International Baccalaureate - Gjøvik Videregående Skole

Extended Essay:
Biology

GROWTH INHIBITION OF MICRO FRESHWATER ALGAE EXPOSED TO CHEMICAL OIL DISPERSANTS.

How does the concentration of the oil dispersing agent "Corexit 9500" affect the growth rate of *Scenedesmus obliquus,* measured by the number of cells left per cm^3 per day?

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Diploma session: May 2011

Candidate Number: 002096-020

Word Count: 3854

Abstract

The aim of this experiment was to investigate if different concentrations of Corexit 9500 affect the growth of the fresh water algae Scenedesmus obliquus. Samples of the algae were statically exposed[1] to different concentrations of the oil dispersing agent Corexit 9500. The concentration varied from 0.04 µl l^{-1} to 100,000 µl l^{-1} and the extracting of data was carried out over a 7 day period were the acute[2] and chronic[3] effects of the dispersant were to be measured. The algae cultures were placed in two North turned windows with natural light. If not cloudy the exposure to direct sunlight was 3-4 hours a day. The number of algae in samples from start, 24 hours, day 4 and day 7 were counted in a converted light microscope and calculated into cells/cm^3. The results for day 4 and day 7 showed a clear trend of higher accelerated growth between concentrations of dispersant of 0.04-0.75 µl l^{-1} than the controls without dispersant. This may indicate that there are substances in the dispersant that works as nutrition for the algae. Exposed to concentrations around 5.00 µl l^{-1} may have little effect on the algae as the acceleration growth was the same as in the controls. For concentrations of dispersant higher than 50 µl l^{-1} on day 4, and 5.0 µl l^{-1} 7 days after exposed to Corexit 9500, the growth was inhibited.

Word count: 298

[1] Exposures for aquatic toxicity tests in which the test organisms are exposed to the same test solution for the duration of the test **(**Ocean Studies Board, 2005)

[2] Toxicity to aquatic organisms estimated from relatively short exposures (i.e., 24, 48, or 96 hr) with death as the typical endpoint. **(**Ocean Studies Board, 2005)

[3] Toxicity to aquatic organisms can be estimated from partial life-cycle tests of relatively short duration (i.e., 7 days).

Table of Contents

Introduction

The effects of oil dispersing agents on micro life in freshwater lakes are a small investigated area of limnology[4]. This is due to the fact that oil catastrophes mainly happen in the ocean, and researchers put their resources into this area. Still small oil leakages occur in fresh water lakes and rivers. Some examples are found in our local area by Norway's biggest lake, Mjøsa. As recent as this spring, it was reported of a minor oil leakage from a high voltage cable isolated with oil outside the town I go to school, Gjøvik, releasing about 30 litres of oil into the lake (Oppland Arbeiderblad, 2010). In 2007, there were two bigger oil leakages in Mjøsa; 9th September it was reported about a 100 meter and 8 meter wide oil flake in the South of the same town (Oppland Arbeiderblad, 2007). 400 litre of heating oil leaked out in city across from Gjøvik, 16th November the same year (Østlendingen, 2007). In all of the mentioned examples the firemen put out lenses to collect the oil. What if the firemen followed British Petroleum's example from the oil catastrophe in the Mexico Gulf, spring 2010, and used the chemical dispersant Corexit 9500 to get rid of the oil? (NALCO, 2010)

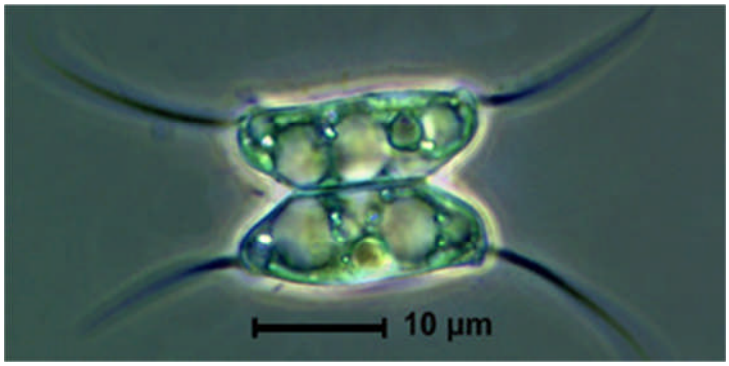
Main ingredients in Corexit 9500:
Sorbitan, Butanedioic acid 1,4-bis(2-ethylhexyl) ester Sodium salt
Propanol
Distillates (petroleum) Hydrotreated light.
(Nalco, 2010)

Corexit 9500 is a chemical dispersant used to minimize the impact of an oil spill. It is made up of a solvent and a surfactant, where the surfactant is hydrophobic[5] in one end and hydrophilic[6] in the other end. When dispersed to oil the molecules arrange themselves so the hydrophilic part is in the water and the hydrophobic part is in the oil, thereby emulsify the oil into tiny droplets when exposed to wind and waves (Nalco, 2010). The liquid has a golden colour and dissolves easily in water.

There is a significant reason for examining the effect of dispersants on algae. Fresh water algae are a primary source of nourishment in limnic food webs. Consequently, the toxicity of the dispersing agent could affect the growth and hence the population size of fresh water algae as well as other parts of the food web. Constituents of the dispersing agent or degradation products from them could also be transferred to other organisms in a food web.

The tested algae Scenedesmus obliquus are common green freshwater algae in the family Scendesmaceae and are flexible and able to withstand harsh conditions (Lürling M. 2003). They are found on the surface of most fresh water lakes in Norway (miljølære 1989-94). The algae are unicellular and form colonies on 2-32 cells aligned. The size varies from 15-20 µm and the shape is usually cylindrical. They are also autotrophic (Connecticut College, 2004).

[4] The study of the biological, chemical, and physical features of lakes and other bodies of fresh water
[5] Having an affinity for oils rather than water
[6] Having an affinity for water rather than oils



Kingdom: Plantae
Phylum: Chlorophycophyta
Class: Chlorophyceae
Order: Sphaeropleales
Family: Scenedesmaceae
Subfamily: Scenedesmoidea
Genus: Scenedesmus
Specie: Scenedesmus obliquus
(Connecticut College, 2004)

Figure 1.1: Scenedesmus obliquus (Copyright: Connecticut College)

A study in marine environment found an observed effect EC_{50}[7] on brown algae when exposed 0.7 µl l^{-1} of Corexit 9500 dispersed on oil in a time span of 72 hours (Ocean Studies Board, 2005). As this study was done in salt water and brown algae and Scenedesmus are two different species it may be difficult to compare the results. It could however, give a hint towards what concentrations of Corexit 9500 to use. It seems natural to think that for high concentrations of Corexit 9500 the growth will be inhibited in the long run, as significant amounts may be toxic for the algae as a result of different environment. However, I do not know for what concentrations of dispersant the growth of Scenedesmus will be affected, and therefore I will choose to leave the hypothesis open. It must also be emphasized that this investigation aims to examine the toxicity of the dispersant itself and not the effect when dispersed to oil. I contacted the oil dispersant company NALCO about my plans and they gave me the desired amount of dispersing agent I needed for the experiment.

Methods

15 different concentrations and 2 control groups were used in the investigation. The concentrations of Corexit 9500 ranged from 0.04 µl l^{-1} to 100,000 µl l^{-1}. Rather than prioritizing having many samples of each concentration to lower the uncertainty, it was focused on for what concentrations the dispersing agent would have an effect. Therefore, many different concentrations and only one sample of each concentration were used. To have an idea of the uncertainty between equal concentrations, two control groups without Corexit 9500 were made.

[7] EC_{50} – concentration causing effect in 50% of test organism

In each flask there was a combination of solutions. Phosphate was added to make sure nutrients would not be a limiting factor and this was done in consultation with teachers (Stabell; Kristiansen; 2010, pers.com). In each flask there was:

- 200 cm^3 of filtrated lake water
- 4 cm^3 of KH_2PO_4 solution (50 mg l^{-1})
- 250 µl of algae sample of Scenedesmus obliquus from Norwegian Institute of Water Research, Norway (NIVA)
- A total of 250 cm^3 (45.75 cm^3) with Corexit 9500 and distilled water.

To get the exact volume in practice, 50 cm^3, 25 cm^3, 20 cm^3, 15 cm^3 and 10 cm^3 pipettes, a mechanical finnpipette (1-10 cm^3) and a mechanical micropipette (50-500 µl) were used.

Filtration of lake water

The lake water was provided by a teacher and taken from a local lake, Sillongen. This water contained sufficient nutrients for algae growth, about 20 mg l^{-1} phosphates (T. Stabell, pers.com). The water was filtrated through Whatman GF/C glass-fiber filter (1,2 µm) by suction so no alien algae, plankton or bacteria would appear in the samples and interfere with the test algae. In a report (Hickel, 1984) it was shown that this filter was as efficient as a 0.4 µm filter. 200 cm^3 of the filtrated water was added in each of the 17 flasks by 50 cm^3 pipettes. To make sure no water would evaporate, the flasks were covered with parafilm while the dilutions were made.

Making dilutions and different concentrations of Corexit 9500

The dispersant Corexit 9500 was provided in undiluted form by the producer (Nalco, 2010). To make the preferred concentrations it was first diluted with distilled water into 5 base concentrations. Pipettes were washed several times in tap water and 2 times in distilled water between the making of each dilution, and the solutions were thoroughly mixed before further transfer.

Table 2.1: The makings of different dilutions of Corexit 9500.

Dilution of dispersing agent:	**10x**	**100x**	**1000x**	**10,000x**	**100,000x**
Method:	25 cm^3 of dispersant added with a pipette together with 225 cm^3 distilled water	25 cm^3 of 10x added with a pipette together with 225 cm^3 distilled water	25 cm^3 of 100x added with a pipette together with 225 cm^3 distilled water	25 cm^3 of 1000x added with a pipette together with 225 cm^3 distilled water	25 cm^3 of 10,000x added with a pipette together with 225 cm^3 distilled water

Calculations were done on what volume of distilled water and diluted dispersant to add, to get the preferred concentrations (table 2.2). The final concentrations were made with dilutions from lowest to highest order, and the pipette tips were dried off when changing between dilutions. To assure more certain concentrations, they were not rinsed with distilled water between each making.

KH_2PO_4 Solution

The phosphate solution was made by teacher T. Stabell. In consultation with him I added 4 cm^3 of a 50 mg l^{-1} potassiumdihydrogen phosphate solution with a mechanical pipette to make sure phosphate would not be a limiting factor.

Table 2.2: Amount of distilled water and diluted dispersant to add in each sample to get the preferred concentrations of dispersant so the total volume would add up to 50 cm^3 after adding 4 cm^3 of $KH_2PO_4^{3-}$ solution and 0.25 cm^3 algae sample.

	Concentration of dispersing agent (µl l^{-1}) in final volume 250 cm^3	**Type of Corexit 9500 dilution to use:**	**Volume of Corexit 9500 dilution to add[8](cm^3):**	**Distilled water left to add[9] (cm^3):**
Control group1	0.00 µl/l	0.00	0.00	45.75
Control group2	0.00 µl/l	0.00	0.00	45.75
1	0.04 µl/l	100 000x	1.0	44.75
2	0.10 µl/l	100 000x	2.5	43.25
3	0.50 µl/l	100 000x	12.5	33.25
4	0.75 µl/l	100 000x	18.75	27.0
5	5.00 µl/l	10 000x	12.5	33.25
6	10.0 µl/l	10 000x	25.0	20.75
7	50.0 µl/l	1000x	12.5	33.25
8	100 µl/l	1000x	25.0	20.75
9	500 µl/l	100x	12.5	33.25
10	1000 µl/l	100x	25.0	20.75
11	5000 µl/l	10x	12.5	33.25
12	10 000 µl/l	10x	25.0	20.75
13	25 000 µl/l	1x	6.25	39.5
14	50 000 µl/l[10]	1x	12.5	33.25
15	100 000 µl/l	1x	25.0	20.75
			= 45.75 cm^3 in each flask	

Monitoring and Collection of Algae Samples

It was chosen to monitor the data instead of having controlled variables to make the investigation as close to reality as possible. The concentrations were placed in two North turned windows. This conclusion was reached mainly because there were too many flasks to fit in one window, but also because it is less direct sunlight during the day from North. The samples were placed on planks so

[8] The decimals are consequently inconsistent as the exact volume (.75) was added with a micropipette
[9] The decimals are consequently inconsistent as the exact volume (0.75, 0.25) was added with a micropipette
[10] 1.4 cm^3 extra of distilled water was added by accident

they would be on equal level with the window, and the end samples were made sure not to be covered by the wall (see picture 2.1).

When the investigation started, at time 0, 250 µl algae sample where added to each flask with a mechanical micropipette. This was after shaking the algae sample well to make sure of an equal distribution of cells. Thereafter, every flask was shaken gently and the control groups were taken samples of. The test sample of Scenedesmus obliquus had been ordered from NIVA, and the amount of sample was chosen to be sure there would be enough algae for all flasks. The control groups were placed in the middle of each window.

Lids were used on all samples to make sure the water would not evaporate, and taken off twice a day so the algae would not suffocate. Twice a day, all 17 flasks were turned so the algae would not attach to the bottom, and light intensity was measured with a luxometer in the middle of each window. The temperature was taken in the middle of each window in control 1, and an additional flask with tap water in window 2. The pH was also to be measured to see if there were any differences in pH between concentrations of dispersant. In the evening, 2 cm^3 of each sample was taken out with a mechanical finnpipette and added 200 µl of Lugol's solution (1%) with a mechanical micropipette, and thereafter stored in a dark and cold room. The sample was taken right after the flasks had been turned to assure an equal distribution of cells. The procedure was repeated every day for 7 days.

Picture 2.1: Test windows. The two North turned windows where the flasks were placed. 9 flasks were placed in each window. Window 1 contained concentrations 0.04-100 µl l^{-1} and control 1. Window 2 contained concentrations 500-100,000 µl l^{-1}, control 2 and one additional flask, where temperature was measured. **(photo: private)**

Picture 2.2: **The position of the flasks in relation to its surroundings.** The flasks are positioned away from the wall and above the window frame. The control groups were placed in the middle (photo: private)

Counting of Algae and Calculation Method

0.4 µl of a sample were to be applied with an automatic pipette into a chamber of 0.400 µl in volume. The samples were first shaken gently to make sure of an even distribution of cells. A glass was placed upon the chamber and the sample was placed under an inverted light microscope. 1-2 minutes was estimated to allow the algae to sink to the bottom. Depending on the number of algae, the samples were counted on magnifications of 100x, 200x or 320x. This was done in diagonals with a mechanical counter to make sure a greater accuracy in number of cells (figure 2.3). 2-5 diagonals were counted from left to right, up and down, and opposite. The same sample was used to count all diagonals, and no diagonal was counted twice.

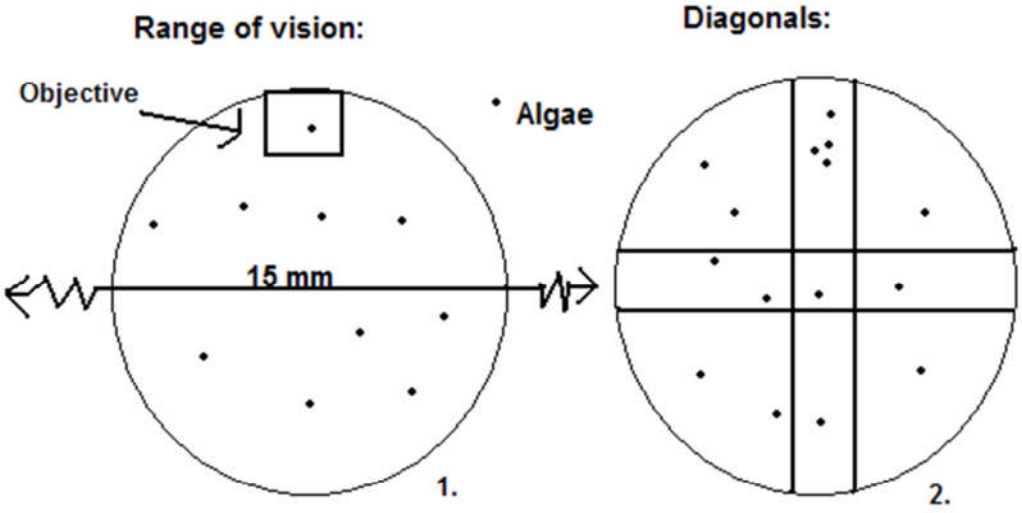


Figure 2.3: 1. Illustration of the range of vision and the width of chamber in the light microscope. **2**. Illustration of how the diagonals were counted either up and down, or from left to right and opposite

A calculation method was used to calculate cells/cm^3. Firstly, the area for one diagonal had to be calculated. Knowing that the diameter was 15 mm, the width of the square was multiplied with the diameter. This depended on the magnification used (table 2.3). Then the total area of the chamber was calculated ($\pi 7.5^2$ mm=176.715 mm^2). Secondly, a multiplication factor was made by dividing the surface area by the area of one diagonal. This was to obtain a number of how many cells in average it would be in the chamber. Thirdly, the number of cells/cm^3 was calculated. The volume in a chamber was 0.400 cm^3 and therefore, cells in the whole chamber were divided by the volume of the chamber. Finally, to get cells/ cm^3 the cells counted in all diagonals were added together and then multiplied with the final multiplier factor. When more than 1 diagonal was counted, this number was divided by the number of diagonals counted.

Table 2.3: Summary of calculation method

Magnification:	**Width of square:**	**Area of 1 diagonal:**	**Multiplication factor (for cells in entire chamber):**	**Final multiplication factor (for cells/cm^3)**
100x	0.911 mm	15 x 0.911 = 13.665 mm^2	176.715÷13.665 = 12.93	12.93÷0.4 =32.33
200x	0.466 mm	15 x 0.466 = 6.990 mm^2	176.715÷6.699 = 25.28	25.28÷0.4 =63.20
320x	0.265 mm	15 x 0.265 = 3.975 mm^2	176.715 ÷ 3.975 = 44.46	44.46÷0.4 =111.14

Results

Because of the limitation of time on the investigation only the control group for day 0, and all the samples after 24 hours, 4 days and 7 days after dispersing were counted.

Table 3.1: Start population (day 0). The number of algae in the control groups right after adding algae in each flask. This correspond to the initial number of algae in all flasks.

Concentration:	No. diagonals:	Total no. of cells counted (magnification):	Cells/cm^3 $\left(= \frac{tot.nr\ of\ cells \times multi.factor}{nr.of\ diagonals} \right)$
Control group 1	4	11 (100x)	89
Control group 2	4	15 (100x)	121

Table 3.2: 24 hours. The data corresponds to the number of diagonals and cells counted on the specific magnification, together with the number of algae per cm^3 in each flask 24 hours after dispersing the given concentrations. The decimals are rounded off to the closest value.

Concentration:	No. diagonals:	Total no. of cells counted (magnification):	Cells/cm^3 $\left(= \frac{tot.nr\ of\ cells \times multi.factor}{nr.of\ diagonals} \right)$
Control group 1	4	28 (100x)	226
Control group 2	4	33 (100x)	267
0.04 µl l^{-1}	4	39 (100x)	315
0.10 µl l^{-1}	4	102 (100x)	824
0.50 µl l^{-1}	4	102 (100x)	824
0.75 µl l^{-1}	4	54 (100x)	436
5.00 µl l^{-1}	4	87 (100x)	703
10.0 µl l^{-1}	4	73 (100x)	590
50.0 µl l^{-1}	4	33 (100x)	267
100 µl l^{-1}	4	23 (100x)	186
500 µl l^{-1}	4	25 (200x)	395
1000 µl l^{-1}	Not Applicable[11]		
5000 µl l^{-1}	Not Applicable		
10,000 µl l^{-1}	4	63 (100x)	509
25,000 µl l^{-1}	4	21 (100x)	170
50,000 µl l^{-1}	4	40 (100x)	323
100,000 µl l^{-1}	2	5 (100x)	81

[11] The samples of concentrations 1000 and 5000 µl l^{-1} were decomposed.

Figure 3.1: Mean cells/cm^3 in each concentration after being exposed to Corexit 9500 for 24 hours. 1000-5000 **µ**l l^{-1} were not applicable, because the samples had been destroyed.

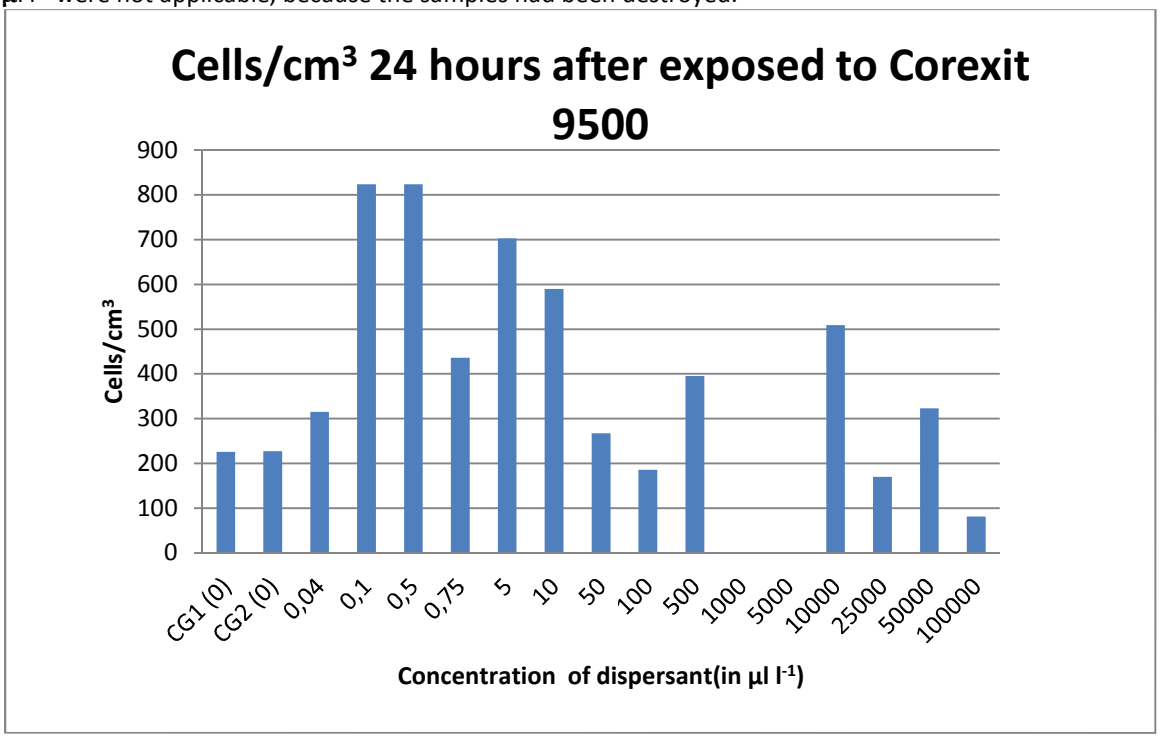


Table 3.3: Day 4. The data correspond to the number of diagonals and cells counted on the specific magnification, together with the calculated number of algae per cm^3 in each flask after being in the given concentration of dispersant for 4 days. The decimals are rounded off to the closest value.

Concentration:	No. of diagonals:	Total nr. of cells counted/ magnification:	Mean of cells/cm^3 $\left(=\frac{tot.nr\ of\ cells \times multi.factor}{nr.of\ diagonals}\right)$
Control group 1	3	311 (320x)	11522
Control group 2	3	527 (200x)	11102
0.04 µl l^{-1}	3	612(200x)	12893
0.10 µl l^{-1}	3	640 (200x)	13483
0.50 µl l^{-1}	3	460 (200x)	9691
0.75 µl l^{-1}	3	284 (200x)	5983
5.00 µl l^{-1}	3	534 (200x)	11250
10.0 µl l^{-1}	3	458 (320x)[12]	16967
50.0 µl l^{-1}	3	138 (320x)	5112
100 µl l^{-1}	3	111 (200x)	2338
500 µl l^{-1}	3	114 (320x)[13]	4223
1000 µl l^{-1}	3	22 (320x)	815

[12] I changed the magnification to count the cells in the chamber easier. This proved more time consuming, so after 50.0 µl l^{-1}, I increased the magnification to 200x again.
[13] At 500 µl l^{-1} I changed the magnification to 320x again to see the cells better.

5000 µl l^{-1}	3	26 (320x)	963
10,000 µl l^{-1}	3	18 (320x)	667
25,000 µl l^{-1}	3	54 (320x)	2001
50,000 µl l^{-1}	3	25 (320x)	926
100,000 µl l^{-1}	3	14 (320x)	519

Figure 3.2: Mean cells/cm^3 in each concentration after being exposed to Corexit 9500 for 4 days (96 hours).

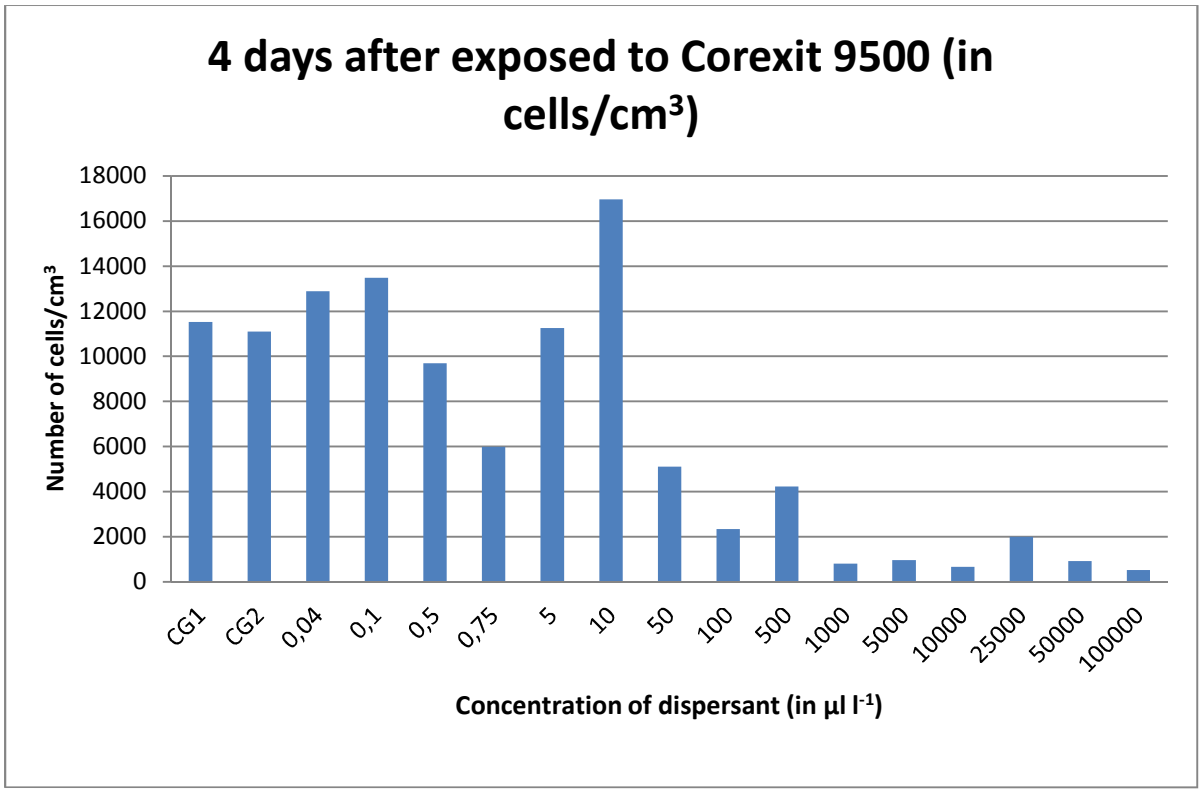
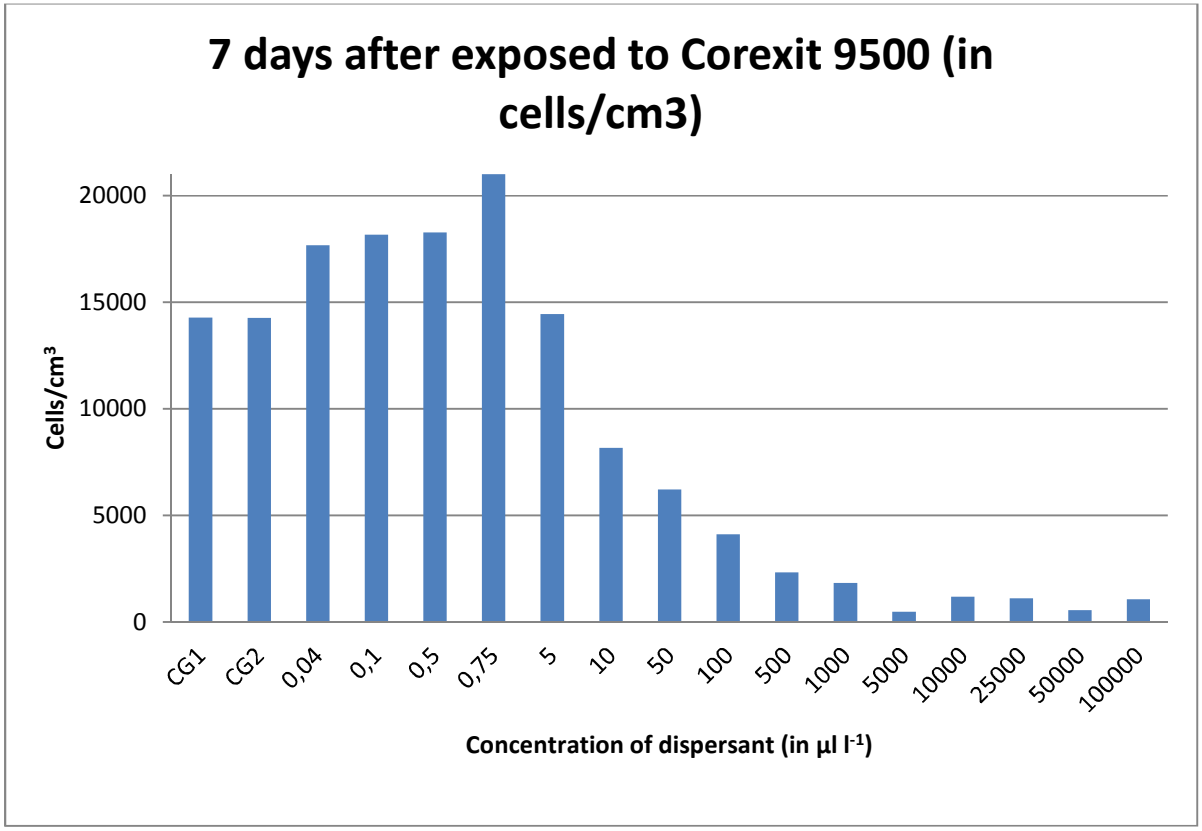


Table 3.4: Day 7. The data correspond to the number of diagonals and cells counted on the specific magnification, together with the number of cells per cm^3 in each concentration after being in the respective concentrations of dispersant for one week. The decimals are rounded off to the closest whole value.

Concentration:	No. of diagonals:	Total of cells (magnification):	Cells/cm^3 $\left(=\frac{tot.nr\ of\ cells \times multi.factor}{nr.of\ diagonals}\right)$
Control group 1	2	257 (320x)	14281
Control group 2	3	385 (320x)	14263
0.04 µl l^{-1}	2	318 (320x)	17671
0.10 µl l^{-1}	2	327 (320x)	18171
0.50 µl l^{-1}	2	329 (320x)	18283
0.75 µl l^{-1}	2	380 (320x)	21117
5.00 µl l^{-1}	2	260 (320x)	14448
10.0 µl l^{-1}	2	147 (320x)	8169

50.0 µl l^{-1}	2	112 (320x)	6224
100 µl l^{-1}	2	74 (320x)	4112
500 µl l^{-1}	2	42 (320x)	2334
1000 µl l^{-1}	2	33 (320x)	1834
5000 µl l^{-1}	3	13 (320x)	482
10,000 µl l^{-1}	3	32 (320x)	1185
25,000 µl l^{-1}	2	20 (320x)	1111
50,000 µl l^{-1}	3	15 (320x)	556
100,000 µl l^{-1}	3	29 (320x)	1074

Figure 3.3: Mean cells/cm^3 in each concentration after 7 days of being exposed to Corexit 9500.



Figures

Figure 3.4: Growth of algae. The growth of Scenedesmus obliquus exposed to the weakest concentrations of Corexit 9500 (0.04, 0.1, 0.5, 0.75 and 5.0 µl l^{-1}) through 7 days.

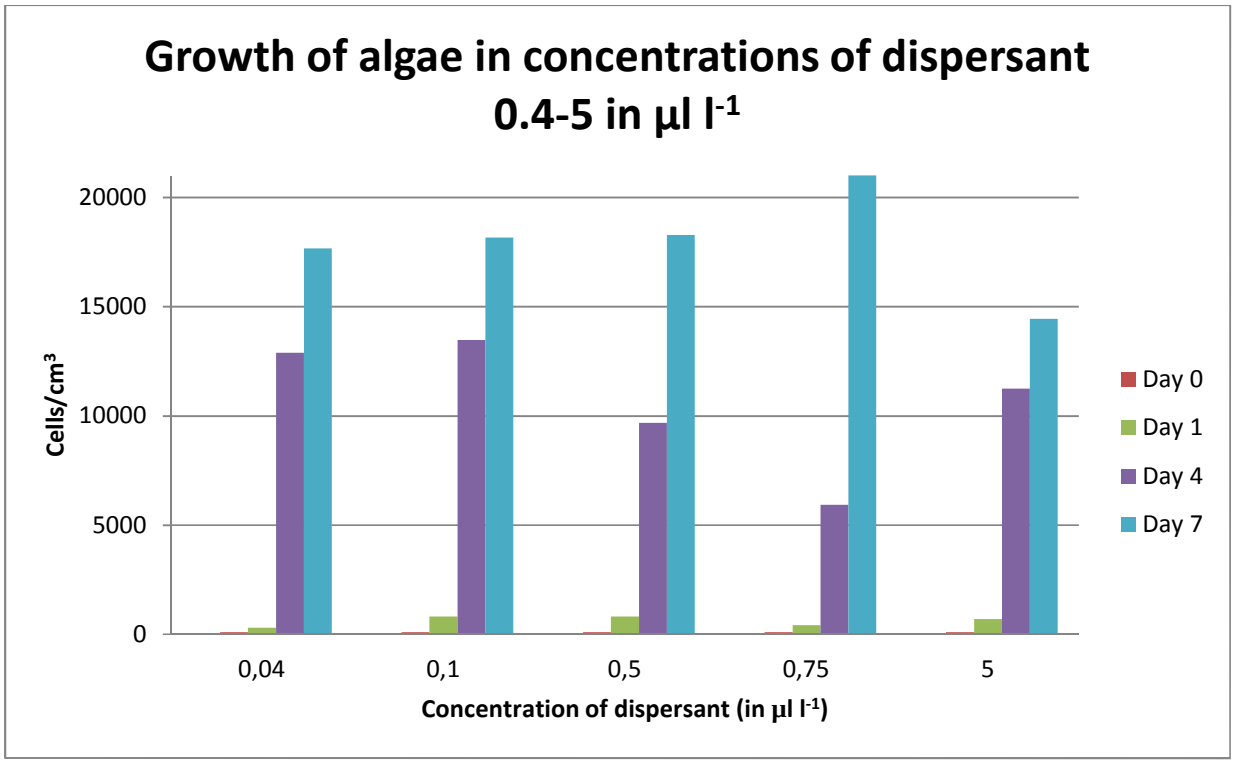


Figure 3.5: Growth of algae. The growth of Scenedesmus obliquus exposed to medium concentrations of Corexit 9500 (10, 50, 100, 500 and 1000 µl l^{-1}) through 7 days.

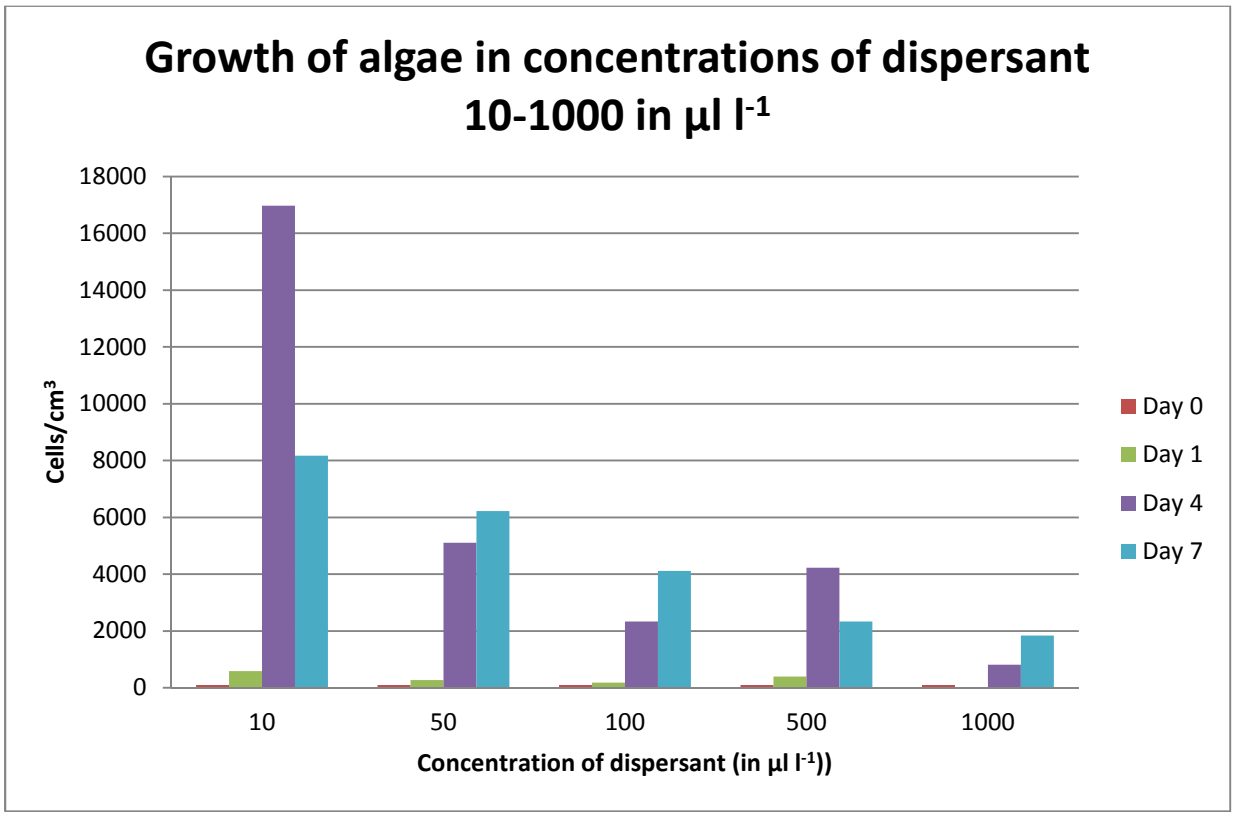
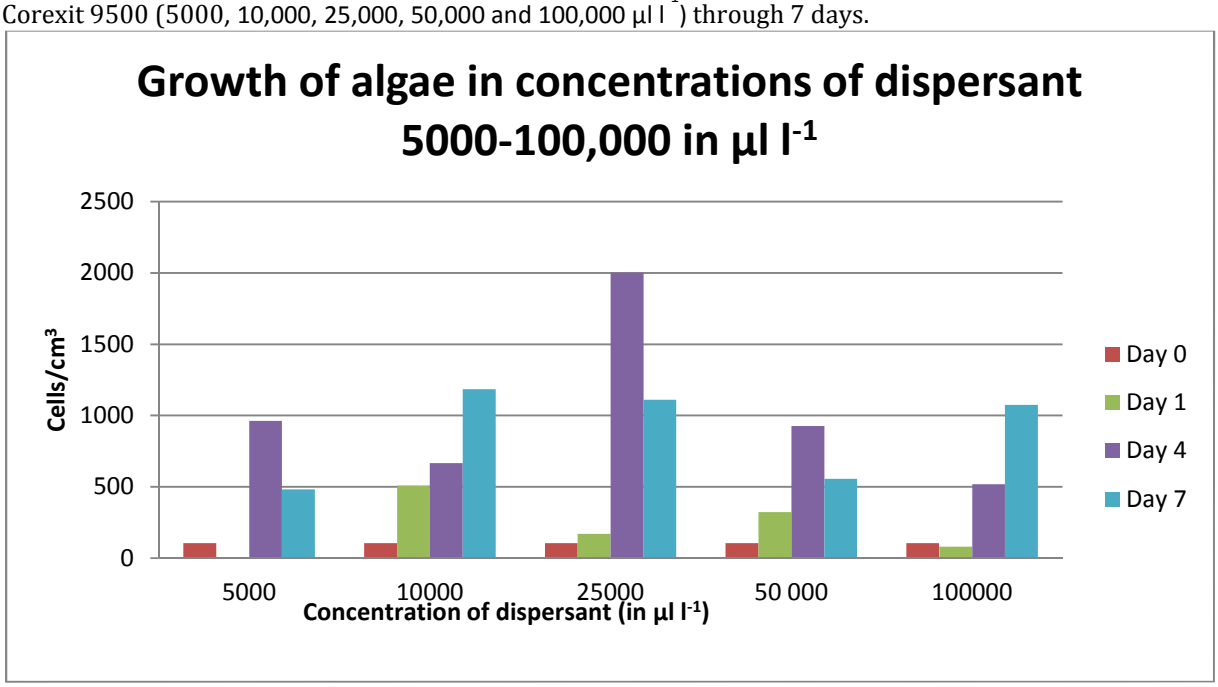


Figure 3.6: Growth of algae. The growth of Scenedesmus obliquus exposed to high concentrations of Corexit 9500 (5000, 10,000, 25,000, 50,000 and 100,000 µl l^{-1}) through 7 days.



MONITORING OF VARIABLES

Table 3.5: Variables. The selected variables were measured twice a day for 7 days. Variables measured were temperature, light intensity, time and pH (V=done, X=not done, 1=window 1, 2=window 2). The weather conditions was also noted.

Day:	Time:	Temp:	Light Intensity (lux):	Sample collected:	Additional Notes:
0	9.30 pm	21.5 °C (1,2)		V	Overcast and raining. pH[14]. Control 1: 7.9 100,000 µl l^{-1} :7.7
1	8.00 am	21 °C (1,2)	1941 (1) 1860 (2)	X	Overcast all day.
	22.00 pm	23°C (1,2)		V	
2	7.00 am	20.5 °C (1,2)	1504 (1) 1450 (2)	X	Some sun in the afternoon, direct sunlight on the flasks in the evening.
	8.00 pm	28°C (1,2) 29°C (1,2)		V	
3	8.00 pm	23.00°C (1,2)	1089[15] (1) 1849[16] (2)	V	Overcast all day.
4	4.00 pm	22.0°C (1,2)	1252(1) 1236 (2)	X	Overcast all day. Starting to see green develop in the bottom
	10.00 pm	22.0°C (1,2)		V	

[14] pH as a variable was not prioritized later in the investigation as it did not differ significantly in value.
[15] Mean light intensity of the middle, right and left side of window 1.
[16] Mean light intensity of the middle, right and left side of window 2.

					of flasks for small concentrations +control groups.
5	11.30 am	21.0°C (1,2)	1464 (1) 1484 (2)	X	Overcast from beginning of day, more sun in the evening. Small concentrations show significantly more green in bottom (control groups, 0.04 ul/l-50ul/l)
	9.00 pm	23.0°C (1,2)	1396 (1) 1429 (2)	V	
6	7.00 am	20°C (1,2)	1513 (1) 1710 (2)	X	Overcast in the morning and in the evening. Had to move flasks to another window because of a scaffold outside the experiment window.
	9.00 pm	23°C (1,2)	1364 (1) 1462 (2)	V	
7	16.00 pm	22.5°C (1,2)	1869 (2) 1873 (1)	X	Sunny outside, warmer in the air. Patches of clouds (changing light intensity). Direct sunlight from 8.00 pm.
	9.30 pm	27.0°C (1,2)	1057 (2) 1510 (1)	V	

Discussion

Summary of results

The initial algae population in all flasks is assumed to be about 105 ±16 cells/cm^3, because this is the mean cells/cm^3 of the controls from day 0. From time 0 to 24 hours after dispersing, the controls without dispersant had increased in mean from 105 cells/cm^3 to 246 cells/cm^3 (figure 3.1) This was a lower growth than in most of the other concentrations, except for 100, 25,000 and 100,000 µl l^{-1} which all had had a negative growth. This indicates an uncertainty in the method or that cells had died and already been decomposed. An earlier investigation (L. Hansen et. al, 1986) found that 34% of dead phytoplankton can decompose and lose their carbon content within 4 hours, and this would make it difficult to see the cells. The most rapid growth was in concentrations between 0.1-10 µl l^{-1} whose population had increased 6-8 times in size. An exception here was 0.75 µl l^{-1} who had an increase about 4 times its original population size. For higher concentrations the population showed high acceleration of growth as well. This goes against the results of (Ocean Study Board, 2005) about the number of cells decreasing for higher concentrations. To summarize, no clear trend developed 24 hours after dispersing. This could indicate uncertainties in the method, or that there might be additional nutrients in the dispersant, or that the algae may not have had time to adapt to the conditions, and not yet affected by the toxicity.

From the initial number of cells at time 0 to 4 days after exposed to Corexit 9500, the algae population without dispersant increased about 108 times its initial size to a mean of 11,312 cells/cm^3 (figure 3.2) For small concentrations between 0.04-0.1 and 10 µl l^{-1} there had with same exception for 0.5 and 0.75 µl l^{-1} been more growth with a mean of 3136 cells/cm^3 more than the controls. The algae population in 5.0 µl l^{-1} had increased about as much as the controls, thereby; the dispersant had no observed effect. The concentrations of 50 µl l^{-1} and higher showed a LC_{50} or higher[17]. 10,000 µl l^{-1} had increased least in comparison with the controls, with a growth of only 562 cells/cm^3 on 4 days. The results showed a trend of an increased population growth for low concentrations of Corexit 9500 in comparison with controls, while for higher concentrations of dispersant above 50 µl l^{-1} there was a decrease in growth.

The clearest trend was obtained after the algae were exposed to Corexit 9500 for 7 days (figure 3.3). The controls without dispersant had increased in size with a mean of 14,272 cells/cm^3, while for concentrations between 0.04-0.75 µl l^{-1} the algae growth had accelerated higher than that of the controls. The algae population in these had now a total of 17,671-21,117 cells/cm^3. This was a mean increase of 24 % higher than the controls. This may indicate substances in the dispersant that are used as nutrition for Scenedesmus obliquus. 5.0 µl l^{-1} had a population growth at about the same level as the controls. This is a similarity from the results obtained on day 4. There was a lower acceleration of growth for all concentrations higher than 5.0 µl l^{-1}. This showed that the toxicity of Corexit 9500 for Scenedesmus may increase with time by inhibiting their growth.

Compared to the report from Ocean Studies Board with brown algae, there is a resemblance in results. In this study there was an observed effect on for concentrations of dispersant on 0.7 µl l^{-1} for brown algae when exposed to Corexit 9500 for 72 hours. My results showed that growth of algae in dispersant with concentration of 0.75 µl l^{-1} after 96 hours had a higher acceleration of growth.

Analysis of Method

Many steps and little time mean also a bigger risk of inaccuracies. I tried to be as exact with measurements as possible, but I still had to make 6 flasks twice because of mistakes in calculations. 50,000 µl l^{-1} had 1.4 cm^3 more distilled water than the others by an accident. After the making of 25,000 µl l^{-1} it looked suspiciously white and I realized later that I may have added phosphate solution instead of distilled water. This was confirmed later (Wetzel, R; Likens, G. 1990, Kristiansen

[17] LC_{50}=Lethal Concentration of 50%. This means there was a 50% reduction in the growth of cells compared to the controls for day 4.

pers.com), as phosphate is poorly soluble and often makes precipitations in contact with other substances. The results from 25,000 µl l^{-1} from day 4 indicated a population growth 12 times higher than growth on day 1, and this was a much higher accelerated growth compared to the closest concentrations in the same time span. The result is atypical and therefore excluded from further evaluation.

At the time of the investigation, I was not aware that the samples should be stored in a cold, dark room, and therefore sample 1000-5000 µl l^{-1} were decomposed before I had time to count them. The samples for Day 0-Day 3 were also decomposed, but I had already counted many of these. This made it impossible to count the results for Day 0 and Day 1 again to increase the accuracy. Having lid or no lid was also an issue, because having no lid may have lead to evaporation of liquid. I chose a combination and let the flasks "air out" twice a day.

The only samples measured at day 0 were the controls. This was assumed to be the start number of cells/cm^3 in all flasks, but as the samples varied in cells/cm^3 for the control groups it is likely that all the flasks contained some differences in start population. The reason for having two controls was to have an indication of how great the uncertainty was between two equal samples. On day 0 there was a variation of $\pm$32 cells/cm^3 in the controls. This may indicate a mean difference of $\pm$ 32 cells, but as there are many other factors that contributes to differences in cell number it is highly variable, for example shaking of samples and flasks before counting.

The fact that the variables were just monitored and not controlled has an impact on the validity of the results. Changes in temperature and light intensity could contribute to increased growth. Using two different windows could have had an impact. As the results from the monitoring of data showed (table 3.5) the light intensity was continuously higher in window 1 in the morning (7.30-8.00 am), but window 2 had higher light intensity in the evening (9.00-10.00 pm). Midday (11.30 am-4.00 pm) it was equal with a difference between 4-20 lux. This may have contributed to the growth as there was direct sunlight on window 1 in the evening, and a possible reason for increased growth at lower concentrations. I noticed that after a few days the flasks with low concentrations (controls-100 µl l^{-1}) collected water vapor on the inside of the flasks, while higher concentrations gradually contained less water vapor. The higher concentrations were placed in window 2 and low concentrations in window 1, and this may indicate different temperatures for the flasks. On day 6 a scaffold was put up outside window 1, and therefore I had to move the samples to a window on the other side of window 2. This window had similar environment and was North faced and since this change only lasted for 1 day it may not have had a major effect on the growth.

The method used to find cells/cm^3 may also have contributed to uncertainties. At the point of counting day 0 and day 1, I found it difficult to distinguish between algae and other particles, and this may be a reason for the uneven trend in the results for Day 1. Later, I made up a rule of what were algae and what to include/not include. To find cells/cm^3 the algae samples were shaken and counted in diagonals, representing the number of algae in the whole chamber. This suggested that the algae were equally distributed, something that may not always have been the case and provides a weakness for the investigation. When there are low concentrations of algae it increases the risk of errors as the algae are more randomly placed through the chamber. This could also have contributed to the unclear trend in the results of day 1. It is known that Scendesmus obliquus most often form colonies of many cells (Connecticut College, 2004), and having colonies increases the risk of miscounting and unequal distribution of cells. However, as the samples were shaken twice a day the cells appeared single in most of the samples.

When microscoping the algae there was another trend worth of evaluation. For higher concentrations above 100 µl l^{-1} the cells looked thinner and smaller, something that may indicate that osmosis had occurred. This was noted on all days counted for these concentrations. At day 7, many of the cells were counted in colonies (50 µl l^{-1}, 10,000 µl l^{-1}). The concentrations from 50,000 µl l^{-1} and higher were very difficult to count as the cells were small and had irregular shape. This made it hard to distinguish from other particles, and one can wonder if these results are applicable at all.

As the investigation was very time consuming, an improvement for next time would be to allow more time. The uncertainty on measurings would then decrease and samples could be counted more times to increase the accuracy. Moreover, it is ideal to have many samples of one concentration to compare the results. I chose to use many different concentrations instead because I did not know at which concentration an effect would be shown. It is also ideal to do the experiment under a controlled environment as it would not be other factors contributing to the growth as well. Another improvement for next time could be to disperse Corexit with oil to provide a more realistic investigation.

Conclusion

There are many uncertainties in this investigation, but the obtained results give an indication of the possible effect on the growth of algae when exposed to Corexit 9500. The results for day 4 and day 7 shows a clear trend of higher accelerated growth between concentrations of 0.04-0.75 µl l^{-1} than the controls without dispersant. This may indicate that there are substances in the dispersant that works as nutrition for the algae. Exposed to concentrations around 5.00 µl l^{-1} have little observed effect as the acceleration of growth was about the same as in the controls. For values above 50 µl l^{-1} on day 4 and 5.0 µl l^{-1} on day 7 the growth was inhibited after being exposed to Corexit 9500. This may also indicate that the effect of the dispersant is more toxic in the long term. It proved difficult to compare the studies from Ocean Study Board with own results, but it gives an indication. For instance, only dispersant seem more nutritious for algae than dispersant applied to oil as it was done in the study. My hypothesis was that the dispersant is toxic and inhibits algae growth for higher concentrations of dispersant. The results from this investigation concluded with this, but an unexpected result was that for low concentrations, the algae accelerate higher in growth.

A limitation of the experiment is that it can only to a certain extent be applied to a real life situation, because Corexit 9500 most often is dispersed on oil. However, it is difficult to know exactly how much dispersant to apply on the oil, and there is always a risk of applying too much. This could have a related impact on algae as the one shown in this investigation.

Bibliography

Books

- Wetzel, Robert G., and Likens Gene E. Limnological Analyses. Table A.1.1, p. 363, 2. Edition. Springer- Verlag, 1990

Definitions

- "Acute Exposure" http://www.nap.edu/openbook.php?record_id=11283&page=R1
- "Chronic Exposure" http://www.nap.edu/openbook.php?record_id=11283&page=R1
- "EC_{50}" http://www.nap.edu/openbook.php?record_id=11283&page=R1
- "Hydrophilic" http://www.biology-online.org/dictionary/Hydrophilic
- "Hydrophobic" http://www.thefreedictionary.com/oleophilic
- "LC_{50}" http://www.nap.edu/openbook.php?record_id=11283&page=R1
- "Limnology" Oxford Dictionary of English, iFinger Version 3.6 (www.ifinger.com)

Internet Sources

- Connecticut College, 2004, *Scenedesmus obliquus* http://silicasecchidisk.conncoll.edu/LucidKeys/Carolina_Key/html/Scenedesmus_Main.html , http://www.algaebase.org/search/species/detail/?species_id=27885, (Cited 20. October)
- Hansen, Lisbeth; Krog, Gudrun F; Søndergaard, Morten; Copenhagen, 1984, *Decomposition of Lake Phytoplankton 1. Dynamics of short-term decomposition* http://www.jstor.org/pss/3565377
- Hickel, Wolfgang; 1984, *Seston Retention by Whatman GF/C glass-fiber filters.* http://www.int-res.com/articles/meps/16/m016p185.pdf *(*Cited 19. October 2010)
- Lührling M.; 2003, *Pheontypic Plasticity in the Green Algae Desmodesmus and Scenedesmus with Special Reference to Induction of Devensive Morhpology*, vol 39, Dept. Environmental Sciences, Aquatic Ecology and Water Quality Management Group, Wageningen University http://cat.inist.fr/?aModele=afficheN& cpsidt=14909891 (Cited 19th October 2010)
- Miljølære 1989-1994, Sør-Trøndelag 1989, Rogaland 1993, Møre og Romsdal 1994, Rogaland 1994 http://miljolare.no/data/ut/art/?vis=omrader&or_id=779
- NALCO COMPANY, 2010. Available from www.nalco.com
    - *Seven Facts About COREXIT 9500 OIL DISPERSANT* http://www.nalco.com/applications/4285.htm (Cited 1. October 2010)

- *NALCO TECHNOLOGY* *http://www.nalco.com/applications/corexit-technology.htm (Cited 1. October 2010)*
- COREXIT Ingredients http://www.nalco.com/news-and-events/4297.htm (Cited 1. October 2010)

- Ocean Studies Board, 2005, *Oil Spill Dispersants: Efficiency and Effects*, Division on Earth and Life Studies, NATIONAL RESEARCH COUNCIL *OF THE NATIONAL ACADEMIES,* Washington D.C http://www.nap.edu/openbook.php?record_id=11283&page=R1 (Cited 19th October 2010)

- Oppland Arbeiderblad
  - Berget, John; *Olje lekker i Mjøsa,* 26.04.2010 http://www.oa.no/nyheter/gjovik_toten/article5088187.ece. (Cited 1. October 2010)
  - Sveen, Torhild; Skretting, Petter*; Olje-forurensing av Mjøsa*, 09.08.2007, http://www.oa.no/nyheter/article2933552.ece. (Cited 1. October 2010)
- Østlendingen
  - Løken, Trine H; *Olje lekket ut I Mjøsa,* 16. november 2007 http://www.ostlendingen.no/nyheter/olje-lekket-ut-i-mjosa-1.4545803 (Cited 1. October 2010*)*

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