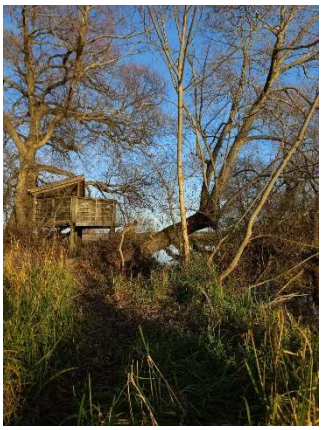
Chlorella sp.

Sampling:



The freshwater sample is collected at the intersection of the rivers Fyrisån and Sävjaån in the south of Uppsala (coordinates 59°49'45.1"N 17°40'02.4"E). The rushes surrounding the sampling area are inhabited by a variety of birds. Before collecting the sample, the sediment is disturbed to collect the maximum number of organisms.

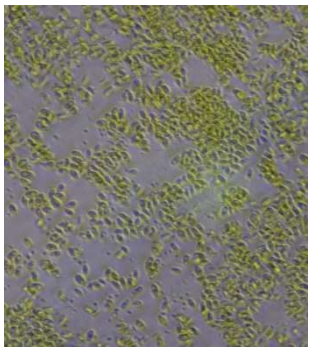
Culturing:

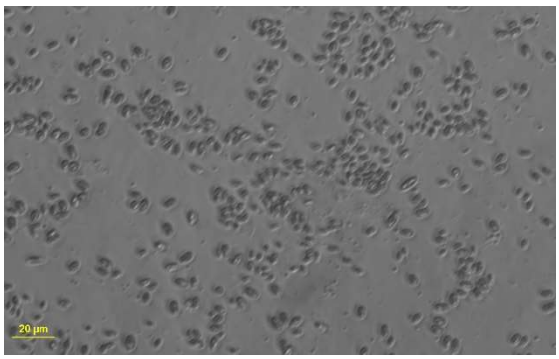
After sampling, the first step is to try to isolate a single species from the collected sample; to do that a dilution to extinction method is applied. A 48 well plate is used: the first column is filled by alternating the sampling material picked from the top, the bottom and the middle of the falcon tube, (excluding the first column) the first 3 rows are then filled with MWC medium and the last 3 rows with Z8 medium; the dilution is then carried out and the plate is observed under an inverse microscope to identify the protists and to draw them. Later the plate is incubated at 18°C under a 12 hours light cycle. After the culturing, a well that seems having a pure culture is chosen and is used for the following steps. To extract the DNA, cold shocks are applied to 2µL of the sample material from the chosen well (B3 – MWC medium): the Eppendorf tube is first dipped in liquid nitrogen and then warmed up to 37°C by holding it in the hands; this is done 3 times. To amplify the desired DNA fragments, the mastermix made of 12.5µL of Go Taq, 2.5µL of 3NDf (forward primer), 2.5µL of V4_euk_R1 (reverse primer) and 5.5µL of nuclease free water for a total of 23µL are added to the Eppendorf tube containing the previously obtained 2µL of the freshwater sample that went through the cold shocks; a negative control tube is also prepared by using the same amount of Go Taq, 3NDf, V4_euk_R1 but 7.5µL of nuclease free water for a total again of 25µL. The PCR procedure is done for all the samples. To check if the PCR is successful, an agarose gel electrophoresis technique is used by loading the wells of the instrument with a solution made of 3µL of the PCR product and 2µL of gel red (one is loaded for the algae and one for the negative control); the ladder is made of 3µL of 1kb Ladder and 2µL of gel red. In this case the negative control doesn't present any line (which means that there is no DNA and that's correct) and the algae has two lines which means that multiple organisms (at least two) were present in the culture and so the DNA sequencing is probably going to give illegible results; because of that the identification of the species of algae will be done only by looking at morphological features.

Description:

By looking at the plate with naked eye, is

possible to notice that there is a thin layer or some spots coloured with green at the bottom of the well (B3). By looking at the chosen well with an inverse microscope is possible to see a lot of subspherical green cells (around 4µm wide) that are present both as solitary cells or aggregated in groups; they don't move and don't present any visible flagella.





Phylogeny:

Since the agarose gel electrophoresis didn't give clear results about only one type of DNA being amplificated, the DNA was not sent for sequencing. The identification only from the morphology for protists can be hard, since morphologies are not unique to one species but are commonly shared between organisms; in this case it is quite reasonable to think that it is a type of green algae. Using the dichotomous keys in the book "Freshwater Algae: Identification, Enumeration and Use as Bioindicators" of Edward G. Bellinger, and David C. Sigee (2015) is possible to define the genus of the majority of the organisms in the sample, that is with high probability *Chlorella* given the features of being spherical to subspherical in shape with cells that measure from 2µm to 10µm wide and presenting a single dark spot which might represents the single pyrenoid of these organisms. The most common species of *Chlorella* is *C. Vulgaris*, so it would be reasonable to say that it might be that, but since there is no DNA sequence that can be consulted, it is not possible to say anything with high certainty; therefore, it is better to define it only with the genus.