*(4), 277-281. PubMed Central. https://doi.org/10.1097/FPC.0b013e3283349e84

Wang, H., Jin, M., Mao, W., Chen, C., Fu, L., Li, Z., Du, S., & Liu, H. (2020, March 10). Photosynthetic toxicity of non-steroidal anti-inflammatory drugs (NSAIDs) on green algae Scenedesmus obliquus. *Science of the Total Environment*, (707). PubMed Central. https://doi.org/10.1016/j.scitotenv.2019.136176

Widdicombe, C. (2011, April 12). *Chains of the centric diatom Skeletonema marinoi, collected at L4*. https://planktonnet.awi.de/index.php?contenttype=image_details&itemid=62110#content

Xin, X., Chen, B., Yang, M., Gao, S., Wang, H., Gu, W., Li, X., & Zhang, B. (2022, November 22). A critical review on the interaction of polymer particles and co-existing contaminants: Adsorption mechanism, exposure factors, effects on plankton species. *Journal of Hazardous Materials*, *445*. ScienceDirect. https://doi.org/10.1016/j.jhazmat.2022.130463

Yaakob, M. A., Radin Mohamed, R. M. S., Al-Gheethi, A., Gokare, R. A., & Ambati, R. R. (2021, February 14). Influence of Nitrogen and Phosphorus on Microalgal Growth, Biomass, Lipid, and Fatty Acid Production: An Overview. *Cells*, *10*(2), 393. PubMed Central. https://doi.org/10.3390%2Fcells10020393

Zhan, D., Ye, A., & Hou, T. (2023, December 20). Research progress on biochar-based material adsorption and removal of ibuprofen. *Frontiers in Environmental Science - Toxicology, Pollution and the Environment*, *11*. Frontiers. https://doi.org/10.3389/fenvs.2023.1327000

Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., Huai, C., Shen, L., Zhang, N., He, L., & Qin, S. (2021, November 26). Cytochrome P450 Enzymes and Drug Metabolism in Humans. *International Journal of Molecular Sciences*, *22*(23). PubMed Central. https://doi.org/10.3390/ijms222312808

Zhou, Z., Yicheng, W., Kuang, Y., Lin, G., Fu, H.-Y., & Wang, Z. (2022, October). Assessment of ibuprofen toxicity and removal potential of Chlorella vulgaris. *Bioremediation Journal*, *28*(4), 1-9. ResearchGate. http://dx.doi.org/10.1080/10889868.2022.21388