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REPORT ON COMPARISON OF CHLOROPHYLL $\underline{a}$

ANALYSES IN SOME LABORATORIES

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CONTENTS

1. ABSTRACT

A comparison exercise for quantitative determination of
chlorophyll a by extraction has been organized by JRC-
Ispra within the EURASEP-PROJECT. For this purpose, 250
samples with 5 different chlorophyll concentrations were
prepared by freeze drying the filtered algae (SCENEDESMUS
OBLIQUUS). The samples were sent to 24 European laborato-
ries for analysis. The results of the analysis from 17
laboratories have been evaluated and compared. Although
the results obtained appear consistent at the level of
the individual laboratories they diverge very widely
between laboratories. This suggests that the analyses
performed by the individual laboratories are accurate
but that small differences in methode and procedure bet-
ween laboratories destroy the effective comparability of
results.

2. INTRODUCTION

Quantitive determination of chlorophyll is important in
certain oceanographic studies. It is also important for
a comparison with remote sensing data of ocean colour.
The laboratory analysis procedures are well established
by UNESCO and in the "Pratical Handbook for Sea Water
Analysis" by Strickland and Parsons.
Considering the importance of accurate and precise deter-
mination of chlorophyll for interpretation of NIMBUS-7
CZCS-data, it was decided to make a comparison of results
from chlorophyll analysis performed in certain laborato-
ries which participate in the EURASEP-PROJECT.
The main tasks of the exercise were:

- To test a freeze-dried algae sample for
  comparison of chlorophyll analysis

- To confirm the comparability of the results
  from the laboratories involved in the exer-
  cise.

Initially, the intention was not to compare the methods
applied, but the scatter of results from different la-
boratories.

However, since the analysed chlorophyll values differed
by a factor of 7, it appeared necessary to carry out a
more detailed study of the chlorophyll standard used and
the analysis procedure.

The available results do not permit a complete analysis.
Nevertheless, some conclusions have been drawn.

3. METHODS

The standard samples sent to the laboratories were pre-
pared by filtering and freeze-drying of a defined quan-
tity of an algae solution. The five different algae
concentrations were obtained through different dilutions
of a single algae culture. Freeze-drying was carried out
by freezing the freshly filtered samples in liquid ni-
trogen and then drying them under vacuum at 10^{-3} Torr.
The filters were divided into 2 halves, packed in dark
plastic bags containing Silicagel to keep them dry and
stored at $-20°$ Celsius before shipping.

A scanning electron microscope examination (Figure 1)
of a sample shows the presentation of the algae.

All 22 laboratories listed in Table 1 received 10 samples.
Three laboratories did not send results from their ana-
lysis, and two have been excluded for incorrect spectro-
photometer calibration.

Some samples were stored at $-20°C$ and analyzed by 2 la-
boratories 6 months later.

4. RESULTS

The chlorophyll values received are shown in Tables 2
to 6. The five different chlorophyll concentrations
were calculated as an arithmetical mean value from
all results received and are represented in column"A".
Column "B" contains the chlorophyll values analysed by
the different laboratories.
For every laboratory the best fit was calculated using
the function

$$y = a + bx.$$

where x is the mean value and y represents the analy-
sed value of chlorophyll. The coefficients a and b for all
laboratories are shown in Table 7 together with the
correlation coefficients r.
To facilitate comparison of the laboratories, the coeffi-
cient b was rescaled setting the b value of labora-
tory 19 equal to 1. Column "b*" in Table 7 shows the
rescaled coefficients.

As an example, a plot of the curves obtained for Lab 05
(highest analysed chlorophyll values), Lab 19 (lowest
analysed chlorophyll values) and Lab 16 (medium analysed
chlorophyll values) is shown in Figure 2.

The results of the control measurements made after 6
months by Lab 17 and Lab 14 are shown in Figure B. They
were carried out on the basis of studying change of the
sample under the given storage conditions. The results
obtained are 25% lower for 14 and 40% higher for 17 than
the results from the first analysis. However these de-
viations are small considering the deviations between
the laboratories from the initial exercise.

5. DISCUSSION

The lack of detailed knowledge about the procedures
applied by the individual laboratories impedes the
interpretation of the results. Neverthless, some con-
clusions can be drawn.

- The results of the measurements can differ
  by a factor of 7 between laboratories.

- The dispersion of the values from a single
  laboratory is around 30%.

- Control measurments in 2 laboratories after
  6 months show a deviation of up to 40%.

The expected analysis values have been calculated, for
a statistical evaluation of the results based on the re-
gression equation of the results for each laboratory (Table 7).
The values are given in the Column "C" of the Tables 2 to 6.
The percentage difference between the expected values
(column "C") and the analysed values (column "B") are
shown in column "D".

The frequency distribution of these differences is
plotted in Figure 3.

This distribution shows that the mean scatter of the
measurements lies within ± 30%, if the characteristic
curve for each laboratory is taken into consideration.
This scatter is insignificant compared to differences
by a factor of 7 found among the laboratories.
The chlorophyll concentration of some samples has been
determined in 3 different ways by W.W. Gieskes at the
Netherlands Institute for Sea Research: spectrophoto-
metric, fluorometric and thin-layer-chromatographic
method (TLC).
The samples analysed by the TLC method contained half
as much chlorophyll b as chlorophyll a. Therefore, chlo-
rophyll a is overestimated by the spectrophotometric
and fluorometric methods.

The chlorophyll a values determined by the fluorometric method were 10% lower than those determined by the spectrophotometric method.

A number of laboratories have commented on the difficulty of extracting the chlorophyll from the fiber-glass-filters. This could be related to the high chlorophyll concentrations and to particular resistance of the cell-structure of Scenedesmus.
It would be beneficial to repeat the exercise to obtain a more detailed assessment of the methods used in the individual laboratories.

6. ACKNOWLEDGMENTS

I am grateful to all laboratories and persons participating in this experiment. Particularly, I would like to express my gratitude to Mr. W. Stephan for preparing all the samples used in the test. Dr. W.W.C. Gieskes made the additional analysis and gave helpful comments, for which I am grateful.

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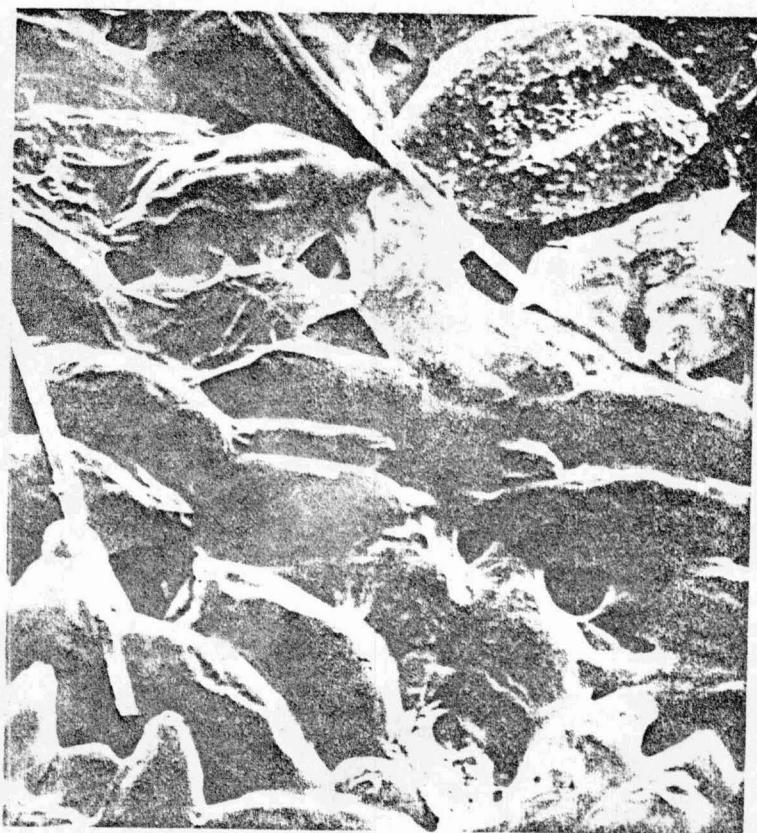
FIGURE 1: Scanning electron microscope picture of a
freezedried sample (SCENEDESMUS OBLIQUUS)
magnified 2600 times.
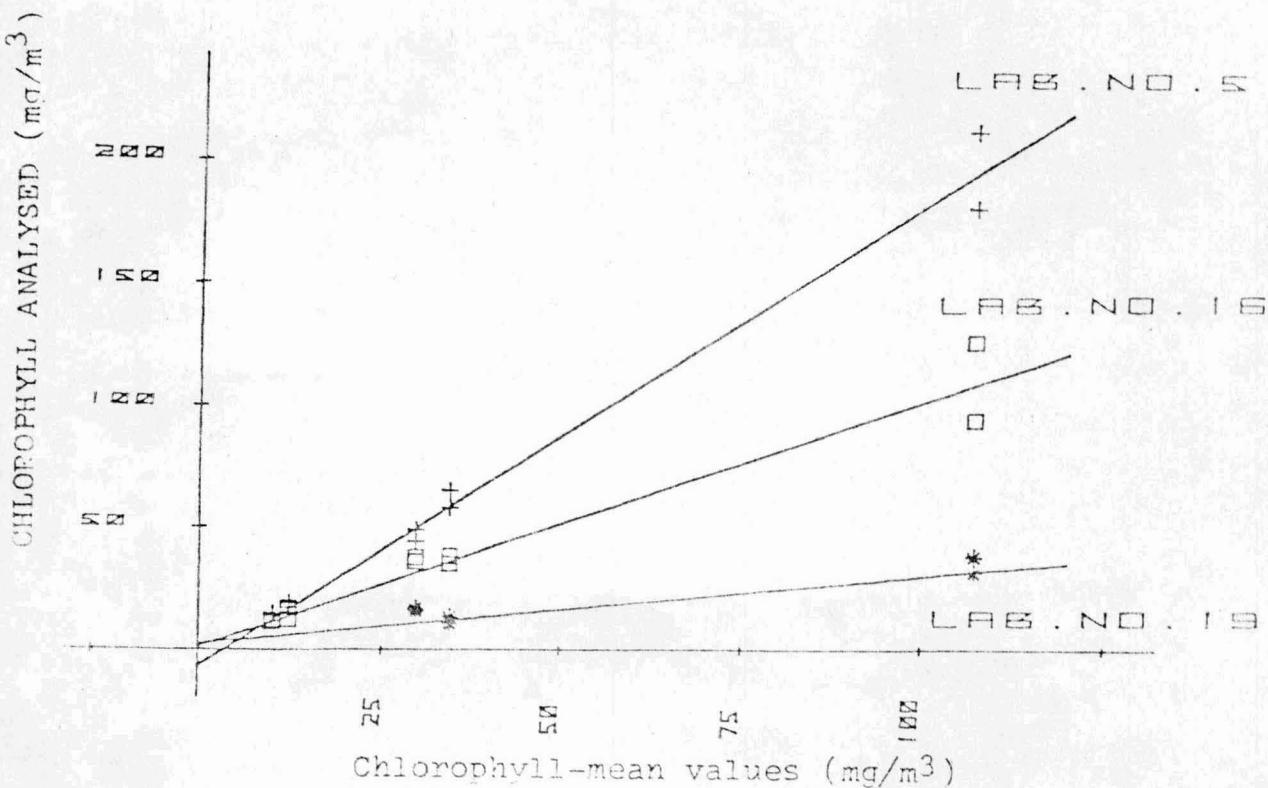
FIGURE 2: Plot of mean values versus analysed chlorophyll values for 3 laboratories

CLASS MIDPOINTS (%)
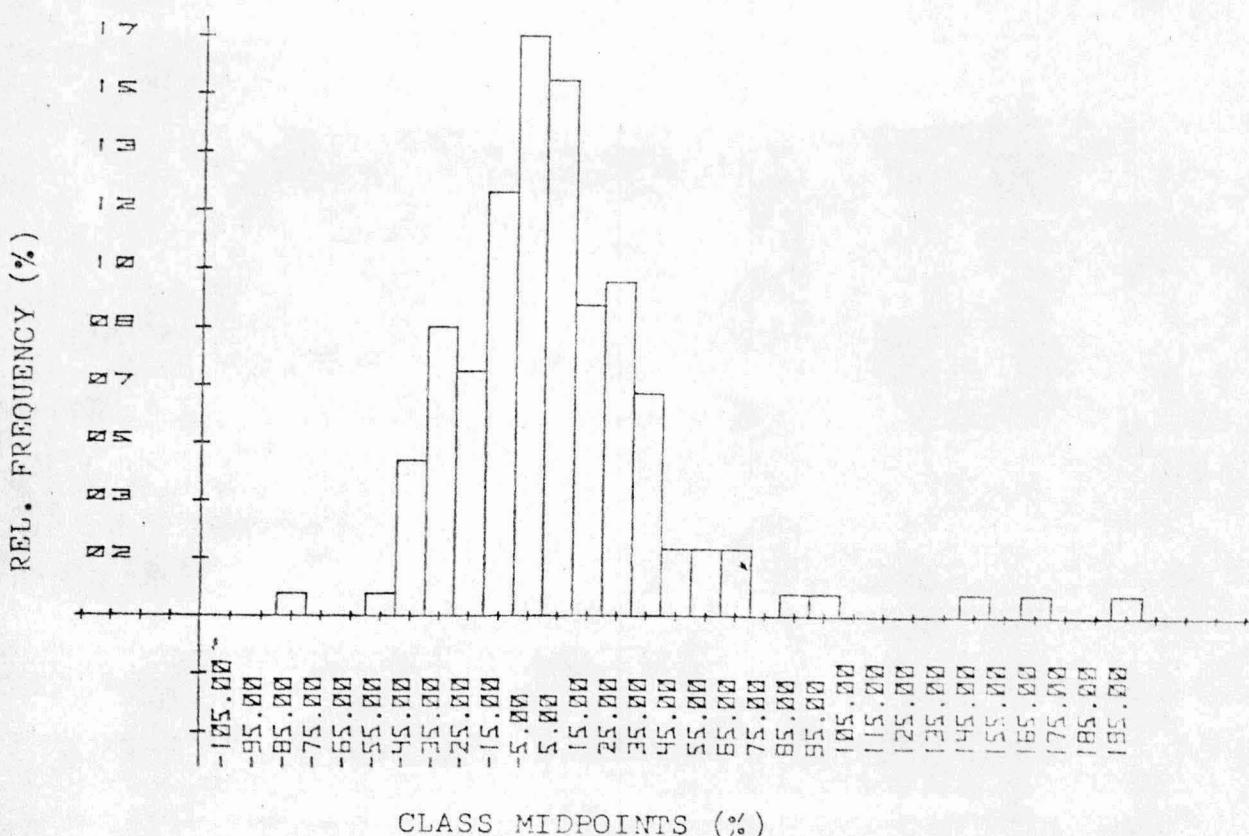
FIGURE 3: Frequency distribution of the differences between the expected values and analysed values (N = 155)

TABLE 1:   Laboratories involved

Ministére de la Santé Publique et de la Famille,
Oostende, Belgium
H. PICARD

GKSS, Geesthacht, W-Germany
R.DORFFER

Landesamt für Wasserhaushalt und Küsten, Kiel
S. BIERNATH-WUPPING

University of Copenahgen
N. HØJERSLEV

JRC-Ispra, Ispra, Italy
G.SERRINI

JRC-Ispra, Ispra, Italy
G.PREMAZZI

C.O.S.P.A.V., Chioggia, Italy
W.J. CANZONIER

Istituto di Idrobiologia, Pallanza, Italy
R. DE BERNARDI

Centre Océanologique de Bretagne Plouzané, France
M. CHAUSSEPIEDS

Marine Science Labs, Gwynedd, N-Wales, UK
J.SIMPSON

Imer, Plymouth, UK
C.W.FAY

University of Dundee, UK
J.A.CHARLTON

CNR, Venezia, Italy
R.FRASSETTO

IPSA, Roma, Italy
A. TODISCO

CNR, Venezia, Italy
L.ALBEROTANZA

Univ. Coll., Galway, Ireland
E.C.MONAHAN

Netherlands Institute for Sea Research, Texel, Netherlands
W.W.C. GIESKES

RIZA, Lelystad, Netherlands
H. VAN DER MARK

University of Stockholm, Stockholm, Sweden
M. NYQUIST

National Swedish Environment Protection Board, Uppsala
Sweden
B.ANDERSSON

TABLE 2: Mean Chlorophyll Concentration 11.10 mg/m^3

FILT. No.	LAB No.	CHLOROPHYLL(mg/m^3) mean	analysed	expected	(B-C)*100/C
		A	B	C	D
1a	01	11.10	8.40	12.66	-33.7
1b	02	11.10	15.10	14.72	2.6
2a	01	11.10	10.40	13.68	-18.0
2b	02	11.10	16.80	14.72	14.1
3a	03	11.10	9.81	8.14	20.6
3b	04	11.10	12.00	20.16	-40.5
4a	03	11.10	15.61	8.14	91.9
4b	04	11.10	12.40	20.16	-38.5
5a	05	11.10	16.00	13.25	20.8
6a	05	11.10	14.00	13.25	5.7
7a	06	11.10	4.01	5.97	-32.9
7b	07	11.10	14.30	16.81	-15.0
8a	06	11.10	5.81	5.97	-2.7
8b	07	11.10	14.60	16.81	-13.2
9b	09	11.10	7.20	6.19	16.4
10b	09	11.10	3.73	6.19	-39.7
11b	11	11.10	8.80	5.70	54.4
12b	11	11.10	7.20	5.70	26.4
13b	13	11.10	13.40	10.17	31.8
14b	13	11.10	10.90	10.17	7.2
15a	14	11.10	17.50	22.50	-22.2
17a	15	11.10	9.16	9.21	-0.5
18a	15	11.10	8.23	9.21	-10.6

TABLE 3: Mean Chlorophyll Concentration 8.85 mg/m^3

FILT. No.	LAB No.	CHLOROPHYLL(mg/m^3) mean	analysed	expected	(B-C)*100/C
		A	B	C	D
20a	01	8.85	8.80	11.15	-21.1
20b	02	8.85	12.20	9.91	23.1
21a	01	8.85	7.60	11.15	-31.8
21b	02	8.85	13.20	9.91	33.2
22a	03	8.85	17.65	5.98	195.3
22b	04	8.85	11.20	19.51	-42.6
23b	04	8.85	10.80	19.51	-44.6
24a	05	8.85	12.00	9.00	33.4
25a	05	8.85	11.00	9.00	22.3
26a	06	8.85	5.87	4.76	23.3
26b	07	8.85	11.40	14.25	-20.0
27a	06	8.85	3.22	4.76	-32.3
27b	07	8.85	9.90	14.25	-30.5
28b	09	8.85	3.73	5.38	-30.6
29b	09	8.85	1.06	5.38	-80.3
30b	11	8.85	6.80	2.82	141.3
31b	11	8.85	7.60	2.82	169.7
32b	13	8.85	10.60	6.37	66.5
33b	13	8.85	9.40	6.37	47.6
36a	15	8.85	5.47	7.95	-31.2
37a	15	8.85	6.23	7.95	-21.6
38b	17	8.85	6.80	5.86	16.1
39b	17	8.85	5.10	5.86	-12.9
42a	18	8.85	5.78	8.50	-32.0
43a	18	8.85	8.32	8.50	-2.1
44a	20	8.85	5.44	10.07	-46.0
44b	21	8.85	13.60	10.06	35.2
45a	20	8.85	7.67	10.07	-23.8
45b	21	8.85	18.20	10.06	80.9

TABLE 4: Mean Chlorophyll Concentration 26.86 mg/m³

FILT. No.	LAB No.	CHLOROPHYLL(mg/m^3) mean	analysed	expected	(B-C)*100/C
		A	B	C	D
46a	01	28.86	29.20	24.75	18.0
46b	02	28.86	47.50	52.73	-9.9
47a	01	28.86	36.80	24.75	48.7
47b	02	28.86	49.20	52.73	-6.7
48a	03	28.86	21.90	25.19	-13.0
48b	04	28.86	33.60	25.31	32.8
49a	03	28.86	14.66	25.19	-41.8
49b	04	28.86	27.60	25.31	9.1
50a	05	28.86	46.00	46.82	-1.7
51a	05	28.86	41.00	46.82	-12.4
52a	06	28.86	19.78	15.56	27.1
52b	07	28.86	38.80	37.06	4.7
53a	06	28.86	21.15	15.56	35.9
53c	07	28.86	37.70	37.06	1.7
54b	09	28.86	9.07	12.58	-27.9
55b	09	28.86	20.29	12.58	61.3
56b	11	28.86	26.40	28.43	-7.1
57b	11	28.86	24.40	28.43	-14.2
58b	13	28.86	36.30	40.18	-9.7
59b	13	28.86	35.30	40.18	-12.2
60a	14	28.86	50.80	48.25	5.3
61a	14	28.86	46.10	48.25	-4.5
62a	15	28.86	29.82	19.15	55.7
63a	15	28.86	24.65	19.15	28.7
64a	19	28.86	12.70	10.55	20.4
64b	17	28.86	16.50	18.26	-9.7
65b	17	28.86	19.00	18.26	4.0
67a	20	28.86	15.08	11.47	31.5
67b	21	28.86	32.00	32.87	-2.6
68a	18	28.86	33.20	35.51	-6.5
69a	18	28.86	25.48	35.51	-28.2
72a	19	28.86	13.90	10.55	31.7
72b	17	28.86	16.40	18.26	-10.2

TABLE 5: Mean Chlorophyll Concentration 33.68 mg/m³

FILT. No.	LAB No.	CHLOROPHYLL(mg/m^3) mean	analysed	expected	(B-C)*100/C
		A	B	C	D
73a	01	33.68	34.40	28.03	22.7
73b	02	33.68	70.40	63.05	11.7
74a	01	33.68	20.00	28.03	-28.7
74b	02	33.68	54.50	63.05	-13.6
75a	03	33.68	22.52	29.81	-24.5
75b	04	33.68	40.40	26.71	51.3
76a	03	33.68	24.81	29.81	-16.8
76b	04	33.68	45.20	26.71	69.2
77a	05	33.68	62.00	55.93	10.9
78a	05	33.68	55.00	55.93	-1.7
79a	06	33.68	9.10	18.17	-49.9
79b	07	33.68	48.80	42.56	14.7
80a	06	33.68	20.20	18.17	11.2
80b	07	33.68	49.20	42.56	15.6
81b	09	33.68	19.22	14.31	34.3
82b	09	33.68	14.15	14.31	-1.2
83b	11	33.68	33.60	34.60	-2.9
84b	11	33.68	24.40	34.60	-29.5
85b	13	33.68	48.90	48.33	1.2
86b	13	33.68	42.40	48.33	-12.3
87a	14	33.68	57.20	55.24	3.6
88a	14	33.68	59.40	55.24	7.5
89a	15	33.68	20.41	21.85	-6.6
90a	15	33.68	12.71	21.85	-41.8
91a	19	33.68	7.60	11.85	-35.9
91b	17	33.68	21.80	21.25	2.6
92a	19	33.68	10.50	11.85	-11.4
92b	17	33.68	23.20	21.25	9.2
93a	20	33.68	14.18	11.81	20.1
93b	21	33.68	32.60	38.37	-15.0
94a	20	33.68	15.33	11.81	29.8
94b	21	33.68	29.40	38.37	-23.4
95a	18	33.68	48.24	42.02	14.0
96a	18	33.68	53.26	42.02	26.8

TABLE 6: Mean Chlorophyll Concentration 106.28 mq/m^3

TILT. Ref.	LAB No.	CHLOROPHYLL(mg/m^3) mean	 analysed	 corrected	(A-C)*100/C
		A	B	C	D
99a	01	106.28	49.20	77.40	-36.4
99b	02	106.28	215.10	218.41	-1.5
100a	01	106.28	102.00	77.40	31.8
100b	02	106.28	224.00	218.41	2.6
101a	03	106.28	100.46	99.51	1.0
101b	04	106.28	44.80	47.76	-6.2
102a	03	106.28	104.29	99.51	4.8
102b	04	106.28	40.40	47.76	-15.4
103a	05	106.28	177.00	193.14	-8.4
104a	05	106.28	208.00	193.14	7.7
105a	06	106.28	56.72	57.37	-1.1
105b	07	106.28	127.60	125.32	1.8
106a	06	106.28	57.89	57.37	0.9
106b	7	106.28	118.80	106.28	11.8
107b	09	106.28	51.26	40.45	26.7
108b	09	106.28	26.70	40.45	-34.0
109b	11	106.28	132.40	127.53	3.8
110b	11	106.28	126.80	127.53	-0.6
111b	13	106.28	164.90	171.02	-3.6
112b	13	106.28	180.50	171.02	5.5
113a	14	106.28	162.30	160.51	1.1
114a	14	106.28	175.20	160.51	9.2
115a	15	106.28	80.19	62.51	28.3
116a	15	106.28	43.82	62.51	-29.9
117a	19	106.28	35.10	31.46	11.6
117b	17	106.28	56.00	66.26	-15.5
118a	19	106.28	28.60	31.46	-9.1
118b	17	106.28	76.00	66.26	14.7
119a	20	106.28	7.71	16.89	-54.4
119b	21	106.28	108.20	121.13	-10.7
120a	20	106.28	24.42	16.89	44.6
120b	21	106.28	138.00	121.13	13.9
121a	18	106.28	137.20	140.03	-2.0
122a	18	106.28	141.62	140.03	1.1
123a	14	106.28	145.00	160.51	-9.7
124a	14	106.28	158.00	160.51	-1.6

TABLE 7: Results of regresssion calculations for the function y = a + bx, where x is the mean value and y represents the analysed value of chlorophyll.
The correlation coefficient r is also given.

Lab N°	a	b	b^{3}	r
01	5.13	0,68	2,52	0,88
02	-9.03	2,14	7,93	1
03	-2.52	0,96	3,56	0,98
04	10,94	0,29	1,07	0,73
05	-7,73	1,89	7,00	0,99
06	-0,02	0,54	2,00	0,98
07	4.16	1,14	4,22	0,99
09	2,19	0,36	1,33	0,89
10	1,62	1.13	4,19	0,9
11	-8,51	1,28	4,74	1,00
13	-8.59	1,69	6,26	1,00
14	6,40	1,45	5,37	0,99
15	2.99	0,56	2,07	0,90
16	1,51	1,00	3,70	0,98
17	0.37	0,62	2,30	0,98
18	-3,45	1,35	5,00	0,99
19	2,76	0,27	1,00	0,95
21	-0,03	1,14	4,22	0,98