Chlorophyll
Application APP-PHM-0002

General
The eutrophication of stagnant and gently fl owing bodies of water is an important subject matter when it comes to ecological observations of water. Here, eutrophication is taken to mean the increased supply of plant nutrients to water bodies caused by human activity and the resulting increase in the productivity of plants and algae.
The chlorophyll-a concentration of these waterbodies is – along with other biomass and bioactivity parameters – an important indicator for assessing the degree of eutrophication of surface waters.
Phaeophytin and phaeophorbide are important degradation products of chlorophyll. The relationship between chlorophyll-a and phaeophytin concentration can provide clues as to the physiological condition of the algae cells.

Method
Chlorophyll-a and phaeophytin concentrations are determined by means of spectrophotometric measurement of an ethanolic extract from the filter residue of a water sample, in accordance with DIN38412 - L16.

Material:

LPV422.99.00001	Spectrophotometer DR 2800 or
LPV424.99.00001	Spectrophotometer DR 3800 or
LPV408.99.00001	Spectrophotometer DR 5000 or
LPV408.99.00001	Spectrophotometer DR 5000 or
LPV408.99.00001	Spectrophotometer DR 5000
2629250	Glass cuvette 50mm

- Filter setup (e.g. water jet pump with suction bottle in accordance with DIN12476)
- Filtration cap with glass or porcelain suction filter
- Filtration device for holding a volumetric fl ask
- Homogenizer, alternatively mortar with nozzle
- Extraction vessel, protected from light
- Glass fibre filter made from borosilicate glass (> 99% for particles > 1 µm)
- 100 ml volumetric fl ask
- Conical flask (Erlenmeyer)
- Filter circle
- Ethanol (90%)
- Hydrochloric acid (2 N)
- (2 N)

When using the DR 2800 / DR 3800 / DR 5000 / DR 3900 / DR 6000 for the first time:
Download the additional evaluation **Chlorophyll APP-PHM-0002** as an application from the Internet.

- Go to www.hach-lange.com and search under **Chlorophyll** and **Documents and Software** the application **Chlorophyll** and save it on your PC.
- Open the zipped file with a double-click and save the folder used for your photometer to an USB stick:
- DR 2800 / DR3800 dbhlc
- DR 5000 dbhl
- DR 3900 dbhlm
- DR 6000 dbhlh

- Take the USB stick and upload the application to your photometer.
- In the PDF file you will find the application note with detailed description.

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Chlorophyll determination procedure

Sample preparation

1. When there is sufficient homogenization 0,5 l (– 2 l) filter the water sample through a suitable filter.
2. Bring the volume of ethanol required for extraction to the boil (78 °C) in a vessel with a condenser placed on it.
3. Take the filter, which will be covered in algae following the filtration process, fold it, tear it into pieces, place in the extraction vessel and pour over approximately 30 ml hot ethanol.
4. After it has cooled, reduce the pieces of filter in the extraction vessel with the help of a homogenizer.
5. The extraction process takes 6–24 hours (usually carried out over night).
6. When the extraction process is complete, filter the homogenate using a paper filter circle.
7. Collect the clear filtrate in a volumetric flask installed in a filtration device protected from light.
8. Rinse filter with ethanol.
9. Top up the clear pigment solution in the volumetric flask with ethanol, up to the mark, then shake it and store it away from light.
10. In order to differentiate the chlorophyll-a and to determine the phaeophytin concentration, a portion of the extract is acidulated by adding hydrochloric acid (0,3 ml per 100 ml extract).
11. In order to produce acidic ethanol, 0,3 ml hydrochloric acid is added to 100 ml ethanol.

Procedure

1. Switch on the spectrophotometer and select **Chlorophyll-a** user program.
2. With the cuvette removed from the cuvette compartment press Zero.
3. Fill cuvette with ethanol (90%) and press Read.
4. Fill cuvette with acidic ethanol (step 11) and press Read.
5. Fill cuvette with sample before acidulating it (step 9) and press Read.
6. Fill cuvette with acidulated sample (step 10) and press Read.

The result is shown in µg/l chlorophyll-a concentration and in µg/lphaeopigment concentration.For additional samples, proceed with point 5.

Procedure

1. Switch on the spectrophotometer and select **Chlorophyll-a_Serie** user program.
2. With the cuvette removed from the cuvette compartment press Zero.
3. Fill cuvette with ethanol (90%) and press Read.
4. 4. Then the total number of samples to be measured is requested. Select the number and press OK. After the measurement “E2-1” is displayed.
5. Insert cuvet with acidic ethanol (step 11) and press READ. The interim result “E4-1” is displayed.
6. Thereafter one after another insert the selected number of sample cuvets (samples before acidification) and press READ. Interim results are displayed as “E6-1”, “E6-2”, “E6-3”, …
7. Then one after another insert the selected number of sample cuvets (samples after acidification) and press READ. The final results are displayed after each measurement.
8. After all measurements have been finalized, a new series could be started with step 2.

Evaluation*:*
The chlorophyll-a-concentration is calculated using the following equation:

$$Chl\text{-}a \text{ in } \mu g/l = (A_V - A_N) * (R/(R-1)) * (V_E/(V_P * d)) * (1000/\alpha) \quad (1)$$

Key:
AV Absorbance before acidification at 665nm, corrected by turbidity adjustment at 750nm
A_N Absorbance after acidification at 665nm, corrected by turbidity adjustment at 750nm
R Relation between von A_V : A_N for pure chlorophyll-a = „acid quotient“
V_E Volume of Extract in ml;
V_P Volume oft he filtered water sample in l;
D Path length oft he cuvette in cm;
α Specific absorption coefficient for chlorophyll-a in ethanol

The following factors have been assumed in the programming:

F1:	29.6	R= 1.7 und α=82	taken together
F2:	100	V_E = 100 ml	
F3:	0.5	V_P = 0.5 l	
F4:	*5*	*d = 5 cm*	

Equation (1) can therefore be simplified to:

$$Chl\text{-}a \text{ in } \mu g/l = 29.6 * (A_V - A_N) * (V_E/(V_P * d)) \quad (2)$$

It is possible to alter factors F2 for extraction volume (V_E= 100 ml), F3 for sample volumee (V_P= 0.5 l) and the pathlength in the cuvette (d= 5 cm), and to adapt these for an individual operating process.

The phaeopigment concentration is calculated on the basis of equation (3):

$$Phaeopigment \text{ in } \mu g/l = 20.8 * A_N * (V_E/(V_P * d)) - Chl\text{-}a \text{ in } \mu g/l \quad (3)$$

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Disposal information

Waste disposal must be carried out in compliance with regional and national regulations.