INTRODUCTION TO ALGAL ASSAYS
IN
WATER RESEARCH

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A B S T R A C T

One of the fruitful advances of experimental water quality research is the algal growth potential (AGP) measurement. Though already used in the beginning of this century it was taken into wider use about 13 years ago. The different possibilities of the use of the method are described. Also some examples are given. Also the detailed description of the algal assay bottle test method and chlorophyll a method are included.

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In addition to that there are also so-called chemostat and dialysis tests. The test described is so-called batch test.

This name derives from the fact, that in the test in a bottle the water "batch" is the same during the whole test period.

2. ALGAL ASSAY PROCEDURES

In the following algal assays will be described in detail.

a. Algal growth potential of waters

In this test a water sample is filtered through a Whatman GF/C glass-fibre filter[1]. Then a volume of 100 ml is poured into a conical flask of 250 ml. This is to ensure, that there is a good volume-surface-ratio. The sample is inoculated by one drop of selenastrum capricornutum culture. A beacer is placed upside down on the flask and the sample is placed under constant light of about 5000 lx (fluorescent lamps, Philips TL 33 or equivalent). The algal are let to grow for about 14 days. The sample must be shaken once a day (National Eutrophication Research Programme, 1971, Nordforsk, 1973). After 14 days the chlorophyll a concentration of the sample is measured (see appendix 2). Also some other parameters can be used; turbidity, cell number, cell volume, dry weight, organic carbon concentration, etc.

The results can be used to compare the nutrient status of the waters. The comparison can also be made against a standard solution cultivation.

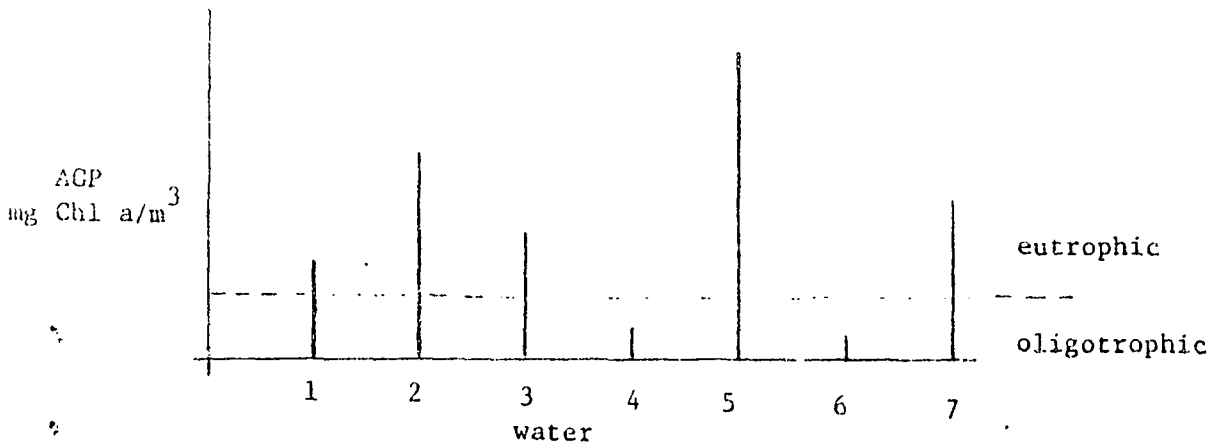

Fig. 1 Status comparison of waters

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1) From the filter the suspended solids can be measured on dry weight basis by weighing.

Until now there has not been enough data to ensure the borderline between oligotrophic and eutrophic waters.

b. The limiting nutrient of a water body

This test is made in the same manner as the lake water test. But instead of one test bottle there are several bottles. The next figure can show an example of this.

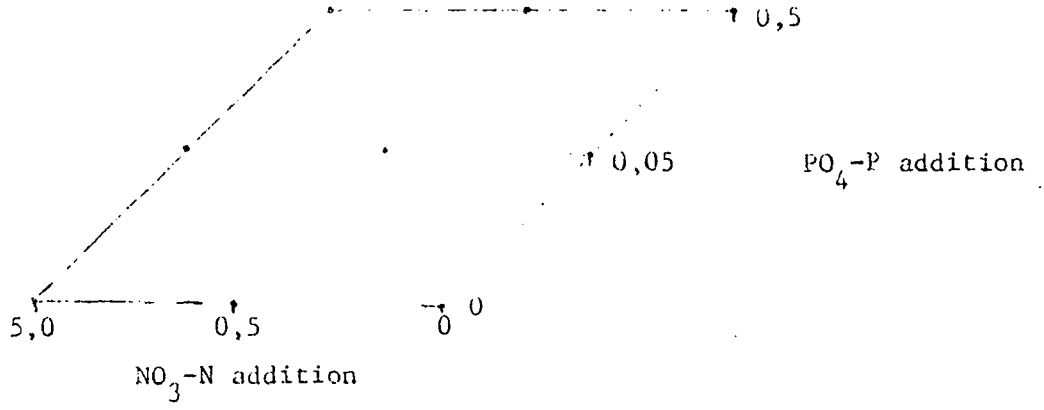

Fig. 2. An example of the scheme of the limiting nutrient test.

After the test a threedimensional figure is drawn according to the results.

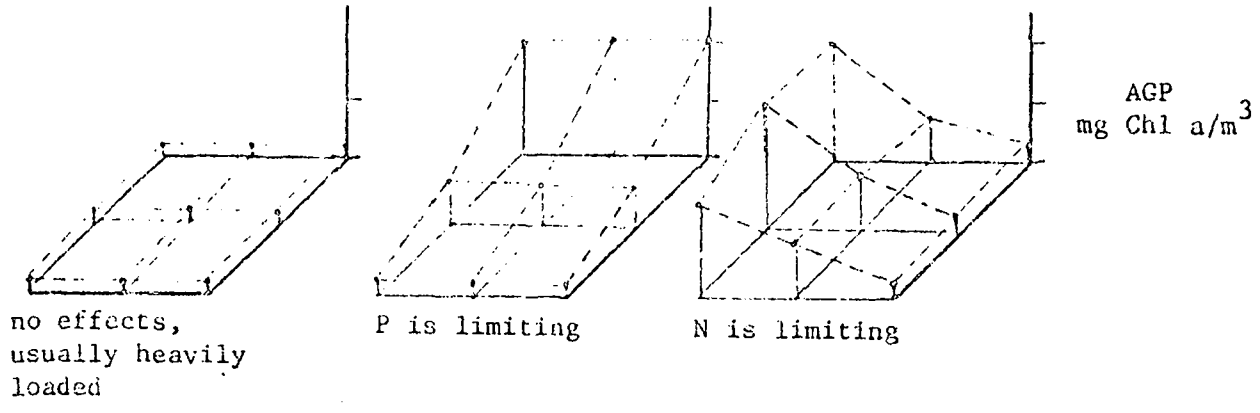

Fig. 3. Examples of some analysis of achieved data

According to these results the sensitivity of receiving water is evaluated and f.ex. the need for nutrient removal from sewage is set.

c. The effectiveness of waste water treatment

One very largely used application of the test is to follow the quality of effluents of the municipal waste water treatment plants. The sample is usually a continuous sample for 24 hours taken according to the effluent flow rate. An AGP-test is then made every day in the way described earlier.

In some cases this method has frequently been used since 1971. In these tests a weekly rhytm has been found. Samples taken on Sundays had the lowest and samples taken on Wednesdays had the highest values. Normally the correlation between PO_4-P and AGP-values was good and no correlation between NO_3-N and AGP-values was found (Forsberg, 1973).

d. The effects of different kinds of waste waters and other components in receiving waters

To test the effects of effluents on the receiving waters the dilution series are usually made.

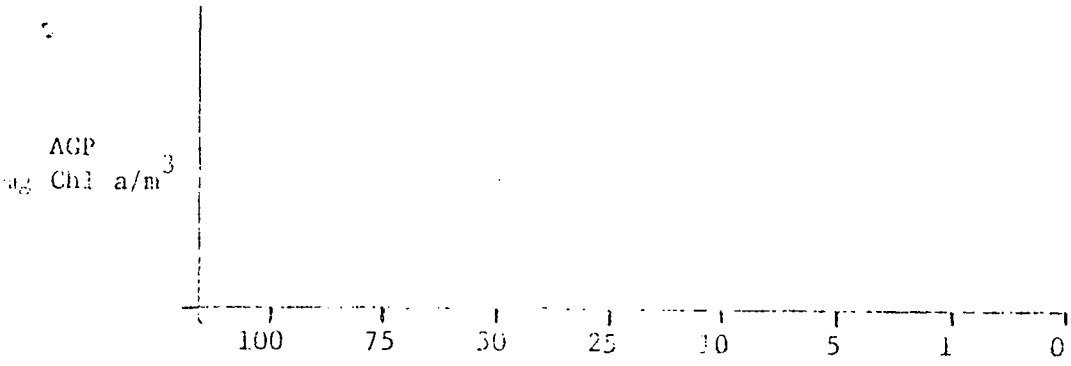

Fig. 4. A scheme of the dilution series

Then the normal AGP-test is made with all the dilutions.

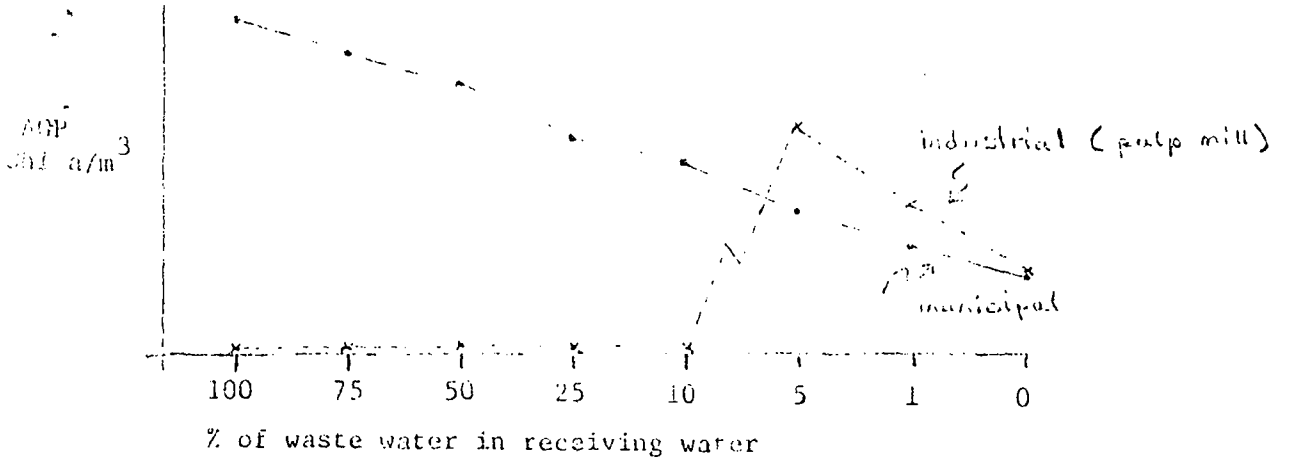

Fig. 5 Examples of the results with municipal and industrial waste waters

Usually municipal waste waters have diminishing effect when the dilution becomes greater. This is not the case with industrial waste waters. For example pulp mill waters have the effect shown on figure 5. The conclusions can also be made comparing the results with the results of similar tests made with a synthetic medium (Lehmusluoto, 1971).

Knowing the hydrology of the receiving water the effects of waste waters can be evaluated by calculating the dilution factors. Also some studies on the fertilizing effects of interstitial water have been made. For example in lake restoration cases tests have been made with lake water, with lake water +10% and with lake water +20% of interstitial water before, during and after the removal of bottom deposit of layer of about 1 meter thick. The effect of bottom deposit removal was quite clear on the nutrient concentration of interstitial water.

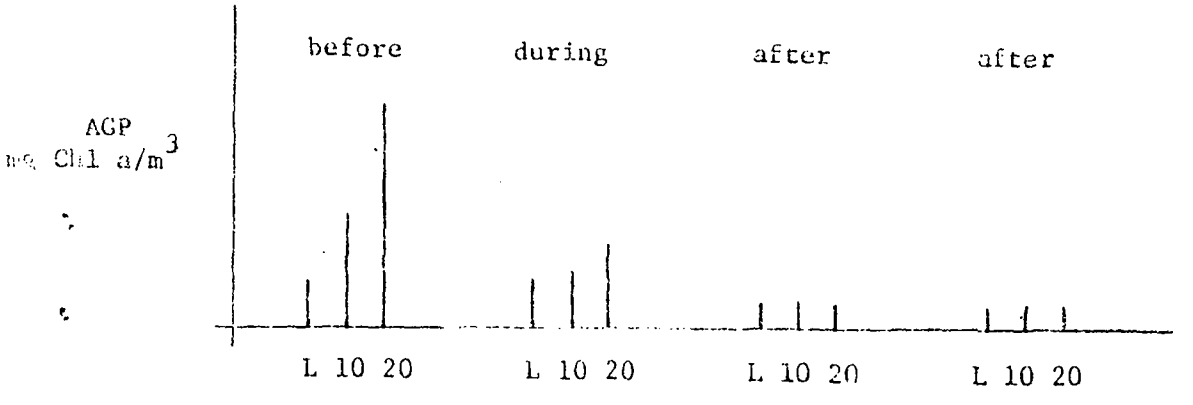

Fig. 6 An example of the effects of bottom deposit removal on the fertilizing capacity of interstitial water (cf. Lindmark, 1973)

3. SOME METHODOLOGICAL PROBLEMS

The method involves some problems in its use. The first one is, that water samples must be filtered through a glass fibre filter. This contributes somewhat to the water itself. But probably this is not so important, because the results are in any case relative.

The use of only one species of algal, may be another factor. But in most cases the total yield of algal is almost the same whether only one or several algal species are used.

When working with areas, which have salinity gradients the application of test results can be more difficult. This is due to the fact, that one algal species has a specific optimal salinity. For example in my own tests the following results have been found.

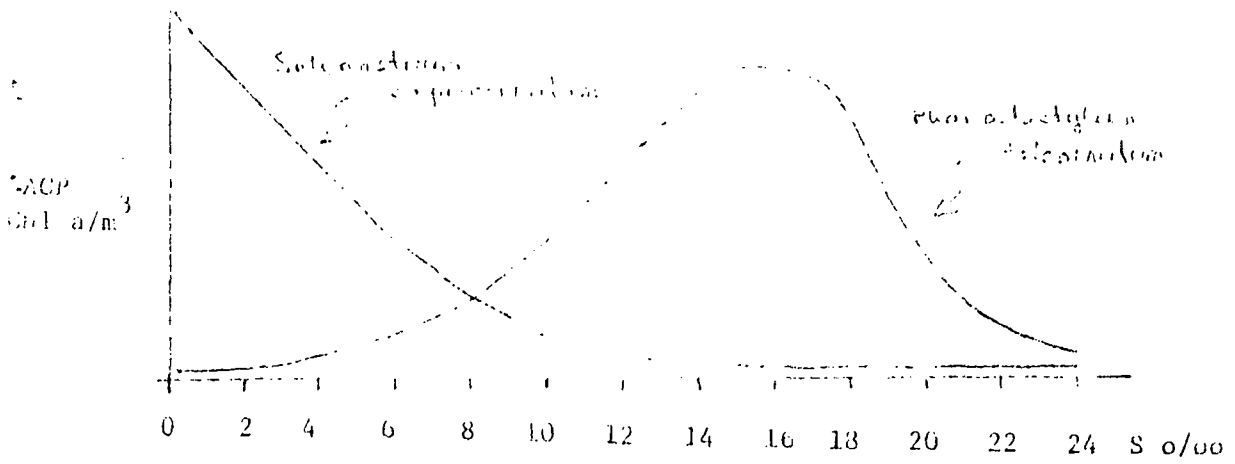

Fig. 7 An example of the effect of salinity on the growth of two algal species.

This means that salinity affects very much on the results (cf. Lehmusluoto, 1977, in print).

Also in different parameter measurements there might be some specific problems. But if we are here going to use the chlorophyll a method, there could probably not be any. The use of algal assays we have found to be of great value. The results of algal assays have the advantage of being very expressive in contrast to most chemical parameters used to characterize the ecological impacts of different kinds. This is an important matter to consider, when the results of an investigation are to be presented to authorities, industry and to the public.

4. REFERENCES

a. Forsberg, C., 1973 : Algal assay procedure. "Mini-test" with lake water (Preliminary Report). - in :Algal assays in water pollution research, Nordforsk, Secretariat of Environmental Sciences, Publication 1973, 2, 81-83.

b. Lehmasluoto, P.O., 1971 : Eutrophication in some lakes and coastal areas in Finland with special reference to polyhumic lakes. - in: International symposium on water pollution control in cold climates.- Environmental Protection Agency, Water Pollution Control Research Series, 16100 EHH 11/77 48 - 60.

c. Lindmark, G., 1973 : Bioassay, with Selenastrum capricornutum to assess the nutrient status of lakes and the fertilizing influence of interstitial water. - in: Algal assays in water pollution research, Nordforsk, Secretariat of Environmental Sciences, Publication 1973; 2, 73-79.

d. National Eutrophication Research Programme, 1971: Algal assay procedure, bottle test. - August 1971, 1-82.

e. Nordforsk, 1973 : Algal assays in water pollution research. - Nordforsk, Secretariat of Environmental Sciences, Publication 1973, 2, 1-128.

f. Oswald, W.S. and C.G. Golueke, 1966: Eutrophication trends in the United States - a problem ? - J.WPCF 38, 964-974.

g. Skulberg, O., 1964: Algal problems related to the eutrophication of European water supplies, and a bio-assay method to assess d fertilizing influences of pollution on inland waters. - in : Algae and Man (ed - D. Jackson), New York, 262 - 299.

A L G A L A S S A Y B O T T L E T E S T

1. INTRODUCTION

Algal assays are mainly used in studying recipients, their status of nutrients and stimulative and inhibitive effects of effluents or other compounds as well as limiting nutrients, mainly nitrogen and phosphorus, of water bodies.

2. APPARATUS

Culture vessels, 250 ml conical flasks

Stoppers for culture vessels, 100 ml beakers

Illumination table, illumination rate 5 klx, temperature +22°C ± 2°C

Glassfibre filters, Whatman GF/C

Filtration apparatus

Sterile pipettes of 1 ml

3. REAGENTS

Stock culture solution, 5% Z 8 (= standard solution)

23.35	mg/l	$NaNO_3$
2.95	"	$Ca(NO_3)_2 . 4H_2O$
1.55	"	K_2HPO_4
1.25	"	$MgSO_4 . 7H_2O$
1.05	"	Na_2CO_3
3.7	"	Komplexon III
140.00	µg/l	$FeCl_3 . 6H_2O$
0.15	"	$Na_2WO_4 . 2H_2O$
0.4	"	$(NH_4)_6Mo_7O_{24} . 2H_2O$

0.6	µg/l	KBr
0.4	"	KI
1.45	"	$ZnSO_4.7H_2O$
0.75	"	$Cd(NO_3)_2.4H_2O$
0.75	"	$Co(NO_3)_2.6H_2O$
0.65	"	$CuSO_4.5H_2O$
0.95	"	$NiSO_4(NH_4)_2SO_4.6H_2O$
0.2	"	$Cr(NO_3)_2.7H_2O$
0.04	"	V_2O_5
2.35	"	$Al_2(SO_4)_3K_2SO_4.24H_2O$
15.5	"	H_3BO_3
12.5	"	$MnSO_4$

See also : Preparation of stock culture solution, Z8 100%, page 17.

$NaNO_3$ - solutions

a. 1 mg NO_3 - N/ml, dissolve 625 mg $NaNO_3$ in 100 ml distilled water.

b. 0.1 mg NO_3 - N/ml, dissolve 62.5 mg $NaNO_3$ in 100 ml distilled water.

K_2HPO_4 - solutions

a. 0.1 mg PO_4 - P/ml, dissolve 55.6 mg K_2HPO_4 in 100 ml distilled water.

b. 0.01 mg PO_4 - P/ml, dissolve 5.56 mg K_2HPO_4 in 100 ml distilled water.

4. STOCK CULTURE

Selenastrum capicornutum (green alga) is maintained in batch culture. 100 ml of stock culture solution is poured into a 250 ml conical flask. The flask is covered by a 100 ml beaker and autoclaved at 120°C for 20 min. After cooling one drop of the two weeks old stock culture is transferred aseptically into this flask. The flask is set onto the illumination table and is shaken once a day. This procedure is done in triplicate every second week.

5. TEST PROCEDURE

The test procedure is divided into four parts depending on the type of the test. The following procedures are the simpliest ones and the additions and dilutions should be kept as examples only.

a. Algal growth potential of waters

- A volume of 200 ml of sample water is filtered through a Whatman GF/C glassfibre filter. This filter can be used to measure the suspended solids on dry weight basis.
- Pour 100 ml filtered water into 250 ml conical flask.
- Transfer one drop of two weeks old stock culture of algae aseptically (not to contaminate the stock culture) into the flask.
- Cover the flask with a 100 ml beaker.
- Set the flask onto the illumination table.
- Shake the flask once a day.
- After 14 days make the chlorophyll a measurement according to appendix 2.

b. The limiting nutrient of a water body

- A volume of 1000 ml is filtered through a Whatman GF/C glass-fibre filter. This filter can be sued to measure the suspended solids on dry weight basis.
- Pour 100 ml aliquots of filtered water into 9 250 ml conical flasks.
- Add NO_3 - N and PO_4 - P into the flasks f. ex. according to the following table :

Flask	NO_3 - N.mg/l	PO_4 - P.mg/l
1	-	-
2	0.5	-
3	5.0	-
4	-	0.05
5	0.5	0.05
6	5.0	0.05
7	-	0.5
8	0.5	0.5
9	5.0	0.5

- Transfer one drop of two weeks old stock culture of algae aseptically (not to contaminate the stock culture) into the flasks.
- Cover the flasks with 100 ml beakers.
- Set the flasks onto the illumination table.
- Shake the flasks once a day.
- After 14 days make the chlorophyll a measurements according to appendix 2.

c. The effectiveness of waste water treatment plants.

- A volume of 200 ml of a continuous sample for 24 hours taken according to the effluent flow rate is filtered through a Whatman GF/C glassfibre filter daily.
- Pour 100 ml filtered water into 250 ml conical flask.
- Transfer one drop of two weeks old stock culture of algae aseptically (not to contaminate the stock culture) into the flask.

- Cover the flask with a 100 ml beaker.
- Set the flask onto the illumination table.
- Shake the flask once a day.
- After 14 days make the chlorophyll a measurements according to appendix 2.

d. The effect of different kinds of waste waters and other compounds in receiving waters.

- Filter a volume of 1000 ml of receiving water through a Whatman GF/C glassfibre filter.
- Make f. ex. the following dilution series with receiving water and waste water or compound in question into 250 ml conical flasks :

	Flask							
	1	2	3	4	5	6	7	8
receiving water, ml	-	25	50	75	90	95	99	100
waste water, ml	100	75	50	25	10	5	1	-
final volume, ml	100	100	100	100	100	100	100	100

- Transfer one drop of two weeks old stock culture of algae aseptically (not to contaminate the stock culture) into the flasks.
- Cover the flasks with 100 ml beakers.
- Set the flasks onto the illumination table.
- Shake the flasks once a day.
- After 14 days make the chlorophyll a measurements according to appendix 2.

6. CALCULATION

Calculation of the results are made according to appendix 2.

7. ADVISORY INFORMATION

National Eutrophication Research Programme, 1971 :
Algal Assay Procedure, Bottle Test. - Environmental Protection Agency, August 1971, 1 - 82.

Nordforsk, 1973 : Algal Assays in Water Pollution Research. - Nordforsk, Secretariat of Environmental Sciences, Publication 1973, 2, 1 - 128.

Nordforsk, 1975 : Jämförelse av algtestmetodik.- Nordforsk, Miljövårdssekretariatet, Publikation 1975, 4, 1 - 20.

PREPARATION OF STOCK CULTURE SOLUTION, Z 8 100%

1. COMPOSITION OF THE NUTRIENT SOLUTION

$NaNO_3$	467,0 mg/l	N	83,90 mg/l
$Ca(NO_3)_2 \cdot 4H_2O$	59,0 "	K	13,90 "
K_2HPO_4	31,0 "	Ca	10,00 "
$MgSO_4 \cdot 7H_2O$	25,0	P	5,51 "
Na_2CO_3	21,2 "	S	3,21 "
$FeCl_3 \cdot 6H_2O$	2,8 "	Mg	2,46 "
Komplexon III	3,7 "	Fe	0,58 "
Trace metals	See note 2.g.		

2. CHEMICALS AND CONCENTRATED SOLUTIONS

The chemicals should be "p.a. Merck" or other brands of equivalent quality.

a. $NaNO_3$:

46,700 g $NaNO_3$ is dissolved in distilled water and diluted to 1000 ml.
(Addition : 10 ml/l).

b. $Ca(NO_3)_2 \cdot 4H_2O$:

59,000 g $Ca(NO_3)_2 \cdot 4H_2O$ is dissolved in distilled water and diluted to 1000 ml. Take 100 ml out of the solution and dilute to 1000 ml.
(Addition : 10 ml/l).

c. K_2HPO_4 :

31,000 g K_2HPO_4 is dissolved in distilled water and diluted to 1000 ml. Take 100 ml out of the solution and dilute to 1000 ml.
(Addition : 10 ml/l).

d. $MgSO_4 \cdot 7H_2O$:

25,000 g $MgSO_4 \cdot 7H_2O$ is dissolved in distilled water and diluted to 1000 ml. Take 100 ml out of the solution and dilute to 1000 ml. (Addition : 10 ml/l).

e. Na_2CO_3 :

21,200 g (without water of crystalisation) Na_2CO_3 is dissolved in distilled water and diluted to 1000 ml. Take 100 ml out of the solution and dilute to 1000 ml.
(Addition : 10 ml/l).

f. "Fe-komplexon":

i. 2,700 g $FeCl_3 \cdot 6H_2O$ is dissolved in 100 ml 0,1 N HCl.

ii. 3,722 g komplexon III is dissolved in 100 ml 0,1 N NaOH.

iii. Transfer 10,5 ml of the $FeCl_3$-solution to a volumetric flask. Add approx. 500 ml distilled water and 10,0 ml komplexon III-solution. Dilute to 1000 ml with distilled water. (Addition : 10 ml/l).

g. Trace metal solution (Gaffron):

i. Weigh out of the following chemicals:

$Na_2WO_4 \cdot 2H_2O$	0,0660 g
$(NH_4)_6 Mo_7O_{24} \cdot 2H_2O$	0,1760 g
KBr	0,2380 "
KI	0,1660 "
$ZnSO_4 \cdot 7H_2O$	0,5740 "
$Cd(NO_3)_2 \cdot 4H_2O$	0,3080 "
$Co(NO_3)_2 \cdot 6H_2O$	0,2920 "
$CuSO_4 \cdot 5H_2O$	0,2500 "
$NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$	0,3960 "
$Cr(NO_3)_3 \cdot 7H_2O$	0,0740 "
$Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$	0,9480 "

The chemicals are dissolved in distilled water and diluted to 5000 ml.

ii. Weigh out the following chemicals:

V_2O_5 0,0447 g

The chemical is dissolved in distilled water and diluted to 1000 ml.

iii. Take H_3BO_3 (3,100 g). Dissolve in 100 ml distilled water (will require heating), add $MnSO_4$ (2,230 g) and dilute to 1000 ml.

iv. Take 250 ml out of the 2.g.i. solution. Transfer to a 1000 ml volumetric flask. Add approx. 500 ml distilled water. Thereafter add 20 ml of the 2.g.ii. solution and 100 ml of the 2.g.iii solution and dilute to 1000 ml.
(Addition : 0,8 ml/l).

h. CO_2-saturated water

Take approx. 10 l distilled water. Bubble with CO_2-gas- 2 hours
If CO_2-saturated water has been standing more than 6 hours it must be bubbled again with CO_2-gas.
(Addition : 30 ml/l).

3. COMPOSITION

a. Take a 1000 ml volumetric flask, fill to ca. half the volume with distilled water and add 30 ml/l CO_2-saturated water (2.h). Then add from 2.a., 2.b., 2.c., 2.d., 2.e., 2.f. solutions 10 ml/l, and from 2.g.iv. is added 0,8 ml/l. Fill to the mark with distilled water.

b. Measure pH. The solution shall have a pH of 6-7.

It is desirable that the solution is autoclaved at once.

4. AUTOCLAVING

Autoclave for 15 minutes at 15 pounds. After autoclaving the solution should be cooled to room temperature. This should take place as soon as possible, preferably by placing the flask in a cold water bath.

5. CHECK UP

The pH should lie between 7 and 8 and the conductivity around 830 micromho/cm (2°C).

6. REFERENCE

Källqvist, T., 1973 : Algal Assay Procedure (Bottle Test) at the Norwegian Institute for Water Research. - in : Algal Assays in Water Pollution Research, Nordforsk, Secretariat of Environmental Sciences, Publication 1973, 2, 5-17.

APPENDIX 2

CHLOROPHYLL a METHOD

1. INTRODUCTION

When measuring chlorophyll a concentration in natural waters we are also getting a relative figure of the phytoplankton concentration. Chlorophyll is extracted into 90% aceton. The optical density is measured by a spectrophotometer with wave lengths near to the absorption maximum for chlorophyll a, b and c (665, 645 and 630 nm). Correction for particulate matter and others is made with a higher wave length (750 nm), where absorption of chlorophyll is at its minimal.

2. APPARATUS

Glassfibre filters Whatman GF/C
Filtering apparatus
Homogenisator with teflon tip (tissue grinder)
Centrifuge
Graded centrifuge tubes (0-10 ml)
Spectrophotometer with 1 cm cyvettes

3. REAGENTS

$MgCO_3$ 1% : 1 g $MgCO_3$ in 100 ml distilled H_2O

Aceton 90% : 100 ml H_2O is poured in a 1000 ml volumetric flask. Fill up to the mark with aceton p.a. To be stored in dark flask.

4. MEASUREMENT

If filtering cannot be made straight after the sample has been taken, the sample must be kept in dark and cool place. 3 ml 1% $MgCO_3$/l is added to stabilize pH. At low pH values chlorophyll will change into phaeophytin.

a. A useful volume of sample is filtered on the glassfibre filter.

b. Filter is folded plankton inside, dried and stored in a desiccator, if the sample cannot be extracted directly after the filtration. They cannot be stored for more than three weeks.

c. Filter is folded again and clipped to stripes into the homogenisator tube.

d. 4 ml 90% aceton is pipetted into the tube .

e. Put the teflon tip of the homogenisator into the tube and start the motor. If it is difficult to grind the sample filter, the tube can be moved vertically a little.

f. Rinse the teflon tip with 3 ml 90% aceton, which also is poured in the tube. Pour the contents into a centrifuge tube and close it tightly.

g. The tube is put in freezer for 24 hours.

h. Centrifuge the tube for ten minutes with a speed of 3000 rpm.

i. Measure the absorbance in 1 cm cyvette with 630, 645, 665 and 750 nm. 90% aceton is used as reference.

5. CALCULATION

Chlorophyll a, $mg/m^3 = (11.6 \times D_{665} - 0.14 \times D_{630} - 1.31 \times D_{645}) \; v/l \times V$

D = reading of spectrophotometer with respective wave lengths when the reading of 750 nm is subtracted.

v = volume of the extract (ml)

l = length of the cyvette (cm)

V = volume of filtered water (l)

6. REFERENCE

Nordforsk, 1975 : Interkalibrering av "mätemetoder" för "bestämning" av klorofyll a. - Nordforsk, "Miljövårdssekretariatet" publikation 1975, 5, 1-21.