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Effects of different process wastes and main sewer effluents from pulp mills on the growth and production of Ankistrodesmus falcatus var. acicularis (Chlorophyta)

VARPU ELORANTA

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The effects of untreated effluents from the cellulose industry were studied by means of algal bioassays and modified BMT tests. In addition, different algal growth monitoring methods (pigment determination, ^{14}C method, O_2 production, cell counts) were compared in respect of their suitability for toxicity and inhibition tests. The results were used to compare the influences of the most closely corresponding process wastes and main sewer effluents from sulphate (Sa) and sulphite (Si) pulp mills.

The algae, *Ankistrodesmus falcatus* var. *acicularis,* were cultivated in constant culture conditions (20 ± 1 °C, 3000 lux given in 24-h light-dark cycles, CO_2 aeration of culture bottles). Twenty-five per cent Holm-Hansen nutrient solution was used in the bioassays, and filtrated and autoclaved oligotrophic humus-poor lake water in the BMT tests. The following effluents were studied: sulphate pulp mill — main sewer effluents I (acid) and II (basic), barking process water, black liquor, secondary condensates and effluents from the bleachery chlorination stage and first alkaline extraction stage; sulphite pulp mill — main sewer effluents I (collective sample) and II (from watercourse just below mouth of main sewer), thin liquor or spent liquor, acid condensate, main sewer effluent from the bleachery, and wastes from its chlorination stage and first alkaline extraction stage.

In the bioassays of the main sewer effluents, algal growth was most strongly inhibited by the acid effluents from the Si mill (toxic at 10 % concentration), but in the BMT tests the basic main sewer effluent from the Sa mill was most toxic to algae (clear inhibition at 1 % concentration). Of the waste waters from the separate processes, the black liquor was most toxic to algae, being followed by the spent sulphite liquor and the condensates. The chlorination effluent was more inhibitory in the Sa than in the Si mill and the reverse was true of the alkaline extraction effluent.

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1. Introduction

Many problems are attached to the study of the effects of effluents from the cellulose industry, since the composition of the waste waters changes rather quickly, owing to such factors as the presence of volatile sulphur compounds (Van HORN *et al.* 1950, HAYDU

et al. 1952, NYLANDER 1964, RUUS 1964, SEPPOVAARA & HYNNINEN 1970) and certain treatments (SEPPOVAARA & HYNNINEN 1970, DAVIS 1973, DAVIS & MASON 1973, ELORANTA 1973, MUELLER & WALDEN 1974, ROGERS *et al.* 1975). It is important to decide exactly what one intends to study. Does one wish to study 1) effluents in the form discharged into the waters, 2) the toxicity of effluents which are neutralized and aerated, or 3) the effects of completely untreated effluents, e. g. the process waters? For example, in Canada neutralized and aerated effluents have been studied for their residual toxicity to fish (cf. BETTS & WILSON 1966, DAVIS & MASON 1973, WALDEN *et al.* 1975). Since experimental laboratory conditions never correspond to natural lake conditions, only relative influences can be examined and elucidated.

Studies have showed that the waste waters of the cellulose industry have both stimulating and inhibiting effects on algal production, and further, that the effluents from sulphate (Sa) and sulphite (Si) pulp mills affect the receiving waters in different ways (KHOBOT'EV & BUCHVAROV 1969, LEHMUSLUOTO & HEINONEN 1970, NUMMINEN 1971, ELORANTA & ELORANTA 1974, SEPPOVAARA & NUMMINEN 1974, VILLA 1975, ELORANTA 1976). Moreover the effects of the waste waters from different processes vary widely (ELORANTA & ELORANTA 1974, ELORANTA 1976). However, little attention has been paid to discovering what kind of algal tests are most suitable for the study of these effluents, and the effects of the effluents from corresponding processes in Sa and Si mills have been not compared with each other. In most cases the waste waters from a Sa or Si pulp mill have been studied as a whole, and in addition, municipal effluents have often been included in the same investigation (LEHMUSLUOTO & HEINONEN 1970, NUMMINEN 1971, SEPPOVAARA & NUMMINEN 1974, VILLA 1975). These studies (with the green alga, *Ankistrodesmus falcatus)* have been performed by the so-called biomass titer method (BMT). The BMT test measures the amount of biomass produced with the nutrients of the test solution in bacteria-free conditions (BRINGMANN & KÜHN, 1956, 1958, 1960, 1962, 1965, 1966, KANGAS 1967, WARTIOVAARA 1971). These experiments were originally developed to elucidate the trophic status of natural waters and the eutrophizing influence of municipal effluents. In general, when the inhibitory and toxic properties of some substance or effluent are studied, a solution of a known nutrient content is used for the cultures, and the toxicity of the substance under study can be assessed from the decline in algal growth (WURTZ 1964). This kind of algal assay study has not been performed earlier with the waste waters from pulp mills.

The purpose of this study was to apply algal tests in research on the effects of effluents from the cellulose industry, and to study the suitability of algal bioassays, BMT tests and various methods of measuring and monitoring algal growth for research of this kind. A farther aim was to compare the effects of untreated process wastes and main sewer effluents from a Sa pulp mill with those of wastes from a Si mill. The research has been performed at the Department of Biology of the University of Jyväskylä, and the effluents and process waters used in the experiments are from Metsäliitto-Union, Äänekoski, and Serlachius Ltd., Mänttä.

2. Methods

2.1. Experimental alga and culture conditions

The green algal strain *Ankistrodesmus falcatus* (Corda) Ralfs var. *acicularis* (A. Braun) G. S. West used in the experiments was isolated by the drop dilution method (FOGG 1966) from the eutrophic lake, Jyväsjärvi. According to KOMARKOVA—LEGNEROVA (1969), the new name of this species is *Monoraphidium griffithii (Berkel.)* Komark.-Legner.

The algae were cultivated in a culture chamber at 20 ± 1°C (+293 °K) and in illumination of approx. 3000 lux, given in 24-h light-dark cycles (14 h light, 10 h dark). The cultures were set up in 150-ml Erlenmeyer flasks in 25 % Holm-Hansen nutrient solution (cf. BRINGMANN & KÜHN 1966). The culture flasks were closed with cotton plugs and supported by a culture stand (cf. ELORANTA 1975a). During the light period a mixture of air and 6 % CO_2 was led into the flasks for 2-min periods at intervals of 1 1/2

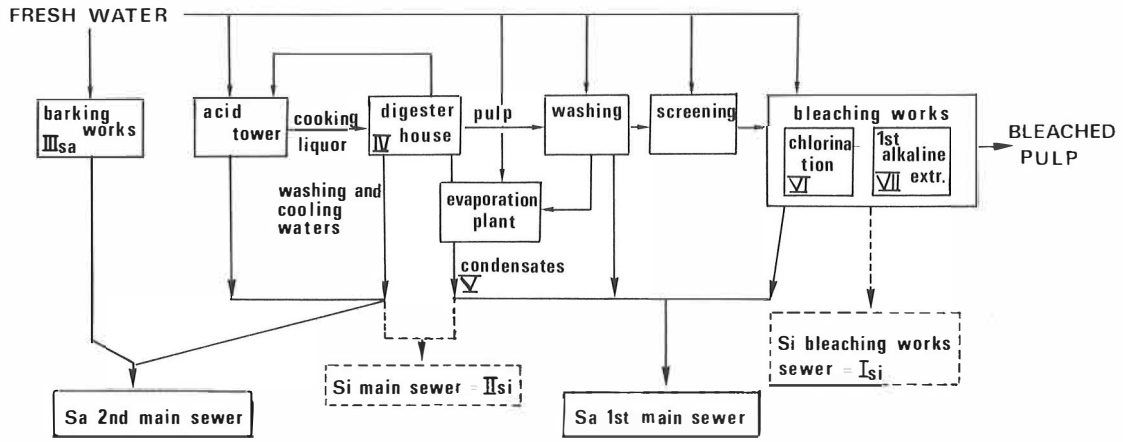

Fig. 1. Schematic diagram of operation of a pulp mill. The process wastes and effluents of the main sewers used in the study are also presented. For details of effluent properties, see text and Table 1.

h with the aid of a time switch (ELORANTA & ELORANTA 1974). The algal cultures were renewed weekly and purified by frequent culture on agar medium.

2.2. The effluents and test conditions

The following effluents from the Sa mill were used in the study (cf. Fig. 1 and also ELORANTA 1976): 1) effluent from the first main sewer (waste waters from the bleachery and condensates, I/Sa), 2) effluent from the second main sewer (waste waters from the barking process and washing waters from the cooking process, II/Sa), 3) effluent from the barking process (III/Sa), 4) black liquor (IV/Sa), 5) secondary condensates (V/Sa), 6) effluents from the chlorination stage (VI/Sa) and 7) the first alkaline extraction stage (VII/Sa), of the bleaching process.

The following effluents were studied from the Si mill: 1) effluent from the main sewer of the bleaching works (I/Si), 2) effluent from the main sewer of the pulp mill (collective sample of 24 hours, II/Si), 3) effluent from the main sewer of the cellulose factory (from watercourse just below mouth of sewer, III/Si), 4) spent liquor of thin liquor (IV/Si), 5) acid condensate from the evaporation plant (V/Si), effluents 6) from the chlorination (VI/Si) and 7) the first alkaline extraction stage (VII/Si) of the bleachery. Table 1 presents the chemical properties of the effluents. The results for partly similar Si effluents have already been presented in an earlier study (ELORANTA & ELORANTA 1974), and also part of the results of analyses of the Sa effluents (ELORANTA 1976).

The storage and treatment of the effluents were restricted to a minimum (no filtering or autoclaving unless otherwise mentioned). They were kept in completely filled air-tight glass bottles at + 4 °C (+ 277°K). In the BMT experiments the effluents were one day old, but in the bioassays they had sometimes been kept one to two weeks.

For the algal bioassays, the effluents were diluted to concentrations of 0.01, 0.1, 1.0 and 10.0 % with cultures which were in the exponential stage of growth, having chlorophyll *a* values of 2.2 µg/ml (cf. ELORANTA & ELORANTA 1974). For the biomass titer tests (BMT) effluents were diluted to concentrations of 0.1, 1.0 and 10. 0% with oligotrophic and oligohumic lake water, which had been filtered and autoclaved for 20 minutes at 130 °C. One drop of prediluted algal suspension was added to Pyrex test-tubes with 10 ml of the mixtures of lake water and effluent. The test-tubes were closed with a paraffin film and no CO_2 was led into these cultures. Each test series had three replicates at each concentration.

Table 1. Composition of effluents from sulphate (Sa) and sulphite (Si) pulp mills and lake water. (The analyses were mainly done by The Hydrobiological Research Institute of Jyväskylä).

		pH	Electr. cond. µS/20°C	$KMnO_4$ cons. mg/l	Tot.N mg N/l	Tot.P mg P/l	Tot.S mg S/l	SO_4 mg/l	H_2S mg/l	CH_3SH mg/l	BOD_7 mg/l	Solids mg/l	Lignin mg/l
Sa pulp mill													
I	1st main sewer	3.1	971	688	6.9	0.27	33.0	33.0	-	-	-	-	-
II	2nd main sewer	9.5	849	2500	20.9	0.64	42.0	42.0	-	-	-	-	-
III	Barking process	7.1	229	5440	25.5	5.31	-	-	-	-	805	2578	-
IV	Black liquor	12.0	27800	272000	129.4	11.97	11000	5100.0	5500	4300	-	-	-
V	Condensates	6.8	246	3600	18.3	20.01	96.0 x)	-	60	64	-	-	-
VI	Chlorination stage	2.3	5742	688	8.3	1.26	-	-	-	-	-	-	-
VII	1st alkaline extraction stage	9.9	445	917	14.8	0.42	-	-	-	-	-	-	-
Si pulp mill													
I	Bleaching works sewer	3.3	898	790	1.09	0.06	-	-	-	-	84	-	-
II	Main sewer I xx)	3.0	960	1201	1.44	0.15	152	280	-	-	195	-	150
III	Main sewer II +++)	4.5	304	1200	0.82	0.30	58	128	-	-	140	-	-
IV	Spent liquor	2.5	8300	369700	-	-	7200	1800	-	-	-	-	78750
V	Acid condensate	2.9	1013	8220	-	-	226	168	-	-	-	-	1400
VI	Chlorination stage	2.5	2113	890	0.94	0.03	-	-	-	-	-	-	-
VII	1st alkaline extraction stage	12.2	6800	4870	6.62	0.31	-	-	-	-	-	-	-
	Lake water	7.0	34	60	0.45	0.011	-	-	-	-	-	-	-

x) inaccuracies caused by evaporation of sulphur compounds
xx) collective 24-h sample
xxx) from watercourse just below mouth of sewer

2.3. Growth monitoring methods

In the bioassay studies the growth of the cultures was followed for four to seven days with cell counts, pigment determinations (chlorophyll *a*) and the ^{14}C method. The inactive chlorophyll *a*, or phaeophytin *a*, content of the cultures was determined after incubation for 24 hours. Further the immediate influence of the effluents was studied by measuring oxygen production in the cultures after incubation for three hours. In the BMT tests, the cell numbers (per unit volume) were counted weekly for three weeks.

For *the cell counts,* daily bioassay samples of 0.5 ml were exposed to ultrasonic vibration for a few minutes to break down the clusters of autospores and the samples were fixed with one drop of concentrated formalin. The BMT tests were run weekly. The bioassay samples were usually diluted 10—20-fold before the cell counts. The number of cells per ml was determined with an inverted microscope and counting chambers. Two transects were counted at magnifications of x600.

The chlorophyll a content of the cultures was determined on a hot methanol extract (90 % methanol) by spectrophotometry (cf. IWAMURA *et al.* 1970, ELORANTA & ELORANTA 1974, ELORANTA 1975 a).

The amount of phaeophytin a was measured on a cold acetone extract (90 % aqueous acetone). First, the absorption of chlorophyll *a* was measured with a spectrophotometer at 665 nm. Next, the extract was acified with 10 % HCl, and then after about 10 minutes it was neutralized with $MgCO_3$ and the optical density was redetermined at 665 nm (cf. MOSS 1967, LORENZEN 1967). The method used is described in more detail in an earlier study (ELORANTA 1975 b). The amount of phaeophytin *a* was calculated according to the equation presented by WETZEL & WESTLAKE (1971).

The ^{14}C technique was used for measuring photosynthetic activity. Five millilitres of algal suspension from flasks of the test series was measured into Pyrex test-tubes. Each effluent concentration had three replicates, two in light, one in dark tubes (the tube covered with black plastic). One millilitre (in a few cases 0.5 ml) of ^{14}C solution, with a radioactivity of about 1 μCi per ml was added to the test-tubes, which were shaken well, closed with cotton plugs and incubated for one hour on the culture stand. Immediately after incubation one to two drops of concentrated formalin were added to the tubes. The algae were collected on Millipore filters (pore size 0.45 μm) and the activity of the samples was measured with a Friesecke-Höpfner GM counter. Only relative impulse values were used in the examination of the results. An attempt was made to perform the ^{14}C tests at the same time of the day, so that they began in the middle of an interval between two CO_2 aeration periods.

In *the determination of the oxygen production* of the algae oxygen was removed as far as possible from the nutrient solution to be used by either boiling 25 % solution or leading nitrogen gas through it for 10 minutes. The pretreated nutrient solution and stock algae were mixed and the algal suspension was poured into 100-ml glass bottles with ground glass stoppers for use with different concentrations of effluents. The oxygen determinations were performed on algal cultures in which the amounts of chlorophyll *a* were 0.6, 1.1 and 2.2 μg/ml. Before the effluents were added the initial oxygen content of the algal suspension was determined by Winkler's method. The effluent concentrations were: 0.01, 0.1, 1.0 and 10.0 % (two replicates in light and one in dark bottles). There were three control flasks. The test flasks were immediately incubated for three hours on the culture stand. Five to seven test series were run for each effluent, with different biomasses. A test series was also run for each effluent in pure 25 % nutrient solution, to measure the chemical oxygen demand of the effluent for the test period. The COD and the oxygen required for respiration were taken into account in calculating the final net assimilation value.

2.4. Acclimation of stock algae in BMT experiments

Before the BMT experiments stock algae grown in the nutrient solution were trans-

Table 2. Influence of pretreatment of Si effluents on growth of algae in BMT tests. The numbers are the ratios of the cell counts of the effluent cultures to those of the controls. (Untreated/autoclaved: F = 5.90*; df = 34, untreated/filtrated: F = 0.0014: df = 34).

Effluent \ weeks	Untreated			Autoclaved			Filtrated		
	1	2	3	1	2	3	1	2	3
0.1 %									
Main sewer I	1.48	.75	.46	6.29	2.24	2.22	1.12	1.17	.48
Bleaching works sewer	.72	1.17	1.04	2.35	.98	1.78	1.24	1.42	.50
1.0 %									
Main sewer I	.40	.53	.88	1.47	1.18	1.52	.52	.50	.48
Bleaching works sewer	.84	.92	.63	.53	1.10	1.48	.68	.58	.48
10 %									
Main sewer I	.04	.11	.12	.70	.39	.35	.24	.25	.44
Bleaching works sewer	.40	.22	.08	.59	.31	.30	.28	.42	.08

ferred to lake water (cf. Table 1) for periods of 2 and 11 days. With the longer acclimation period, the algae were transferred after one week to new lake water. The stock algae used in the BMT tests of the Si effluents were acclimated for 11 days.

2.5. Treatment of the results

Common statistical methods were used in the treatment of the results. The dependence between results obtained by different measuring methods was studied by correlation analysis. Differences between the effects of the effluents were studied by one-way variance analysis. The chlorophyll values and ^{14}C fixation results obtained after 24- and 96-hour incubation were compared separately. Relative values (ratio between results and the mean of the controls of the test series)

Table 3. Acclimation of stock algae to lake water before Si BMT tests and its influence on algal growth. Incubation periods used for acclimation: two days and seven + four days. Ratios of cell counts of cultures to those of controls. The results are the means of 3—4 parallel tests. The strength of the influence of acclimation was studied with the analysis of variance (F). For composition of effluents, see Table 1.

Effluents	No acclimation			2 days			F	7 + 4 days			F
	1	2	3	1	2	3		1	2	3 weeks	
0.1 %											
Main sewer I	1.19	1.04	1.09	1.48	.75	.46		1.07	1.04	.85	
Bleaching works	.90	1.05	1.06	.72	1.17	1.04		.98	1.16	1.41	
Spent liquor	.43	.25	.16	-	-	-		.84	.80	1.02	
Acid condensate	.45	.32	.37	-	-	-		1.13	1.04	.80	
Chlorin. stage	1.12	1.14	1.13	.96	.92	.76		.85	1.07	1.22	
1st alkal. extr.	.92	.97	1.10	1.20	.75	.44		.93	.88	1.20	
							0.31				4.44^{+}
1.0 %											
Main sewer I	.51	.39	.39	.40	.53	.88		.85	1.05	1.12	
Bleaching works	.92	1.06	1.03	.84	.92	.62		.79	.87	1.20	
Spent liquor	.06	.06	.06	-	-	-		.17	.11	.15	
Acid condensate	.06	.05	.05	-	-	-		.17	.55	.78	
Chlorin. stage	1.02	1.03	1.07	1.60	1.08	.92		.91	1.30	1.68	
1st alkal. extr.	.43	.41	.42	.84	.83	.72		1.06	1.01	1.35	
							6.28^{+}				5.51^{+}
10.0 %											
Main sewer I	.06	.05	.04	.04	.11	.12		.03	.40	.17	
Bleaching works	.07	.04	.04	.40	.22	.08		.17	.13	.09	
Spent liquor	.04	.03	.03	-	-	-		.04	.02	.01	
Acid condensate	.05	.04	.02	-	-	-		.10	.08	.09	
Chlorin. stage	.06	.05	.04	.20	.14	.08		.26	.10	.15	
1st alkal. extr.	.05	.04	.06	.24	.55	.21		.17	.28	.51	
							19.99^{++}				12.27^{++}
$\bar{x}$											
Main sewer I	.59	.49	.51	.64	.46	.49		.65	.83	.71	
Bleaching works	.63	.72	.71	.65	.77	.58		.65	.72	.90	
Spent liquor	.18	.11	.08	-	-	-		.35	.31	.39	
Acid condensate	.19	.14	.15	-	-	-		.47	.56	.56	
Chlorin. stage	.73	.74	.75	.92	.71	.59		.67	.82	1.02	
1st alkal. extr.	.47	.47	.53	.76	.71	.46		.72	.72	1.02	
							5.83^{+}				8.05^{++}

were generally used in the comparison of the results obtained by the different measuring methods. This greatly facilitated the treatment and interpretation of the results, because such values immadiately showed the direction and strength of the influence of the effluent.

3. Results

3.1. Influence of pretreatments in BMT tests

3.1.1. Effluents

The changes in the influence of the effluents caused by autoclaving and filtering were elucidated (Table 2). After autoclaving the relative cell counts of the test cultures were generally higher ($F = 5.90^{*}$, df = 34) than those of the cultures with untreated effluent. At the lowest concentrations of the effluent, the difference was on the average three to four-fold. Autoclaving 1 % and 10 % Si effluent (II/Si) clearly increased the growth of the cultures more than autoclaving the bleachery effluent (I/Si) (Table 2).

Filtering the effluents did not cause significant changes in the growth of the algal cultures ($F = 0.0014$, df = 34). However, filtering the effluent from the bleaching works clearly decreased growth at a concentration of 1 %.

3.1.2. Stock algae

The cell counts were significantly higher ($F = 8.05^{**}$, df = 34) in the cultures in which the stock algae had grown 1 1/2 weeks in lake water before the BMT experiment than in the unacclimated cultures (Table 3). The stimulating effect of acclimation was more clearly apparent at the higher effluent concentrations than lower ones, but also showed well at low concentrations (0.1 %) of the acid effluents from separate processes, spent liquor and acid condensate, and in the cultures with 1 % effluent from the alkaline extraction stage and Si (II/Si). On the other hand, acclimation decreased algal growth in some test cultures with bleachery effluent (I/Si) and chlorination effluent (Table 3). Culturing in lake water for a short period (2 days) also generally increased the cell counts ($F = 5.83^{*}$, df = 28), although the opposite effect was also noted (I/Si, VI/Si). The growth-stimulating effect was greatest at the highest concentrations of effluent (Table 3).

3.2. Results of new monitoring methods used in bioassays

3.2.1. ^{14}C fixation experiments

When 1 ml of ^{14}C solution was used in the experiments, the carbon fixation values were clearly greater than when 0.5 ml ^{14}C solution was used but were not always double (Fig. 2). The fixation rate of the algae also depended to some extent on whether the algae were transferred to the test-tubes just before aeration of the cultures or just after. The difference was particularly clear when 1 ml of ^{14}C solution was used on the third and fourth day of the test period, in the phase of declining relative growth rate. At this time the fixation ability of the algae was gratest just after aeration with CO_2, differing in this respect from the other results (Fig 2). The pH values of the cultures were always about 0.15 — 0.40 pH unit greater before aeration than after it.

Measured as ^{14}C fixation, the assimilation of the experimental cultures was, generally greatest on the third or fourth test day (Fig. 3). The strongly inhibitory influence of the effluents was apparent in a shift in the ^{14}C fixation maximum (Fig. 3: B), and their toxic influence decreased fixation clearly (Fig. 3: F) or completely prevented it (Fig. 3: A, C).

The dark ^{14}C fixation in the cultures was very low, averaging 0.7—0.8 % of the light ^{14}C fixation.

3.2.2. Oxygen production

The decline of photosynthesis caused by the toxic effluents (for example spent sulphite liquor or black liquor) was evident as a sudden or gradual diminution of O_2 production (Fig. 4: E, F, H). Some effluents (Si effluent and condensates) slightly stimulated algal oxygen production at certain concentrations (Fig. 4: A, G). Some effluents had no visible influence on O_2 production (cf. bleachery effluent, Fig. 4: D).

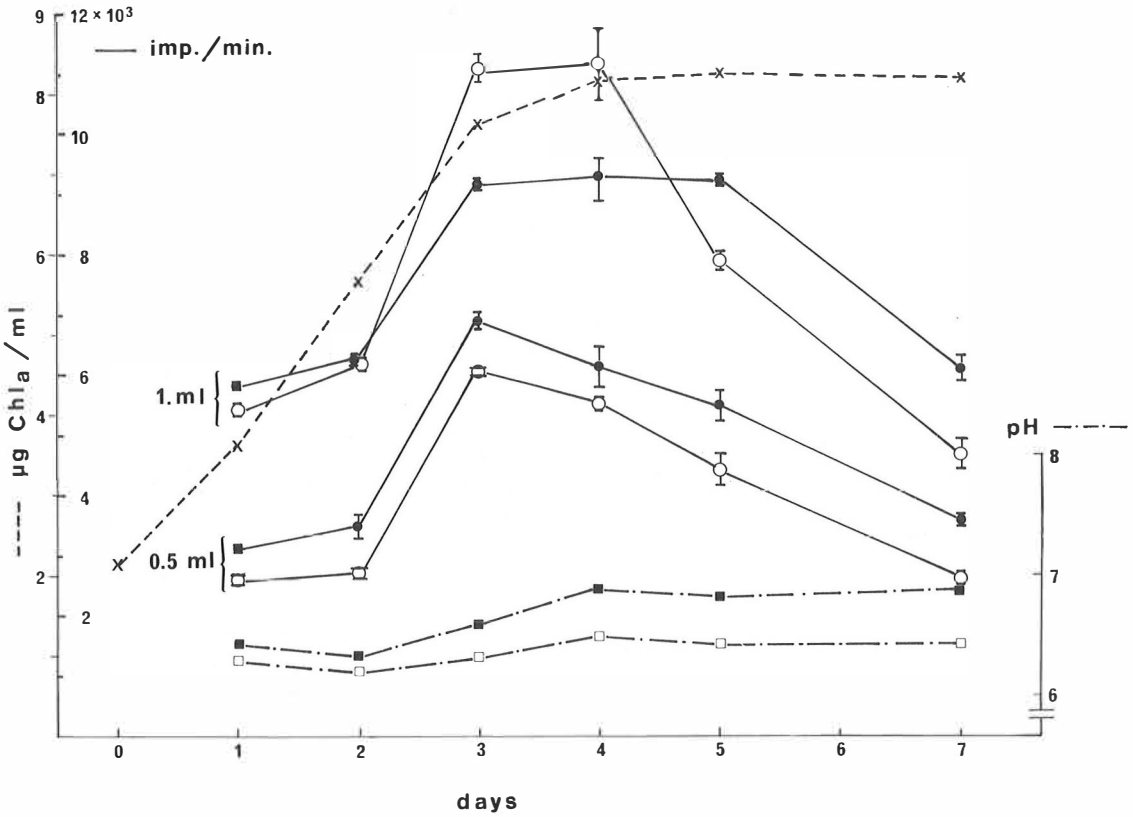

Fig. 2. Growth curves of *Ankistrodesmus* in 25 % Holm-Hansen nutrient solution as shown by chlorophyll *a* values (x — — — x) and ^{14}C fixation (——————: black dots = measured before CO_2 aeration, open rings = measured after it) of six parallel cultures. 0.5 ml or 1.0 ml of ^{14}C solution used. The standard errors of the means are presented as vertical lines. The pH values of the cultures (—.—.—) before (black square) and after (white square) CO_2 aeration are given.

3.3. Comparison of the methods used for measuring algal growth

During the lag and exponential growth phases, there were highly significant positive linear correlations between the chlorophyll *a* values and ^{14}C fixation ($r = .967^{***}$, $n = 78$; Fig. 5: 0—6 days), between the cell counts and the chlorophyll *a* contents ($r = .984^{***}$, $n = 31$) and between the cell counts and ^{14}C fixation ($r = .990^{***}$, $n = 33$). During the phase of declining relative growth rate and the stationary phase, the various methods gave differing results. The chlorophyll values showed a clear cessation of growth, while the ^{14}C fixation values decreased sharply and the cell counts continued to rise (Fig. 5).

Cell size diminished significantly ($r = .936^{**}$, $n = 7$) as the culture became older (Fig. 6). At the beginning of the growth stage (Fig. 6: 2nd day), the algal cells were significantly longer ($t = 3.255^{**}$, $n = 60$) than at the end of exponential growth (6th day); the average lenghts were 55.22 µm and 47.19 µm. The difference in algal cell length between the second and the last experimental day was highly significant ($t = 3.555^{***}$, $n = 60$).

In the effluent tests the chlorophyll values and ^{14}C fixation changed in the same way during the test period as in the control cultures (Fig. 7: A, C), although some differences were apparent (Fig. 7: B). The ^{14}C fixation rate of the cultures was clearly more sensitive to the effluents than the chlorophyll *a* contents, especially if the effluent was toxic to algae or strongly inhibited algal growth (Fig. 7: E, F).

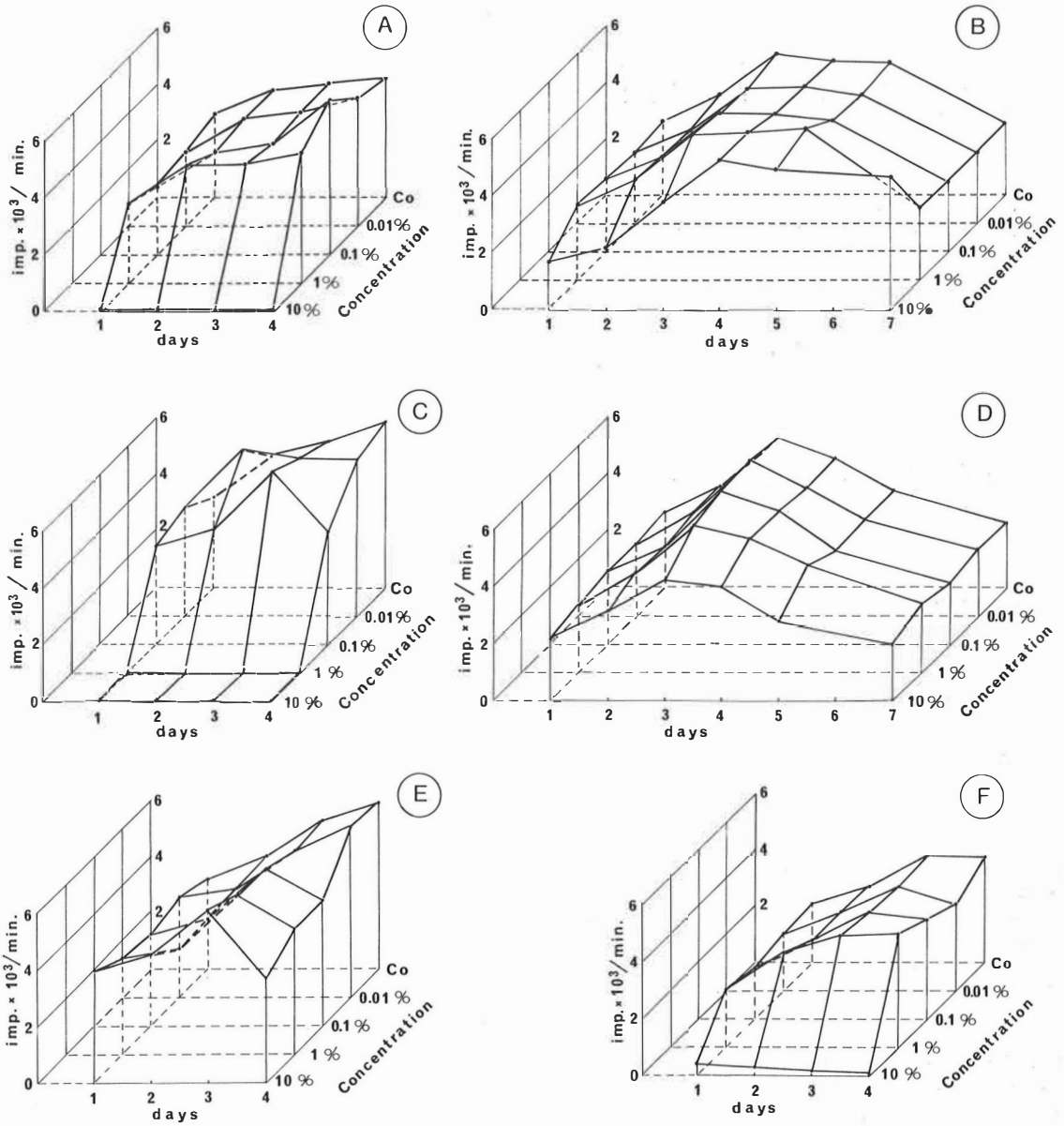

Fig. 3. Influence of the effluents on ^{14}C fixation of *Ankistrodesmus* cultures. Means of two parallel tests (+ one dark fixation). A = Si main sewer I, B = Sa 1st main sewer, C = spent sulphite liquor, D = Sa 2nd main sewer, E = Si chlorination stage, F = Sa chlorination stage.

The diagrams (Fig. 8 a and b) for the chlorophyll *a* values and ^{14}C fixation in the different effluent tests show that the values increased almost linearly during the growth stage of the cultures, but that their courses later varied fairly widely between the different tests. In some test series (Fig. 8 a, b: I/Sa, II/Sa, III/Sa) ^{14}C fixation began to decline while the chlorophyll values were still increasing or just beginning to level out (cf.

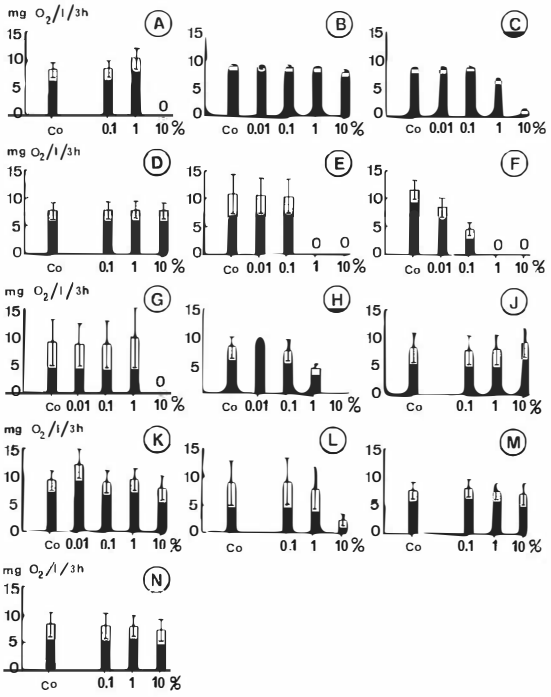

Fig. 4. Influence of the effluents at different concentrations on O_2 production of *Ankistrodesmus* cultures. Means of five ... seven parallel test series with a partly differing biomass. The standard errors of the means are presented as vertical lines. Incubation period 3 hr. A = Si main sewer I, B = Sa 2nd main sewer, C = Sa 1st main sewer, D = Si bleaching works sewer, E = spent sulphite liquor, F = black liquor, G = Si acid condensate, H = Sa secondary condensates, J = Si chlorination stage, K = Sa chlorination stage, L = Si 1st alkaline extraction stage, M = Sa 1st alkaline extraction stage, N = Sa barking process.

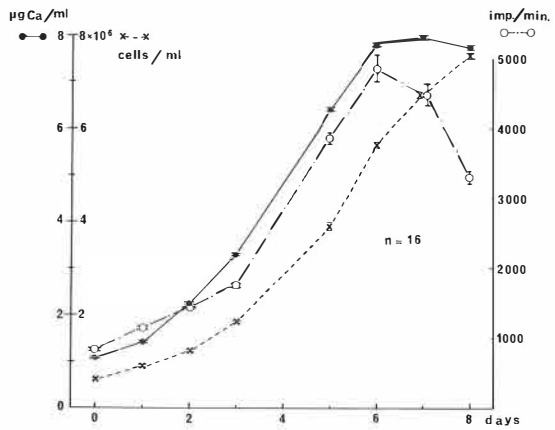

Fig. 5. Growth curves of *Ankistrodesmus* in 25 % Holm-Hansen nutrient solution as shown by chlorophyll *a* values (solid line and black dots), ^{14}C fixation (dotted line and open rings) and cell counts (broken line and crosses) of 16 parallel cultures. The standard errors of the means are presented as vertical lines.

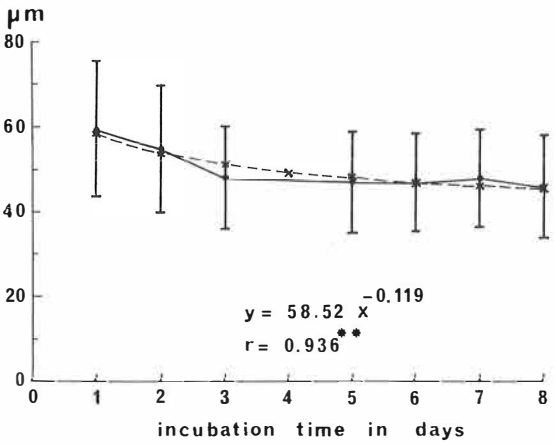

Fig. 6. Means and standard deviations of algal cell lenghts (black dots; n = 60) in *Ankistrodesmus* cultures during incubation, and correlation between cell lenghts and incubation time in days (broken line).

Fig. 5); in other series ^{14}C fixation was still clearly increasing (Fig. 8 a, b: I/Si, V/Sa, VII/Si), while the chlorophyll values were levelling out.

The results obtained by the different methods in the various test series are presented together with those of the controls in Table 4. The incubation period was 24 hours, except for the oxygen production measurements, where it was three hours. Clear positive correlations were found between the chlorophyll *a* values and the ^{14}C fixation values (Fig. 11: A, r = .843***, n = 55), between the chlorophyll *a* values and those for oxygen production (r = .743***, n = 48), between the chlorophyll *a* values and the cell counts (Fig. 13, r = .777***, n = 22), between the results of the methods measuring the rate of photosynthesis (cf. Fig. 11: B, r = .909***, n = 55) and between the cell counts and ^{14}C fixation (Fig. 12: A, r = .746***, n = 22).

After the incubation period of 96 hours, there was a highly significant positive correlation between the chlorophyll *a* values and ^{14}C fixation, and between the chlorophyll *a* values and the cell counts (Table 5 and Figs. 13, 14). Only the correlation between the cell counts and ^{14}C fixation was weaker (Fig. 12).

3.4. Si effluents and inactivation of chlorophyll a

The inactivation of chlorophyll *a*, or its change to phaeophytin *a*, after a 24-h incubation period was strongest in acid test waters (Fig. 9). Ten per cent spent liquor inactivated chlorophyll *a* completely; 10 % acid condensate changed ca. 60 % the total chlorophyll *a* to phaeophytin (Fig. 9: D, E). The 10 % effluents of the Si main sewer and the first alkaline extraction stage inactivated about 30 % of the total chlorophyll *a* (Fig. 9: A, F).

Phaeophytin *a* constituted at least 10 % of the total chlorophyll *a* of the control cultures at the beginning of expontetial growth.

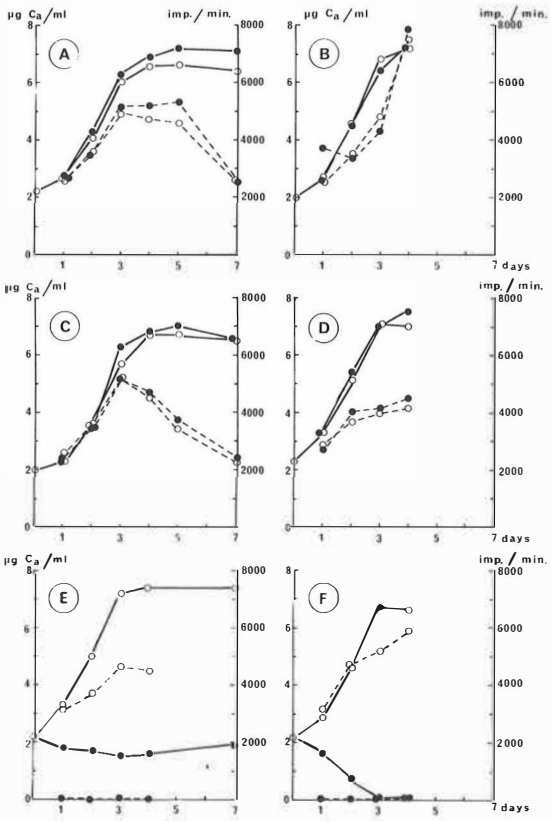

Fig. 7. Growth curves of *Ankistrodesmus* at 1 % concentrations of the effluents as shown by chlorophyll *a* values (solid line, n = 3) and ^{14}C fixation (broken line, n = 2). Black dot = test, open ring = control. A = Sa 1st main sewer, B = Si bleaching works sewer, C = Sa 2nd main sewer, D = Si main sewer (I), E = black liquor, F = spent sulphite liquor.

3.5. The BMT experiments with Si effluents

The pH and conductivity values for the effluent dilutions (0.1, 1.0, 10.0 %) are presented in Table 6.

The Si main sewer effluent (II/Si) was slightly toxic to algal growth at 10 % concentration, whereas the effluent taken from the receiving water, just below the mouth of the main sewer (III/Si) stimulated the growth of the cultures, this effect increasing with its concentration (Fig. 10: A, B). The spent liquor and acid condensate were toxic even at 1 % concentration, although the condensate ceased to have a toxic effect after two weeks (Fig. 10: C, D). Growth was also inhibited in the cultures with 0.1 % spent liquor. The effluent from the bleaching works (I/Si) was toxic at 10 % concentration, inhibitory at 1.0 % and slightly growth-stimulating at 0.1 % concentration. The chlorination effluent had a slight initial inhibitory effect at 1.0 % concentration, after which its influence differed from that of I/Si in being clearly growth-stimulating (Fig. 10: E, F). At 10 % concentration the alkaline extraction effluents had a toxic effect, which diminished towards the end of the experiment. At the lower concentrations its influence was more or less neutral (Fig. 10: G).

4. Discussion

4.1. Algal tests in research on the effect of effluents from the cellulose industry

4.1.1. Effluent age, storage and pretreatments

In many studies on the biological effects of waste waters from the cellulose industry (HOWARD & WALDEN 1965, DAVIS 1973, DAVIS & MASON 1973, JACOBS & GRANT 1974, ROSEHART *et al.* 1974) the toxicity of the effluents has been found to change, generally declining as they age,

Fig. 8 a

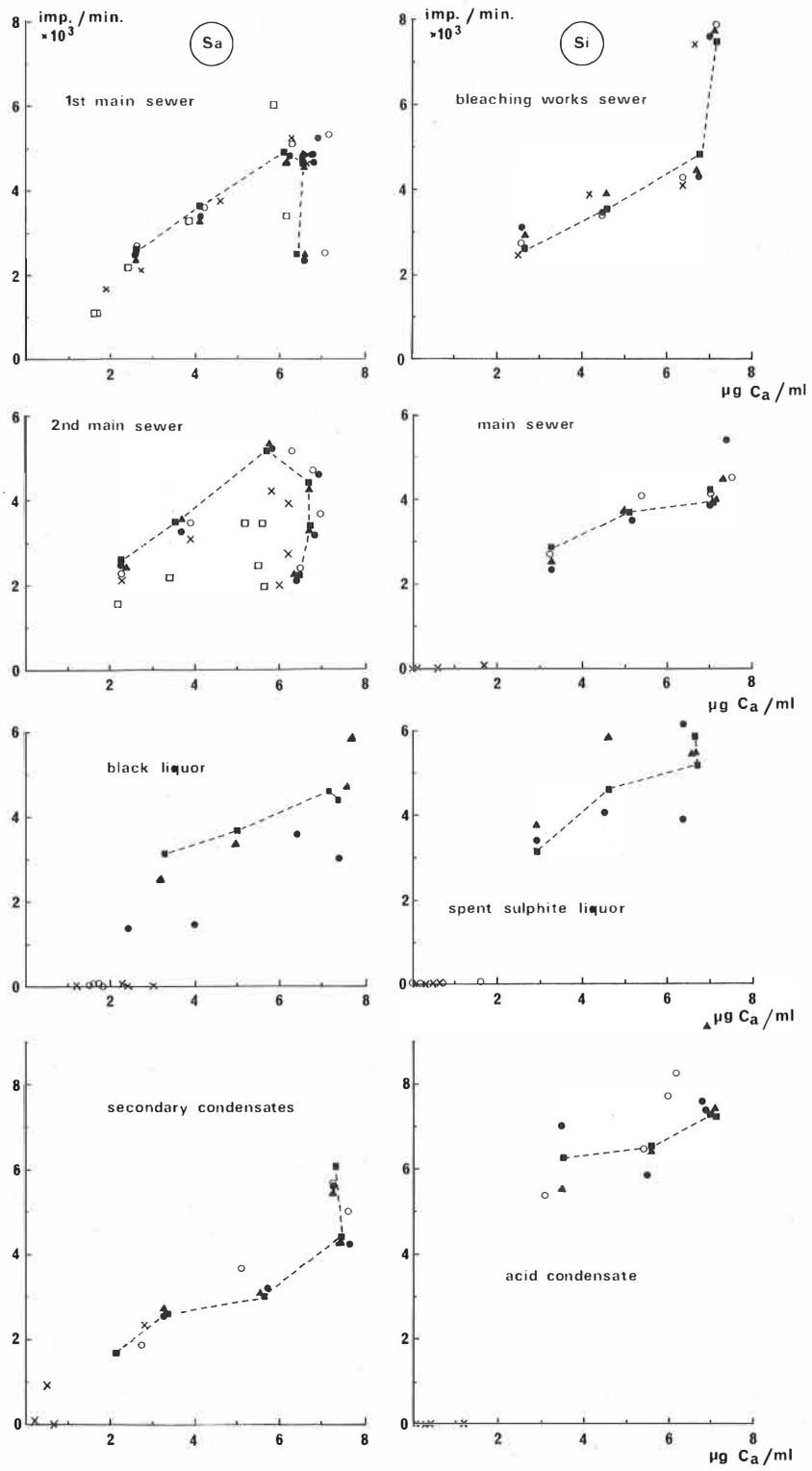
imp. / min. ×10³
Sa
1st main sewer
imp. / min. ×10³
Si
bleaching works sewer
µg Ca / ml
2nd main sewer
main sewer
µg Ca / ml
black liquor
spent sulphite liquor
µg Ca / ml
secondary condensates
acid condensate
µg Ca / ml

Fig. 8 b

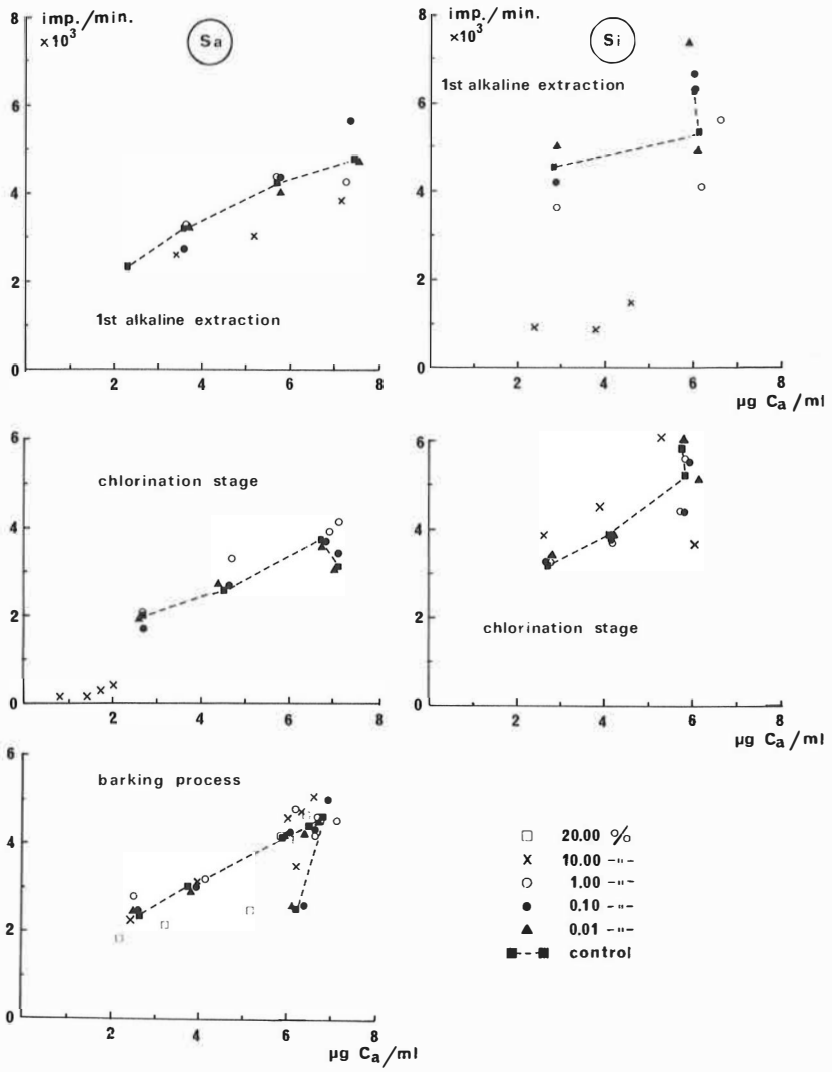

Fig. 8 a and b. Relation between chlorophyll *a* values and ^{14}C fixation in bioassays of effluents. Mean values of control series joined by a broken line.

even at low temperatures. The effluents should therefore be used when they are as fresh as possible. DAVIS and MASON (1973) found that their toxicity changed unpredictably with storage, even at 2 °C (storage 1—15 days): in some effluent samples toxicity declined, in some it increased and in a few it remained unchanged. According to JACOBS and GRANT (1974), unbleached kraft mill effluent did not lose its toxicity when stored for 5 to 7 days at 4 °C. In the study of ROSEHART *et al.* (1974), the BOD_5 and toxicity of unneutralized waste bisulphite liquor changed only slightly over one month. In the studies of WALDEN *et al.* (1975), the effluents were stored in completely filled, tightly sealed plastic jerricans at either 15 or 4 °C for 0 to 4 days.

Pretreatment also has a considerable influence on the toxicity of industrial waste waters (SEPPOVAARA & HYNNINEN 1970, ELORANTA 1973, MUELLER & WALDEN 1974, ROGERS *et al.* 1975). According to SEPPOVAARA and HYNNINEN (1970), aeration and steamstripping of condensates reduced their toxicity considerably. Conventional biological treatment systems can also

Table 4. Bioassay results after incubation of 24 h compared with those of controls. The effluents: I/Sa = Sa 1 st main sewer, II/Sa = Sa 2nd main sewer, III/Sa = barking process, IV/Sa = black liquor, V/Sa = condensates, VI/Sa = chlorination stage, VII/Sa = 1 st alkaline extraction, I/Si = bleaching works sewer, II/Si = Si main sewer I (collective sample), III/Si = Si main sewer II (from watercourse just below mouth of sewer), IV/Si = spent sulphite liquor, V/Si = acid condensate, VI/Si = chlorination stage, VII/Si = 1st alkaline extraction.

Effluent concn.	Effl.	Sulphate pulp mill: Chl a	Assimilation: ^{14}C-fix.	O_2 prod. x)	Cell count	Effl.	Sulphite pulp mill: Chl a	Assimilation: ^{14}C fix.	O_2 prod. x)	Cell count
0.01 %	I_{Sa}	1.00	.94	1.00	1.10	I_{Si}	1.00	1.13	1.04	-
	II	1.04	.95	1.01	.99	II	1.00	.89	1.09	-
	III	.96	1.03	1.00	.96	III	1.00	1.02	-	1.09
	IV	.97	.83	.70	-	IV	1.00	1.21	1.00	-
	V	1.00	1.03	.99	-	V	.99	.87	.97	-
	VI	.99	.96	.96	-	VI	1.04	1.11	.92	-
	VII	1.03	1.00	1.00	-	VII	1.04	1.10	1.03	.98
0.1 %	I_{Sa}	1.00	.99	1.00	.99	I_{Si}	.96	1.20	1.01	-
	II	1.00	.99	1.01	.95	II	1.00	.83	1.01	-
	III	1.00	1.16	.99	.97	III	1.00	.96	1.00	1.01
	IV	.74	.44	.40	-	IV	1.00	1.10	.99	-
	V	1.00	.96	.94	-	V	.99	1.11	.95	-
	VI	1.00	.88	.92	-	VI	1.00	1.01	.97	-
	VII	1.00	.86	1.05	-	VII	1.04	.92	1.04	1.02
1.0 %	I_{Sa}	1.00	1.02	.74	1.03	I_{Si}	.96	1.46	1.03	-
	II	1.00	.92	.99	.96	II	1.00	.95	1.23	-
	III	1.00	1.18	1.01	.96	III	1.00	.99	.99	1.23
	IV	.55	.02	.00	-	IV	.55	.02	.00	-
	V	.82	.71	.59	-	V	.87	.87	1.01	-
	VI	1.00	1.01	.96	-	VI	1.00	1.06	1.00	-
	VII	1.00	1.03	.98	-	VII	1.04	.80	.90	.79
10.0 %	I_{Sa}	.73	.63	.10	.66	I_{Si}	.93	.94	1.03	-
	II	1.00	.83	.86	.97	II	.52	.02	.00	-
	III	.92	.97	.89	(1.62)	III	1.00	1.14	.94	1.06
	IV	.36	.00	.00	-	IV	.24	.00	.00	-
	V	.18	.01	.00	-	V	.34	.00	.00	-
	VI	.74	.20	.72	-	VI	.96	1.23	1.18	-
	VII	.94	.80	.88	-	VII	.86	.22	.21	.57
20.0 %	I_{Sa}	.62	.45	-	.57					
	II	.96	.63	-	1.01					
	III	.85	.78	-	.61					

x) incubation of 3 h

Table 5. Bioassay results after incubation of 96 h compared with those of controls. For explanation of effluent symbols, see Table 4. Correlations between the results: chl a/^{14}C fix.; r = .895*** (n = 59), chl a/cell count; r = .754*** (n = 23), cell count/^{14}C fix.; r = .587** (n = 23). →

Sulphate pulp mill					Sulphite pulp mill			
Effluent concn.	Effluent	Chl_a	^{14}C fix.	Cell count	Effluent	Chl_a	^{14}C fix.	Cell count
0.01 %	I_{Sa}	1.00	1.02	.97	I_{Si}	.99	1.03	-
	II	1.00	1.00	1.05	II	1.04	1.06	-
	III	.98	.98	.97	III	1.03	.99	.96
	IV	1.04	1.32	-	IV	1.00	.95	-
	V	.99	.91	-	V	.99	1.28	-
	VI	.99	.96	-	VI	1.02	1.03	-
	VII	1.01	1.02	-	VII	.98	1.18	.98
0.1 %	I_{Sa}	1.03	1.02	1.02	I_{Si}	.99	1.01	-
	II	1.03	1.04	1.02	II	1.06	1.28	-
	III	1.02	1.01	1.00	III	1.03	1.00	.97
	IV	1.00	.68	-	IV	.97	.68	-
	V	.99	.92	-	V	.97	1.04	-
	VI	1.00	1.11	-	VI	1.02	.76	-
	VII	.99	1.20	-	VII	1.00	1.06	.99
1.0 %	I_{Sa}	1.05	1.10	1.05	I_{Si}	1.00	1.05	-
	II	1.01	1.05	1.10	II	1.07	1.06	-
	III	1.03	1.07	1.00	III	1.03	.98	1.37
	IV	.21	.01	-	IV	.00	.00	-
	V	.99	.92	-	V	.89	1.13	-
	VI	1.00	1.30	-	VI	1.00	.76	-
	VII	.97	.91	-	VII	1.10	.90	1.00
10.0 %	I_{Sa}	.95	1.10	.69	I_{Si}	.93	.99	-
	II	.93	.88	1.08	II	.00	.00	-
	III	.97	1.10	.79	III	.96	.94	1.18
	IV	.42	.00	-	IV	.00	.00	-
	V	.07	.15	-	V	.01	.00	-
	VI	.11	.04	-	VI	1.05	.62	-
	VII	.96	.81	-	VII	.77	.25	.44
20.0 %	I_{Sa}	.58	.71	.50				
	II	.84	.78	.65				
	III	.92	.97	.67				

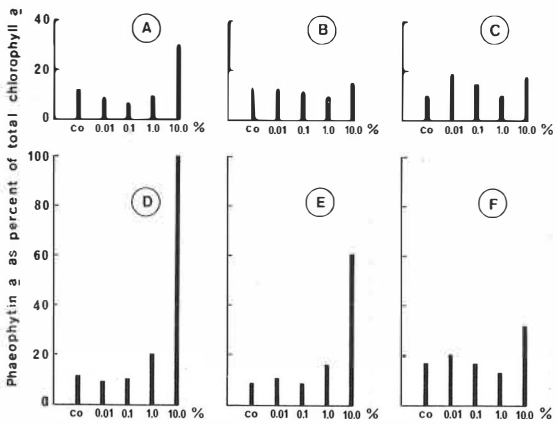

Fig. 9. Inactivation of chlorophyll *a* in bioassays during incubation of 24 h at different concentrations of effluents of sulphite pulp mill. Means of three parallel tests. A = Si main sewer (I), B = bleaching works sewer, C = chlorination stage, D = spent sulphite liquor, E = acid condensate, F = 1st alkaline extraction stage.

In the present experiments autoclaving the Si main sewer effluent clearly changed and decreased its effect (Table 2). A growth-stimulating effect, due to the nutrients contained by the effluent, became apparent, because autoclaving had evidently removed toxic compounds from the effluent (for example SO_2). The autoclaved Si bleachery effluent did not stimulate algal growth quite so clearly as the Si main sewer effluent. It evidently does not have such high contents of volatile and changing toxic compounds as the former, and is also less rich in growth nutrients (cf. Table 1). The effect of autoclaving on the pH of the test cultures was not studied. VILLA (1975) noted in her BMT diminish the toxicity of bleached kraft mill effluents (MUELLER & WALDEN 1974, ROGERS *et al.* 1975). ROGERS *et al.* (1975) found that the biological treatment time greatly affected the decline of the toxicity, BOD, and resin acids of bleached kraft mill effluents. Deep-freezing effluents also diminished their toxicity (ELORANTA 1973).

Table 6. pH and electrolytic conductivity at different concentrations of effluents of sulphite pulp mill diluted with lake water.

	0.1 %		1.0 %		10.0 %		100.0 %	
	pH	$\varkappa_{20}$ µS	pH	$\varkappa_{20}$ µS	pH	$\varkappa_{20}$ µS	pH	$\varkappa_{20}$ µS
Lake water	-	-	-	-	-	-	7.2	39
Si main sewer I	7.2	38	6.2	44	3.8	119	3.0	960
- " - II[x)]	7.2	38	6.8	42	5.7	70	4.5	304
Spent liquor	5.2	85	3.9	230	3.1	1600	2.5	8300
Acid condensate	7.0	37	4.8	49	3.7	149	2.9	1013
Bleaching works sewer	7.2	41	6.8	45	4.6	92	3.3	898
Chlorination stage	7.2	41	6.4	41	3.5	185	2.5	2113
1st alkaline extraction stage	7.2	43	9.4	78	11.0	631	12.2	6800

x) = from watercourse just below mouth of main sewer

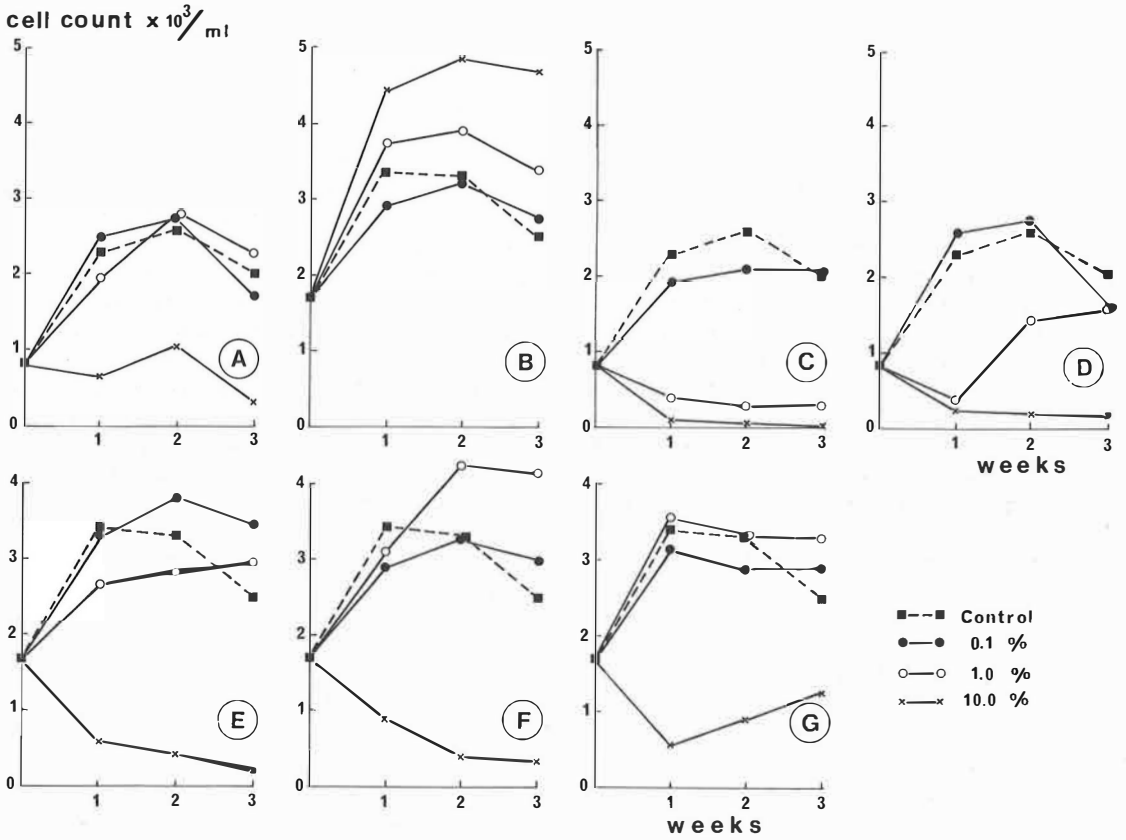

Fig. 10. Growth of *Ankistrodesmus* in oligotrophic lake water (BMT test) at different concentrations of effluents from sulphite pulp mill. Means of three parallel tests. A = Si main sewer (I), B = Si main sewer (II; from watercourse just below mouth of main sewer), C = spent sulphite liquor, D = acid condensate, E = bleaching works sewer, F = chlorination stage, G = 1st alkaline extraction stage.

tests that autoclaving may cause changes in waste water.

It is thus questionable whether typical BMT tests (pure cultures) should be used to study the effects of untreated effluents of the wood-processing industry, because the properties of the effluents may be greatly changed by autoclaving.

Since filtering the effluents did not have a significant effect on algal growth (Table 2), the solid matter or bacteria in the effluents evidently do not have a notable influence on the test results. There were, however, some signs that filtering the effluent from the bleaching works decreased algal growth, which would suggest that the bacteria have a growth-stimulating effect.

4.1.2. Pretreatment of stock algae

It has often been noted in algal tests that acclimation of the stock algae to the experimental conditions is essential for realiable test results (cf. OSWALD & GAONKAR 1969, LANDNER & ÖSTERMAN 1970, RŮŽIČKA 1971, FORSBERG 1972, CLAESSON 1975). When the nutrient contents of effluents (or lake water) are studied in BMT tests, the test algae must first use up their own stored nutrients, with which they may be able to grow for at least a few days (cf. RODHE 1948). In the present studies of toxic effluents, the acclimation of the stock algae to the BMT experimental conditions was also found to be important (Table 3). In the tests of Si effluent acclimation generally increased

algal growth. During acclimation the algae became accustomed to the nutrient-poor lake water. The effluents from the bleachery sewer and the chlorination stage sometimes inhibited algal growth more in the acclimated than the unacclimated cultures. A starved algal strain may be more sensitive to certain toxic effects than a healthy one. The above-mentioned waste waters may also be too poor in nutrients for the algae (cf. Table 1).

The acclimation period of 11 days used in this study was evidently long enough. The algae transferred to the lake water had time to form many new generations and renew themselves completely. The period of two days was probably too short, although it clearly changed the growth of the algal cultures compared with the untreated cultures (Table 3). The periods recommended in the literature for the acclimation of stock algae vary greatly. OSWALD and GAONKAR (1969) suggest many weeks, RŮŽIČKA (1971) 2—6 days, or 2—3 weeks for pronounced changes. FORSBERG (1972) used 7 days.

4.1.3. Methods for measuring and monitoring growth

General

Algal growth can be determined by many different methods in static bioassays. Four methods are accepted for standardization in PAAP (the Provisional Algal Assay Procedure): 1) gravimetric, 2) absorbance, 3) cell count and 4) ^{14}C. In addition, two additional methods have been presented for more careful study, 5) fluorescence and 6) volumetric (OSWALD & GAONKAR 1969). LANDNER and ÖSTERMAN (1970) have compared many different methods for monitoring algal growth, including most of the above. The gravimetric method was not satisfactory, mainly because of the poor repeatability of the results. The absorbance method proved to be fairly useful, particularly with special culture bottles which can also be used in measuring absorbance, though the results are purely relative. Cell counting with the haemocytometer was found to be suitable as basic method used together with some other technique (absorbance or fluorescence). Measuring the chlorophyll fluorescence of cultures *in vivo* took large amounts of culture solution, which limits the number of measurements that can be made during a long monitoring period. LANDNER and ÖSTERMAN (1970) determined the chlorophyll content only in cultures of filamentous blue-green algae using 90 % acetone extract. CLAESSON found (1975) good correlation between cell volume *(Selenastrum capricornutum)* and dry weight in six samples. SKULBERG (1964) recommends determination of chlorophyll content in preference to cell counts or measurements of absorbance or turbidity in algal bioassays undertaken in studies of eutrophication. According to the literature, cell counting is the most general method of monitoring the growth of algal cultures. It is performed with either the microscope and haemocytometer, or the Coulter Counter.

The methods of measuring algal growth used in this study can be divided into two groups: 1) the ^{14}C fixation and O_2 production methods, which measure the physiological activity of the cultures, and 2) pigment determinations and cell counts, which measure the biomass of the cultures.

^{14}C method

The ^{14}C method is fairly easy and is satisfactory if only relative values are needed. A further advantage of the method, especially in the toxicity and inhibition tests, is its sensitivity (cf. Fig. 7 and Table 4). However, the method demands very carefully standardized and controlled culture conditions, because, it reflects all the changes in the state of the culture (cf. Fig. 2, the periodic CO_2 aeration). The variation in the pH of the cultures between the aeration periods was so small (cf. Fig. 2) that it should not have any noticeable effect on algal photosynthesis or on the form in which the carbon used in photosynthesis occurs (cf. ÖSTERLIND 1947, 1948, ROUND 1970, DUBINSKY & ROTEM 1947).

It is generally assumed that when two isotopes of carbon are available at the same time as a source of inorganic carbon, plants fix them photosynthetically in proportion to the partial pressures at which they occur (VOZNESENSKII *et al.* 1971). This is evidently the main reason for the difference between the amounts of radiocarbon fixed before and after CO_2 aeration (Fig. 2). Although many studies (HOLM-HANSEN *et al.*

1958, YEMM & BIDWELL 1969) have shown a certain discrimination (2—5 %) against carbon-14 dioxide during photosynthesis, it is possible that some external source of error in the test conditions has influenced the results (cf. VOZNESENSKII *et al.* 1971). The apparent discrimination against radiocarbon may be due mainly to dilution of the $^{14}CO_2$ by respiratory $^{12}CO_2$.

The maximum values for ^{14}C fixation were measured at the time when, according to the chlorophyll values, the exponential growth of the cultures was ending (cf. Figs. 2, 5, 7). The physiological scenescence of the algal cultures was much more clearly revealed by the ^{14}C fixation values than by the chlorophyll values. Changes in the composition of the nutrient salts and the accumalation in the cells of fat or assimilation products in general cause a decline in photosynthesis in an aging culture (cf. HOLM-HANSEN 1967, STEEMANN NIELSEN *et al.* 1969, ROUND 1970).

The differences in the ratios between the chlorophyll and ^{14}C fixation values presented in Fig. 8 a and b may be due mainly to technical variation in the ^{14}C method, chiefly in the amount of ^{14}C solution used (cf. Fig. 2). The amount of radio-carbon available caused the clearest difference the ^{14}C fixation and chlorophyll values at the end of the exponential growth period. In most cases ^{14}C fixation was used to follow the growth of the cultures only up to the fourth day, and thus it does not give a really complete picture of the development of the cultures.

Measurement of oxygen production

Measurements of the O_2 production of cultures are rather awkward to perform and require abundant algal test solution. The repeatability was good in the replicates but the arrangement of the test conditions may give rise to many errors. When comparing biological tests for industrial waste waters, WARTIOVAARA (1971) concluded that the accurate arrangements required in the oxygen method limit its application in practice. The rather great deviation in the absolute amounts of oxygen measured was mainly due to differences in the amounts of algae used in the experiments (Fig. 4).

Determination of pigments

Determination of the chlorophyll *a* content of the cultures is a fairly easy and rapid method of monitoring algal growth (cf. ELORANTA 1976). It can be used during a long test period, too, but its chief disadvantage is that a certain amount of the algal suspension under study must be taken for each measurement. The results of the replicates deviated very little (Fig. 5 and ELORANTA 1976). The chlorophyll values of the culture measured in the test period form a clear sigmoid growth curve (cf. FOGG 1966). The chlorophyll content of the cultures does not reflect changes occurring in the culture conditions so sensitively as the measurements of the assimilation rate. This is both an advantage and a disadvantage, and makes the method unsuitable for studying rapid changes in the cultures.

In tests of toxic, and especially acid, effluents the chlorophyll determinations can be accompanied by determinations of inactive chlorophyll, phaeophytin *a* (Fig. 9 and ELORANTA 1975 b). This study showed that many problems are still encountered in the determination of phaeophytin *a,* although many methods were tested, alone and together (cf. MOSS 1967, LORENZEN 1967, WETZEL & WESTLAKE 1971, MARKER 1972). The methods of determining phaeophytin *a* evidently require further development, but that used in the study gave a fairly good picture of phaeophytin values in the different effluent cultures (Fig. 9). At 10 % concentration, the acid chlorination effluent decreased the pH of the cultures to only 5.7, which explains the relatively low measurement for phaeophytin.

Cell counts

The microscopic counting of cell numbers has already been criticized as a very laborious method of monitoring growth (ELORANTA 1976). CLAESSON's study (1975) showed very clearly how widely cell numbers may vary between different laboratories. As the size, form and condition of the algal cell may change when circumstances become critical for growth (cf. STEEMANN NIELSEN *et al.* 1969, RŮŽIČKA 1971), it is questionable whether the number of algal

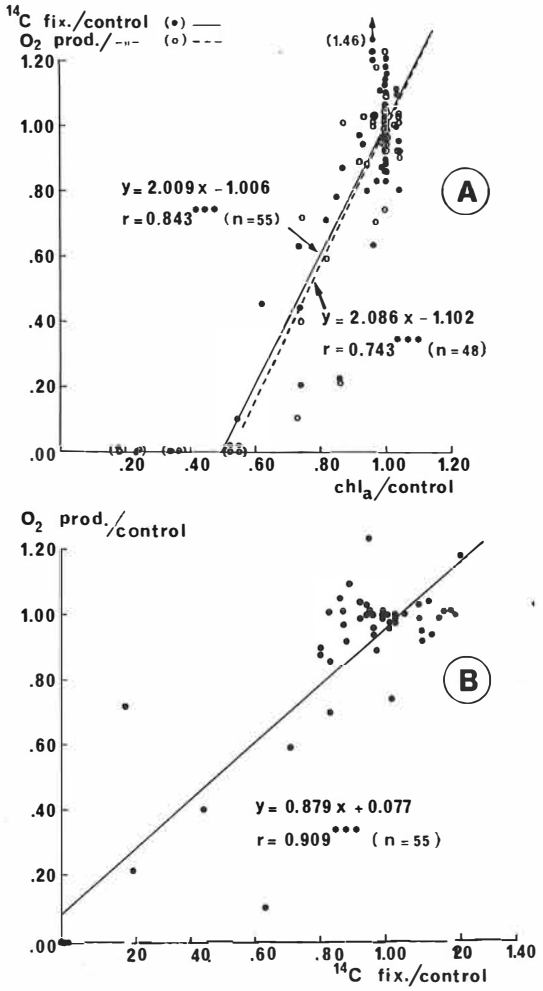

Fig. 11. A) Correlations between chlorophyll *a* values and the ^{14}C fixation (solid line) and between chlorophyll *a* values and oxygen production (broken line) in bioassays of different effluents after incubation of 24 h. Cf. Table 4. The values within parentheses have not been taken into consideration. B) Correlation between ^{14}C fixation and oxygen production in bioassays of different effluents after incubation of 24 h. Cf. Table 4.

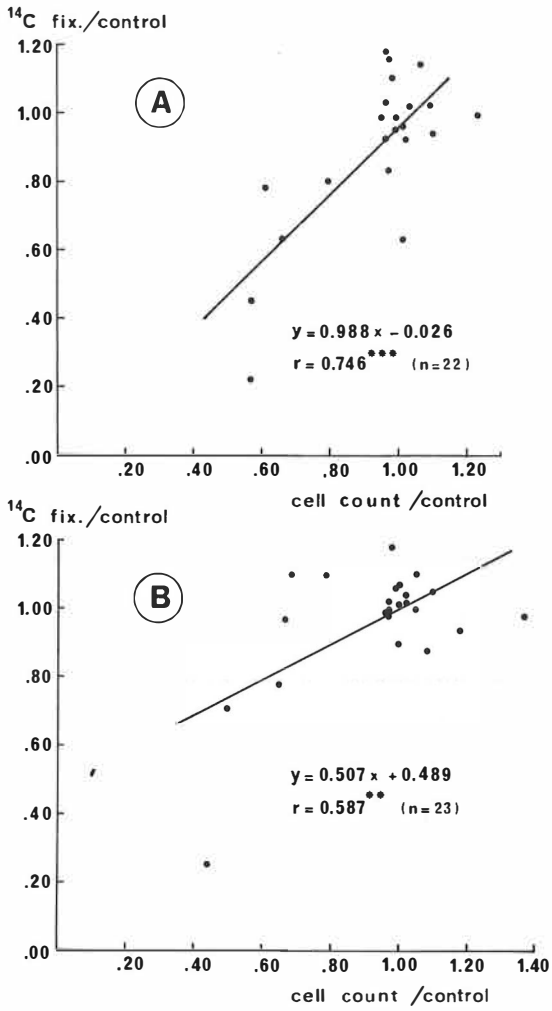

Fig. 12. A) and B) Correlations between cell counts and ^{14}C fixation in bioassays of different effluents after incubation of 24 h (A) and 96 h (B). Cf. Tables 4 and 5.

cells should be used as the only index, particularly in studies of toxic effluents. VILLA (1975) has also drawn attention to this question (cf. also LANDNER & ÖSTERMAN 1970).

In algal cultures certain relations obtain between cell division and cell size and between cell size and the nutrient content of the culture. When one *Ankistrodesmus* cell divides, it can give rise to 2 to 16 autospores or daughter cells (cf. KOMARKOVA—LEGNEROVA 1969), which only gradually reach the normal cell size. For this reason in a rapidly multiplying culture there are on average smaller cells than in a more slowly multiplying culture (cf. Fig. 6). When a shortage of nutrient salts, for example nitrogen, begins to limit growth, the algal cells can still multiply for some time with the aid of their own "stores", but the cell size becomes smaller

and their chlorophyll content decreases (RODHE 1948, and Figs. 5, 6).

Conclusions

When used to monitor growth at the exponential growth phase, ^{14}C fixation, chlorophyll *a* determinations and cell counts give completely parallel results under normal culture conditions (cf. Fig. 5) but if growth-limiting factors occur (e.g. lack of nutriens, presence of inhibitory or toxic substances), differences and limitations become apparent in the measuring methods. In spite of the difference in sensitivity between the methods measuring the physiological processes of the cultures and those measuring the biomass, the results obtained by them in the wastewater tests paralleled each other closely and were highly significantly correlated with each other (cf. Tables 4, 5 and Figs. 11, 12, 13, 14). The slopes of the correlation lines show the difference in sensitivity between the methods. The inhibition evident in the chlorophyll values after 24 hours is reflected twofold in ^{14}C fixation and O_2 production (Fig. 11 A). Thus chlorophyll values showing 50 % inhibition indicate that photosynthesis had stopped completely. After the incubation period of 96 hours, the ^{14}C fixation and chlorophyll content of the cultures showed growth inhibition almost equally sensitively and strongly (Fig. 14). The effluents had decreased cell division somewhat more strongly (1.3—1.5-fold) than the chlorophyll contents of the cultures after both 24 and 96 hours (Fig. 13 and cf. also ELORANTA 1976). At first the cell numbers and ^{14}C fixation of the cultures reacted almost equally sensitively to the effluents (Fig. 12 A), but later the inhibitory effect appeared much more clearly in the cell numbers, even being twofold that revealed by the ^{14}C fixation values (Fig. 12 B). Thus algal assimilation recovered relatively quickly compared with cell division.

The following conclusions can be drawn regarding that suitability of these growth-monitoring methods for algal assays of waste waters from the cellulose industry and toxic effluents in general: 1) the ^{14}C method is

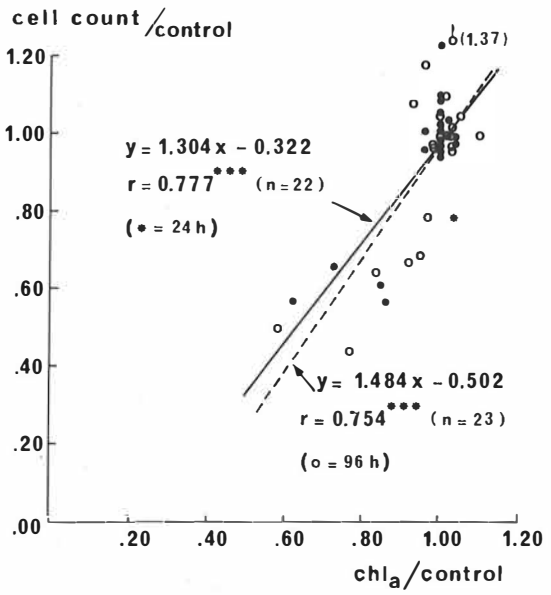

Fig. 13. Correlations between chlorophyll *a* values and cell counts in bioassays of different effluents after incubation of 24 h (solid line) and 96 h (broken line). Cf. Tables 4 and 5.

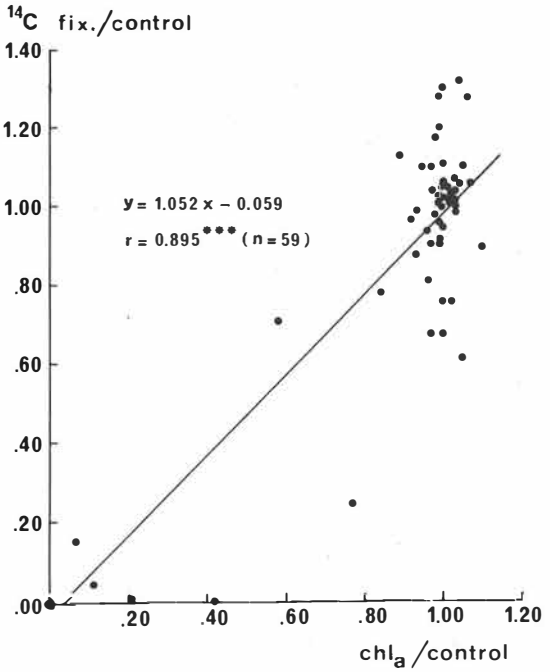

Fig. 14. Correlation between chlorophyll *a* values and ^{14}C fixation in bioassays of different effluents after incubation of 96 h. Cf. Table 5.

very suitable for studying the short-term effect. Being a sensitive method, it registers all the changes in the state of the culture, and thus requires carefully controlled test conditions. 2) The principle of the method of measuring oxygen production is comparable to that of the ^{14}C method, but it must still be developed much farther. 3) Cell counts are suitable for studying both short-term and long-term effects, but, especially in the latter case, should be used together with some other monitoring method or supplemented with studies relating to cell morphology. 4) The determination of the chlorophyll *a* content of the cultures is too insensitive for studying the short-term effects of the effluents. On the other hand, it is a very suitable monitoring method or longer incubation periods and gives a good picture of the condition of the cultures. Naturally the best solution in studies of inhibitory effluents is to choose two growth-monitoring methods that complement each other. Although this study deals with effluents of the cellulose industry, the more general expressions toxic and inhibitory waste waters are often used, because these are the chief effects of such effluents.

4.1.4. Sources of error in algal tests

The algal strains used in tests have generally been grown in laboratories, and their physiological properties differ from those of the corresponding algal strains in nature (cf. FOGG 1964, PRESCOTT 1964). A laboratory strain raised in optimal growth conditions is generally clearly more sensitive to certain stresses. For example, STOKES *et al.* (1973) found in experiments with heavy metals that strains isolated from metal-polluted waters were more tolerant than closely related laboratory strains. In addition, since the culture conditions never correspond exactly to the natural ones, it is self-evident that the results do not give a completely true picture of the effect of industrial effluents on algal growth in the waters receiving and transforming them. However, the natural condition can be simplified in the tests, which makes it possible to find out the probable effect of the effluents and to identify the components most strongly inhibiting or stimulating algal growth. Perhaps flowing-water algal bioassays would give a better picture of the effect of effluents, and especially of their toxicity; they have already proved useful with fish (ROGERS *et al.* 1975).

4.2. Comparison of effects of Sa and Si effluents

4.2.1. Eearlier studies

According to NUMMINEN (1971) and SEPPOVAARA and NUMMINEN (1974), Si effluents inhibited plankton production much more strongly in BMT experiments *(Ankistrodesmus falcatus)* than Sa effluents. A definite inhibitory effect was evident in the effluents at 15 % concentration (SEPPOVAARA & NUMMINEN 1974). According to NUMMINEN (1971), Sa effluents stimulated growth at 2—10 % concentration. In VILLA's BMT experiments (1975) Si and Sa effluents had an inhibitory effect at all the concentrations used (5, 15, 25 %) and no great differences were found between the effects of the effluents or the different concentrations. The secondary growth of the cultures was best at 5 % concentration. According to LEHMUSLUOTO and HEINONEN (1970), in BMT experiments combined Si and Sa effluents had an inhibitory effect at 10 % concentration, a strongly eutrophizing effect at 1 % and a still clearly eutrophizing effect at 0.1 %.

The toxicity of effluents of the cellulose industry and the effects of the toxic compounds that they contain have been studied much more in fish than in algae. SEPPOVAARA and HYNNINEN (1970) studied the toxicity of sulphate mill condensates. The untreated evaporation plant condensate was less dangerous than a similar digester house condensate. The results of their tests, especially those with *Daphnia,* showed that, although the condensates contained other toxic components, their toxicity was chiefly due to certain sulphur compounds. DAVIS (1973) has studied sublethal effects of bleached kraft mill effluent (BKME) on respiration and circulation in sockeye salmon *(Oncorhynchus nerka);* the effluent used was aerated, neutralized and filtered; its threshold concentration appeared to be around 20 % of the 96-h TL_{50} (static bioassay). Young sockeye salmon seemed to be most sensitive, and pink salmon *(O. gorbuscha)* and coho salmon *(O. kisutch)* somewhat more resistant to neutralized and filtered BKME solutions (DAVIS & MASON 1973). LEACH and THAKORE (1973) studied the toxicity of kraft pulping effluent on juvenile coho salmon *(O. kisutch).* Over 80 % of the toxicity of the nonvolatile constituents was caused by three resin acid soaps. The remaining toxicity

was contributed by sodium salts of the unsatured fatty acids. When ROGERS (1973) isolated and identified the toxic components of kraft mill wastes, he found that the toxicity of whole mill effluents to fish is mainly due to resin acids, with some additional toxicity arising from long-chain polyunsatured fatty acids. Resin acids were toxic to young sockeye salmon at 2.0 ppm in static biossay. Such acids are still detectable in biologically oxidized wastes. The only chlorinated compound detected was trichloroveratrole in minor yield. The toxicity contributions of heavy metals were considered negligible. MÄENPÄÄ *et. al.* (1968) found the 4-h TL_{50} for *Daphnia pulex* to be 2 mg/l resin acid sodium salts.

According to SEPPOVAARA (1973), fish *(Salmo gairdneri and Carassius carassius)* survived the toxic effects of bleaching effluents other than those of the chlorination stage in dilutions of 1:10 to 1:25, but the chlorination stage effluents killed rainbow trout in a dilution of 1:150. BETTS and WILSON (1966) also observed that the bleachery component from the chlorination stage was considerably more toxic to young Atlantic salmon *(Salmo salar)* than the other components. DAS *et al.* (1972) identified the organic chlorine compound tetrachloro-o-bentzoquinone, which is toxic to fish, in the chlorination stage effluent of a kraft pulp mill.

JACOBS and GRANT (1974) have studied the acute toxicity of unbleached kraft mill effluent (UKME) to the opossum shrimp *(Neomysis americana)*. The 96-hour TL_{50} values of unbleached kraft mill effluent were 3.3—6.9 per cent at 26—28 °C, and 3.9—7.3 per cent at 16—18 °C. Toxicity did not appear to be correlated with the BOD of individual batches of raw effluent. ROSEHART *et al.* (1974) examined the origins of toxicity in sulphite pulping, and observed in the preliminary bioassays *(Salmo gairdneri)* that the three most toxic streams were the sulphite waste liquor, the paper mill press water and the pulp washer overflow. Sulphite waste liquors were very toxic, with yield and wood species affecting the toxicity. The type of inorganic base did not seem to be significant. The results indicated the relatively toxic nature of the bark compared to the wood, and the high toxicity of the softwood (spruce) compared to hardwood (poplar). Several chemical additives were also found to be highly toxic.

ROGERS *et al.* (1975) found in their study of the toxicants in kraft mill effluents that the neutral component, which was mainly derived from the unsaponifiable fraction of lodgepole pine wood extractives and frequently caused fish mortalities, contained a series of diterpene alcohols and aldehydes related to the pimaric- and abietic-type resin acids. These compounds arise in living trees and have been identified in wood extract from many pine species.

4.2.2. The effluents of separate processes

The spent liquors from both cellulose factories were found to be very toxic to algae in both the bioassays and the BMT experiments (cf. Tables 4, 5, 7 and Figs. 8 a and b, 10, ELORANTA 1976 Fig. 6). The basic black liquor from the sulphate cellulose factory was more toxic than the acid spent sulphite liquor, because in the BMT tests it was toxic to algae at its lowest concentration (0.1 %), while the thin liquor was only inhibitory at that concentration. The acid Ca-bisulphite spent liquor destroyed the chlorophyll in the algal cultures (Fig. 9), which gradually killed them completely. In basic culture solutions, algal growth is limited by the shortage of carbon dioxide, used in photosynthesis, precipitation of phosphorus and the possible occurrence of toxic ammonium nitrogen (cf. ÖSTERLIND 1948, FITZGERALD 1964). In addition a too acid or basic nutrient solution increases the deleterious effect of any toxicants that may occur. The presence of compounds toxic to algae was especially clear with the black liquor, which was to be expected, in view of the studies demonstrating the toxicity of pine wood extract, resin acids, diterpene alcohols and aldehydes (cf. MÄENPÄÄ *et al.* 1968, LEACH & THAKORE 1973, ROGERS 1973, ROGERS *et al.* 1975). With the thin liquor, the acidity of the cultures was the main cause of their death. Spent sulphite liquor contains abundant sugars, and lignin and its derivativs (RENNERFELT 1963, and Table 1). Pure lignosulphonic acids have not been found to be toxic to algae (ELORANTA & ELORANTA 1974), and according to CHOPIN (1959), lignin and its derivatives are practically non-toxic to fish.

The Sa and Si condensates were growth-inhibiting or toxic to algae. Their effect was less evident in the bioassays than in the BMT tests (cf. Tables 4, 5, 7 and Figs. 8 a, 10). In the BMT experiments, the acid condensate was toxic at first at 1 % concentration, but this effect gradually declined (cf. ELORANTA & ELORANTA 1974). The Sa condensates stimulated growth slightly at 0.1 % concentration and inhibited it slightly at 1 % concentration. The toxicity of these condensates is due to more or less volatile organic sulphur compounds, and resin and fatty

Table 7. Comparison of effects of most closely corresponding process wastes and main sewer effluents from Sa and Si mills. Analysis of one-way variance applied to chlorophyll *a* values and ^{14}C fixation of bioassays after incubation of 24 h and 96 h. Calculations made to three decimal places. The effluents: I/Sa = 1st main sewer, I/Si = bleaching works sewer, II/Sa = Sa 2nd main sewer, II/Si = Si main sewer (I), III/Sa = black liquor, III/Si = spent sulphite liquor, IV/Sa = secondary condensates, IV/Si = acid condensate, V/Sa = Sa chlorination effluent, V/Si = Si chlorination effluent, VI/Sa = Sa 1st alkaline extraction effluent, VI/Si = Si 1st alkaline extraction effluent.

	24 h				96 h			
	Chl $_a$		^{14}C fixation		Chl $_a$		^{14}C fixation	
Effluent / Concn.	I_{sa}	I_{si}	I_{sa}	I_{si}	I_{sa}	I_{si}	I_{sa}	I_{si}
0.01 %	.99 ± .013	1.00 ± .027	.93 ± .017	.99 ± .008	1.00 ± .009	.99 ± .010	1.01 ± .006	1.03 ± .008
0.10 %	.99 ± .011	.98 ± .018	.99 ± .084	1.06 ± .086	1.03 ± .009	.98 ± .004	1.02 ± .014	1.01 ± .008
1.00 %	.98 ± .010	.98 ± .030	1.02 ± .045	1.28 ± .039	1.05 ± .006	1.00 ± .001	1.10 ± .030	1.05 ± .140
10.00 %	.74 ± .011	.94 ± .011	.63 ± .037	.83 ± .013	.95 ± .021	.93 ± .004	1.10 ± .042	.00 ± .060
$\bar{x}$	.93 ± .116	.98 ± .028	.89 ± .169	1.04 ± .176	1.01 ± .040	.97 ± .027	1.06 ± .049	1.02 ± .071
	F = 2.03		F = 2.90		F = 6.21*		F = 1.28	
	II_{sa}	II_{si}	II_{sa}	II_{si}	II_{sa}	II_{si}	II_{sa}	II_{si}
0.01 %	1.01 ± .007	1.00 ± .008	.96 ± .006	.87 ± .039	1.00 ± .001	1.04 ± .028	1.00 ± .005	1.07 ± .151
0.10 %	.98 ± .009	.99 ± .008	.99 ± .140	.83 ± .020	1.02 ± .004	1.05 ± .001	1.04 ± .040	1.28 ± .035
1.00 %	1.00 ± .008	.99 ± .018	.92 ± .030	.95 ± .012	1.02 ± .004	1.06 ± .005	1.05 ± .013	1.06 ± .120
10.00 %	1.01 ± .019	.52 ± .011	.83 ± .085	.02 ± .004	.93 ± .002	.00 ± .000	.88 ± .042	.002± .002
$\bar{x}$	1.00 ± .014	.88 ± .212	.92 ± .089	.67 ± .405	.99 ± .040	.79 ± .475	.99 ± .076	.85 ± .538
	F = 4.21		F = 1.49		F = 2.23		F = 1.89	
	III_{sa}	III_{si}	III_{sa}	III_{si}	III_{sa}	III_{si}	III_{sa}	III_{si}
0.01 %	.98 ± .029	1.00 ± .049	.83 ± .078	1.21 ± .146	1.04 ± .019	1.00 ± .021	1.32 ± .375	.95 ± .116
0.10 %	.75 ± .042	.98 ± .021	.45 ± .040	1.06 ± .054	1.00 ± .021	.97 ± .016	.67 ± .023	.68 ± .093
1.00 %	.55 ± .025	.56 ± .020	.02 ± .006	.02 ± .004	.21 ± .050	.004± .001	.01 ± .002	.001± .0002
10.00 %	.38 ± .060	.25 ± .038	.00 ± .000	.002± .002	.31 ± .013	.04 ± .003	.01 ± .006	.00 ± .000
$\bar{x}$	.67 ± .236	.70 ± .329	.33 ± .370	.57 ± .607	.64 ± .399	.50 ± .502	.50 ± .599	.41 ± .450
	F = 14.29**		F = 1.04		F = 1.79		F = 7.14*	
	IV_{sa}	IV_{si}	IV_{sa}	IV_{si}	IV_{sa}	IV_{si}	IV_{sa}	IV_{si}
0.01 %	1.01 ± .008	1.00 ± .000	1.03 ± .036	.87 ± .028	.99 ± .005	.99 ± .015	.91 ± .068	1.28 ± .042
0.10 %	1.00 ± .027	1.00 ± .002	.98 ± .062	1.07 ± .054	.98 ± .034	.98 ± .006	.92 ± .107	1.04 ± .091
1.00 %	.83 ± .011	.88 ± .011	.71 ± .002	.87 ± .049	.98 ± .036	.89 ± .015	.92 ± .027	1.13 ± .173
10.00 %	.19 ± .014	.35 ± .012	.01 ± .004	.001± .001	.07 ± .002	.01 ± .004	.15 ± .008	.00 ± .000
$\bar{x}$	.76 ± .350	.81 ± .281	.68 ± .436	.70 ± .443	.76 ± .413	.71 ± .429	.73 ± .356	.86 ± .545
	F = 6.67*		F= 4.55		F = 16.67***		F = 2.85	
	V_{sa}	V_{si}	V_{sa}	V_{si}	V_{sa}	V_{si}	V_{sa}	V_{si}
0.01 %	.97 ± .003	1.03 ± .023	.98 ± .023	1.11 ± .024	.98 ± .009	1.02 ± .014	.96 ± .268	1.03 ± .020
0.10 %	1.01 ± .011	1.02 ± .013	.88 ± .038	1.01 ± .008	1.00 ± .013	1.00 ± .049	1.11 ± .052	.76 ± .001
1.00 %	1.00 ± .003	1.02 ± .006	1.01 ± .012	1.06 ± .003	1.00 ± .008	1.00 ± .041	1.30 ± .242	.76 ± .118
10.00 %	.73 ± .007	.96 ± .013	.20 ± .001	1.23 ± .105	.12 ± .005	1.04 ± .018	.04 ± .002	.62 ± .107
$\bar{x}$	.93 ± .119	1.01 ± .030	.77 ± .355	1.10 ± .095	.78 ± .396	1.02 ± .035	.85 ± .536	.79 ± .169
	F = 5.11*		F = 6.64*		F = 4.31*		F = 12.50**	
	VI_{sa}	VI_{si}	VI_{sa}	VI_{si}	VI_{sa}	VI_{si}	VI_{sa}	VI_{si}
0.01 %	1.02 ± .021	1.03 ± .009	1.00 ± .010	1.10 ± .013	1.01 ± .010	.97 ± .009	1.02 ± .108	1.19 ± .101
0.10 %	1.00 ± .003	1.02 ± .001	.86 ± .061	.91 ± .008	.98 ± .022	.99 ± .033	1.20 ± .105	1.06 ± .030
1.00 %	.99 ± .018	1.03 ± .016	1.03 ± .045	.80 ± .051	.97 ± .020	1.09 ± .021	.91 ± .141	.90 ± .052
10.00 %	.94 ± .008	.83 ± .018	.80 ± .091	.22 ± .038	.95 ± .041	.76 ± .001	.82 ± .074	.25 ± .024
$\bar{x}$	.99 ± .034	.98 ± .091	.92 ± .111	.76 ± .352	.98 ± .030	.95 ± .123	.98 ± .174	.35 ± .388
	F = 7.69*		F = 1.64		F = 1.82		F = 1.27	

acids (cf. SEPPOVAARA & HYNNINEN 1970). The acidity and SO_2 of the Si condensate were the main factors limiting algal growth. According to RUUS (1964), in addition to SO_2 the acid condensate contains organic alcohols, aldehydes and acids, mainly acetic acid. Their deleterious effect lies mainly the high BOD loading imposed by the condensates on the receiving waters.

The effect of the Si chlorination stage effluent on algal growth was clearly less harmful than that of the water from the corresponding process in the Sa factory (cf. Tables 4, 5, 7 and Fig. 3). As the effluents of the chlorination stage are always acid, both waste waters were too acid for the algae at 10 % concentration in the BMT experiments and were thus toxic, but at 1 % concentration they stimulated growth. The Si waste water stimulated growth more strongly (cf. Fig. 10 and ELORANTA 1976: Fig. 6) than the Sa process water. The reason for this is obscure; the opposite result could be expected from, for example, the nutrient contents of the waste waters (cf. Table 1). The main reason for the death of the cultures with 10 % Sa process water in the bioassays was also the excessive acidity of the culture solutions (pH 3.5). The results did not give a clear picture of the effect on the algae of the toxic organic chlorine compounds arising during the chlorination stage of the bleaching process (cf. BETTS & WILSON 1966, DAS *et al.* 1972, SEPPOVAARA 1973).

The alkaline extraction effluents from the bleachery of the Si mill limited algal growth more strongly the corresponding effluents from the Sa mill (Table 4, 5, 7 and Fig. 10 and ELORANTA 1976: Fig. 6). In the BMT tests both were toxic at first at 10 % concentrations, but this effect declined during the test period. The Sa process water was slightly growth-stimulating at lower concentrations (cf. Table 1). The Si alkaline extraction effluent used was very basic (pH 12.2). However, this was not the sole cause of its inhibitory effect, because throughout the test period in the bioassays, the pH values of the culture solutions were the same as those of the controls, even at 10 % concentration. SEPPOVAARA (1973) assumes that alkaline extraction removes the organically bound chlorine from the compounds formed in the chlorination stage and thus eliminates the most toxic components. However, in the present study the alkaline extraction stage effluent from the Si bleachery was considerably more harmful to algal growth than the chlorination stage effluent. The result is thus the opposite to that obtained with the Sa bleachery effluents and to those of the fish assays of BETTS & WILSON (1966) and SEPPOVAARA (1973). On the other hand, HOWARD and WALDEN (1971) found that the most toxic process water was the first caustic extraction from the major process stream of bleached kraft mills.

4.2.3. The effluents of the main sewers

The acidity of the effluents from the first main sewer of the Sa mill (bleaching process effluents and condensates) and from the Si bleachery appeared to be the primary factor limiting algal growth (cf. Tables 1, 6 and ELORANTA 1976: Table 2). However, the growth inhibition in the cultures with 1 % effluents in the BMT tests and strongest bioassay cultures showed that the effluents have other properties limiting algal growth besides their acidity (Tables 4, 5 and Fig. 10, ELORANTA 1976: Fig. 11). In the Sa effluent these were evidently the more or less stable sulphur compounds, and resin and fatty acids with their related compounds (cf. SEPPOVAARA & HYNNINEN 1970, ROGERS 1973, BRUYNESTEYN & WALDEN 1974). According to HOWARD's and WALDEN's bioassays (1965), the effluents of the bleaching process of a kraft pulp mill were, in fact, still toxic after neutralization. Studies on the process waters suggest that the inhibition of the Si bleaching effluent may be due to chemical additives used in the bleaching (cf. ROSEHART *et al.* 1974).

The effluent of the second main sewer of the Sa mill (waste waters of the barking process and washing waters of the cooking plant) was very basic (Table 1). At strong concentrations in the bioassays, it caused slight but steady algal growth inhibition (cf. Tables 4, 5), and in the BMT experiments it was clearly inhibitory even in the 1 % cultures (ELORANTA 1976: Fig. 11). The effluent did not stimulate algal growth in the BMT tests, although its nitrogen and phosphorus contents were many times as great as

those of the waste waters of the other main sewers (Table 1). It is uncertain whether the inhibition caused by the effluent is due wholly to the toxic compounds it contains (cf. ROGERS 1973, ROGERS *et al.* 1975), or whether algal growth in the BMT tests is also limited by the alkalinity of test solution (cf. ELORANTA 1976: Table 2).

The effluent of the main sewer in the Si mill (condensates and washing waters) was very acid (pH 3.0). This clearly inhibited algal growth at strong concentrations (Tables 4, 5, 7 and Fig. 10). In the BMT tests the pH of the cultures with 10 % effluent was 3.8 (Table 6), thus the cell counts used as a measure of the biomasses of the cultures are surprisingly high. This is evidently due to difficulties in separating live and dead cells. Slight growth stimulation was apparent at the lowest concentrations of the effluent, and the studies of Si effluent II (sample from receiving waters just below mouth of main sewer) revealed a much stronger stimulative effect (Fig. 10, Tables 1, 4, 5). In the BMT experiments the cell counts rose with increasing effluent concentration. Thus in conditions favourable to algal growth, when acidity (10 %: pH 5.7) or oxygen shortage (oxidation of SO_2) have not yet become limiting the effluent is seen to have a eutrophizing effect (cf. LEHMUSLUOTO & HEINONEN 1970, ELORANTA 1972).

4.2.4. Conclusions

The analysis of variance revealed considerable differences in the effects of the effluents on algal growth and biomass. Of the process waste waters, the spent liquors were the most toxic to algae, the effect of black liquor being stronger than that of spent sulphite liquor. The next most toxic were the condensates, the effect of the acid condensate (Si) being mainly due to its acidity. Of the chlorination stage effluents, those of the Sa mill inhibited algal growth clearly, while the effects f the Si wastes were more or less stimulative, unless their acidity was lethal. The alkaline extraction effluent from the Si bleachery inhibited algal production more than that from the Sa bleaching works.

According to the bioassays, the acid main sewer effluents, Si effluent (I) and Sa first main sewer effluent, were the most harmful to algae; next came the basic effluent from Sa second main sewer and last the acid effluent from the Si bleachery. However, the effect of Si effluent (I) changed rapidly from growth-inhibiting to growth-stimulating after aeration and "aging". In the modified BMT tests, the basic effluent from Sa second main sewer inhibited algal growth the most strongly of all. In the literature, the waste waters from sulphate pulp mills are generally said to be more toxic than those of sulphite mills, whose greatest disadvantage is considered to be their high BOD. However, in such cases the effect of the acidity of the effluents has been eliminated.

In this study, the waste waters inhibited algal growth at much lower concentrations than, for example, in those performed by NUMMINEN (1971) and SEPPOVAARA & NUMMINEN (1974). The difference may be due not only to the composition of the effluents used, but also to the autoclaving of the waste waters in the typical BMT experiments. In the other studies both the Si and Sa effluents were acid, which indicates that the Sa effluents contained the condensates and waste waters from the bleaching process. In addition, in the present study the Si waste water contained much less nutrients than the Sa effluents, which increased its inhibitory effect.

Comparison of the results of the bioassays and the BMT experiments shows that the effluents are neutralized in the nutrient solution and their influence is weakened, while in the BMT tests both their inhibitory and stimulating effects are more clearly revealed (cf. ELORANTA 1976). Bioassays in which suitably low nutrient solution is used permit us to study only certain effects of the effluents from the wood-processing industry on algal growth. The advantages of bioassays are the relatively short test periods, the opportunity to use many growth-monitoring methods and to examine the immediate effects of toxic effluents. In the BMT experiments, which generally last many weeks, the biomasses may stay very small under the influence of the effluents, which limits the selection of growth-monitoring methods and the interpretation of the results. For example, chlorophyll *a* could not always be measured in the BMT tests, because of the shortage of biomass.

Comparison of the results of this study with, for example, the studies of pulp mill effluents performed earlier with fish is difficult and for many reasons of questionable value (differences in test conditions, and composition, treatment and concentrations of effluents, etc.). When the tolerance of different links in the food chain (fish, shrimps and diatoms) was examined in one bioassay study, it was found that diatoms tolerated about 1.3 times as great concentrations of spent sulphite liquor as juvenile silver salmon, but that the tolerance of shrimps was 6.7—fold that of fish (LEA 1959). However, it must be remembered that different algal groups have very different resistance to, e.g., algicides and other toxicants. Diatoms have been found to be one of the most susceptible algal groups, while green algae generally tolerate toxicants better than the others (MALONEY & PALMER 1956, UKELES 1962, NIEMI 1972). *Ankistrodesmus falcatus* generally occurs in strongly eutrophicated lakes and some of its varieties (var. *mirabile, setiformis* and *spirilliformis)* have been found to be relatively numerous even in waters polluted by pulp mills (JÄRNEFELT 1961).

5. Summary

1. Algal bioassays and BMT tests were examined for their suitability in studies of the effects of untreated effluents from the pulp industry. Various methods of monitoring algal growth were compared. The results were used to compare the effects of different waste streams from Sa and Si mills.

2. In the BMT experiments the cell counts of the cultures were greater ($F = 5.90^*$, $df = 34$) when the effluents had been autoclaved. Filtering of the effluents did not caused significant changes ($F = 0.0014$, $df = 34$) in algal growth.

3. Acclimation of the stock algae to lake water before the BMT tests promoted the growth of the cultures. An acclimation period of one and a half weeks increased growth more clearly ($F = 8.05^*$, $df = 34$) than one of two days ($F = 5.83^*$, $df = 28$).

4. In the bioassays biomass measurements obtained by different methods (chlorophyll *a* determination, ^{14}C fixation and cell counts) showed highly significant positive correlations at the exponential growth phase. But when the growth of the cultures became slower the measuring results differed markedly; the chlorophyll values clearly levelled out, the ^{14}C fixation values fell steeply and the cell counts rose. Cell size decreased significantly as the cultures aged.

5. The ^{14}C method proved to be very sensitive for growth measuring and monitoring and is thus particularly suitable for investigating toxic and inhibitory effects, but it demands very carefully controlled test conditions. When the immediate effects of the effluents on algal cultures were studied, a highly significant positive correlation was found between oxygen production and ^{14}C fixation. However, the method of measuring O_2 production is awkward and requires farther development.

6. With an effluent concentration of 10 % and 24-h incubation period, spent sulphite liquor inactivated 100 % of the total chlorophyll *a* of the bioassay cultures, acid condensate inactivated 60 % and Si effluent (I) and the alkaline extraction effluent 30 %.

7. The inhibitory effect revealed by the chlorophyll values of the bioassay cultures after 24-h incubation was shown twice as clearly by ^{14}C fixation and O_2 production. After 96 hours, the inhibitory effect was reflected almost equally. Cell division was a somewhat (1.3—1.5 x) more sensitive index of the inhibitory effect than the chlorophyll *a* content of the cultures. The cell counts and ^{14}C fixation reflected the effect of the effluent almost equally sensitively, but the disturbance was evident in the cell counts for a much longer time.

8. Of the waste waters from the separate processes, the spent liquors were most toxic to algae (black liquor more strongly), being followed by the condensates. The chlorination effluent was more inhibitory in the Sa than in the Si mill and the reverse was true of the alkaline extraction effluent. The Si chlorination effluent had a more or less stimulating effect on algal growth when acidity was not a limiting factor. Both the waste streams of the Sa bleachery stimulated growth slightly at low concentrations.

9. According to the bioassays, the acid main sewer effluents, Si (I) and the Sa first main sewer effluent, were most toxic to algae. Next came the basic effluent from Sa second main sewer and finally the acid effluent from

the Si bleachery. The toxicity of the Si main sewer effluent was due mainly to its acidity and volatile compounds, because its effect changed rapidly, becoming stimulating to algal growth (cf. Si II). In the modified BMT tests, the basic waste water from Sa 2nd main sewer inhibited algal growth most strongly of all, being clear even at 1 % concentration.

10. In the modified BMT experiments (no autoclaving of effluents), the effects of the effluents were revealed much more sensitively than in the bioassays. Advantages of the algal bioassays are the short test periods, the great biomasses, and the opportunities for using many different monitoring methods and examining the immediate effects of the effluents. The typical BMT tests are not as suitable for studies of untreated cellulose industry effluents as the modified BMT experiments. A general disadvantage of BMT tests of toxic effluents is that the small biomasses limit the use of growth-monitoring methods. Cell counts alone are not sufficient in studies of the effects of toxic waste waters.

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Selostus

Selluloosatehtaiden prosessi- ja pääkanaalijätevesien vaikutuksia Ankistrodesmus falcatus var. acicularis (Chlorophyta) kasvuun ja tuotantoon.

VARPU ELORANTA

Käsittelemättömien seulluloosateollisuuden jätevesien vaikutuksia *Ankistrodesmus falcatus* var. *acicularis*-populaation kasvuun ja tuotantoon tutkittiin levätestien ja muunnettujen BMT-kokeiden avulla. Tulosten pohjalta vertailtiin lähinnä toisiaan vastaavien sulfaatti- (Sa) ja sulfiitti- (Si) selluloosatehtaan prosessi- ja pääkanaalijätevesien vaikutuksia keskenään. Lisäksi tutkittiin käytettyjen levätestien ja leväkasvun seurantamenetelmien (klorofylli *a*:n määritys, ^{14}C-menetelmä, O_2-tuotanto, solulaskenta) soveltuvuutta toksisten jätevesien vaikutusten selvittelyssä. Tutkimuksessa käytetyt sulfaattiselluloosatehtaan jätevedet olivat seuraavat: I pääkanaalin jätevesi (valkaisimon jätevesiä ja lauhteita), II pääkanaalin jätevesi (kuorimon jätevesiä ja keittämön pesuvesiä), kuorimon jätevesi, mustalipeä, sekundaariset lauhteet, valkaisimon kloorausvaiheen ja I alkalivaiheen jätevedet, sekä sulfiittiselluloosatehtaan jätevesistä valkaisimon pääkanaalin jätevesi, selluloosatehtaan pääkanaalin jätevesi I (vuorokauden kokoomanäyte), jätevesi II (purkuvesistöstä heti viemäriputken alapuolelta), ohutliemi, haihduttamon hapanlauhde, valkaisimon kloorausvaiheen ja I alkalivaiheen jätevedet.

Biotesteissä pääkanaalijätevesistä hapan Si-jätevesi I inhiboi levien kasvua kaikkein voimakkaimmin, mutta sovellettujen BMT-testien tulosten mukaan emäksinen Sa-jätevesi (II pääkanaali) oli leville kaikkein myrkyllisin. Si-jäteveden kasvua rajoittava vaikutus muuttui kasvua lisääväksi jäteveden ilmastuksen ja "vanhenemisen" jälkeen. Tehtaiden vastaavien prosessivesien vaikutusten keskinäisessä vertailussa suurimmat erot olivat kloorausvaiheiden jätevesillä, lauhteilla ja keittoprosessin jätevesillä. Mustalipeä oli prosessivesistä leville selvästi myrkyllisin, seuraavina olivat sulfiittijätelipeä ja lauhteet. Sa-kloorausvaiheen ja Si-alkalivaiheen jätevedet inhiboivat leväkasvua enemmän kuin toisen prosessin vastaavat jätevedet.

Jätevesien kasvua rajoittavat ja kasvua lisäävät sekä myrkylliset vaikutukset näkyivät huomattavasti herkemmin sovelletuissa BMT-testeissä (ei jätevesien autoklaavausta) kuin ravintoliuoslevätesteissä. Ravintoliuoskokeiden etuja ovat lyhyet koejaksot, suuret biomassat, mahdollisuus useiden kasvun mittausmenetelmien käyttöön sekä jätevesien välittömien vaikutusten tutkimiseen. BMT-kokeet vaativat huomattavasti edellisiä pitemmät koejaksot ja niiden niukat biomassat, etenkin toksisuus- ja inhibitiivisyystesteissä rajoittavat kasvun seurantamenetelmien valintaan ja tulosten tulkintaan. Sovelletut BMT-testit sopivat käsittelemättömien jätevesien vaikutusten tutkimiseen paremmin kuin tyypilliset BMT-testit, koska autoklaavauksen todettiin muuttavan jätevesien vaikutuksia jokseenkin merkitsevästi ($F = 5.90^*$, $df = 34$). Kasvun seurantamenetelmistä ^{14}C- ja happimenetelmä olivat kaikkein herkimmät. Myös solujen jakautumisessa näkyi jätevesien aiheuttama kasvun inhibitio herkemmin kuin viljelmien klorofylliarvoissa. 96 tunnin koejakson jälkeen viimemainitut menetelmät mittasivat viljelmissä esiintyvän inhibition lähes yhtä voimakkaana. ^{14}C-menetelmän herkkyys asettaa tietyt vaatimukset koeolosuhteille, koska menetelmä reagoi herkästi kaikkiin viljelytilanteessa tapahtuviin muutoksiin. O_2-tuotannon mittaamisessa käytetty menetelmä vaatii vielä käytännön suorituksena kehittämistä. Pelkkä solumäärien laskenta ei yksin riitä kasvun seurantamenetelmäksi toksisuus- ja inhibitiivisyystesteissä, koska solujen morfologiset ominaisuudet voivat koetilanteen aikana suuresti muuttua. Viljelmien klorofyllipitoisuuden määrittäminen ei sovi jätevesien välittömien vaikutusten tutkimiseen, mutta pitemmän inkubation aikana se on sopiva seurantamenetelmä.

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A comparison of littoral periphyton in some lakes of Central Finland

PERTTI ELORANTA and SOILE KUNNAS

ELORANTA, P. and KUNNAS, S. 1976: A. comparison of littoral periphyton in some lakes of Central Finland. — Biol.Res.Rep. Univ. Jyväskylä 2: 34—50.

The accumalation of seston, algal growth, energy content, species composition, and diatom cell densities of the periphyton were studied by means of artificial substrates in littoral zones of different lake types in Central Finland in 1974. The differences in the degree of the eutrophication were clearly seen in the growing rates and chlorophyll *a* contents per unit area. The maximum values of the periphyton mass and chlorophyll content per unit area were reached after 3 weeks exposition in summer, but the growth became slower in the autumn, especially in the eutrophic areas. The mean percentage of inorganic matter of the periphyton was 56.8, that of the algae 13.6 and that of the other organic matter 29.4. The amount of mineral particles usually decreased during the exposition, while the percentage of the algae increased. The positive correlation between the total periphyton mass and chlorophyll *a* content was very high. The energy content of periphyton dry weight matter increased at most stations during the exposition. On the contrary, the energy content of the organic matter of the periphyton decreased at all stations during the exposition. The mean energy content of the periphyton was 9.58 J/ mg dry weight and 22.43 J/ mg ash free dry weight. The most important algal groups in the periphyton were filamentous green algae and diatoms. The most dominant diatom genera were *Tabellaria, Synedra, Eunotia, Achnanthes, Gomphonema,* and *Cymbella.* The evenness and species richness of the diatom community was greatest at the oligotrophic station. The diversities decreased during the whole exposition time. In summer the cell densities increased exponentially the first two weeks and the maximum densities were at all stations 2—8 $\times$ 10^5 cells/cm^2, but in the eutrophic areas the increase of the cell densities was very slow in the autumn.

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1. Introduction

Attached or periphyton algae have been studied especially in the flowing waters. Many of the periphyton algae are stationary and sensitive to the properties of the water and thus they have been used as indicator organisms in the pollution studies of the rivers (cf. e.g. FJERDINGSTAD 1964, SLADEČEK 1973). Periphyton algae have a great importance as primary producers also in the shallow lakes together with phytoplankton and macrophytes (WETZEL 1964). The studies concerning periphytic algae usually deal with the species composition, biomass, chlorophyll content or production of the communi-

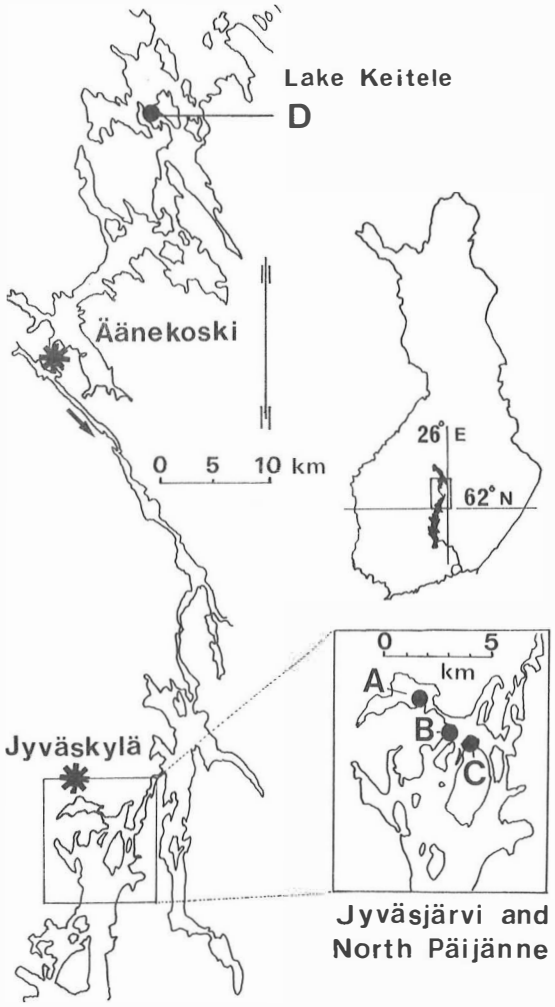


Fig. 1. The study area and the location of the sampling stations.

ties (ROUND 1956, CASTENHOLZ 1961, HOHN & HELLERMAN 1963, WETZEL 1964, HICKMAN 1971, STOCKNER & ARMSTRONG 1971, EVANS & STOCKNER 1972, LOWE 1972, STICKNEY & CAMPBELL 1972, BAHLS 1973, ERTL 1974). However, the comparison of the results between different water bodies is difficult since there are environmental dissimilarities and methodical differences. The studies on the periphyton algae of the inland waters in Finland are very sparse (cf. e.g. MÖLDER & TYNNI 1966, KUNNAS 1974, ÖVERLUND 1974).

The aim of this study was to compare the growth rate, biomass, contents of chlorophyll and energy of periphyton as well as species composition during the growing season in the littoral zones of lakes representing different lake types. The study is a part of a more comprehensive investigation of the periphyton in lakes and rivers of Central Finland performed by the Department of Hydrobiology, University of Jyväskylä.

2. The study area

Samples for analyses were collected at four localities (A—D) situated in Lake Jyväsjärvi, in the northern part of Lake Päijänne and in the central part of Lake Keitele (Fig. 1). Lake Jyväsjärvi is heavily eutrophicated by domestic sewage from the town of Jyväskylä, but occasionally the primary production of phytoplankton of the lake is inhibited by effluents from a paper mill containing sulphuric acid (GRANBERG 1973, ELORANTA 1976). The northern part of Lake Päijänne is mesotrophic and influenced by the waste waters from cellulose factories and by waste

Table 1. Some chemical properties of water in the study area (+ ELORANTA 1976, station D according to National Board of Waters, Finland 1972 and others GRANBERG 1975).

Station	pH	Water colour mg Pt/l	Alkal. mval/l	$KMnO_4$ cons. mg/l	Electr. conduct. µS	Tot.P µg/l
A	5.8+)	91	0.09+)	54	78	145
B - C	6.6+)	46	0.14	45	48	25
D	6.9	18	0.16	25	33	<10

waters which come into the area from Lake Jyväsjärvi. The central parts of Lake Keitele are oligotrophic with low humus content of water.

The sampling station A in Lake Jyväsjärvi was situated in the outer border of a dense *Phragmites*-association in a sparse *Nuphar-Sparganium*-association. The depth of the water was about 1 meter and the bottom consisted of hard clay. The sampling station B in Lake Päijänne was situated in a *Nuphar*-association (depth 1 m, silt bottom) in an area through which the waters from Lake Jyväsjärvi flow. The station C in North Päijänne was situated in a sparse *Nuphar-Polygonum amphibium*-association. The depth was 1 m and the bottom was hard clay. The sampling station D in Lake Keitele was situated in the open littoral zone without macrophytic vegetation. Some values of the properties of the water in the study area are given in Table 1 (ANONYMUS 1972, GRANBERG 1975, ELORANTA 1976).

3. Methods

The study was started in May 14th in 1974 and it finished 4.X.1974. The samples were collected by means of colourless celluloid plates which were fastened on stands anchored in the littoral (Fig. 2). The stands were floating in a constant depth. The size of the celluloid plates was 10 × 15 cm and 8 plates were fixed on each stant at the same time. The distance of the upper edges of the plates from the water surface was 20 cm. The exposure to light of different plates in the stand was regarded as equal because the stands were turning round in the waves. The gathering of the falling detritus and sediment on the plates was minimized by setting the plates vertically (cf. CASTENHOLZ 1960, 1961).

Two of the plates in each locality were removed after 1—5 weeks exposition. The previous plates were always replaced by new ones during the whole growing season. The two parallel samples were treated according to the schedule represented in Fig. 3. The chlorophyll *a* content was calculated according to the following formula (IWAMURA *et al.* 1970):

(1) Chl a = (17.12 D_{666} — 8.68 D_{653}) × v/(l × A) $\mu g/cm^2$, where D_{666} and D_{653} are the optical densities at the corresponding wavelenghts after lessening the turbidity measured at 720 nm, v is the amount of the solvent (90 % methanol) in cm^3, l is path lenght in cm, and A the area of the sample in cm^2. The dry weights were determined after drying the samples in oven at 60°C (RICHMAN 1971). Some parallel samples were dried first at 60 °C and then at 105 °C. On the average the mass of the samples decreased during the latter drying about 5 %. The content of in-

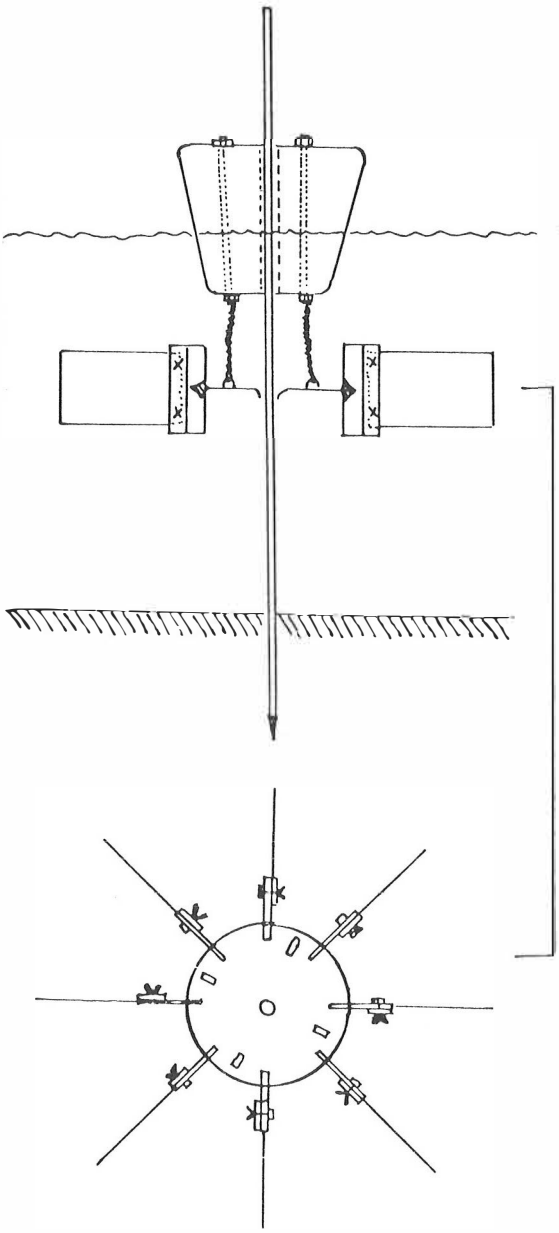


Fig. 2. The floating stand for the artificial plates (for further explanations see text).

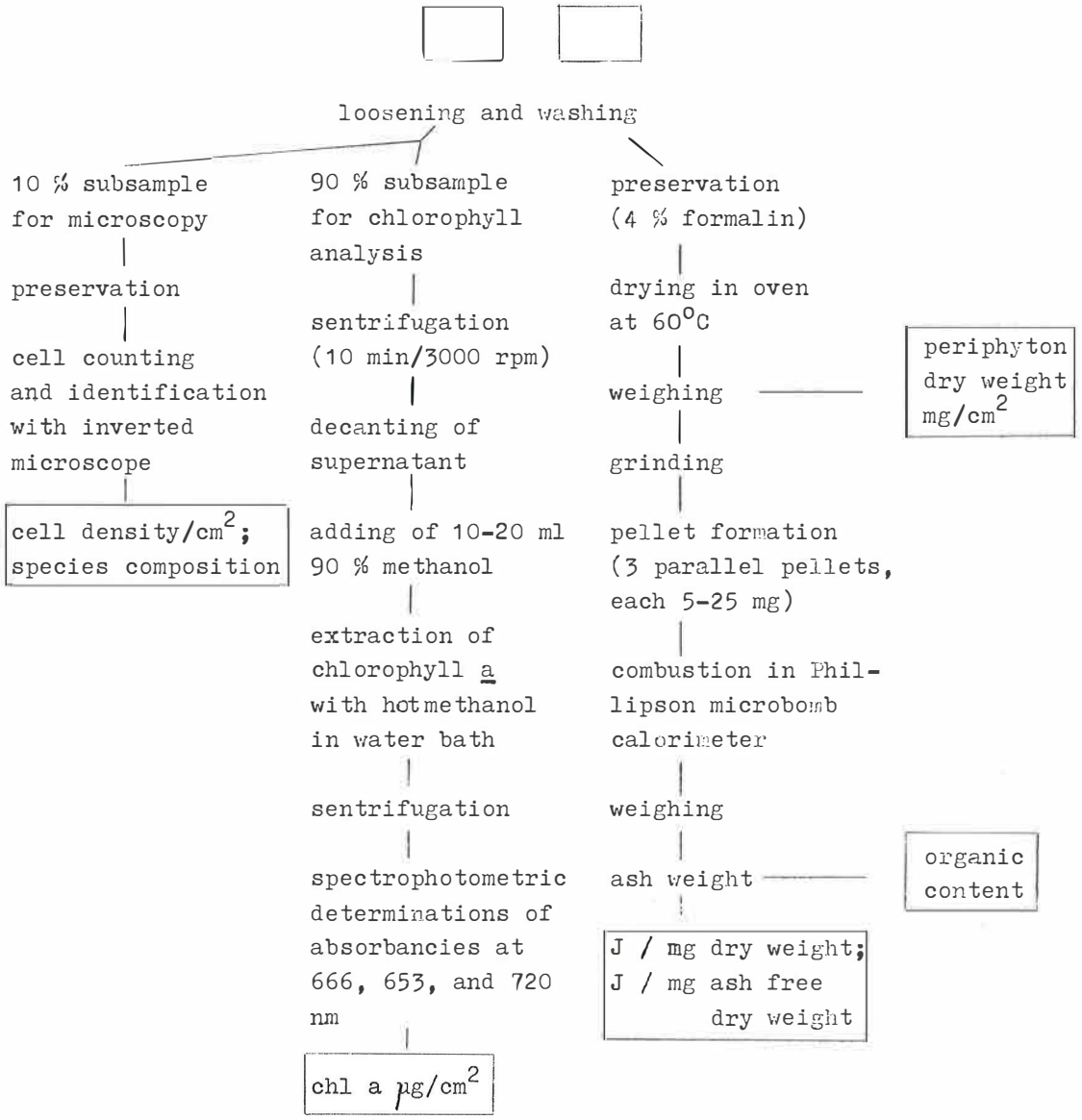


Fig. 3. The schematic diagram for handling of the periphyton samples.

organic matter was so great in some samples that the pellets did not combust in the calorimeter without the addition of a certain amount of benzoic acid (about 2 mg/pellet; cf. RICHMAN 1971). Also the calibration of the calorimeter was made by the aid of benzoic acid. The relative portions of algae, detritus and other organic and inorganic matter were calculated according to the following equations:

$$(2)\ \text{Organic material} = \frac{\text{dry weight} \times 100 - \text{ash-\%}}{100}$$

mg/cm², where the dry weight is given in mg/cm².

(3) Dry weight of algae A = chl *a* × 0.033 mg/cm², where chl *a* is the chlorophyll *a* content of the sample in µg/cm² and 0.033 is a constant, which is based on an assumption that the chlorophyll *a* content of algae is 1 % of their organic

dry weight (cf. HAGMEIER 1961, ROUND 1965). The portion of organic matter from the dry weight was assumed to be 30 % (CUMMINS & WUYCHECK 1971).

(4) Dry weight of other organic matter (Z) = 1.064 × organic matter — 0.3 × dry weight of algae) mg/cm², where 1.064 is based on the ash-% value of 6 % of the dry weight for the periphyton animals and detritus (CUMMINS & WUYCHECK 1971).

(5) Dry weight of inorganic material (M) M = total dry weight of sample — (A + Z) mg/cm².

The taxonomical identifications and cell countings were made with an inverted microscope. A subsample of 0.2—1 cm³ was taken from a homogenized sample into the counting chamber and the algal cells (mainly diatoms) were counted on 10—15 random fields or on some transverse strips. The comparison of the numbers of taxa in different samples has the weakness that the size of the counted samples varied. The identification of the species was carried out mainly according to HUSTEDT (1927—1966), CLEVE-EULER (1951—1955) and KALBE (1973). The diversities of the diatoms were calculated on the basis of cell numbers according to the following aquations:

$$(6)\quad \bar{H} = -\Sigma\ n_i/N\ \ln n_i/N$$

(SHANNON & WEAVER 1949), wehere n_i/N is the portion of each species of the total cell number.

$$(7)\quad e = \frac{\bar{H}}{\ln S}$$

(LLOYD & GHELARDI 1964), where *e* is the proportion of the species diversity ($\bar{H}$) to the maximum diversity (ln S) or the evenness of the community; ln S is the natural logarithm of the number of taxa in the sample.

4. Results

4.1. Dry weight masses of periphyton

Periphyton (expressed as dry weight) accumulated on the plates usually logarithmically (Fig. 4 a). On the average, the maximum of the weekly increment of the periphyton fass was reached after 3 weeks exposition at all stations (Fig. 4 b), in spite of the differences in the increments of periphyton which were great between the stations. The increase of the periphyton mass per cm² continued longer than 3 weeks in the oligotrophic area and a clearly retarded growth was seen first after 5—6 weeks exposition. The maximum masses per cm² were noted in eutrophic waters in early summer but in oligotrophic conditions (station D) in the middle of the summer (Fig. 5, Table 2).

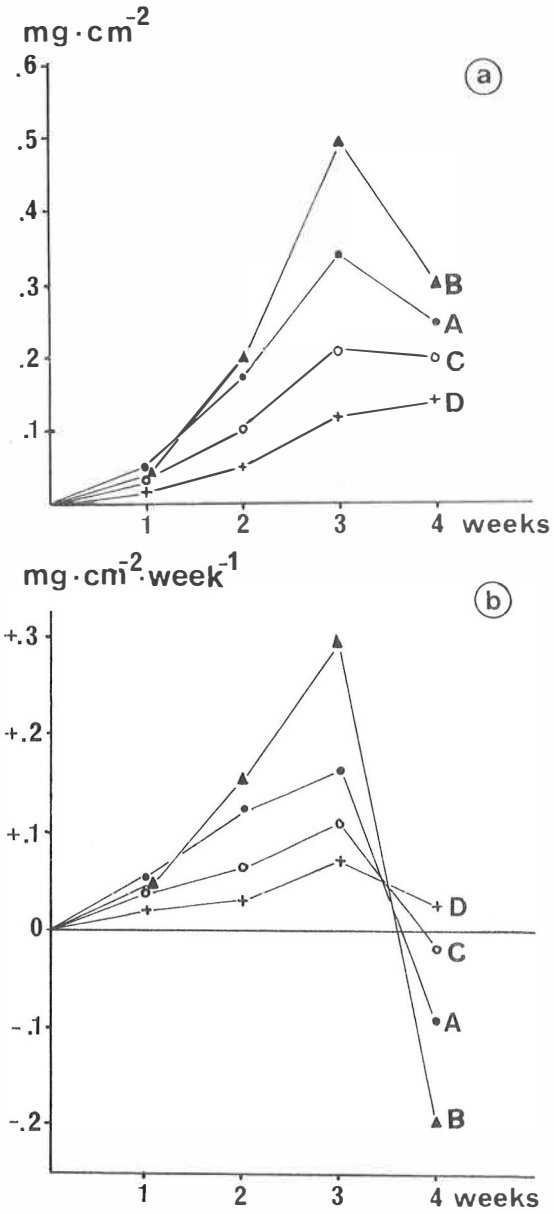


Fig. 4. *a.* The mean dry weights at different stations after 1—4 weeks exposition. *b.* The mean weekly increments of the periphyton matter at different stations.

If the portions of inorganic matter, algae and other organic matter (animals and det-

Table 2. Periphyton dry weight masses as mg/cm² at different stations and seasons. (ES = early summer; V—VI, MS = middle of summer; VII—VIII, AU = autumn; IX—X).

	STATIONS											
	A			B			C			D		
Weeks	ES	MS	AU	ES	MS	AU	ES	MS	AU	ES	MS	AU
1	.16	.06	.03	.12	.06	.01	.11	.10	.01	.01	.01	.04
2	.35	.17	.11	.26	.34	.03	.20	.24	.03	.03	.05	.05
3	.53	.38	.17	.92	.44	.21	.43	.27	.05	.12	.17	.11
4	.49	.36	.09	-	.36	.27	.63	.24	.16	.13	.25	.15
5	.36	.26	-	1.08	-	-	.83	.33	-	-	.34	.21
6	-	-	-	-	-	-	-	-	-	-	.44	.28

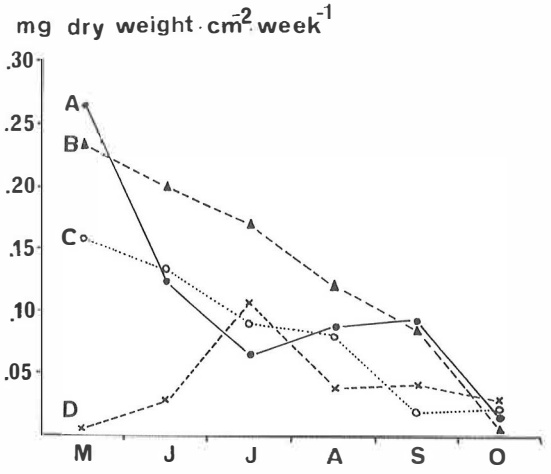


Fig. 5. The mean dry weight masses of the periphyton in different months per cm² and week.

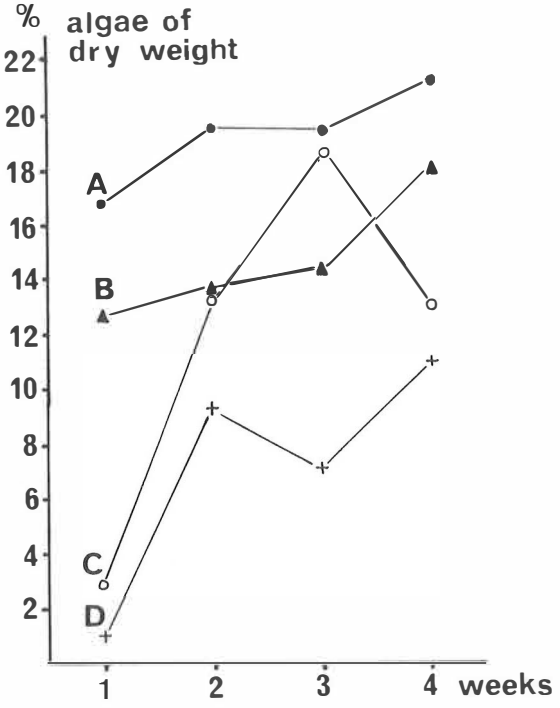


Fig. 6. The mean percentages of algae of the periphyton dry weight matter after 1—4 weeks exposition.

ritus) are compared it is evident that the percentage of the algae increased on the plates during the exposition (Fig. 6). The mean percentage of the algae was greatest at the most eutrophic station (A: 19.4 %) and smallest at the oligotrophic station (D: 8.4 %). The portions of detritus and animal matter were, on the average, greatest at station D (34.5 %) and the smallest at station A (26.1 %). The relative differences of the portions between the stations were not great (mean inorganic content varied from 53.3 % to 61.7 %, Fig. 7).

4.2. Chlorophyll a

A very clear positive correlation was found between the dry weights and the chlorophyll *a* contents of the periphyton (Fig. 8). Thus

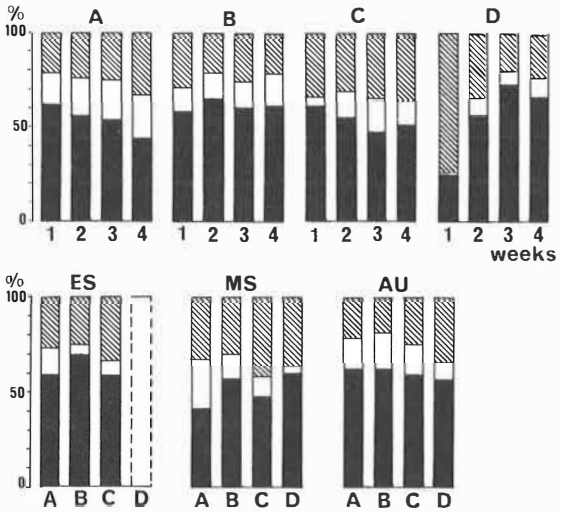


Fig. 7. The mean percentages of the inorganic material (black), algae (white) and other organic matter (ruled parts of the columns) at the different stations after 1—4 weeks exposition and means in the different seasons (ES = early summer, MS = middle of the summer, AU = autumn).

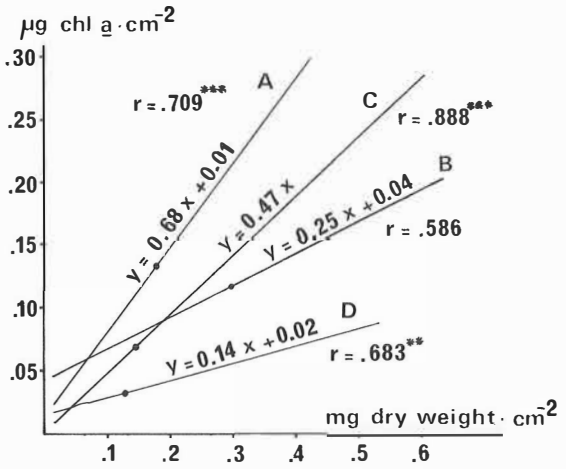


Fig. 8. The regressions and correlations between the dry weight matter and the chlorophyll *a* content of the periphyton at different stations.

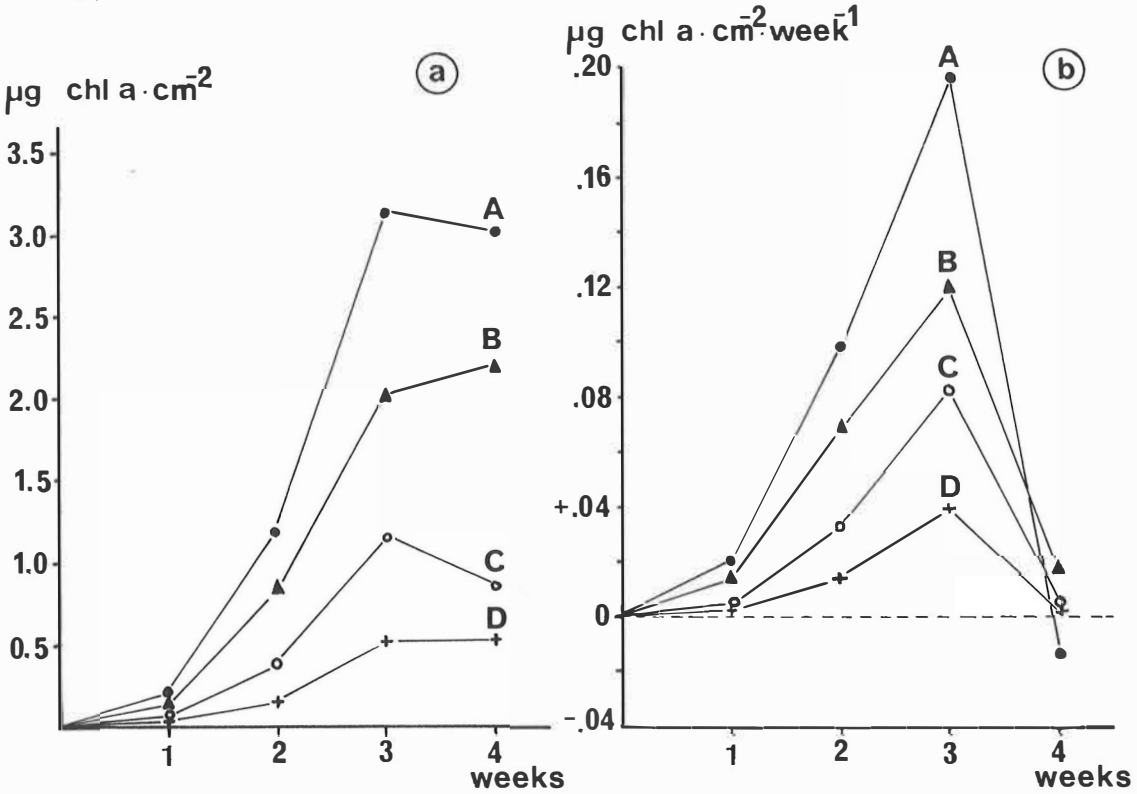


Fig. 9. *a.* The mean chlorophyll *a* content of the periphyton at different stations after 1—4 weeks exposition. *b.* The weekly increment of the chlorophyll *a* content after 1—4 weeks exposition.

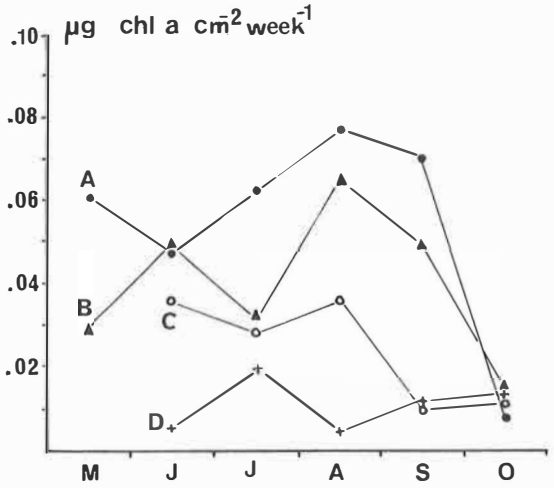


Fig. 10. The mean chlorophyll *a* contents of the periphyton per cm² and week in different months.

the growth of the algae obviously played the main role in the increment of the whole periphyton in the littoral samples. The differences in the percentages of the algae between the stations were, however, great as seen in the regressions of the dry weight masses and chlorophyll *a* contents.

The exponential growth of the algae was seen in the chlorophyll contents of the periphyton on the plates, which were measured weekly, but the differences between the stations were great according to the productivity of the water (Fig. 9 a). The weekly increment was greatest after 3 weeks exposition after which the increment decreased most rapidly in the eutrophic areas and least in the oligotrophic area (Fig. 9 b). The chlorophyll *a* contents were greatest in August at stations A—C and in July at station D (Fig. 10). The growth decreased rapidly in the eu-

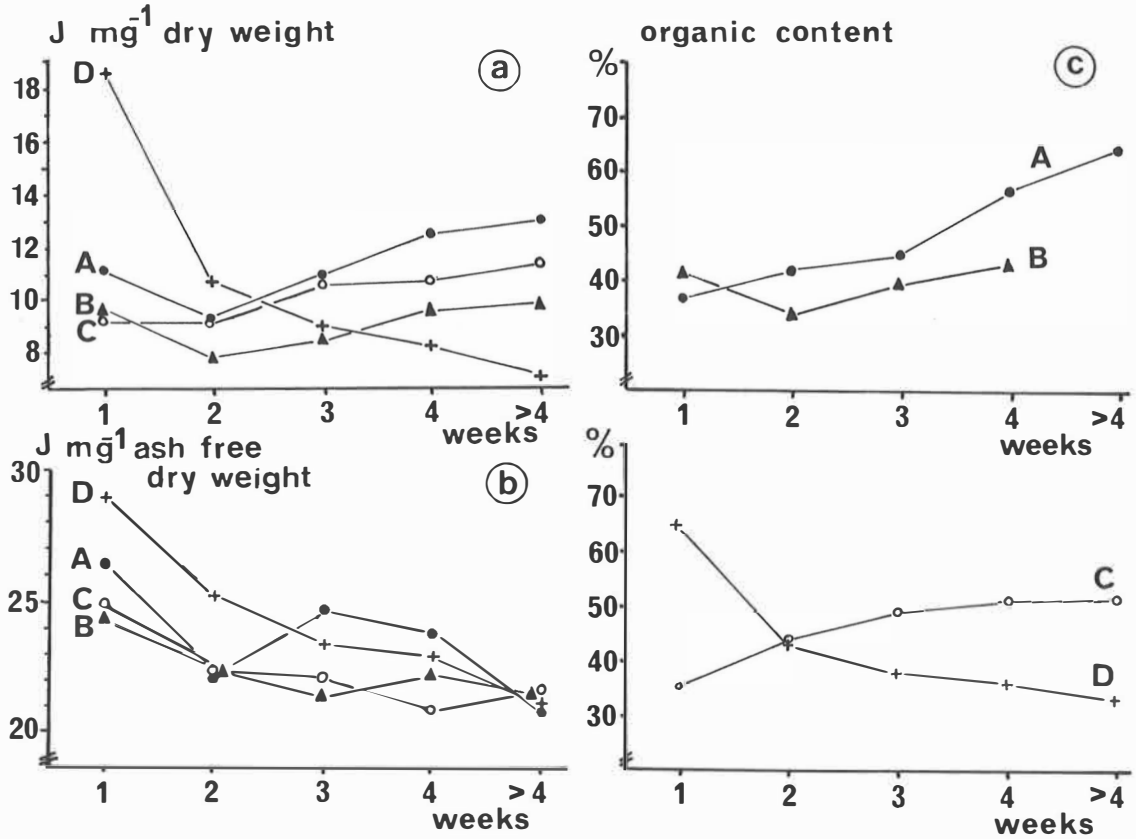


Fig. 11. *a.* The energy content of the dry weight matter of the periphyton. *b.* The energy content of the ash free dry weight of the periphyton matter. *c.* The organic content of the periphyton after different exposition time at different stations.

trophic areas in October but remained almost unchanged at the oligotrophic station and the means of the chlorophyll *a* contents were quite equal at all stations in October.

4.3. The energy content of periphyton

Since the periphyton contains both animals, organic detritus, algae and inorganic matter, the relative portions of these have an effect on the energy content of the samples. As has shown earlier the organic content ot the periphyton usually increased during the exposition affecting increase also in the energy content per dry weight unit (Figs. 7 and 11 a). Many individuals of water fleas (especially *Sida crystallina)* were attached on the plates at the oligotrophic station (D) during the first weeks exposition. The energy content of water fleas is rather great and on the other hand the content of the inorganic matter in these samples was low. Later the algal vegetation grew denser and also the portion of the inorganic matter increased at the same time as the portion of the animals decreased. The energy content of the ash free dry weight unit of the periphyton decreased at all the stations when the exposition time increased (Fig. 11 b). The energy content was greatest, on the average, in late summer from August to September (Table 3).

4.4. Composition of species and cell densities

The main interest in this study was paid on epibiontic diatoms but some observations were made on the taxa of the other algal groups. Expect epibiontic algae, also some planktonic species occurred frequently in the samples. The blue green algae were not very abundant at any station, the most common taxa being *Oscillatoria tenuis, O. limosa* and *Lyngbya* spp. The big *Chlamydomonas* species

Table 3. The energy content of the periphyton samples at different stations and in different months.

Station	V	VI	VII	VIII	IX	X	Mean
			J/mg dry weight				
A	5.44	8.95	11.80	13.10	10.13	7.74	10.38
B	6.07	6.69	6.78	9.37	9.33	6.65	8.08
C	-	8.20	10.04	11.51	9.41	9.54	9.79
D	-	13.35	6.86	9.25	11.51	7.41	10.00
Mean	5.73	9.29	8.87	10.79	10.08	7.82	9.58

Station	V	VI	VII	VIII	IX	X	Mean
			J/mg ash free dry weight				
A	18.49	21.92	19.83	22.64	25.44	23.64	22.84
B	22.64	22.26	18.03	22.01	24.98	18.07	21.76
C	-	20.54	20.67	22.51	22.72	21.51	21.38
D	-	29.83	23.30	22.38	23.35	20.67	23.72
Mean	20.59	23.64	20.46	22.38	24.14	20.96	22.43

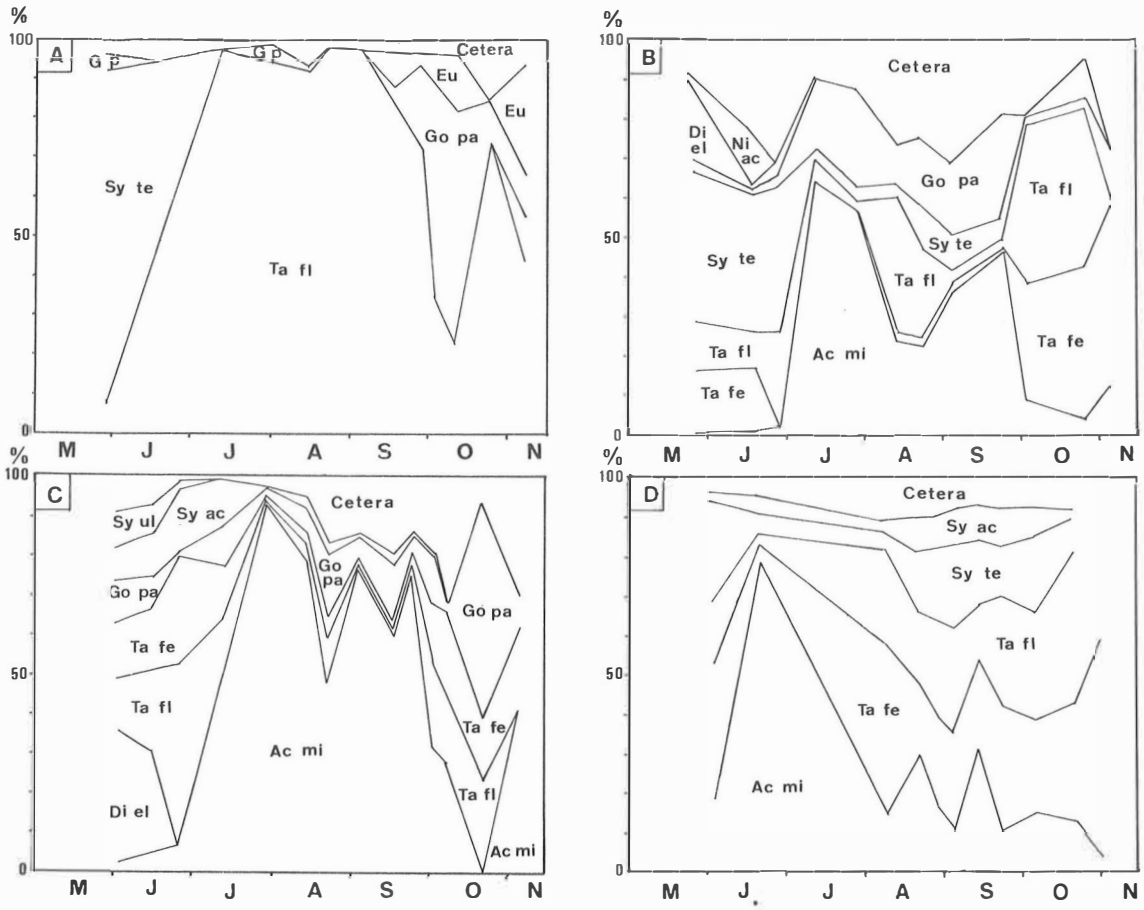


Fig. 12. The dominant species at different stations after 2 weeks exposition during the study period. (Go pa/G p = *Gomphonema parvulum,* Eu = *Eunotia,* Sy te = *Synedra tenera,* Sy ul = *Synedra ulna,* Sy ac = *Synedra acus,* Ta fe = *Tabellaria fenestrata,* Ta fl = *Tabellaria flocculosa,* Di el = *Diatoma elongatum,* Ac mi = *Achnanthes minutissima,* Ni ac = *Nitzschia acicularis).*

(Chlorophyta, Volvocales) which dominated in the phytoplankton of Jyväsjärvi and North Päijänne in the beginning of August (ELORANTA 1976) was also very common in the periphyton samples during the same time. The most common chlorococcal genera in the periphyton samples were *Scenedesmus* and *Pediastrum* and the commonest desmids belonged to the genera *Cosmarium* and *Closterium.* The filamentous green algae were most abundant during the middle of the summer and the most frequent genera at station A were *Mougeotia, Stigeoclonium* and *Microthamnion.* At the stations of North Päijänne (stations B and C) the genera *Oedogonium, Chaetophora, Mougeotia, Bulbochaete* and occasionally *Draparnaldia,* and *Spirogyra* were most frequent. In Lake Keitele (station D) the most frequent filamentous green algae were *Spirogyra, Bulbochaete, Mougeotia, Geminella,* and *Zygnema.*

Table 4. The identified diatom species, percentages of frequencies and some ecological valencies (MERILÄINEN 1976, BREITIG 1970, KUKKONEN & TYNNI 1973, SLADEČEK 1973; x = xenosaprobic, o = oligosaprobic, b = beta-mesosaprobic, a = alfa-mesosaprobic, acb = acidobiontic, acf = acidophilic, ind = indifferent, alkf = alkaliphilic, alkb = alkalibiontic). Sides 44—45 →

Species	Frequencies (%) A	B	C	D	saprobic valency	pH group
Melosira ambigua (Grun.) Müller	-	3.1	3.0	31.3		alkf
M. distans (Ehr.) Kütz.	2.7	12.5	9.1	50.0		acf
M. granulata (Ehr.) Ralfs	-	3.1	-	-		alkf
M. islandica Müller	-	-	-	3.1		alkf
M. italica (Ehr.) Kütz.	5.4	25.0	-	6.3		alkf
M. varians Ag.	2.7	6.3	9.1	3.1		alkf
Cyclotella spp.	2.7	9.4	3.0	56.3		
Tetracyclus lacustris Ralfs	2.7	3.1	-	-	x	acf
Tabellaria binalis (Ehr.) Grun.	2.7	3.1	-	12.5		
T. fenestrata (Lyngb.) Kütz.	86.5	93.8	97.0	100.0	o-b	acf
T. flocculosa (Roth)	100.0	100.0	100.0	100.0	o-x	acf
Diatoma elongatum (Lyngb.) Agardh.	-	28.1	30.3	100.0		
D. hiemale (Lyngb.) Heiberg	5.4	31.3	15.9	-		alkf
Meridion circulare (Grev.) Agardh.	10.8	-	-	-		alkf
Asterionella formosa Hassal	-	6.3	6.1	50.0	o-b	alkf
Ceratoines arcus (Ehr.) Kütz.	-	9.4	-	6.3	x-o	
Synedra acus Kütz.	2.7	90.6	84.8	90.6	b	alkf
S. amphicephala Kütz.	24.3	9.4	-	15.6	x	
S. parasitica (W. Sm.) Hust.	8.1	-	-	3.1		
S. rumpens Kütz.	-	62.5	36.4	-		alkf
S. tenera W. Sm.	70.3	96.9	81.4	100.0		
S. ulna (Nitzsch.) Grun.	43.2	93.8	97.0	96.9	b	alkf
Fragilaria capucina Desm.	-	3.1	15.2	9.4	o-b	alkf
F. construens (Ehr.) Grun.	13.5	-	-	-	b	
F. crotonensis Kitton	-	9.4	42.4	6.3	o-b	
F. pinnata Ehr.	-	3.1	9.1	9.4		alkf
F. virescens Ralfs.	-	3.1	18.2	12.5		ind
Eunotia alpina (Naeg.) Hust.	-	9.4	24.2	-		
E. arcus Ehr.	2.7	-	-	3.1		ind
E. attenuata A. Cleve	2.7	-	-	-		
E. bigibba Kütz.	-	-	-	3.1		
E. exigua (Breb.) Rabenh.	2.7	-	-	-		acb
E. faba (Ehr. Grun.)	-	3.1	-	-		
E. flexuosa (Breb.) Kütz.	-	3.1	-	-		acf
E. gracilis (Ehr.) Rabenh.	-	-	-	3.1		ind
E. lunaris (Ehr.) Grun.	40.5	31.3	63.6	43.8	o	acf
E. meisteri Hust.	40.5	6.3	-	-		acf
E. pectinalis Rabenh.	70.3	18.8	39.4	9.4	x	acf
E. praerupta Ehr.	51.4	31.3	30.3	43.8		acf
E. robusta Ralfs	-	-	-	3.1		acf
E. veneris (Kütz.) O.Müller	10.8	-	21.2	-		acf
Cocconeis placentula Ehr.	-	3.1	6.1	-	b	alkf
Achnanthes clevei Grun.	2.7	-	3.0	-		alkf
A. exigua Grun.	-	6.3	3.0	3.1		
A. kolbei Hust.	-	-	3.0	-		
A. lanceolata (Breb.) Grun.	-	-	3.0	-	x-b	alkf
A. levanderi Hust.	-	3.1	-	-		
A. linearis (W. Sm.) Grun.	16.2	87.5	78.8	18.8	x-o	ind
A. lutheri Hust.	-	3.1	-	-		
A. microcephala Kütz.	5.4	-	-	46.9		
A. minutissima Kütz.	35.1	96.2	97.0	100.0	o-b	ind
Amphipleura pellucida Kütz.	-	-	6.1	-		alkf
Frustulia rhomboides (Ehr.) De Toni	5.4	3.1	18.2	21.9	o-x	acf
F. vulgaris (Thwait.) De Toni	-	-	6.1	-		alkf
Gyrosigma sp.	-	3.1	-	6.3		alkf
Caloneis sp.	-	3.1	-	-		alkf
Diploneis sp.	-	-	-	3.1		

Table 4. (continued)

Stauroines anceps Ehr.	21.5	-	9.1	15.6	b	ind
S. javanica (Grun.) Cleve	-	-	-	3.1		
S. phoenicenteron (Nitzsch.) Ehr.	8.1	-	-	3.1	b	ind
S. smithii Grun.	2.7	-	3.0	-		
Anomoeoneis exilis (Kütz.,Grun.)Cleve	-	34.4	15.0	-		ind ?
A. serians (Breb.) Cleve	8.1	3.1	-	-	x	acf
Navicula cryptocephala Kütz.	35.1	9.4	48.5	18.8	a	alkf
N. dicephala (Ehr.) W. Sm.	2.7	-	-	3.1	o-b	
N. hustedti Krasske	2.7	-	-	15.6		acf
N. pupula Kütz.	2.7	3.1	-	3.1	b	ind
N. radiosa Kütz.	5.4	9.4	42.4	28.1	o-b	ind
N. rhyncocephala Kütz.	2.7	34.4	36.0	50.0	b	alkf
N. subtilissima Cleve	8.1	-	-	-		acb
Pinnularia appendiculata (Ag.) Cleve	27.0	9.4	-	-		
P. brebissonii (Kütz.) Cleve	-	-	3.0	-		
P. divergens W. Sm.	16.2	-	-	-		ind
P. gibba Ehr.	5.4	-	9.1	6.3	x	ind
P. interrupta W. Sm.	-	3.1	3.0	-		acf
P. mesolepta (Ehr.) W. Sm.	16.2	6.3	3.0	6.3	o	ind
P. microstauron (Ehr.) Cleve	10.8	-	3.0	9.4	b	ind
P. subcapitata Gregory	13.5	-	6.1	-	o	ind
P. viridis (Nitzsch.) Ehr.	2.7	-	-	-	b	ind
Amphiprora paludosa W. Sm.	-	-	-	6.3		
Amphora ovalis Kütz.	2.7	12.5	12.1	50.0	o-b	alkf
Cymbella affinis Kütz.	-	-	3.0	3.1	o-b	
C. amphicephala Naegeli	-	-	-	3.1		
C. cesatii (Rabenh.) Grun.	-	6.3	-	81.3	x	
C. cistula (Hemprich.) Grun.	5.4	3.1	60.6	37.5	b	alkf
C. cymbiformis (Ag., Kütz.) v. Heurck	2.7	3.1	-	46.9		ind
C. gracilis (Rabenh.) Cleve	-	-	24.2	34.4	x	ind
C. helvetica Kütz.	2.7	-	3.0	21.9	x-o	alkf
C. lanceolata (Ehr.) v. Heurck	-	6.3	6.1	3.1	b	
C. microcephala Grun.	-	-	-	9.4		alkf
C. naviculiformis Auerswald	16.2	3.1	-	6.3	b	ind
C. tumida (Breb.) v. Heurck	-	3.1	33.3	15.6		
C. ventricosa Kütz.	16.2	53.1	72.7	71.9	b	ind
Gomphonema acuminatum Ehr.	2.7	34.4	90.0	90.5	b	alkf
G. angustatum (Kütz.) Rabenh.	10.8	43.8	42.4	50.0	o	alkf
G. constrictum Ehr.	5.4	71.9	90.9	68.8	b	alkf
G. intricatum Kütz.	2.7	3.1	15.2	59.4	o	alkf
G. lanceolatum Ehr.	-	-	18.2	3.1	b	alkf
G. olivaceum. (Lyngb.) Kütz.	5.4	6.3	6.1	15.6	b	
G. parvulum (Kütz.) Grun.	83.8	84.4	100.0	93.8	b	ind
Epithemia sorex Kütz.	-	-	3.0	3.1	b	alkf
Nitzschia acicularis W. Sm.	10.8	12.5	24.2	37.5	a	alkf
N. palea (Kütz.) W. Sm.	8.1	-	15.2	6.3	a	ind
N. parvula Lewis	2.7	6.3	3.0	-	b	
N. vermicularis (Kütz.) Grun.	-	-	-	9.4	b	alkf
N. spp.	2.7	6.3	-	21.9		
Stenopterobia intermedia Lewis	-	-	-	6.3		acf
Surirella angustata Kütz.	5.4	3.1	6.1	21.9	b	
S. cf. biseriata	5.4	3.1	-	6.3	b	

The percentages of frequency of the identified diatom taxa and some notices on their saprobic valencies and the relations to the pH of the water of the species (HUSTEDT 1939, BREITIG 1970, SLADEČEK 1973) are presented in Table 4. The relative abundances of the dominant species in the samples after 2 weeks exposition during the growing season are presented in Fig. 12. Some factors llustrating the structure of the diatom communities in the different areas and after different exposition times are seen in Fig. 13. The most dominant species were *Tabellaria fenestrata, T. flocculosa* (especially at the station A), *Synedra* spp., *Achnanthes minutissima* and *Gomphonema parvulum*. The species richness and the evenness values were greatest in the oligotrophic areas and the richness of species increased during the first three weeks of exposition and then it decreased (Fig. 13). The densities of diatom cells varied widely, depending especially on the month and on the duration of the exposition (Figs. 13 a and 14). The maximum of the cell density was usually reached within 2 weeks, after which the increase of the density became slower and then began to decrease. In autumn, the cell density increased slowly during the whole exposition time, especially in the eutrophic areas while at the more oligotrophic stations (C and D) the increment of the cell densities was almost the same as in the summer (Fig. 14). The species composition has, of course, its effect on the cell densities. Consequently, the occurrence of the large-sized *Tabellaria flocculosa* accounts for the small cell densities at station A, while such small-sized species as *Achnanthes minutissima* and *Gomphonema parvulum* dominated at the other stations.

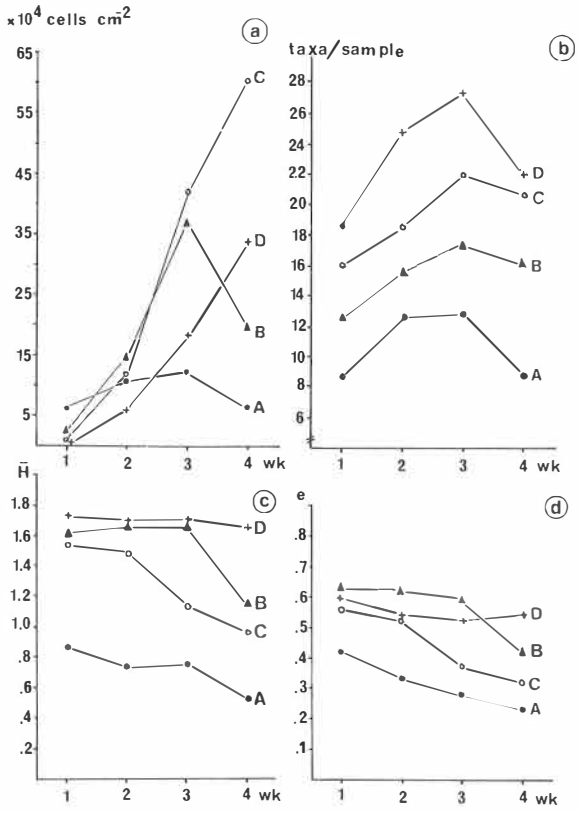


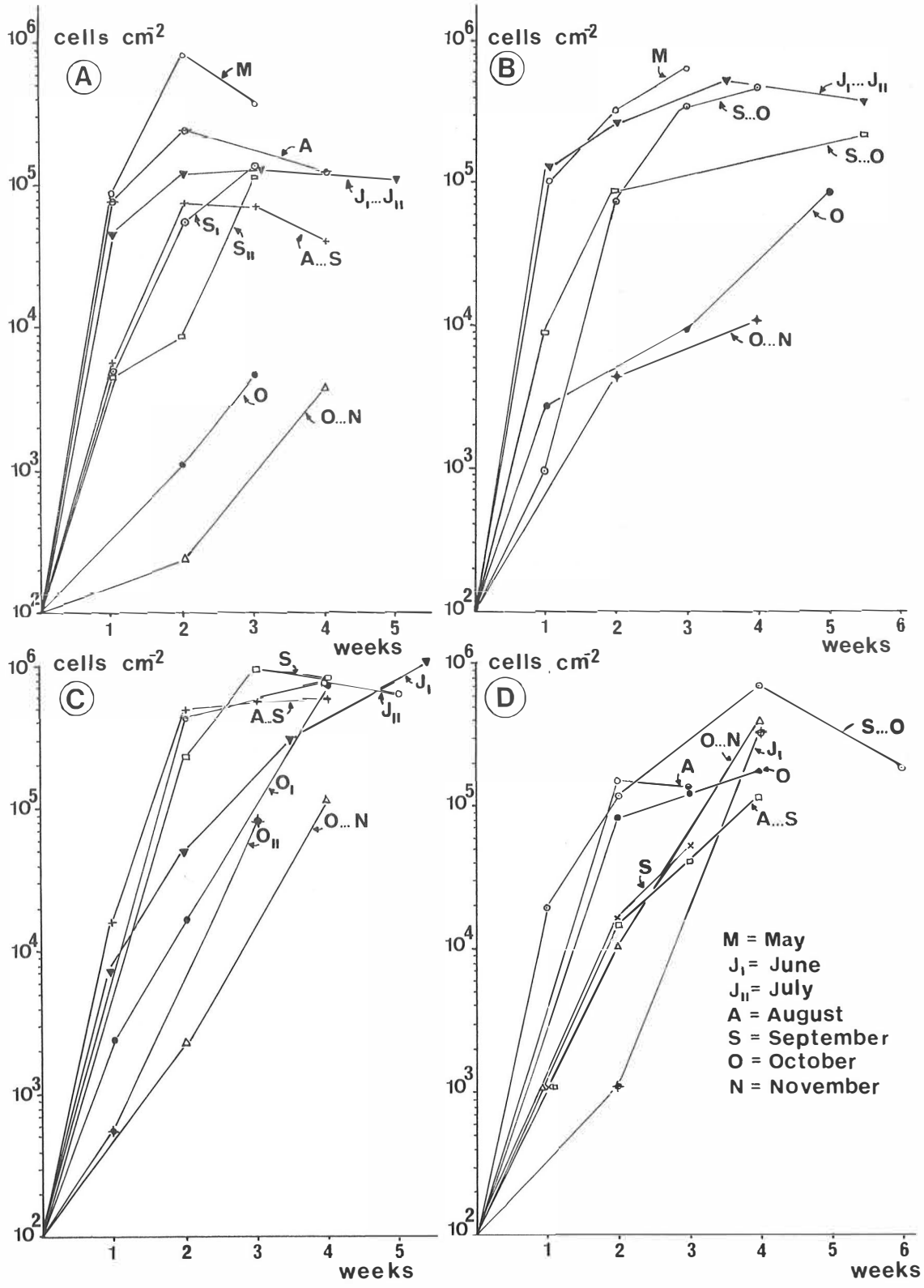
Fig. 13. The mean diatom cell densities, numbers of taxa, species diversity and evenness indices at different stations after 1—4 weeks exposition.

5. Conclusions

The dry weight masses of the periphyton in the lake littorals are primarily correlated with the growth of the attached algae and not so much with the allochthonous material as in flowing waters. Thus no great seasonal variations of the periphyton mass are seen in lake littorals as in the flowing waters especially when the fluctuations of the discharge are great. The water usually contains less inorganic matter in the sheltered shores with rich vegetation than it does in the open and wavy shores and in the streams. Many water fleas (especially *Sida)* occurred on the plates in oligotrophic areas, whereas in the eutrophic areas, where the algae grew rapidly, grazing herbivores, such as small chironomid larvae and gastropods, occurred on the plates.

The increasing of the amount of the periphyton per unit area ended usually after 3 weeks exposition since the older parts of the periphyton were lost and the grazing increased. During slow growth of algae, especially in autumn, the retarded increment of

Fig. 14. The developments of the cell densities on the artificial plates at different stations during the growing season. →


A
cells cm⁻²
M
A
J_I...J_II
S_I
S_II
A...S
O
O...N
weeks
B
cells cm⁻²
M
J_I...J_II
S...O
S...O
O
O...N
weeks
C
cells cm⁻²
S
J_I
J_II
A...S
O_I
O_II
O...N
weeks
D
cells cm⁻²
S...O
O...N
J_I
A
O
A...S
S
M = May
J_I = June
J_II = July
A = August
S = September
O = October
N = November
weeks

periphyton mass was seen first after 4—5 weeks exposition, probably due to a low nutrient content of the water and low water temperature and decreasing of the illumination. Also CASTENHOLZ (1960, 1961) and ERTL (1974) have verified that the periphyton mass per unit area reaches its maximum after 3—4 weeks exposition owing to the reasons mentioned above. Like the total periphyton mass also the chlorophyll *a* content increased exponentially on the plates during the first three exposition weeks, but the growth rate was different in different localities depending on the degree of the eutrophication (cf. also CASTENHOLZ 1960, 1961, ERTL 1974, TILLEY & HAUSHILD 1975).

In the autumn, the chlorophyll contents were rather greater in the oligotrophic than in the eutrophic localities, because the growth continued in the oligotrophic areas at the same time as the growth slowered strongly in the eutrophic areas. The growth rate of the periphyton algae corresponds in late summer the degree of the eutrophication determined by the phytoplankton production and biomass at different stations (Fig. 9; ELORANTA 1976). Thus the maximum growth rate of the periphyton at the eutrophic stations A and B occurred in the early summer when also the production of phytoplankton was highest. The inhibition of the phytoplankton production caused by the acid effluents in Lake Jyväsjärvi (station A) was also seen in the growth of the periphyton algae and in the total dry weight masses of the periphyton (Figs. 5 and 10).

The energy content of the periphyton dry matter depends primarily on its organic content (Fig. 15; cf. also RYBAK 1969 ref. CUMMINS & WUYCHECK 1971). On the other hand, the energy content of the organic matter of the periphyton is dependent on the portions of animals, algae and detritus. Some values illustrating the energy content of different fractions of the periphyton given in the literature are shown below (CUMMINS & WUYCHECK 1971):

— periphyton	18.91	J/mg ash free dry weight
— filamentous green algae + diatoms	16.88	„
— Cladocera	22.22	„
— Chironomidae (larvae)	35.77	„
— aquatic detritus	21.62	„

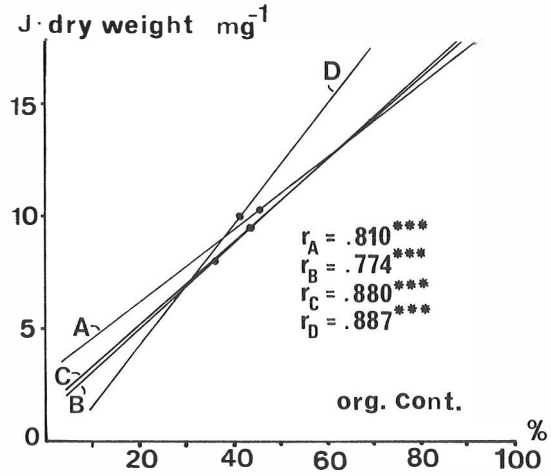


Fig. 15. The correlations between the organic content of the periphyton matter and its energy content.

The energy content of the animals and detritus is higher than that of the algae. Thus the relative increase of the algae on the plates during the exposition was one reason for the decrease of the energy content of the organic matter in the older samples.

The species composition found on the plates was typical for the epiphytic and epilithic diatom communities usually found in lakes (see e.g. ROUND 1956). The diatom floras at the different stations reflected the degree of the pollution and eutrophication and especially the pH of the water. The acidophilic species (especially *Tabellaria* spp.) occurred in the most polluted and eutrophic Lake Jyväsjärvi where the pH of the water was also lowest and where, at the same time, the lowest species richness and evenness were found. The alkaliphilic genera *Cocconeis* and *Epithemia* occurred only occasionally in the acid waters of the study area and the frequencies and species richness of the genera *Achnanthes, Cymbella, Gomphonema* and *Synedra* were greatest at the oligotrophic, but least acid station D. The saprobic system based on the diatoms was not relevant to the description of the conditions of the waters in the study area.

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Selostus

Järvilitoraalin perifytonista eräissä Keski-Suomen järvissä

PERTTI ELORANTA ja SOILE KUNNAS

Tekoalustoja käyttäen tutkittiin kesällä 1974 erityyppisten järvien litoraalin perifytonin kasvua, energiasisältöä sekä piilevien solutiheyksiä ja lajistoa.

Tutkittujen järvien väliset rehevyyserot näkyivät selvästi perifytonin kasvunopeudessa ja klorofyllipitoisuuksissa pinta-alayksikköä kohti. Perifytoaineksen määrän maksimi kuivapainona ja klorofyllimääränä mitaten saavutettiin kesällä 3 viikon eksspositioajan jälkeen, mutta kasvu hidastui syksyllä erityisesti rehevämmillä alueilla.

Epäorgaanisen aineksen osuus kuivapainosta oli keskimäärin 56,8 %, levien osuus 13,6 % ja muun orgaanisen aineksen osuus keskimäärin 29,4 %. Eksspositioajan pidentyessä mineraaliaineksen suhteellinen osuus pieneni, ja levien osuus yleensä kasvoi. Levyille kerääntyneen aineksen kuivapainon ja klorofyllimäärän välinen korrelaatio oli erittäin vahva. Useimmilla havaintopaikoilla perifytoaineksen energiasisältö kuivapainoyksikköä kohti laskettuna kasvoi eksspositioajan pidentyessä, mutta toisaalta energiasisältö aleni laskettuna tuhkatonta kuivapainoyksikköä kohti eksspositioajan pidentyessä. Keskimääräinen energiasisältö oli 9,58 J/kuivapaino-mg ja 22,43 J/tuhkaton kuivapaino-mg.

Tärkeimmät alustoilla kasvaneet leväryhmät olivat rihmamaiset viherlevät sekä piilevät. Dominoivat piileväsuvut olivat *Tabellaria, Synedra, Eunotia, Achnanthes, Gomphonema* ja *Cymbella*. Lajimäärä ja piileväyhteisön tasaisuus oli suurin oligotrofisella alueella. Vaikka lajimäärät kasvoivat eksspositioajan alkuvaiheessa, aleni diversiteetti koko eksspositioajan erityisesti eutrofisilla alueilla. Kesällä solutiheydet kasvoivat eksponentiaalisesti kaiden ensimmäisen ekspositioviikon ajan, maksimin ollessa 2—8 $\times$ 10^5 solua/cm^2, mutta syksyllä solumäärät kasvoivat eutrofisilla alueilla hyvin hitaasti, kun taas oligotrofisimmalla havaintopaikalla kasvu oli yhtä voimakasta kuin kesällä.

Biol. Res. Rep. Univ. Jyväskylä 2: 51—66. 1976

Phytoplankton and primary production *in situ* in the lakes Jyväsjärvi and North Päijänne in summer 1974

PERTTI ELORANTA

ELORANTA, P. 1976: Phytoplankton and primary production *in situ* in the lakes Jyväsjärvi and North Päijänne in summer 1974. — Biol. Res. Rep. Univ. Jyväskylä 2: 51—66.

The phytoplanktonic production by ^{14}C method, biomasses by cell countings and chlorophyll *a* contents were studied in two eutrophicated and polluted lakes in Central Finland. The subsurface light conditions were measured for the calculations of the primary production efficiency at the different depths. The thickness of the photosynthetically productive layer was about the same as the Secchi disc visibility when the colour of the water was more than 50 mg Pt/l and about 1.5—fold in the more transparent waters. The phytoplanktonic primary production was in very significant positive correlation to the phytoplankton fresh weight biomass and to the chlorophyll *a* content of the water. The correlation between the primary production and the incident solar radiation energy was positive, but weak. The fresh weight phytoplankton biomass was in high positive correlation to the chlorophyll *a* content of the water and the correlation between the photosynthetically available radiation and the activity coefficient (P/B) at the depth of the maximum cubic meter primary production was positive and very significant. The efficiency of the primary production increased rapidly from 0 to 1 meter, but the increase became slower in the deeper layers.

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1. Introduction

There are today in Finland several studies concerning the primary production connected with the state control of watercourses. Thus the bulk of the observations are concerned with the primary productivity whereas fewer studies have been made on the primary prodution *in situ*. The first named method has the particular advantage of constant temperature and illumination. Thus the results of different times are better comparable but the comparison with the results of the primary production measurements *in situ* are very difficult because of the methodical reasons (TALLING 1971 a). When measuring the primary production *in situ* the underwater light conditions and the penetrating of the solar radiation must be studies because the fluctuation of the solar radiation energy is often the most important factor affecting the variation of the primary production during the growing season. Also the particles in the water and the dissolved coloured substances have their effect on the penetration and the quality of light, so that it is important to ascertain also the amount of the photosynthetically active radiation at various depths. The production and the activity of the phytoplankton also depend on the species composition and other ecological factors (cf. RODHE 1958, TALLING 1971 b, HILLBRICHT-ILKOWSKA *et al.* 1972, PECHLANER *et al.* 1972, GÄCHTER 1972,

JAVORNICKY & KOMARKOVA 1973, TILZER 1973).

In this study the qualitative and quantitative variation of the phytoplankton has been followed as well as the primary production *in situ* at different conditions. At the same time the correlations between the above mentioned parameters were observed in two receiving waters, loaded by industrial effluents and sewage.

2. The study area

Lake Jyväsjärvi is heavily eutrophicated primarily by the sewage of the town of Jyväskylä (GRANBERG 1973 a). The effects of the industrial effluents containing large amounts of sulphuric acid from a paper mill are also characteristic of the lake.

A rich algal production and the great quantity of fish in the lake revealed that the influence of these industrial effluents was not so heavy in the summer 1974. The injurious effects of these effluents were moderated by the abundant rains in the summer and autumn of 1974 and by the plentiful discharge into the North Päijänne (Fig. 1). The waters discharging from Lake Jyväsjärvi flow in the North Päijänne through the west side of the lake and become diluted quite quickly at the same time. This situation was followed at station B in North Päijänne. The waters discharging from northern water-courses are loaded especially with the effluents of wood processing industry, but these effluents are already clearly diluted when flowing into North Päijänne. The main flow of these waters flows through the eastern lakeside, where the third sampling station (C) was situated.

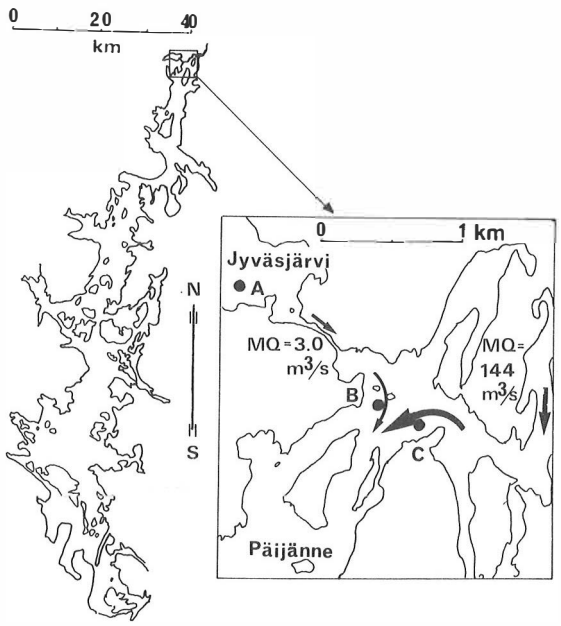

Fig. 1. The study area and the sampling stations (A—C). The arrows indicate the main flowing directions (MQ values according to LAPPALAINEN 1972).

3. Methods

The phytoplankton samples for the determinations of the biomass and the species composition were taken from the water column of 0—2 m with a 2-meter-long tube-sampler. The samples were preserved with some drops of IKI-solution with Na-acetat (UTERMÖHL 1958). 1 cm³ of concentrated formalin was added later. The counts were made with an inverted microscope and phase contrast optics. The smaller algal species were counted on three transverse strips at the magnifications of × 600 and the larger algal species were counted on the whole of the counting chamber floor at the magnifications of × 150 (UTERMÖHL 1958). The counts of the samples from Lake Jyväsjärvi were made with counting chambers of 10 cm³ and those from other sampling stations with chambers of 50 cm³.

The total volumes (fresh weight biomasses) of the phytoplankton were mainly calculated with the volume tables of NAULAPÄÄ (1972) but for the most abundant species mean volumes were also calculated for different samples.

The species diversities were calculated on the basis of the biomasses of each species according to the following formula (SHANNON & WEAVER 1949):

(1) $\bar{H} = -\Sigma\ Bm/BM \ln (Bm/BM)$,

where Bm/BM is the portion of each species of the total phytoplankton biomass.

The estimation of chlorophyll *a* content of the water was made by filtering through a paper filter (Macherey-Nagel & Co MN 640 md) 0.5—1.0 l of a water sample taken as collective sample from the water layer of 0—2 m. The filters were cut into pieces for the centrifuge tubes and 10—20 cm³ of extract solution was added into the tubes. The extraction was made by cold 90 % acetone or by boiling the samples in 90 % methanol in a water bath for one minute. Then the samples were centrifugated and the extinction was mea-

sured with a spectrophotometer in 5 cm pathlenght cells against 90 % methanol as a reference at the wavelenghts of 666, 653, and 720 nm (IWAMURA *et al.* 1970, cf. also TALLING 1971 b). In the correlative determinations with 90 % acetone the samples were kept in a refrigerator to the next day, and the extinction was measured against 90 % acetone at the wavelenghts of 665, 645, 630, and 750 nm (STRICKLAND & PARSONS 1968). The comparisons showed that the estimation with 90 % methanol gave 1.18-fold contents when compared with the results of acetone extraction (cf. also MARKER 1972). The comparative studies concerning different pigment estimation methods are still being continued.

The primary production was measured with the ^{14}C-method according to LASSIG & NIEMI (1972). The water samples for incubation were taken from the depths of 0.2, 0.5, 1, 2, 3, and 5 meters and poured into three parallel bottles (130 cm^3); 1 cm^3 of $NaH^{14}CO_3$ solution having a specific activity of 1 µCi was injected into one dark and one light bottle. These six parallel dark and light bottles were exposed for 24 h in the lake. The experiment began in the morning. After the incubation the photosynthetic activity was stopped by adding 1 cm^3 of concentrated formalin into the bottles. No decrease in the activity was noted when parallel samples handled and not handled with formalin were compared (JUSSILAINEN, personal comm., cf. ILMAVIRTA 1974). The whole contents of the bottles were filtered with a membrane filter (Millipore HAWP 04700, 0.45 µm) which was not handled with HCl (cf. LASSIG & NIEMI 1972). The radioactivity of the residues on the filters was measured with an end window GM counter (Frieseke & Hoepfner Type FH 49).

Total alkalinity was measured by titration of samples with 0.02 N HCl to pH 5.1 against mixed indicator of brom-cresol green and methyl red. The free CO_2 was determined by titration of samples with 0.02 N NaOH using phenolphthalein as an indicator. The inorganic carbon in the water was calculated from the total alkalinity and from the free carbon dioxid content by the following equation (cf. VOLLENWEIDER 1971 a):

(2) C inorg. = 12 × alkalinity (meq/l) + 0.273 × free CO_2 (mg/l).

The approximate net primary production values have been calculated by subtracting the dark fixation values from the light bottle values.

The light attenuation was measured by lowering the photocell from the water surface into 0.25 m, 0.5 m, 0.75 m, 1 m and then at depht intervals of 0.5 meter. The photoelement was equipped with a green filter (VG 9) and an opal glass and connected to a microammeter. The variation of incident light was controlled with a second photocell (VOLLENWEIDER 1971 b). Comparative measurements were also made at the northern part of Lake Keurusselkä with high humus content and in the middle of Lake Keitele with low humus content.

The total values of solar irradiance were measured at the meteorological station of Luonetjärvi about 15 km from the study area to NNW (Department of Aerology of the Institute of Meteorology, personal comm.). These values were multiplied by 0.45 when the photosynthetically active part of the radiation was calculated (cf. VOLLENWEIDER 1971 b, GÄCHTER 1972); the values are shown in the diagrams illustrating the vertical primary production (Figs. 8—10).

4. Results

4.1. The light conditions

In spite of the long summer days the underwater light conditions in Finnish lakes are not generally good because of the great angle of incidence of the radiation and the high humus content of the waters. Especially in Lake Jyväsjärvi the water was very turbid in the early summer of 1974, but during the summer the water became clear. The light conditions in Lake Jyväsjärvi were comparable with those in a polyhumic dystrophic Lake Keurusselkä, in which 1 percent of the incident surface illumination is measured at the depth of 1.5 m (Fig. 2). In the northern

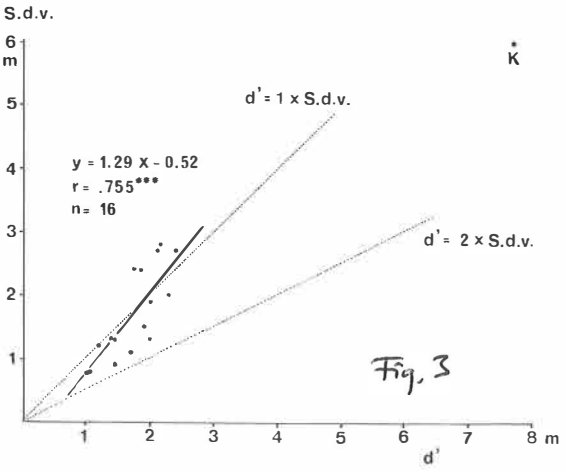

Fig. 2. The mean relative penetration of green light in Lakes Jyväsjärvi, North Päijänne, Keitele (K, 17.VIII.1974), and Keurusselkä (20.VI.1974; PC-type = water with high humus content, MC-type = water with medium high humus content; cf. JÄRNEFELT 1963).

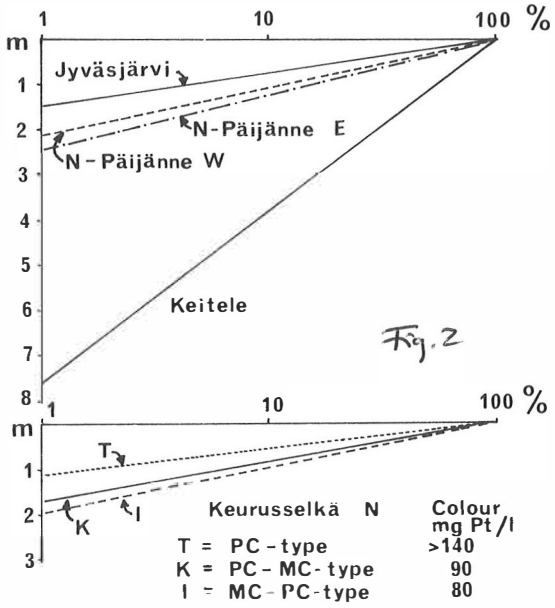

Fig. 3. The correlation between the depth with 1 % of the incident green light (d') and the Secchi disc visibility at the sampling stations and in Lake Keitele.

parts of Lake Päijänne the corresponding depth was 2.1—2.3 m and in Lake Keitele 7.7 m. SCHEGG (1971) has verified that the Secchi disc visibility values correspond to the depth with 20 % of the incident green light and at the depth of 2—3 × Secchi disc visibility value is about 1 % of incident light. In the study area the Secchi disc value corresponds to the depth of 1 % of the incident green light (Fig. 3). In waters with high humus content the Secchi disc values were even the depth of 1 % of incident light. In Lake Keitele the Secchi disc visibility was however much smaller than the depth of 1 % of surface illumination.

4.2. Biomasses and chlorophyll a contents

The fresh weight biomasses as well as the chlorophyll *a* concentrations clearly reflected the differences in the degree of the eutrophication at the stations (Table 1, Fig. 4). In early and middle summer the chlorophyll content of the water layer of the water layer of 0—2 m was about 10—20 % higher than in the water layer of 2—4 m. This difference disappeared when the thermal stratification weakened in late summer. There was a clear positive correlation between the biomass and the chlorophyll *a* concentration ($r = .940^{***}$, Fig. 5). In Lake Jyväsjärvi the mean chlorophyll content of algal biomass was 0.32 % ranging from 0.15 to 0.46 % (Table 2). At stations B and C (North Päijänne) the chlorophyll contents were somewhat higher, at station B the mean was 0.40 % (0.09—0.75) and at station C 0.47 % (0.20—0.82). In Lake Keitele the chlorophyll content of the fresh weight biomass was 1.13 % in the

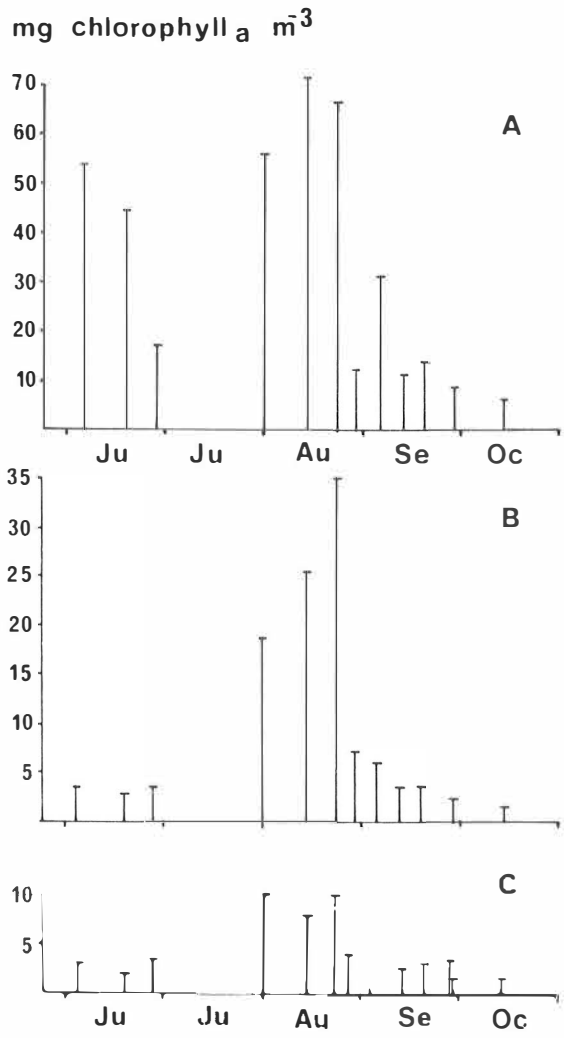

Fig. 4. The chlorophyll *a* concentrations at the sampling stations in the summer 1974.

Table 1. Temperature, photosynthetically available incident radiation (J/cm²/day), pH of the water, and values illustrating the phytoplanktonic primary production, biomass and ecological efficiency (V/O, P/B, 1/R) at the sampling stations in the summer 1974 (BM = phytoplankton fresh weight biomass as g/m³ in the water layer of 0—2 m, Chl a = chlorophyll *a* content of the water in the layer of 0—2 m as mg/m³, $\bar{H}v$ = species diversity on the biomass basis, ^{14}C ass = net primary production as mg C ass/m²/day, ^{14}C ass max = maximum ^{14}C production per cubic metre as mg C ass/m³/day. Dark fix. % = percentage of the ^{14}C dark fixation of the net primary production, 1/R = the renewal time of the phytoplankton as days).

Station A

Date	t_C	I_o	pH	BM	Chl_a	$\bar{H}_v$	$^{14}C_{ass}$	$^{14}C_{ass\ max.}$	Dark fix. %	V/O	P/B	1/R
28.V.	12.0	1131	-	13.29	-	1.53	383	335	11.4	0.87	.142	7.0
05.VI.	12.6	854	6.7	20.10	54.0	1.15	812	697	5.8	0.86	.185	5.4
17.VI.	20.0	1404	-	9.68	44.9	2.06	818	803	4.6	0.98	.406	2.5
27.VI.	23.0	729	6.5	4.64	20.9	1.52	428	606	9.5	1.42	.435	2.3
01.VIII.	18.9	432	4.4	19.41	56.4	1.91	468	690	11.4	1.47	.116	8.6
12.VIII.	16.8	520	4.9	18.40	72.0	1.11	118	164	10.8	1.39	.031	32.3
19.VIII.	17.5	-	-	-	67.4	-	-	-	-	-	-	-
28.VIII.	18.1	549	5.9	8.09	11.9	1.63	196	292	9.4	1.49	.085	11.8
02.IX.	16.5	-	-	14.91	30.6	1.73	-	-	-	-	-	-
10.IX.	15.6	423	5.9	3.62	11.0	1.97	37	42	33.0	1.14	.049	20.4
17.IX.	12.5	-	-	3.87	14.4	1.42	-	-	-	-	-	-
26.IX.	10.7	-	-	2.38	8.8	2.10	-	-	-	-	-	-
14.X.	8.9	189	6.5	2.17	6.8	1.73	10.6	13.2	57.7	1.25	.015	66.7

Station B

28.V.	10.0	1131	-	2.98	-	1.77	150	140	8.7	0.94	.232	4.3
05.VI.	10.4	854	6.8	2.83	2.5	1.33	122	190	10.3	1.55	.205	4.9
17.VI.	17.1	-	-	1.53	2.8	1.57	-	-	-	-	-	-
27.VI.	17.5	641	-	1.02	3.6	2.16	61	53	17.9	0.88	.273	3.7
01.VIII.	19.3	432	6.1	7.48	18.8	1.65	170	247	12.1	1.46	.109	9.2
12.VIII.	16.8	186	6.7	4.84	25.5	2.01	29	42	16.1	1.43	.018	55.6
19.VIII.	17.5	-	-	10.54	35.2	1.57	-	-	-	-	-	-
28.VIII.	18.1	549	6.5	3.76	7.0	1.97	121	149	6.5	1.23	.149	6.7
02.IX.	16.2	-	-	1.59	6.2	2.30	-	-	-	-	-	-
10.IX.	15.9	423	6.3	0.64	3.6	2.58	11	11	67.7	0.98	.080	12.5
17.IX.	12.1	-	-	0.54	3.7	2.42	-	-	-	-	-	-
26.IX.	10.2	-	-	0.46	2.4	2.40	-	-	-	-	-	-
14.X.	9.7	189	6.8	0.24	1.8	2.86	6.4	3.6	8.9	0.56	.109	9.2

Station C

05.VI.	10.3	854	6.7	1.28	3.2	2.11	110	88	11.1	0.80	.361	2.8
17.VI.	16.1	1404	-	1.02	2.1	1.89	73	59	14.4	0.80	.333	3.0
27.VI.	18.2	1035	6.6	0.45	3.7	1.68	54	45	17.7	0.82	.539	1.9
01.VIII.	18.9	432	7.1	2.27	9.9	2.40	73	66	19.7	0.91	.143	7.0
12.VIII.	17.0	186	6.7	1.85	8.3	2.28	16	19	32.1	1.21	.040	25.0
19.VIII.	17.2	-	-	1.57	10.2	1.90	-	-	-	-	-	-
28.VIII.	17.5	548	6.5	1.37	3.9	2.35	26	31	39.7	1.18	.080	12.5
02.VIII.	16.2	-	-	0.76	3.6	2.63	-	-	-	-	-	-
10.IX.	16.0	423	6.4	0.46	2.4	2.45	11	11	51.9	0.98	.111	9.0
17.IX.	12.1	-	-	0.52	2.9	2.18	-	-	-	-	-	-
26.IX.	10.3	-	-	0.37	1.8	2.86	-	-	-	-	-	-
14.X.	9.7	189	6.8	0.33	1.8	2.17	4.1	3.0	10.5	0.72	.050	20.0

Keitele

21.VIII.	16.5	610	6.9	0.52	3.7	2.72	17.1	6.8	9.1	0.41	.100	10.0

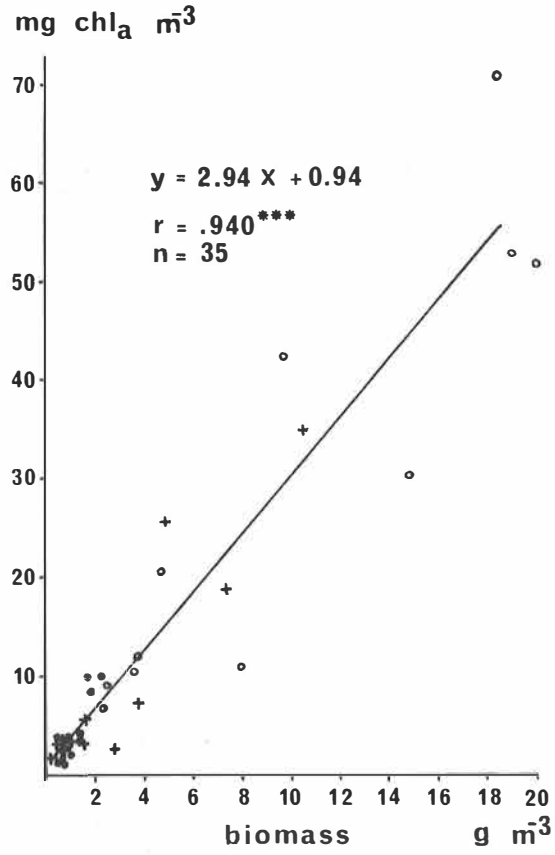

Fig. 5. The correlation between the phytoplankton fresh weight biomass and the chlorophyll *a* concentration in the study area in summer 1974 (circles = station A, + = station B, and black dots = station C).

water layer of 0—2 m and only 0.44 % in the depth of 2—4 m (20.VIII.1974).

4.3. The structure of the phytoplankton community

The high degree of eutrophy in Lake Jyväsjärvi was clearly reflected in the structure of the phytoplankton community in spite of the low pH caused by the industrial effluents (cf. Table 1). It was true that the number of the species belonging to the groups Chlorococcales and Euglenophyta, which are characteristic of eutrophy (JÄRNEFELT 1952), was quite low with regard to the level of eutrophy.

Table 2. The percentages of the chlorophyll *a* of the phytoplankton fresh weight biomass at the stations A—C in the summer 1974.

	STATION		
DATE	A	B	C
05.VI.	.27	.09	.25
17.VI.	.46	.18	.21
26.VI.	.45	.35	.82
01.VIII.	.29	.25	.44
12.VIII.	.39	.53	.45
19.VIII.	—	.33	.65
28.VIII.	.15	.19	.28
02.IX.	.21	.39	.47
10.IX.	.30	.56	.52
17.IX.	.37	.69	.56
26.IX.	.37	.52	.49
14.X.	.31	.75	.55
Mean ± S.D.	.32 ± .10	.40 ± .21	.47 ± .17

In the early summer the most dominant species were the diatoms *Melosira italica, Asterionella gracillima, Tabellaria* spp. and *Fragilaria* spp. (Table 3, Fig. 6). At the same time and especially after the diatom maximum the genus *Scenedesmus* (Chlorococcales) became dominant in the phytoplankton. Cryptomonads began to increase in dominance rapidly later in the summer which is typical especially of the dyseutrophic lakes (ELORANTA 1974). A unusual maximum of the group Volvocales in the middle of the summer (Fig. 6) was caused by a *Chlamydomonas* species which alone consisted of nearly 3/4 of the biomass at station A (Lake Jyväsjärvi) in August 1974 (Table 3). The abudance of the chrysomonads, especially of the species *Mallomonas caudata* and *Synura uvella,* increased at the end of August.

The relative portion of *Rhodomonas minuta* in the whole biomass was greater at station C than in the more eutrophic areas. The dominance of this species decreases with the increase of eutrophy, especially dyseutrophy (cf. GRANBERG 1973 a, ELORANTA 1974). The species occurred only occasionally in Lake Jyväsjärvi. This taxon is obviously the same as that identified as *Chroomonas acuta* in Lake Pääjärvi (ILMAVIRTA & KOTIMAA 1974).

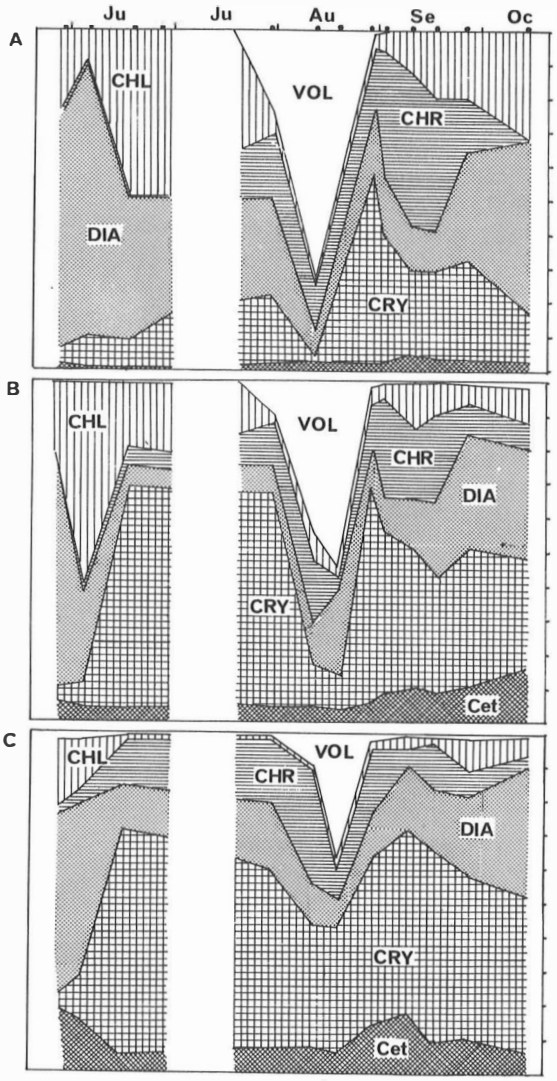

Fig. 6. Percentages of the biomasses relating to various phytoplankton groups at the sampling stations in summer 1974 (CHL = Chlorococcales, VOL = Volvocales, CHR = Chrysomonadinae, DIA = Diatomae, CRY = Cryptomonadinae, and Cet = cetera).

In the autumn there was another increase in the dominance of the diatoms but no clear diatom maximum occurred during the end of the study period.

4.4. Diversity

The species diversity reflects the structure of the phytoplankton but not the degree of the eutrophy, in late summer. The correlation between the fresh weight biomass and the species diversity was negative and very significant in late summer (Fig. 7).

The increase of the dominance of the most abundant species charcteristic of eutrophic waters was also seen in the study area. The biomass of the three most abundant species was, on the average, 80 % of the total biomass at station A and at stations B and C 72.0 % and 66.7 % respectively. The mean species diversity in the growing season of 1974 was 1.66 ± 0.33 at station A and in North Päijänne 2.05 ± 0.46 (station B) and 2.24 ± 0.33 (station C).

4.5. The primary production

4.5.1. The quantity of production and dark fixation

The times of the most copious production were early summer and the beginning of August; the maxima of the biomasses also occurred at the same times (Table 1). Admittedly, the production in Lake Jyväsjärvi in August was not as high as could be expected on ground of the biomass. The most important reason for this was obviously the low pH of the water (1.VIII.1974 pH 4.4 and 12.VIII.1974 pH 4.9; cf. ELORANTA & ELO-

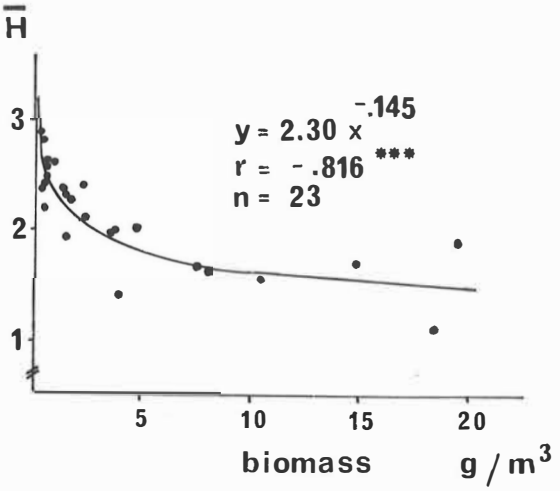

Fig. 7. The correlation between the phytoplankton fresh weight biomass and the species diversity.

Station A	Vol.%
28.V.	
Melosira italica	56.6
Scenedesmus pecsensis	19.6
Asterionella gracillima	5.1
	81.3
05.VI.	
Melosira italica	70.8
Cryptomonas spp.	8.9
Scenedesmus pecsensis	8.5
	88.2
17.VI.	
Scenedesmus armatus	35.7
Melosira italica	29.8
Scenedesmus pecsensis	9.1
	74.6
31.VII.	
Chlamydomonas sp.	24.7
Tabellaria fenestrata	23.8.
Cryptomonas spp.	20.0
	68.5
12.VIII.	
Chlamydomonas sp.	73.4
Synura uvella	11.8
Tabellaria flocculosa	3.7
	88.9
19.VIII.	
29.VIII.	
Cryptomonas spp.	55.5
Synura uvella	15.4
Melosira italica	13.5
	84.4
02.IX.	
Cryptomonas spp.	34.5
Synura uvella	33.4
Melosira italica	15.4
	83.3
10.IX.	
Synura uvella	40.5
Cryptomonas spp.	23.9
Melosira italica	10.8
	75.2
17.IX.	
Synura uvella	35.2
Cryptomonas spp.	23.8
Scenedesmus armatus	18.3
	77.3
26.IX.	
Cryptomonas spp.	28.7
Melosira italica	27.8
Scenedesmus armatus	18.6
	75.1
14.X.	
Melosira italica	47.7
Scenedesmus opoliensis	20.7
Cryptomonas spp.	14.5
	82.9

Station B	Vol.%
28.V.	
Melosira italica	47.6
Scenedesmus pecsensis	23.3
Tabellaria flocculosa	6.3
	77.2
05.VI.	
Scenedesmus pecsensis	59.8
Melosira italica	24.5
Cryptomonas spp.	7.0
	91.3
17.VI.	
Cryptomonas spp.	59.0
Scenedesmus pecsensis	17.6
Rhodomonas minuta	6.3
	82.9
01.VIII.	
Cryptomonas spp.	61.0
Chlamydomonas sp.	10.1
Synura uvella	6.2
	77.3
12.VIII.	
Chlamydomonas sp.	44.7
Synura uvella	16.7
Cryptomonas spp.	11.4
	72.8
19.VIII.	
Chlamydomonas sp.	54.6
Melosira italica	20.8
Cryptomonas spp.	9.8
	85.2
29.VIII.	
Cryptomonas spp.	57.7
Synura uvella	7.4
Melosira italica	6.6
	71.7
02.IX.	
Cryptomonas spp.	42.8
Synura uvella	20.9
Rhodomonas minuta	4.4
	68.1
10.IX.	
Cryptomonas spp.	35.1
Synura uvella	16.0
Scenedesmus armatus	6.9
	58.0
17.IX.	
Cryptomonas spp.	31.2
Synura uvella	20.3
Melosira italica	12.5
	64.0
26.IX.	
Cryptomonas spp.	36.1
Melosira italica	21.4
Rhodomonas minuta	4.8
	62.3
14.X.	
Cryptomonas spp.	25.9
Melosira italica	20.7
Rhodomonas minuta	6.8
	53.4

Station C	Vol.%
28.V.	
05.VI.	
Melosira italica	39.5
Scenedesmus pecsensis	14.4
Cryptomonas spp.	13.2
	67.1
17.VI.	
Cryptomonas spp.	58.5
Mallomonas caudata	8.4
Rhodomonas minuta	6.3
	73.2
01.VIII.	
Cryptomonas spp.	41.0
Rhodomonas minuta	11.9
Synura uvella	8.0
	60.9
12.VIII.	
Cryptomonas spp.	32.9
Synura uvella	27.7
Chlamydomonas sp.	10.4
	71.0
19.VIII.	
Chlamydomonas sp.	36.8
Cryptomonas spp.	36.0
Mallomonas caudata	8.2
	81.0
29.VIII.	
Cryptomonas spp.	47.7
Mallomonas acaroides	9.5
Rhodomonas minuta	7.8
	65.0
02.IX.	
Cryptomonas spp.	41.0
Synura uvella	10.9
Rhodomonas minuta	7.9
	59.8
10.IX.	
Cryptomonas spp.	48.9
Synura uvella	5.6
Rhodomonas minuta	5.3
	59.8
17.IX.	
Cryptomonas spp.	49.3
Synura uvella	10.7
Rhodomonas minuta	7.5
	67.5
26.IX.	
Cryptomonas spp.	38.7
Rhodomonas minuta	9.3
Melosira italica	8.7
	56.7
14.X.	
Cryptomonas spp.	41.6
Melosira italica	25.7
Rhodomonas minuta	4.4
	71.7

Table 3. The percentages of the three most dominant species of the phytoplankton samples of the total biomass.
←

RANTA 1974). The inhibition of the acid waters caused the decrease in the biomass, too. However, it began to increase again, when pH became more favourable.

The maximum quantity of the dark fixation was noted in the study area at the same time as the maximum of the photosynthetic production (the correlation between the dark fixation and the photosynthetic production was very significant, r = .811***; cf. ILMAVIRTA *et al.* 1974). The vertical distribution of the dark fixation corresponded also to the depth distribution of primary production and the maximum was usually found in the uppermost metre (cf. also ILMAVIRTA 1974). The dark fixation percentages of the light fixation were greater than those in the unpolluted waters (the means of the growing season values were 12.0—23.0 %). KUZNETSOV (1958) gives the values below 3.3 % for the chemosynthetic fixation of the natural waters.

In Lake Pääjärvi the mean values of dark fixation percentages in the growing seasons of 1972 and 1973 were 6.1 % and 4.0 % respectively (ILMAVIRTA *et al.* 1974). An increase of the dark fixation percentage is known to indicate pollution of waters by cellulose factory effluents as well as eutrophication (LEHMUSLUOTO 1967, BAGGE & LEHMUSLUOTO 1971, GRANBERG 1973 a). These factors are obviously the reason for the increase of the dark fixation in the study area. The increase of the dark fixation percentage could be noted when there was a decrease in the primary production (correlation coefficient r = —.459*, n = 25).

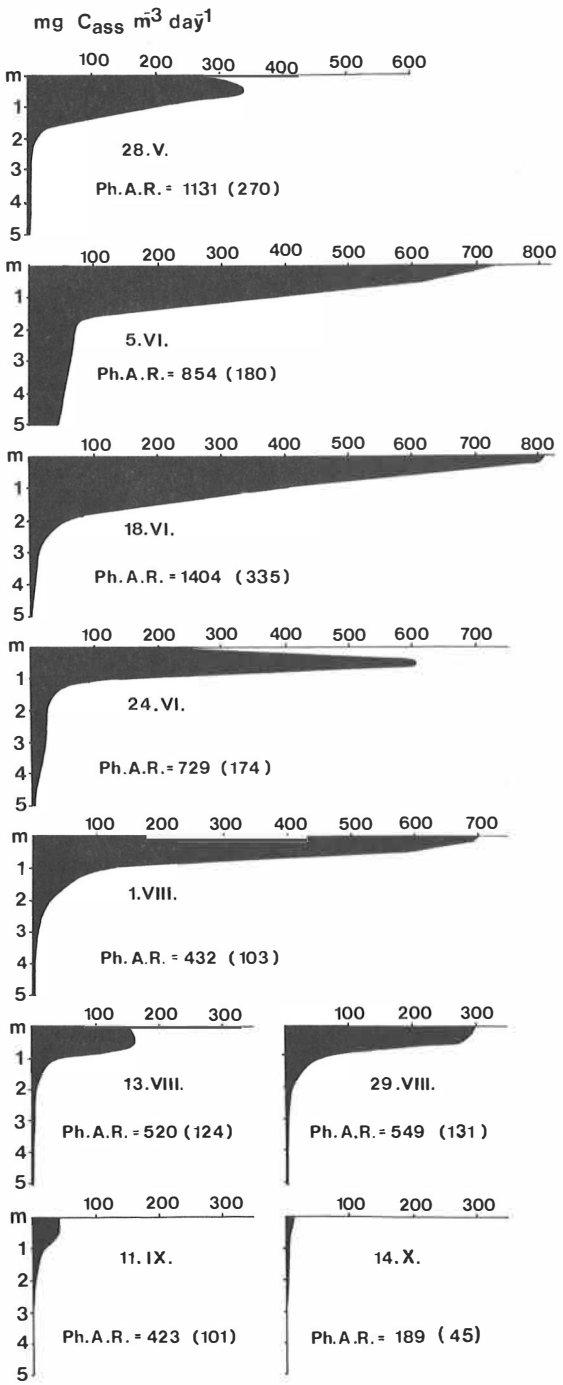

Fig. 8. Depth curves of daily "net" primary production of phytoplankton in Lake Jyväsjärvi (station A) in summer 1974. The dates are the days on which exposure was commenced (Ph A.R. is the photosynthetically available radiation energy as J/cm²/day and as cal/cm²/day during each incubation periods). →

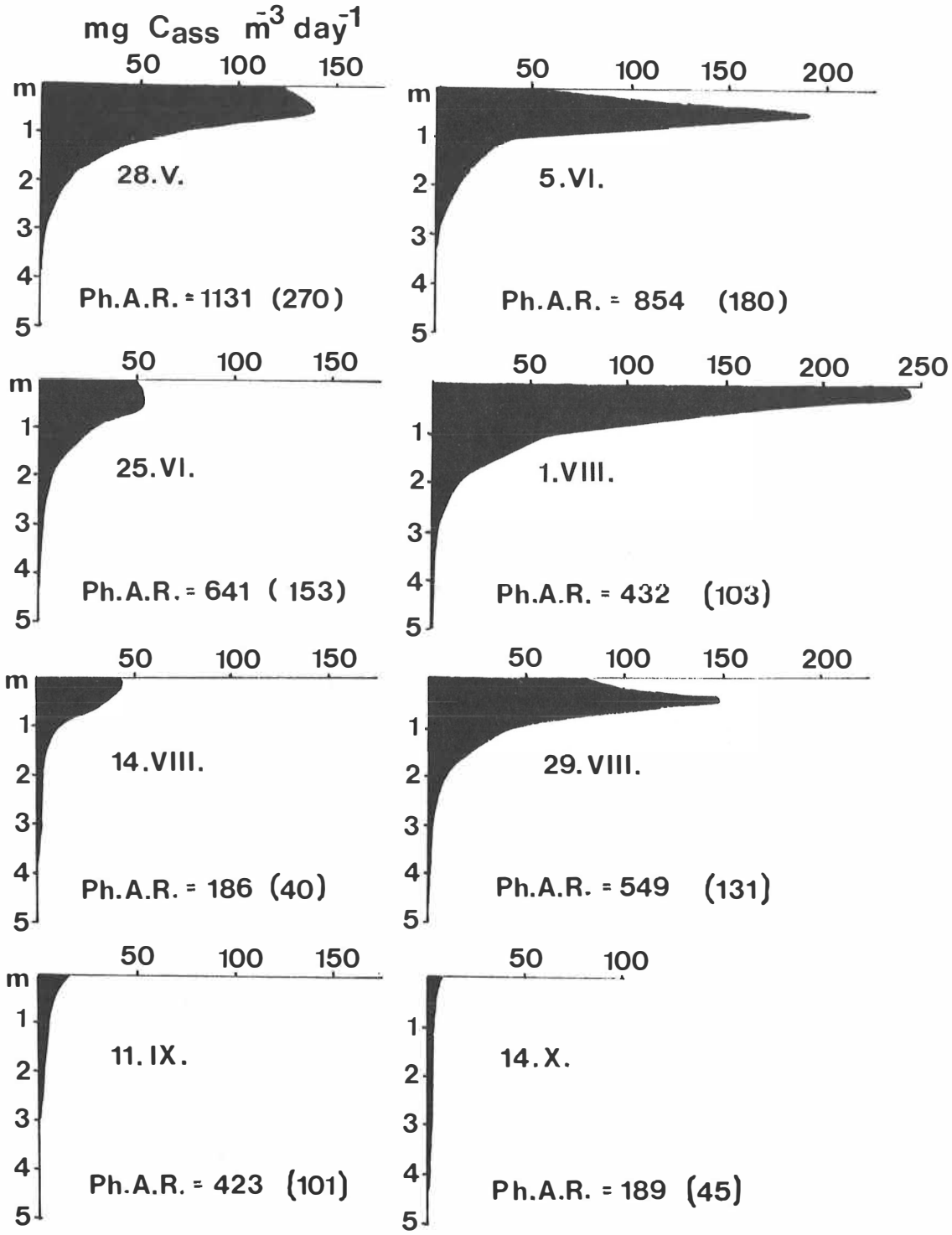

Fig. 9. Depth curves of daily net primary production of phytoplankton at station B (North Päijänne) in summer 1974 (cf. Fig. 8).

4.5.2. The vertical distribution of the primary production

The strong concetration of the algal production in the surface layers was clearest at station A (Fig. 8), but also at the other stations (Figs. 9 and 10). The restriction of the primary production to the uppermost water layer is especially characteristic of the eutrophic waters, in which the selfshading of the phytoplankton causes a decrease in the wate layer suitable for the photosynthetic production (RODHE 1958). On the other hand the rapid extinction of the illumination caused by the high humus content of the water increases the ratio of the maximum production and total production per square meter (V/O quotient; LEHMUSLUOTO 1969). In summer 1974 the mean V/O quotient at station A was 1.21 ± 0.25 (range 0.86—1.49) and in North Päijänne at station B 1.13 ± 0.34 (0.56—1.55) and at station C 0.93 ± 0.18 (0.72—1.21). In Lake Keitele with low humus content the V/O quotient was only 0.41 (21.VIII.1974).

The inhibition of the production due to the intense radiation was noted at all the stations during the sunny days in the summer (Figs. 8—10). Owing to the humus and the suspended particles in the water the extinction of the illumination was rapid and thus the inhibition was observed only in the uppermost 20—30 cm layer. In the middle of Lake Keitele the depth of the maximum production was 1 m, which corresponds to the situation in Lake Konnevesi which is also a lake with low humus content (GRANBERG 1973 b).

4.5.3. P/B and 1/R coefficients

The activity coefficient of the phytoplankton (P/B coefficient or R/m² values; TALLING 1971 c) was very clearly positively correlated to the quantity of the suitable radiation energy (r = .800***; Fig. 11). The mean of the activity coefficient at station A was 0.163 (range 0.015—0.435) and in the North Päijänne at station B 0.147 (range 0.018—0.273) and at station C 0.207 (range 0.040—0.539) (Table 1). It was a matter of course that the production maxima were measured in the early summer during the richest incident radiation energy.

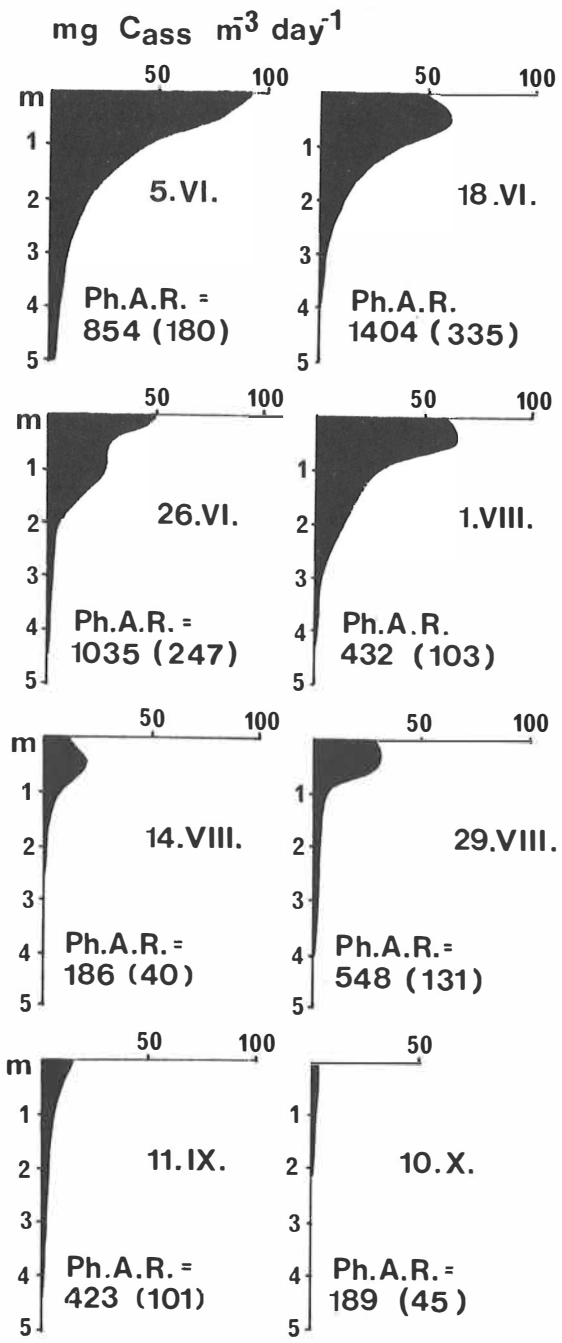

Fig. 10. Depth curves of daily net primary production of phytoplankton at station C (North Päijänne) in summer 1974 (cf. Fig. 8).

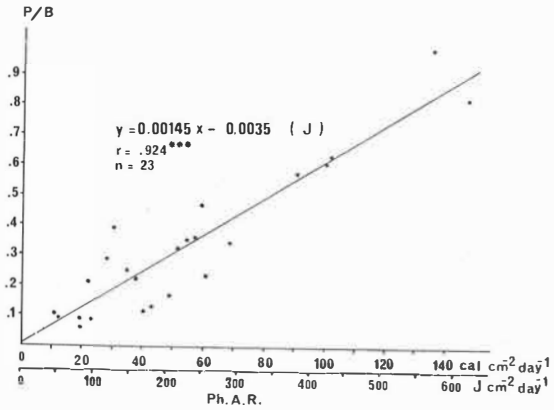

Fig. 11. The correlation between the photosynthetically available radiation (Ph.A.R.) and the activity coefficient (P/B) at the depth of maximum cubic meter primary production.

The reneval times calculated according to the activity coefficients varied in the study area in the water layer of 0—2 m from 2 to 7 days in the early summer increasing clearly in the autumn. The inhibition caused by the acid industrial effluents at station A was also seen in the values of the P/B coefficient and the reneval time (Table 1).

4.5.4 Utilization of light energy

The comparison of the light energy utilization at the different depths is made with the presumption that the plankton is homogenously distributed in the illuminated water layer. The ground for this presumption is that thermocline is below the water layer with photosynthetic production (0—2 m (—3

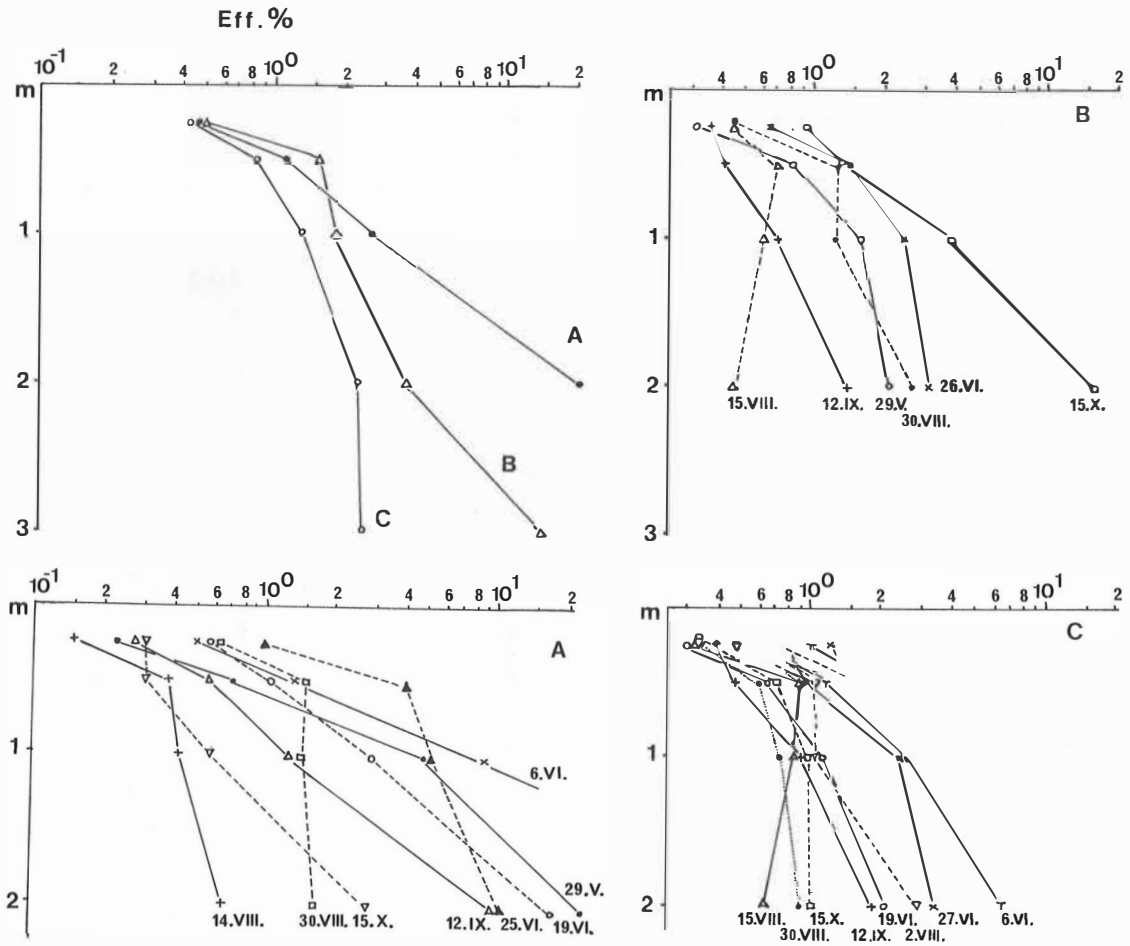

Fig. 12. Depth curves of photosynthetic efficiency in the study area in 1974.

m)). Thus the mixing of water caused by the winds provides against the concentration of the algae in the surface water layers, the chemical propertes of which also indicated homogeneity.

The light energy utilization increased towards the deeper water layers at all sampling stations, the more rapidly the thinner the photosynthetically productive layer was (Fig. 13; cf. TILZER 1973). On the contrary the mean efficiency was at station C in the depth of 2 m nearly the same as in the depth of 3 m (Fig. 12 C).

5. Conclusions

The extinction of the green light is very rapid in the waters with great humus content. Consequently, the layer of production is very thin, often below 5 m because 1 % of the incident light intensity reaches the depth of not more than 1.5—2.5 meters. This intensity was found at the depth of 7—8 meters in the oligotrophic and oligohumic waters (cf. also GRANBERG 1973b).

In Lake Jyväsjärvi (station A), the concentration of the algal producton in surface water was reflected as great V/O quotient values as was noted by GRANBERG (1973 a). The mean values in North Päijänne were greater than the values given for this area by GRANBERG (1973 a: 0.60), and greater than those in Lake Pääjärvi (0.60—0.69; ILMAVIRTA 1974) in the polyhumic Lake Hakojärvi (0.68; LEHMUSLUOTO 1969). The corresponding values of the V/O quotient were measured in Lake Lovojärvi (mean 1.05). The colour of this lake was about the same as that in the study area.

The illumination measurements indicate that a collective sample for the determination of primary productivity must be taken with a tube-sampler from the water layer of 0—4 or 0—5 meters in coloured (> 50 mg Pt/l) humic waters. to obtain a right view of the mean primary productivity in the above mentioned layer (cf. GRANBERG 1973 a). The samples for the analyses of the photosynthetic pigments must also be taken with the tube-sampler in order to avoid the errors caused by the strong gradients of the phytoplankton (cf. TOLSTOY 1972). It is possible to get good information of the total primary production with only one pair of

Table 4. The correlations between the temperature of the surface water, net primary production (P m^2), maximum production per cubic metre (P max), phytoplankton fresh weight biomass (BM), chlorophyll *a* content (Chl a), activity coefficient (P/B) and the photosynthetically active incident radiation (Io).

	P_{m^2}	P_{max}	BM	Chl_a	P/B	I_o
t	.229	.345	.248	.348	.296	.060
P_{m^2}	-	.961***	.716***	.912***	.333	.486*
P_{max}		-	.732***	.695***	.327	.505**
BM			-	.940***	-.149	.159
Chl_a				-	-.047	.131
P/B					-	.924***

the ^{14}C bottles when V/O quotient is near the value 1. Then the pair of bottles must be settled to the depth of the maximum production or 0.3—0.5 m. The thickness of the photosynthetically productive layer is in such waters (the colour > 50 mg Pt/l) about the same as the Secchi disc visibility and in more transparent waters about 1.5—fold Secchi value. Analogously the samples for the determinations of the algal biomass and the contents of the photosynthetically active pigments must be taken from the illuminated water column.

The chlorophyll content of the algae in the study area corresponded to the values in Lake Pääjärvi (KOTIMAA 1974) and in Lake Lilla Ullevifjärden (TOLSTOY 1972). The maxima of the chlorophyll content did not occur at the same time as the maxima of different algal groups.

The mean values of the species diversity were at station A greater in 1974 than in 1969 and 1970 (cf. GRANBERG 1973 a) but corresponding to the diversities at the stations B and C in the northern Päijänne. It is, however, difficult to compare the values of the year 1974 with those of 1969 and 1970, because GRANBERG has calculated the values on the basis of the 'individual numbers' of each species. The diversity indices based on the biomasses of each species seem to give a better view on a phytoplankton community within the seasons than those calculated on the ground of the 'individual num-

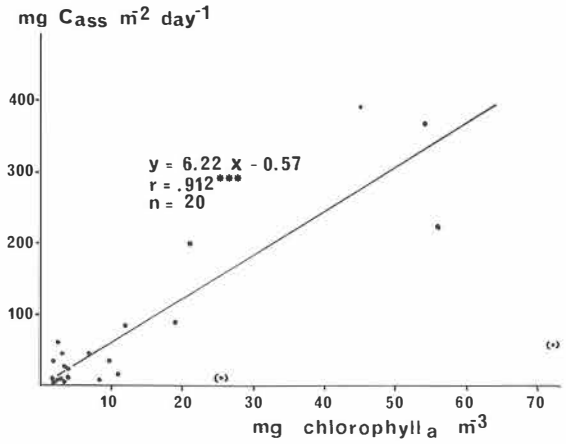

Fig. 13. The correlation between the chlorophyll *a* concentration and the 'net' primary production in the surface water layer (0—2 m) in the study area in summer 1974. The dots in parentheses are not included in the regression because the inhibition of production caused by acid waters (see text).

bers' (ELORANTA 1976). The correlation between the temperature of water and production or fresh weight biomass which was noted in Lake Pääjärvi (ILMAVIRTA 1974, 1975, ILMAVIRTA *et al.* 1974) was not seen in the study area (Table 4), obviously due to the lack of winter observations. JAVORNICKY & KOMARKOVA (1973) did not observe any significant correlation between the surface temperature of water and primary production, biomass, turnover rate, chlorophyll *a* concentration or utilization of solar energy or incident solar energy during the open water periods in Slapy Reservoir 1960—1967. The actual incident solar radiation energy correlated weakly to the primary production per square meter ($r = .486^{*}$) but more highly to the maximum production per cubic meter ($r = .505^{**}$). The same correlation in Lake Pääjärvi varied in different years (ILMAVIRTA *et al.* 1974). JAVORNICKY & KOMARKOVA (1973) did not find any correlation between the actual incident solar radiation energy and the production, biomass, chlorophyll, or assimilation numbers.

The production per square meter correlated highly to the cubic meter production at the maximum production depth ($r = .961^{***}$), chlorophyll *a* content ($r = .912^{***}$, Fig. 13), or fresh weight biomass ($r = .716^{***}$). The fresh weight biomass correlated also to the chlorophyll *a* concentration ($r = .940^{***}$). Corresponding correlations were also found by JAVORNICKY & KOMARKOVA (1973), ILMAVIRTA *et al.* (1974), ILMAVIRTA (1974, 1975), and KOTIMAA (1974). The activity coefficient (P/B quotient) correlated very significantly to the actual incident solar radiation energy (Io) ($r = .800^{***}$). KAJAK *et al.* (1972) found a negative correlation between the activity coefficient and fresh weight biomass. In this study this correlation was negative but very weak and not significant.

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Selostus

Jyväsjärven ja Pohjois-Päijänteen kasviplanktonista ja perustuotannosta *in situ* kesällä 1974

PERTTI ELORANTA

Kasviplanktonin tuotantoa, biomassaa ja lajikoostumusta seurattiin kesällä 1974 Jyväsjärvellä ja Pohjois-Päijänteellä taustatutkimuksena alueella samanaikaisesti tehdylle litoraalin perifytonia koskevalle tutkimukselle. Samalla tehtiin myös menetelmiä koskevia vertailuja.

Perustuotanto määritettiin radiohiilimenetelmällä havaintopaikoilla *in situ* 0—5 m:n vesikerroksessa, biomassat solulaskentamenetelmällä käänteismikroskooppia käyttäen sekä määrittämällä klorofylli *a*:n pitoisuus vedessä. Tuotannon tehokkuuden selvittämiseksi mitattiin myös vihreän valon sammumisnopeus syvemmälle mentäessä.

Valaistusmittausten perusteella voitiin todeta, että humusvesissä, missä veden väri on yli 50 mg Pt/l oli valaistun kerroksen paksuus suunnilleen sama kuin näköetäisyys ja vähähumuksisissa vesissä noin 1,5 × näkösyvyys. Tämä olisi huomioitava kokoomanäytteitä otettaessa perustuotantokyvyn määrityksiä varten. Perustuotanto on tummissa vesissä keskittynyt voimakkaasti ylimmän metrin vesikerrokseen ja valaistusolosuhteista riippuen jopa ylimmän puolen metrin vesikerrokseen. Täten tummemmissa vesissä planktonnäytteet olisi otettava putkinoutimella keskimääräisen kuvan saamiseksi.

Tutkimuksessa todettiin erittäin merkitsevät ja positiiviset korrelaatiot perustuotannon ja biomassan sekä klorofylli *a*:n pitoisuuden välillä sekä pintaan tulevan valon säteilyenergian ja levien aktiivisuuskertoimen (P/B) välillä. Myös veden klorofyllipitoisuuden ja solulaskennan avulla määritetyn biomassan välinen riippuvuus oli hyvin merkitsevä ja positiivinen. Tuotannon tehokkuus kasvoi pintavesikerroksessa nopeasti ylimmän metrin syvyydessä, mutta tehokkuuden kasvu hidastui ja pysähtyi syvemmälle mentäessä.