RESULTS

Experimental Results
Part I

Growth curve experiment:

Mass culture data for ***Chlorella vulgaris*** was grown on Bold's basal (BBM) medium under controlled conditions (pH 7 under 5000 lux for 14hrs light - 10hrs dark photoperiod at temperature 25 °C±2) are presented in Table (5) and Fig (1).

The growth curve showed that the lag phase in which the cells in culture have just begun to grow at 2 days incubation. The log phase (or exponential phase) in which reproduction is extremely fast at 4-6 days incubation periods. The transitional phase or phase of declining growth comes next after 6 days incubation.

Table (5): Growth data for ***Chlorella vulgaris*** was grown on Bold's basal (BBM) medium at pH 7, 5000 lux for 14hrs light - 10hrs dark photoperiod and temperature 25 °C±2) in indoor cultures.

Incubation periods ⇒ Parameters ⇓	0 day	2 days	4 days	6 days	8 days	10 days
Cell numbers (x 10^5 cells ml^{-1})	15.5	46.4	190.2	440	450.3	394.3
Dry weight gain (mg L^{-1})	114.28	153.25	334.6	649.7	662.6	592.01
Chlorophyll "a" content (µg L^{-1})	1021.3	1277.2	1879.6	2925.9	2969.1	2734.53

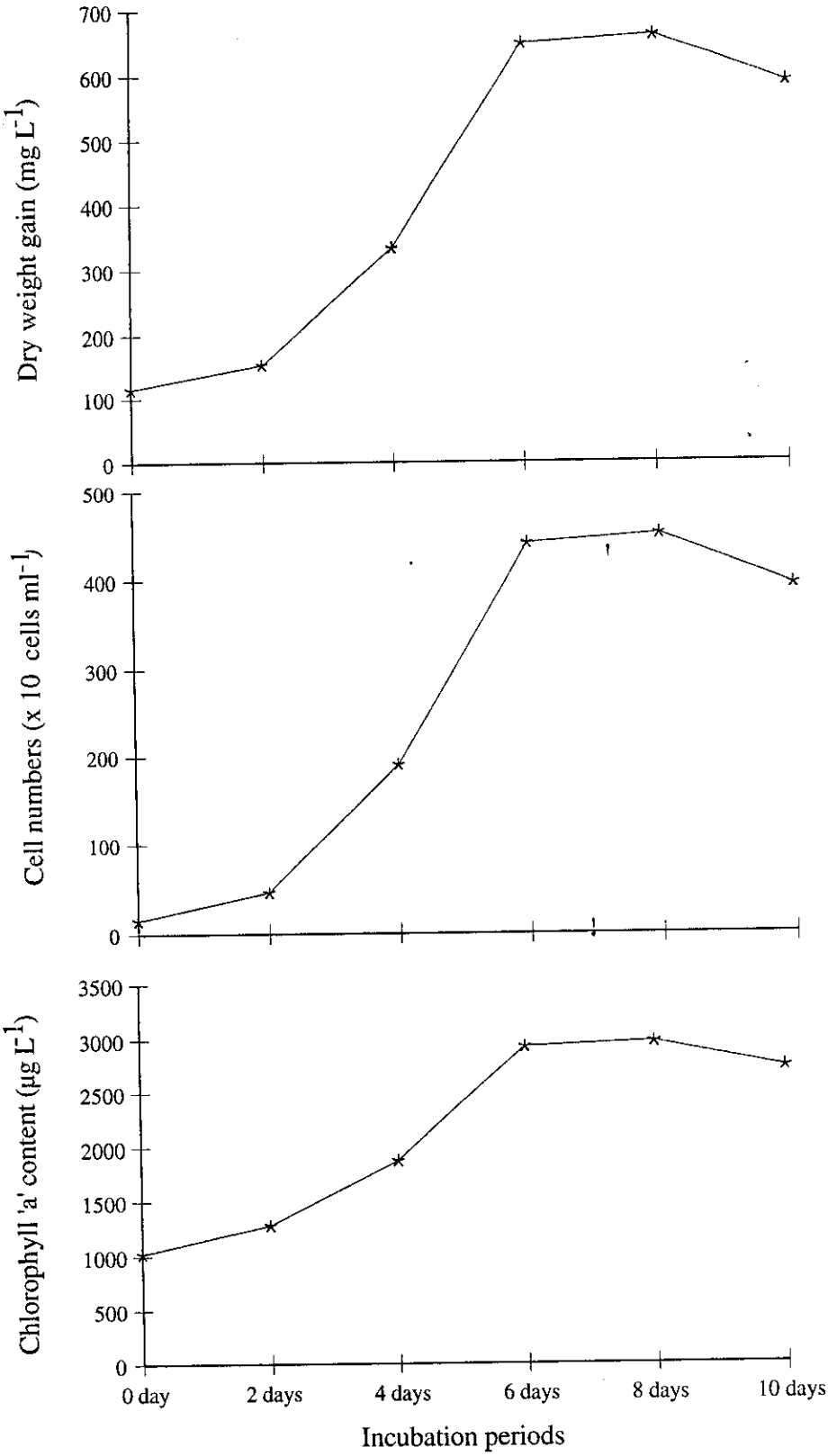


Fig (1): Growth data for ***Chlorella vulgaris*** was grown on Bold's basal (BBM) medium at pH 7, 5000 lux for 14hrs light - 10hrs dark photoperiod and temperature 25 °C±2) in indoor cultures.

I- Indoor Experiments

Exp. 1- Growth of *Chlorella vulgaris* at different media:

The unicellular green alga, ***Chlorella vulgaris***, was cultivated in three different nutrient media (Bold's basal (BBM), Beijerinck (BM) and Chu no. 10 (CM), under controlled conditions (pH 7 under 5000 lux for 14 light - 10 dark photoperiod at temperature 25 °C±2). The composition of these media as mentioned elsewhere in the material and methods.

Dry weight gain:

The results presented in Table (6) and illustrated in Fig (2) show clearly that utilizing Bold's Basal medium increased the growth of ***Chlorella vulgaris***, as dry weight gain , a phenomenon that was highly intensified at the end of incubation period (8 days), which amounted to 736.7 mg l^{-1}. Culluring on Chu no. 10 showed the lowest growth represented as dry weight gain at all days of incubation periods. Dry weight gain of ***C. vulgaris*** was significantly different between the three used media with high significant differences at 6th days incubation periods LSD = 42. (α = 0.05).

Cell count

Chlorella cells recorded less cell number, when cultivated in Chu no. 10 medium throughout the experiment (Table 6 and Fig 2). However, ***Chlorella*** recorded higher growth as cell number, when cultivated in Bold's basal medium which amounted 5.53 x 10^6 cells ml^{-1} at end of incubation period (8 days). ***Chlorella*** cells grown on Beijerink medium recorded higher cell number of all the media used in the 6th day of incubation (423.3 x 10^4) cells ml^{-1}. Cell numbers were significantly different between media with high significance difference at 6 days incubation periods LSD = 286.4 (α = 0.05).

Chlorophyll "a" content

Table (6) and Fig (2) show that chlorophyll "a" content of ***C. vulgaris*** was significantly increased when cultures the alga in BBM. In the meantime, maximum accumulation of chlorophyll "a" was maintained on using BBM which amounted 3382.5 µg l-1 at 6 days incubation periods. It is worthy to notice that Chu no. 10 medium caused highly significant drop in chlorophyll "a" accumulation. It could be seen that there were no significant differences between the Beijerink medium and Chu no. 10 medium with respect to the chlorophyll "a" biosynthesis during the days of the experiment, except at 8 days incubation. The differences were significant. The chlorophyll "a" content reaching high significantly difference at 4 days. LSD = 376.5 ($\alpha = 0.05$).

Table (6): Growth response of ***Chlorella vulgaris*** using different media (14hrs light / 10hrs dark photoperiod, 5000 lux, pH 7 and temperature 20 °C) at 2 days interval for a period of 8 days incubation in indoor cultures.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Media type ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
Bold's basal (BBM)	206.7	49.0	1496.6	474.7	233.3	2602.0	717.0	341.0	3382.5	736.7	553.3	2862.6
Significance	A	A	A	A	A	A	A	A	A	A	A	A
Beijerink (BM)	155.3	41.7	1161.4	349.0	203.3	2187.9	694.3	423.3	2947.4	699.3	483.3	2844.9
Significance	B	B/A	B	B	B/A	B	A	A	B	A	A	A
Chu no. 10 (CM)	117.0	36.3	1035.7	256.3	146.7	1838.1	341.0	253.3	2629.0	386.7	296.7	2420.5
Significance	C	B	B	C	B	B	B	A	B	B	B	B
LSD	23.1	8.1	229.4	35.8	58.8	376.5	42	286.4	329.0	40.2	83.2	218.2

Initial (0 day): Dry weight = 62.3 mg l^{-1}, algal count x 10^4 ml^{-1} = 23, chl "a" µg l^{-1} = 604.1

For LSD:

Alpha = 0.05 df = 6 Critical value of T = 2.45

Means in the same columns with the same letter are not significant.

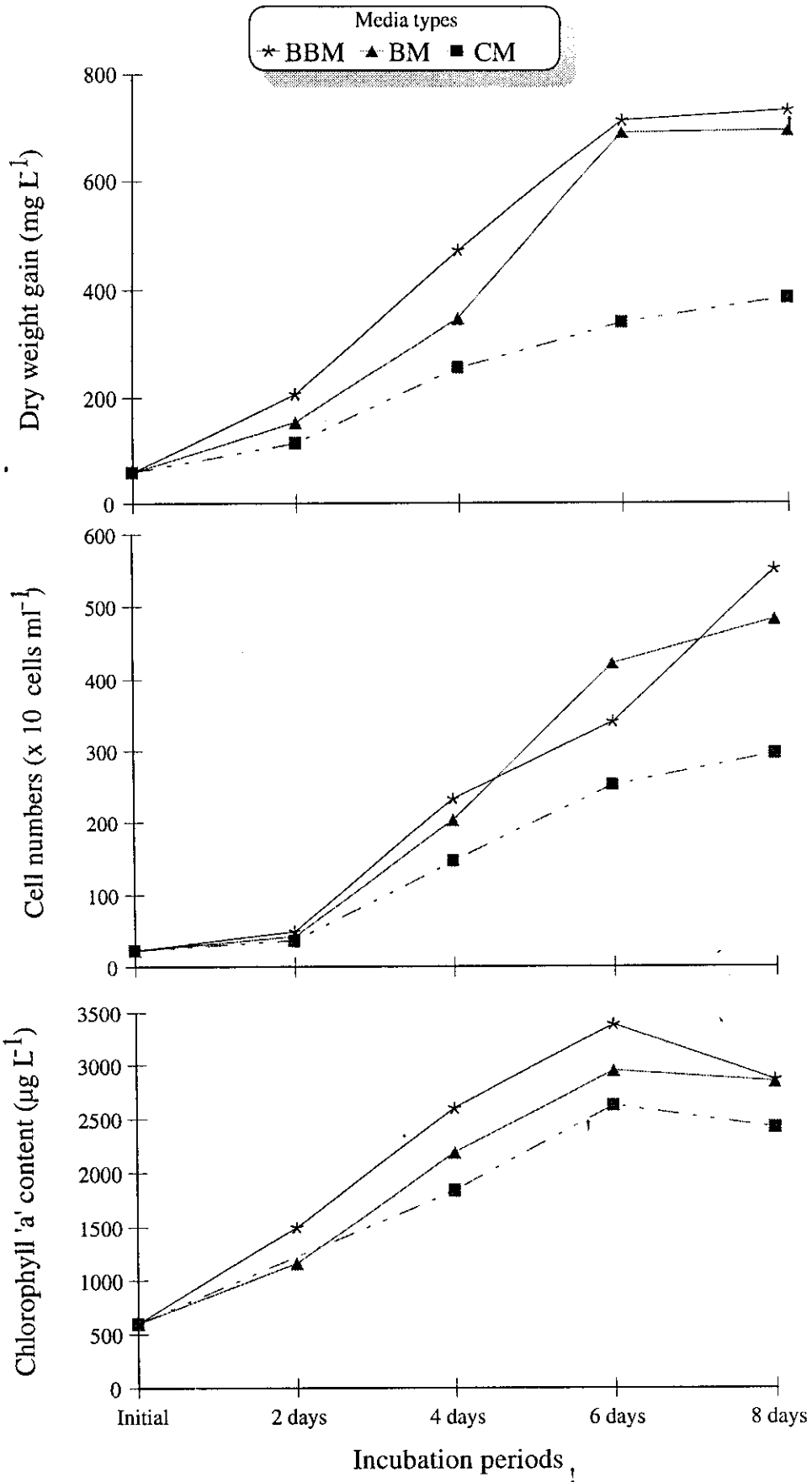


Fig (3) : Effect of different media on dry weights, cell numbers and chlorophyll "a" contents of ***Chlorella vulgaris*** at 2 days interval for a period of 8 days incubation in indoor cultures .

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for this experiment in Table (7). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

The dry weight gain was positively correlated with the count of ***Chlorella*** cells at 2 days incubation periods ($r = 0.77$) with an regression line: $y = 1.26\ x + 105.82$, $r^2 = 0.89$ in all treatments of incubation periods (Fig 3). In addition, the dry weight gain showed a significant correlation ($r = 0.86$) with the chlorophyll "a" content at the same day of incubation periods. Regression line $y = 3.75\ x + 1328.67$, $r^2 = 0.75$ for the mean values of all treatments of culture age (Fig 3).

Also, the dry weight gain showed strong positive correlation with the content of chlorophyll "a" ($r= 0.89$) at 4th days incubation periods.

while the cell number of the organism showed correlation with the mount of chlorophyll "a" ($r = 0.65$) at 2 days incubation periods. Regression line: $y = 0.28\ x - 221.33$, $r^2 = 0.85$ for all treatments during the days of the experiment (Fig 3). The other correlation of other variables at other incubation periods are shown in Table (7).

Table (7): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** at different media at 2 days interval for a period of 8 days incubation in indoor cultures.
Data listed are only the coefficient of the significant correlations (P< 0.05).

Time days ⇓			:	d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
Day 2	Dry weight	d. wt. 2	:	1											
	Cell number	no. 2	:	0.77	1										
	Chlorophyll "a"	Chl. 2	:	0.86	0.65	1									
Day 4	Dry weight	d. wt. 4	:	0.96	0.84	0.87	1								
	Cell number	no. 4	:	0.87	0.74	0.57	0.78	1							
	Chlorophyll "a"	Chl. 4	:	0.87	0.57	0.94	0.89	0.62	1						
Day 6	Dry weight	d. wt. 6	:	0.81	0.73	0.67	0.83	0.79	0.78	1					
	Cell number	no. 6	:	0.16	-0.02	0.28	0.23	0.1	0.47	0.46	1				
	Chlorophyll "a"	Chl. 6	:	0.88	0.74	0.8	0.93	0.73	0.87	0.79	0.48	1			
Day 8	Dry weight	d. wt. 8	:	0.83	0.74	0.7	0.86	0.79	0.81	0.1	0.45	0.81	1		
	Cell number	no. 8	:	0.86	0.81	0.67	0.89	0.79	0.73	0.92	0.36	0.86	0.93	1	
	Chlorophyll "a"	Chl. 8	:	0.71	0.67	0.6	0.8	0.64	0.75	0.93	0.63	0.84	0.93	0.88	1

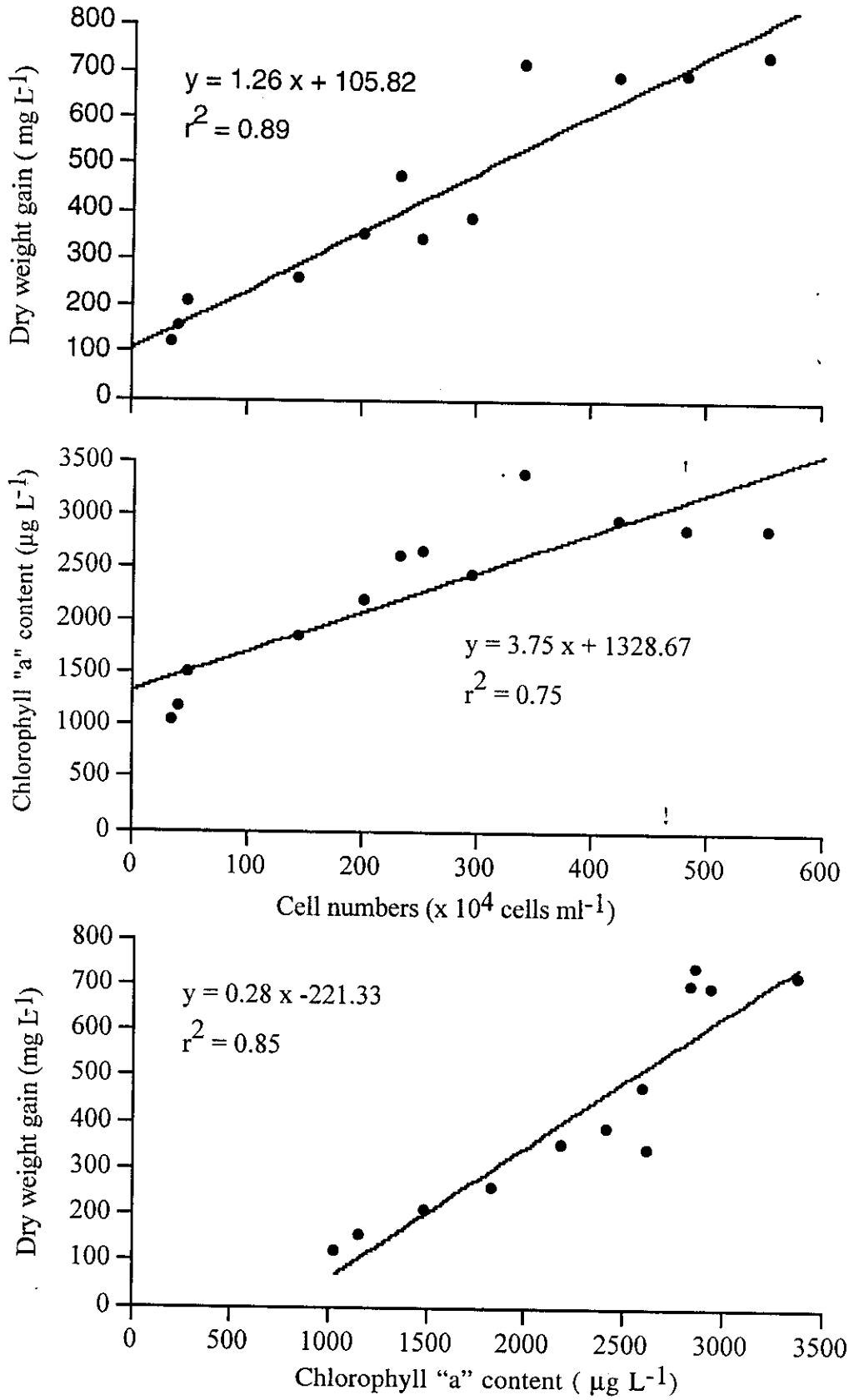


Fig. (3): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content for ***Chlorella vulgaris*** at different media at all incubation periods in indoor cultures.

Chlorophyll "a" content:

It is also obvious from the data presented in Table (8) and Fig (4) that chlorophyll "a" content in the samples of the culture incubated at pH 7 recorded maximum values. It reached a maximum of 3290.5 μg l^{-1} at the 6th day of culture age being highly significant LSD = 277.6 ($\alpha = 0.05$).

pH 5 showed an inhibitory effect on chlorophyll "a" biosynthesis at all time intervals except at 2 days incubation where pH 6 was more inhibitory.

Generally, at pH 5 and pH 6 levels, the growth of ***Chlorella vulgaris*** represented as dry weight gain, cell number as well as chlorophyll "a" content increased during culture life (8 days). But at pH 7, 8, 9, the growth represented as dry weight gain, cell number and chlorophyll "a" content were intense and high during exponential phase of the culture until 6 days incubation periods remained steady around the same level with slight decrease at the end of incubation periods (8 days).

Exp. 2- Growth of *Chlorella vulgaris* at different pH values:

Chlorella vulgaris was grown in normal growth conditions (BBM under 5000 lux for 14 light - 10 dark photoperiod at temperature 25 °C±2), using pH values 5, 6, 7, 8, 9. The pH values of the cultures were controlled by KOH pellets or HCl. The pH values were measured and adjusted daily by pH meter Orion model 420A, accuracy of ±0.1.

Dry weight gain:

From Table (8) and Fig (4) , growth of ***Chlorella vulgaris*** after 8 days of culturing showed that, pH 7 value proved to provide the most agreeable growth pH to that strain. Data also show that algal yield as milligrams of dry weight was increased by aging of culture in all pH values cultures. However, the growth was more intensive at 6 days incubation periods. The acceptable pH levels fell between 6-8 in culture of ***Chlorella vulgaris*** which show highly growth represented in dry weight gain. ***Chlorella*** dry wt was lower at pH 9 in the second day of incubation while the lowest values for other incubation periods were recorded at pH 5. But the growth of ***Chlorella vulgaris*** was more high in pH 7. Dry weight gain was more intensive at 6 days incubation periods which amounted 823 mg l^{-1} and showed highly significantly different LSD = 38 (α = 0.05). Table (8) and Fig (4).

Cell count:

Cell numbers in the culture of ***Chlorella vulgaris*** at pH 7 showed highly count at 6 days of culturing age which amounted 450 x 10^4 cells ml^{-1} and also showed highly significantly different LSD = 101.1 (α = 0.05).Algal cell count increased with increasing pH values from 5 to 7 after which cell number began to decrease recording its lowest values at pH 9 at all incubation periods.

Chlorophyll "a" content:

It is also obvious from the data presented in Table (8) and Fig (4) that chlorophyll "a" content in the samples of the culture incubated at pH 7 recorded maximum values. It reached a maximum of 3290.5 μg l^{-1} at the 6th day of culture age being highly significant LSD = 277.6 ($\alpha = 0.05$).

pH 5 showed an inhibitory effect on chlorophyll "a" biosynthesis at all time intervals except at 2 days incubation where pH 6 was more inhibitory.

Generally, at pH 5 and pH 6 levels, the growth of ***Chlorella vulgaris*** represented as dry weight gain, cell number as well as chlorophyll "a" content increased during culture life (8 days). But at pH 7, 8, 9, the growth represented as dry weight gain, cell number and chlorophyll "a" content were intense and high during exponential phase of the culture until 6 days incubation periods remained steady around the same level with slight decrease at the end of incubation periods (8 days).

Table (8): Growth response of ***Chlorella vulgaris*** at different pH values(on Bold's basal medium,14hrs light / 10hrs dark photoperiod, 5000 lux, and temperature 20 °C) at 2 days interval for a period of 8 days incubation in indoor cultures.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ pH value ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
pH 5	183.0	25.0	640.4	324.0	69.3	1165.2	388.7	286.7	1234.6	444.0	350.0	1260.7
Significance	C	D	C	E	C	D	D	C	E	B	A	D
pH 6	195.7	32.3	639.7	367.3	79.7	1655.6	440.0	323.3	2152.7	458.0	350.0	2194.5
Significance	C	C	C	C	C	C	C	B/C	D	B	A	C
pH 7	303.3	49.0	1474.0	561.3	196.7	2541.6	823.3	450.0	3290.5	823.0	453.3	3172.5
Significance	A	A	A	A	A	A	A	A	A	A	A	A
pH 8	244.7	37.3	1076.8	456.7	109.0	2182.0	795.3	393.3	2917.3	789.3	360.0	2902.1
Significance	B	B	B	B	B	B	A	B/A	B	A	A	A
pH 9	182.3	28.7	1047.2	347.3	78.0	1840.2	560.0	246.7	2631.8	502.7	230.0	2576.7
Significance	C	D/C	B	D	C	C	B	C	C	B	B	B
LSD	14.2	4.8	284.8	14.7	26.6	240.8	38.0	101.1	277.6	97.7	104.0	325.0

Initial (0 day): Dry weight = 54.3 mg l^{-1}, algal count x 10^4 ml^{-1} = 15.3, chl "a" µg l^{-1} = 518.9

For LSD:

Alpha = 0.05 df = 10 Critical value of T = 2.23

Means in the same columns with the same letter are not significant.

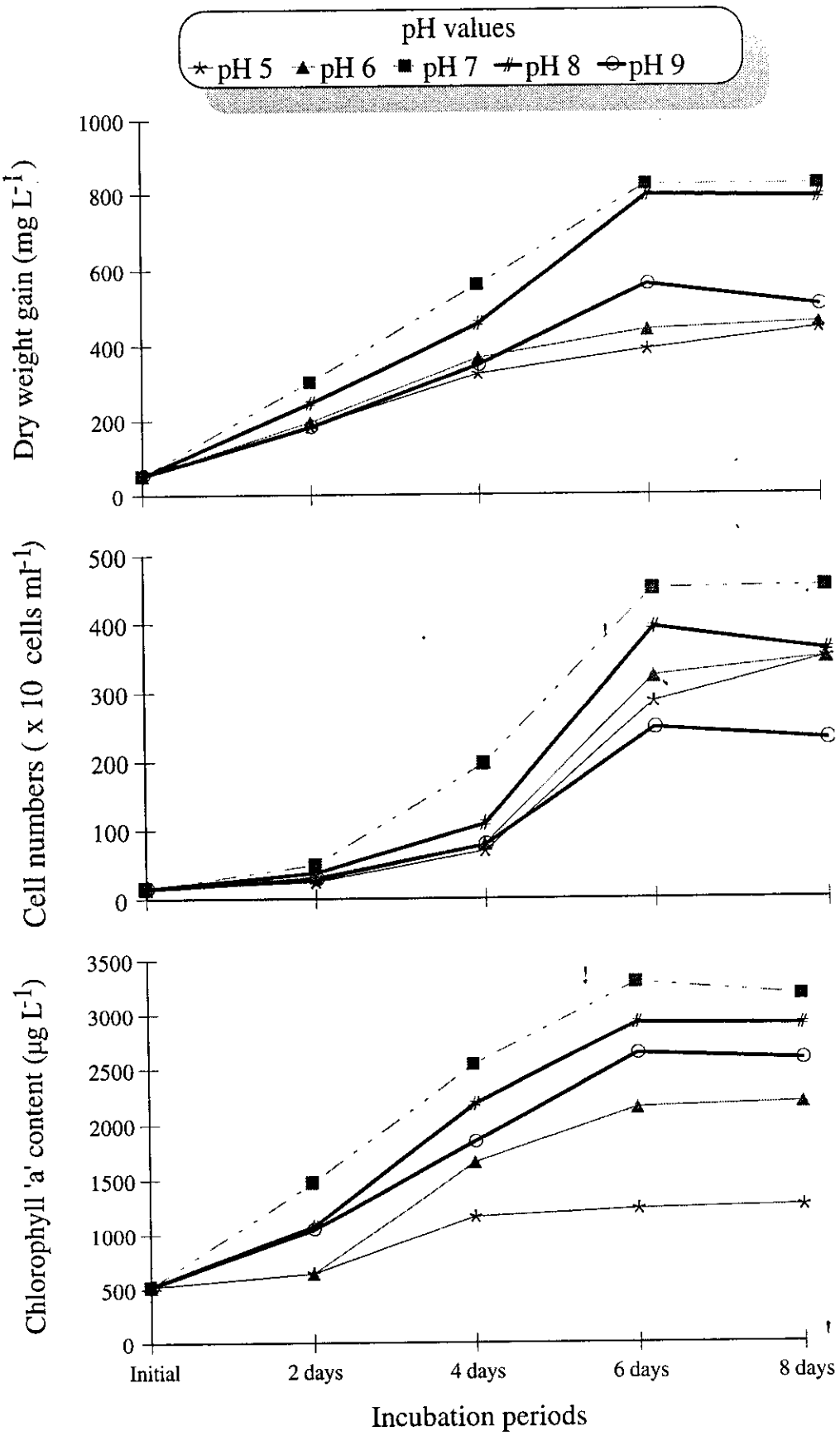


Fig (4): Effect of different pH values on dry weights, cell numbers and chlorophyll "a" contents of ***Chlorella vulgaris*** at 2 days interval for a period of 8 days incubation in indoor cultures.

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for the experiment and represented in Table (9). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

The dry weight gain was very strong positively correlated with the count of ***Chlorella*** cells at 2 days incubation periods ($r = 0.95$). Regression line $y = 1.19\ x + 212.92$, $r^2 = 0.75$ in all pH value cultures of experiment time (Fig 5). In addition, strong positive correlation was found between dry weight gain and chlorophyll "a" content ($r = 0.78$) at the same day of incubation periods. Regression line: $y = 4.92\ x + 1176.9$, $r^2 = 0.86$ at all incubation periods (Fig 5). Also, the dry weight gain showed strong positive correlation with the cell count and the content of chlorophyll "a"(r= 0.93 & 0.9 respectively) at 4th days incubation periods.

The cell number of the organism showed positive correlation with the amount of chlorophyll "a" ($r = 0.8$) at 2 days incubation periods. Regression line: $y = 0.23\ x + 6.88$, $r^2 = 0.87$ of all culture age (Fig 5). The other correlation of other variable at other incubation periods are shown in Table (9).

Table (9): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** at different pH values at 2 days interval for a period of 8 days incubation in indoor cultures.
Data listed are only the coefficient of the significant correlations (P< 0.05).

Time days ⇓				d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
Day 2	Dry weight	d. wt. 2	:	1											
	Cell number	no. 2	:	0.95	1										
	Chlorophyll "a"	Chl. 2	:	0.78	0.8	1									
Day 4	Dry weight	d. wt. 4	:	0.98	0.96	0.82	1								
	Cell number	no. 4	:	0.94	0.9	0.74	0.93	1							
	Chlorophyll "a"	Chl. 4	:	0.85	0.89	0.91	0.9	0.77	1						
Day 6	Dry weight	d. wt. 6	:	0.86	0.81	0.81	0.89	0.79	0.91	1					
	Cell number	no. 6	:	0.82	0.79	0.49	0.81	0.73	0.62	0.68	1				
	Chlorophyll "a"	Chl. 6	:	0.74	0.78	0.82	0.81	0.69	0.95	0.89	0.55	1			
Day 8	Dry weight	d. wt. 8	:	0.88	0.8	0.75	0.89	0.81	0.85	0.96	0.73	0.79	1		
	Cell number	no. 8	:	0.66	0.58	0.26	0.64	0.66	0.35	0.41	0.84	0.26	0.58	1	
	Chlorophyll "a"	Chl. 8	:	0.72	0.75	0.76	0.79	0.69	0.93	0.88	0.55	0.99	0.8	0.28	1

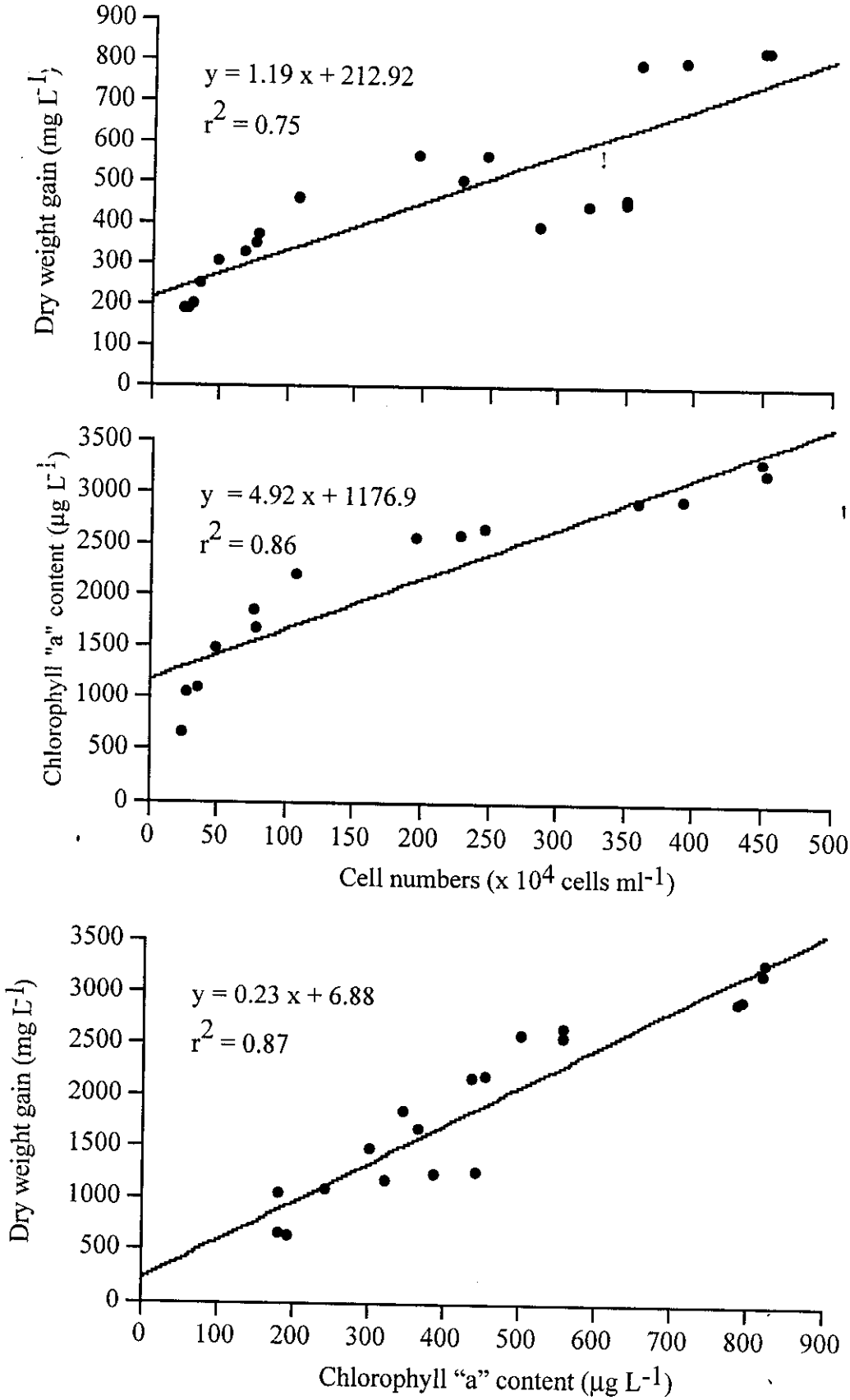


Fig. (5): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content of different pH values for ***Chlorella vulgaris*** at all incubation periods in indoor cultures.

Exp. 3-Growth response of *Chlorella vulgaris* to various photoperiods:

The ***Chlorella vulgaris*** cultures were grown in normal growth conditions (on BBM at pH 7 under 5000 lux at temperature 25 °C ±2), by using 10/14, 14/10, 18/6hrs light-dark cycle and continuos light which controlled by electricity timer which conducted with illumination lights (fluorescent lamps).

Dry weight gain

From data shown in Table (10) and Fig (6), growth of ***Chlorella vulgaris*** in continuos light was more intense than other photoperiod cycles, which recorded maximum production during the period of 4 days of culture age. Afterward, it fluctuated below that level. Production of dry matter showed maximum weight in continuos light at 4 days incubation periods which amounted 516.0 mg l^{-1} and showed significantly different LSD = 26.2 ($\alpha = 0.05$). The dry weight gain followed 10hrs light / 14hrs dark and 14 light / 10 dark photoperiod cycle increased during the culture age of experiment (8 days) which amounted 226.3 and 300 mg l^{-1} respectively at the end of incubation periods. However, the dry weight under 18 light / 6 dark photoperiod cycle reached its highest rate at 4 days of incubation periods which amounted to 313 mg l^{-1}.

Cell count:

Cell number of ***Chlorella*** cells cultivated in continuos light recorded maximum production during the period of 4 days of culture age which amounted to 420 x 10^4 cells ml^{-1} and showed significantly different LSD =79.5 ($\alpha = 0.05$). The cell number of cultures subjected to 10 light / 14 dark and 14 light / 10 dark photoperiod cycle (hrs) were increasing during the culture age of experiment (8 days) which amounted to 180 and 190 x 10^4

cells ml^{-1} respectively at the end of incubation periods. However, the cell number of algal cultures followed 18 light / 6 dark photoperiod cycle (hrs) was characterized by highest rate at 4 days of incubation periods which amounted to 290 x 10^4 cells ml^{-1}. Table (10) and Fig (6).

Chlorophyll "a" content:

Efficiency of cells to produce chlorophyll "a" during culturing time at different photoperiod cycles, are illustrated in Table (10) and Fig (6). A maximum production of chlorophyll "a" took place at 4 days of culturing time under continuos light which amounted to 4618.e µg l-1 and showed significantly different LSD = 628 (α= 0.05). Thereafter, it started decreasing up to the end of incubation period. The chlorophyll "a" biosynthesis under 10 light / 14 dark and 14 light / 10 dark photoperiod cycle (hrs) were increasing during the culture time of experiment (8 days) which amounted to 3841.7 and 3872.3 µg l^{-1} respectively at the end of incubation periods. However, at 18 light / 6 dark photoperiod cycle (hrs), pigment accumulation was characterized by highest rate at 4 days of incubation periods which amounted to 4112.6 µg l^{-1}. Table (10) and Fig (6).

Table (10): Growth response of ***Chlorella vulgaris*** to different photoperiod cycles (on Bold's basal medium, 5000 lux, pH 7 and temperature 20 °C) at 2 days interval for a period of 8 days incubation in indoor cultures.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Photoperiod cycles ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. m g l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
10 light:14 dark	169.0	50.7	1373.7	195.7	73.7	3114.2	215.7	113.3	3871.1	226.3	180.0	3841.7
Significance	D	C	C	C	C	C	D	C	B	D	B	B/A
14 light:10 dark	204.3	63.3	1534.5	212.0	82.3	3642.7	272.7	163.3	4435.0	300.0	190.0	3872.3
Significance	C	C	C	C/B	C	B/C	C	C/B	A	C	B	A
18 light:6 dark	221.7	89.0	1959.8	313.0	290.0	4112.6	226.0	210.0	3443.0	206.0	203.3	3438.6
Significance	B	B	B	B	B	B/A	B	B	B	B	B	B
24 light	271.3	136.7	2443.5	516.0	420.0	4618.2	416.0	386.7	3421.1	405.3	350.0	3166.3
Significance	A	A	A	A	A	A	A	A	B	A	A	B
LSD	13.0	21.0	278.0	26.2	79.5	628.0	23.6	85.8	1527.9	20.7	78.4	1258.7

Initial (0 day): dry weight = 120.0 mg l^{-1}, algal count x 10^4 ml^{-1} = 40.0, chl "a" µg l^{-1} = 1124.9

For LSD:

Alpha = 0.05 df = 8 Critical value of T = 2.31

Means in the same columns with the same letter are not significant.

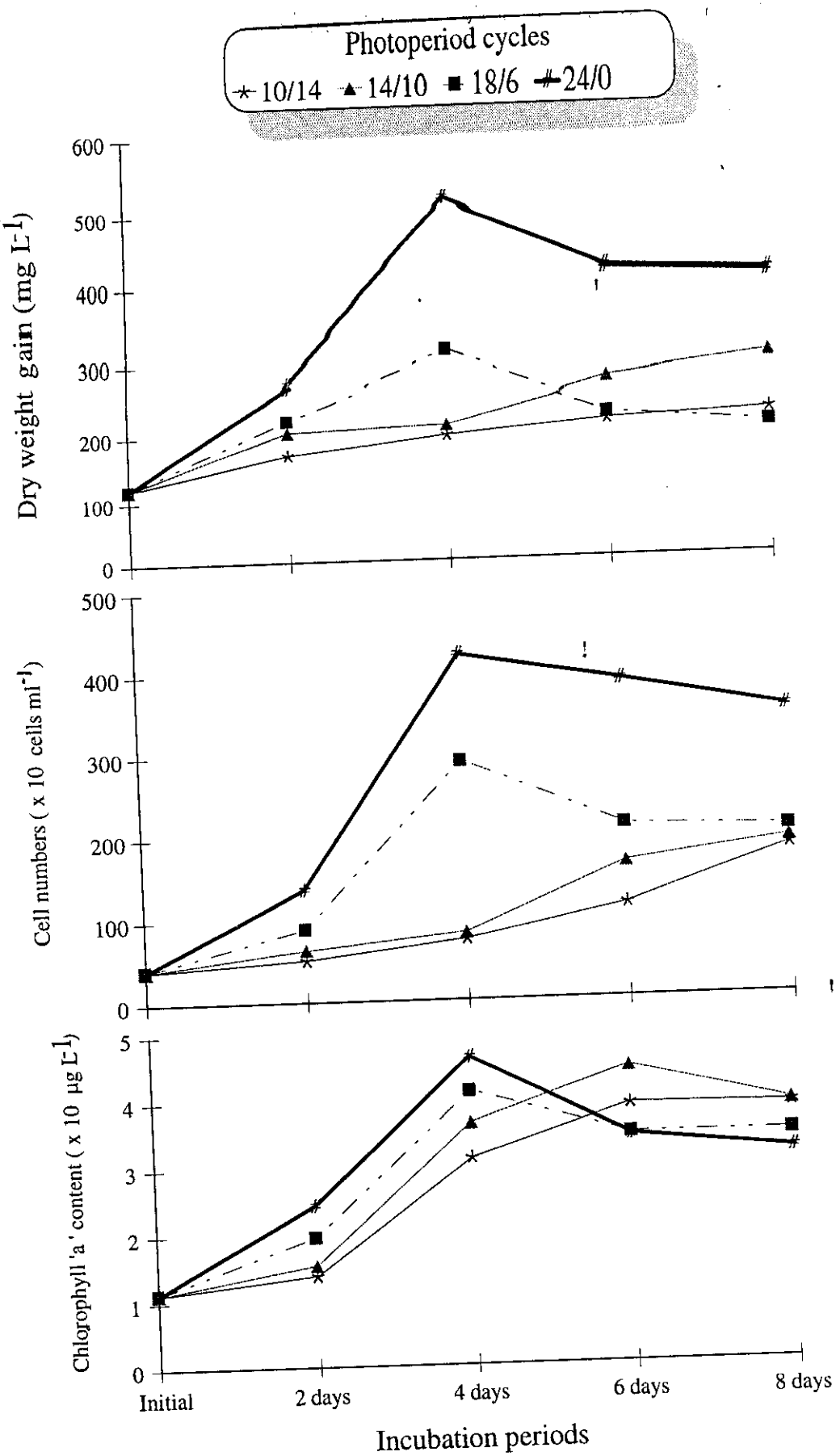


Fig (6): Effect of photoperiods on dry weights, cell numbers and chlorophyll "a" contents of ***Chlorella vulgaris*** at 2 days interval for a period of 8 days incubation in indoor cultures .

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for the experiment and expressed in Table (11). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

The dry weight gain was very highly positively correlated with count of the organism at 2 days incubation periods ($r = 0.93$). Regression line: $y = 0.84\ x + 136.84$, $r^2 = 0.91$ for all culture age (Fig 7). In addition, very strong positive correlation was existed between dry weight gain and chlorophyll "a" content ($r = 0.92$) at the same day of all incubation periods. Regression line: $y = 5.82\ x + 2019.5$, $r^2 = 0.49$ for all incubation periods (Fig 7).

Also, the dry weight gain showed positive correlation with the cell count and the chlorophyll "a" content (r= 0.84 and 0.83 respectively) at 4th days incubation periods.

The cell number of the organism showed positive correlation with the amount of chlorophyll "a" ($r = 0.89$) at 2 days incubation periods.). Regression line: $y = 1.79\ x + 3128.58$, $r^2 = 0.93$ for all experiment time (Fig 7). The other correlation of other variable at other incubation periods are shown in Table (11).

Table (11): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** to various photoperiod cycles at 2 days interval for a period of 8 days incubation in indoor cultures.
Data listed are only the coefficient of the significant correlations ($P< 0.05$).

Time			d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
days ⇓			---	---	---	---	---	---	---	---	---	---	---	---
	Dry weight	d. wt. 2 :	1											
Day	Cell number	no. 2 :	0.93	1										
2	Chlorophyll "a"	Chl. 2 :	0.92	0.89	1									
	Dry weight	d. wt. 4 :	0.91	0.92	0.95	1								
Day	Cell number	no. 4 :	0.89	0.89	0.92	0.84	1							
4	Chlorophyll "a"	Chl. 4 :	0.89	0.89	0.84	0.83	0.79	1						
	Dry weight	d. wt. 6 :	0.96	0.96	0.92	0.97	0.90	0.86	1					
Day	Cell number	no. 6 :	0.89	0.89	0.84	0.87	0.88	0.84	0.94	1				
6	Chlorophyll "a"	Chl. 6 :	-0.30	-0.34	-0.50	-0.39	-0.58	-0.13	-0.34	-0.24	1			
	Dry weight	d. wt. 8 :	0.97	0.95	0.91	0.97	0.88	0.85	0.99	0.94	-0.30	1		
Day	Cell number	no. 8 :	0.78	0.80	0.74	0.81	0.77	0.7	0.88	0.96	-0.20	0.87	1	
8	Chlorophyll "a"	`Chl. 8` :	-0.42	-0.45	-0.60	-0.50	-0.67	-0.24	-0.46	-0.32	0.96	-0.42	-0.25	11

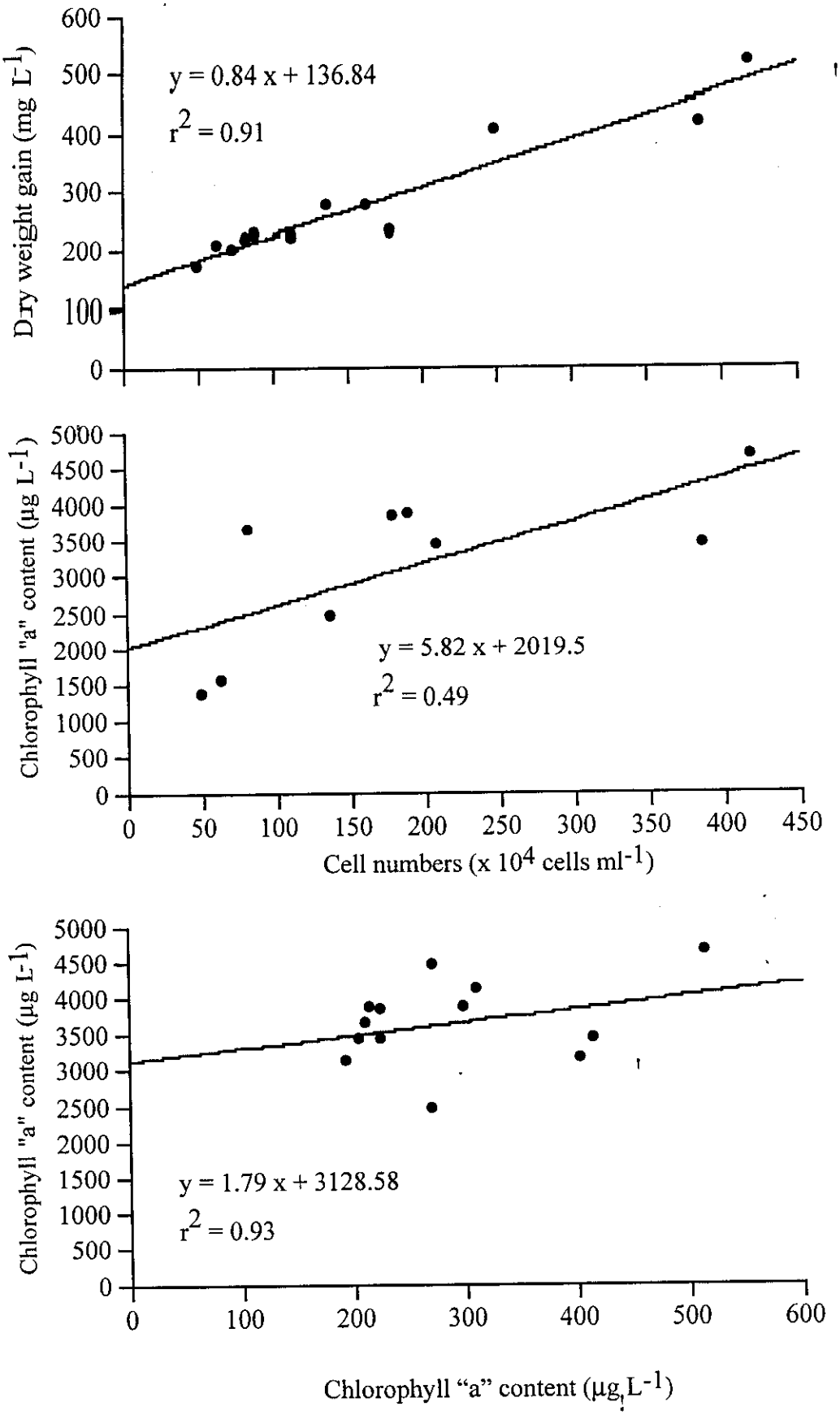


Fig. (7): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content of photoperiod cycles values for ***Chlorella vulgaris*** at all incubation periods in indoor cultures.

Exp. 4- Growth response of *Chlorella vulgaris* to various light intensities:

The organism was grown in normal growth conditions (on BBM at pH 7 followed a system of 14 light / 10 dark photoperiod cycle (hrs) at temperature 25 °C ±2) under different light intensities: 3000 , 5000 , 7000, 9000 , and 12000 lux . Illumination for indoor ***Chlorella*** cultures was provided by fluorescent lambs which conducted by electricity timer .

The growth of the organism represented as dry weight gain, cell number and chlorophyll "a" content were favoured by aging of culture at all light intensities. However, the growth was more intensive at high light intensities than low light intensities.

Dry weight gain:

Data in Table (12) and Fig (8), show that algal yield as milligrams of dry weight was increased by aging of culture in all light intensities up to 6 days of incubation , afterward they showed steady growth up to the end of culture time (8 days). The only exception was at 3000 lux when the dry weight gain was stimulated until the end of incubation periods. It is also obvious that the dry weight gain exhibited high values at 12000 lux and reached maximum at 6 days incubation periods which amounted to 1808.7 mg l^{-1}. It shows high significantly differences LSD = 124.4 ($\alpha = 0.05$).

Cell count:

The cell number of ***Chlorella*** cells was high during exponential phase of the culture and still steady around the same level with slight gradual decrease at the end of incubation periods (8 days).

At low light intensities it is obvious from Table (12) and Fig (8) that the growth of the organism represented as cell number was intensive during culture life and was increasing up to the end of incubation periods (8 days). The cell count reached maximum in culture incubated under 12000 lux. It amounted to 710 x 10^4 cells ml-1 at 6 days of incubation which showed significantly differences LSD = 72.6 (α = 0.05).

Chlorophyll "a" content:

Chlorophyll "a" content of ***Chlorella*** was increased during the culture life up to 6 days of incubation and still steady around the same level with slight gradual decrease at the end of incubation period (8 days).

At 3000 lux (Table 12 and Fig 8) the chlorophyll "a" content of the organism was intensive during culture life and was increasing up to the end of incubation periods (8 days). The amount of chlorophyll "a" reached maximum cultures grown under 12000 lux. It amounted to 6068.9 µg l^{-1} at 6 days of incubation which showed significantly differences LSD = 689.3 (α = 0.05).

Table (12): Growth response of ***Chlorella vulgaris*** to different light intensities (grown on Bold's basal medium, 14hrs light / 10hrs dark photoperiod, pH 7 and temperature 20 °C) at 2 days interval for a period of 8 days incubation in indoor cultures.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Light intensity (lux) ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
3000 lux	101.7	51.7	1078.8	197.7	90.3	1492.3	452.3	183.3	2792.0	464.0	216.7	2811.9
Significance	D	E	D	C	D	D	D	E	D	E	E	D
5000 lux	140.0	61.7	1340.9	259.3	130.0	1865.2	716.7	330.0	3516.1	714.3	336.7	3508.5
Significance	C	D	C	C	D	D/C	C	D	C	D	D	C
7000 lux	227.7	73.3	1509.8	376.3	223.3	2079.5	834.7	420.0	5234.7	833.3	420.0	5184.7
Significance	B	C	C	B	C	C	C	C	B	C	C	B
9000 lux	251.6	82.3	1801.0	456.3	286.7	2572.1	1169.0	513.3	5493.5	1170.0	523.3	5443.6
Significance	A	B	B	B	B	B	B	B	B/A	B	B	B
12000 lux	271.7	91.7	2004.7	611.0	393.3	3292.2	1808.7	710.0	6068.9	1801.0	706.7	6051.2
Significance	A	A	A	A	A	A	A	A	A	A	A	A
LSD	21.4	6.3	195.9	97.4	39.9	393.7	124.4	72.6	689.3	112.8	48.4	496.6

Initial (0 day): Dry weight = 69.0 mg l^{-1}, algal count x 10^4 ml^{-1} = 29.3, chl "a" µg l^{-1} = 604.1

For LSD:

Alpha = 0.05 df = 10 Critical value of T = 2.23

Means in the same columns with the same letter are not significant.

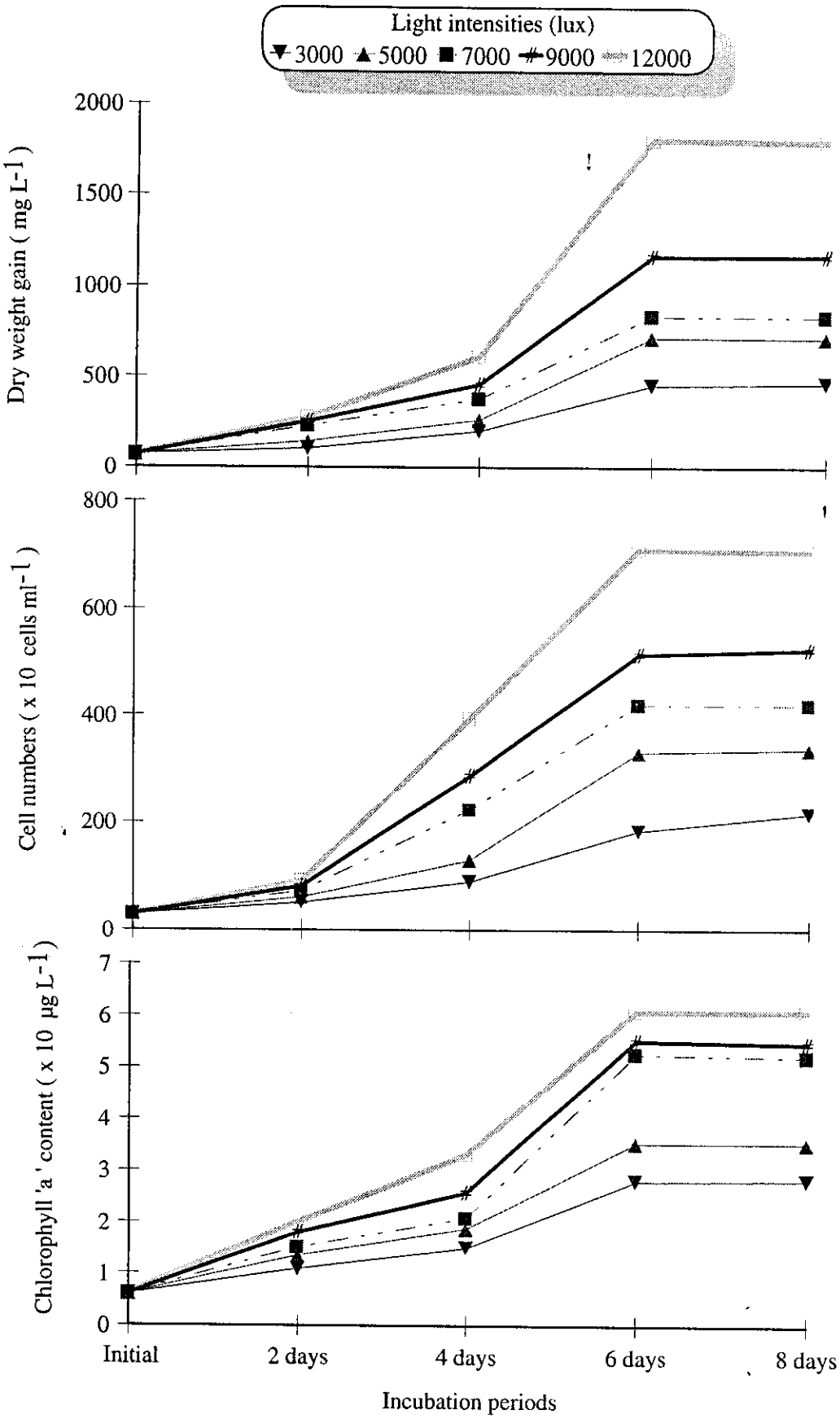


Fig (8): Effect of various light intensities on dry weights, cell numbers and chlorophyll "a" contents of ***Chlorella vulgaris*** at 2 days interval for a period of 8 days incubation in indoor cultures .

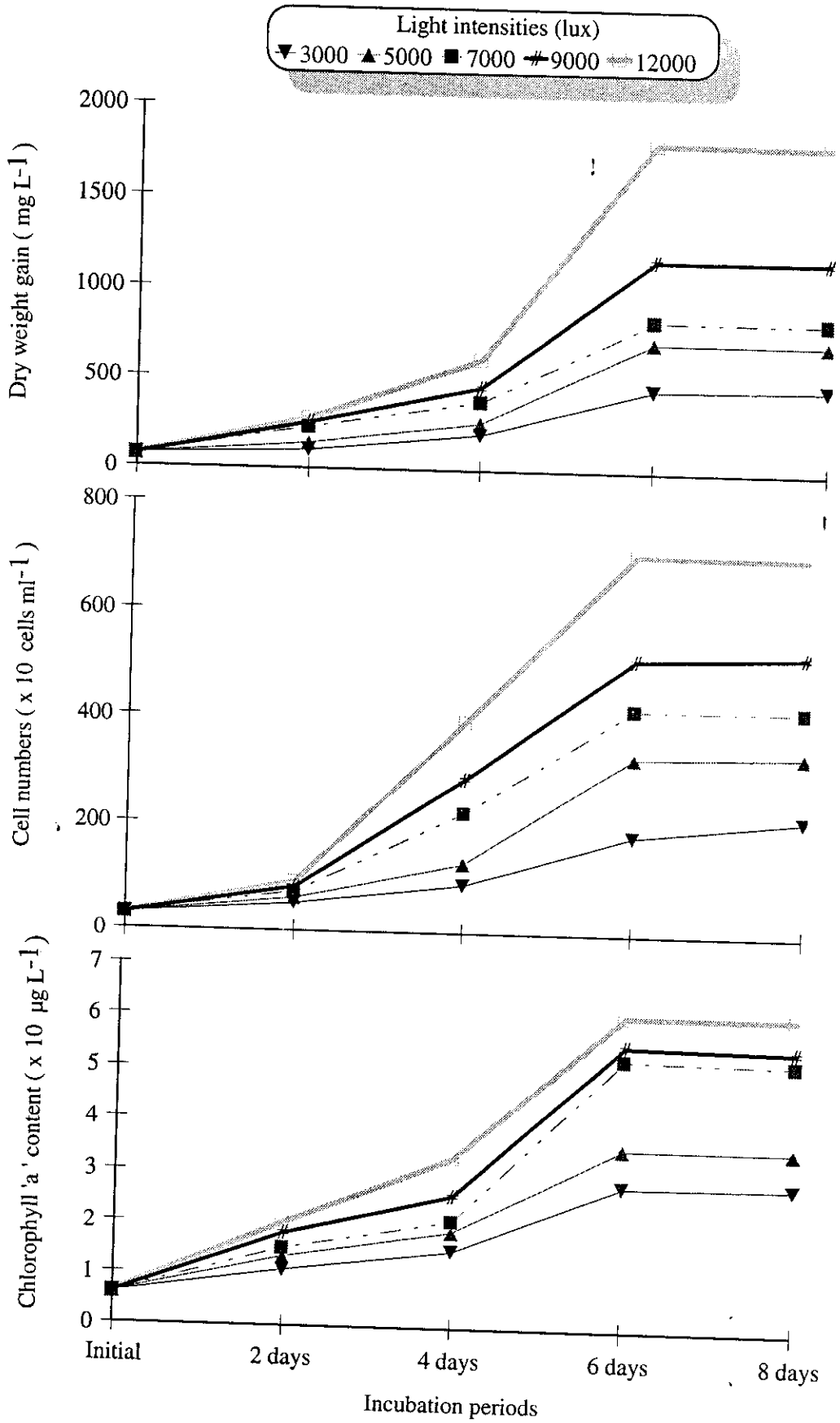


Fig (8): Effect of various light intensities on dry weights, cell numbers and chlorophyll "a" contents of ***Chlorella vulgaris*** at 2 days interval for a period of 8 days incubation in indoor cultures .

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for the experiment and represented in Table (13). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

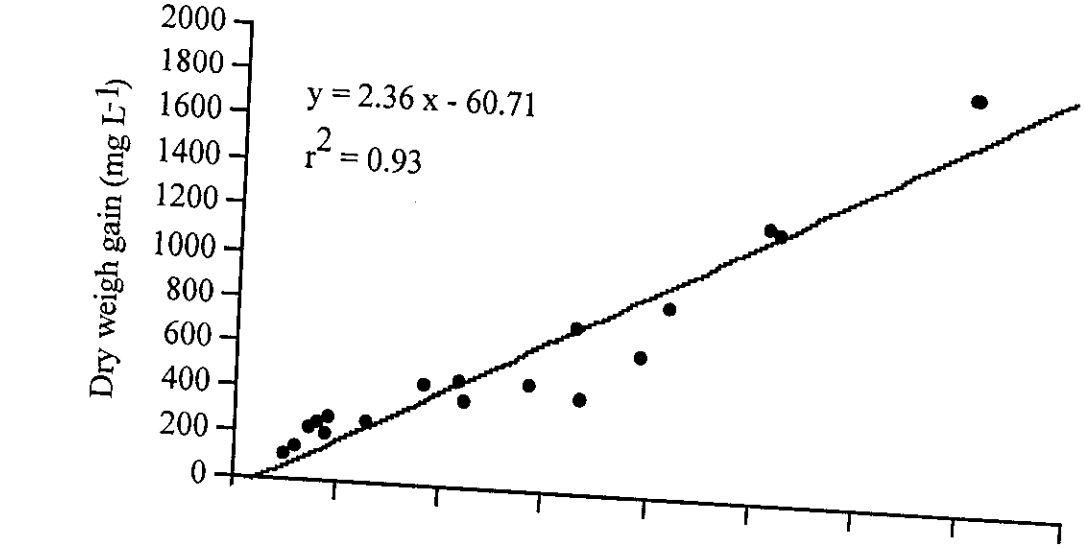
The dry weight gain was high positively correlated with the count of ***Chlorella*** cells at 2 days incubation periods ($r = 0.95$). Regression line: $y = 2.36 x - 60.71$, $r^2 = 0.93$ for all culture time of the experiment. In addition, strong positive correlation was found between dry weight gain and chlorophyll "a" content ($r = 0.93$) at the same day of incubation periods which showed linear relationship at all experiment time regression line: $y = 7.87 x + 957.52$, $r^2 = 0.92$ (Fig 9).

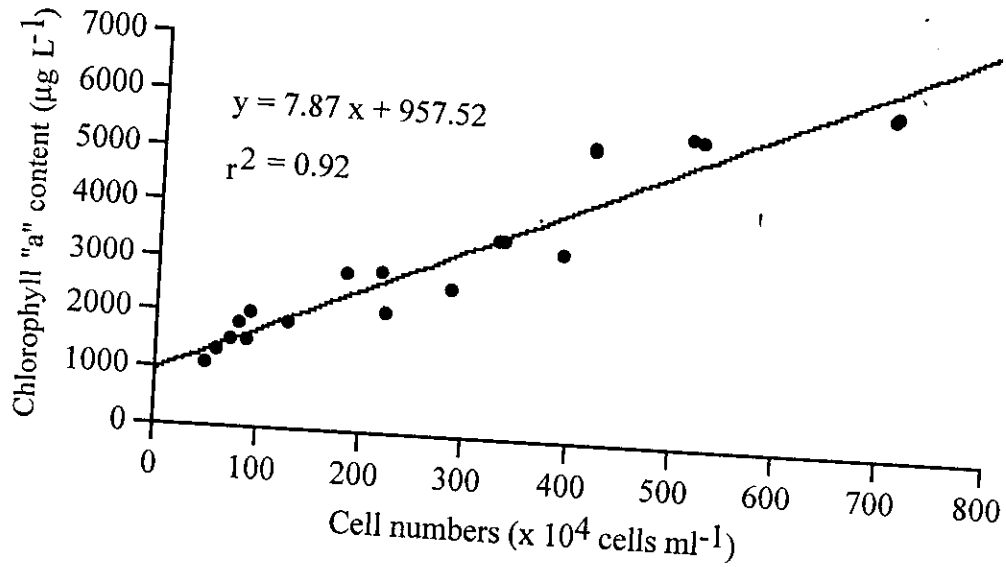
Also, the dry weight gain showed very strong positive correlation with the cell count and chlorophyll "a" content (r= 0.97 and 0.91 respectively) at 4th days incubation periods.

The cell number of the organism showed strong positive correlation with the amount of chlorophyll "a" ($r = 0.94$) at 2 days incubation periods. Regression line: $y = 0.28 x - 274.82$, $r^2 = 0.87$ for all incubation periods. The other correlation of other variable at other incubation periods are shown in Table (13).

Table (13): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** to various light intensities at 2 days interval for a period of 8 days incubation periods .
Data listed are only the coefficient of the significant correlations ($P < 0.05$).

Time days ⇓			:	d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
Day 2	Dry weight	d. wt. 2	:	1											
	Cell number	no. 2	:	0.95	1										
	Chlorophyll "a"	Chl. 2	:	0.93	0.94	1									
Day 4	Dry weight	d. wt. 4	:	0.91	0.92	0.95	1								
	Cell number	no. 4	:	0.93	0.96	0.95	0.97	1							
	Chlorophyll "a"	Chl. 4	:	0.85	0.92	0.92	0.91	0.95	1						
Day 6	Dry weight	d. wt. 6	:	0.85	0.93	0.92	0.91	0.95	0.94	1					
	Cell number	no. 6	:	0.92	0.94	0.95	0.96	0.98	0.96	0.94	1				
	Chlorophyll "a"	Chl. 6	:	0.95	0.91	0.89	0.87	0.90	0.86	0.83	0.9	1			
Day 8	Dry weight	d. wt. 8	:	0.85	0.93	0.92	0.91	0.95	0.95	0.1	0.95	0.83	1		
	Cell number	no. 8	:	0.92	0.95	0.94	0.96	0.98	0.95	0.97	0.99	0.89	0.97	1	
	Chlorophyll "a"	Chl. 8	:	0.96	0.94	0.89	0.88	0.91	0.85	0.85	0.9	0.98	0.86	0.91	1





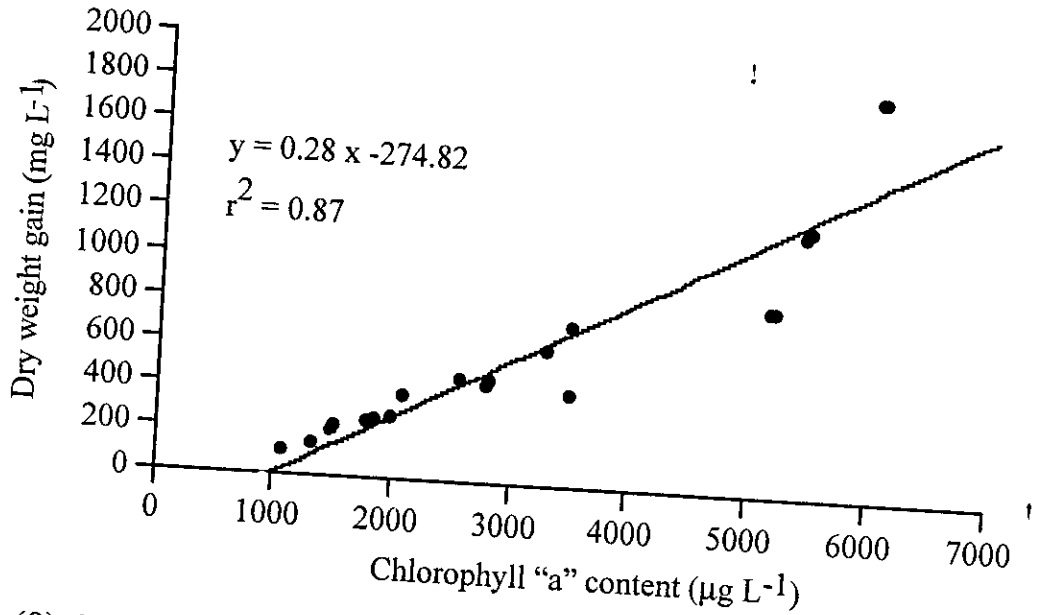


Fig. (9): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content of various light intensities for ***Chlorella vulgaris*** at all incubation periods in indoor cultures.

Exp. 5- Growth response of *Chlorella vulgaris* to various temperature degrees:

Growth of ***Chlorella*** at higher temperature was more intense than lower temperature. At 25 °C the growth of ***Chlorella*** was faster than at lower temperature and it recorded high dry weight gain, cell number as well as chlorophyll "a" content during the course of culturing time. The relative high temperature (at 35 °C) led to a depression in growth of ***Chlorella*** cells expressed as dry weight, cell number as well as chlorophyll "a" content compared with data obtained intially. Table (14) and Fig(9).

Dry weight gain

It is shown from data in Table (13) and Fig (4), that growth of ***Chlorella vulgaris*** at 20 °C was more intensive than other temperatures degree, which recorded maximum production during the period of 6 days of culture age which amounted to 663.3 mg l^{-1} and showed significantly different LSD = 92 (α= 0.05). The dry weight gain at 15 °C was increasing during the culture age of experiment (8 days) which amounted to 396.7 mg l^{-1} at the end of incubation periods. However, the dry weight in cultures incubated at 25 and 30 °C reached highest yield at 6 days of incubation periods which amounted to 609.7 and 201.7 mg l^{-1} respectively. Table (14) and Fig (9).

Cell count:

The cell number of ***Chlorella*** cultures incubated at 20 °C recorded maximum crop during the period of 6 days of culture age which amounted to 533.3 x 10^4 cells ml-1 and showed significantly different LSD = 63 (α = 0.05). The cell number at 15 °C further increase by time up to the 8th day which amount 353.3 x 10^4 cells ml-1. However, the cell number at 25 & 30 °C reached highest rate at 6 days of incubation periods which amounted to 503.3 and 223.3 x 10^4 cells ml-1 respectively, Table (14) and Fig (9).

Chlorophyll "a" content:

The efficiency of cells to produce chlorophyll "a" during culturing time at different temperature values, are illustrated in Table (14) and Fig (9). A maximum chlorophyll "a" biosynthesis took place at 6 days of culturing time at 20 °C which amounted to 4074 µg l-1 and showed significantly different LSD = 558.4 (α = 0.05). After word, it started decreasing up to the end of incubation period. Chlorophyll "a" content of algal cells at lower temperature (15 °C) was increasing during the culture time of experiment (8 days) which amounted to 2475.2 µg l-1 at the end of incubation periods. However, at higher temperature 25 and 30 °C, the stationary phase was attained at 6 day incubation periods which amounted to 3566.8 and 1249.8 µg l-1 respectively. Table (14) and Fig (9). At 35 °C chlorophyll "a" content was hardly detected indicating that it is highly supervise for chlorophyll "a" biosynthesis.

Table (14): Growth response of ***Chlorella vulgaris*** at different temperatures (grown on Bold's basal medium, 14hrs light / 10hrs dark photoperiod, 5000 lux, and pH 7) at 2 days interval for a period of 8 days incubation in indoor cultures.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Temperatures °C ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
15 °C	98.7	60.0	940.8	175.3	143.3	1332.1	371.7	213.3	2437.1	396.7	353.3	2475.2
Significance	C	C	B	C	B	B	C	B	B	B	C	C
20 °C	159.7	83.3	1211.1	326.3	320.0	2238.0	663.3	533.3	4074.0	618.3	503.0	3961.9
Significance	B	B	A	B	A	A	B	A	A	A	A	A
25 °C	206.7	93.7	1337.6	451.7	310.0	2208.7	609.7	503.3	3566.8	600.0	416.7	3289.5
Significance	A	A	A	A	A	A	A	A	A	A	B	B
30 °C	68.7	49.3	629.2	110.0	85.3	915.0	201.7	223.3	1249.8	147.7	193.3	1230.2
Significance	D	D	C	D	C	C	D	C	C	C	D	D
35 °C	46.0	20.3	487.4	39.3	15.0	342.5	31.3	10.0	196.8	23.0	6.3	166.3
Significance	E	E	C	E	D	D	E	D	D	D	E	E
LSD	15.7	8.0	142.0	61.3	54.4	385.8	92.0	63.0	558.4	55.3	48.4	487.0

Initial (0 day): Dry weight = 59.0 mg l^{-1}, algal count x 10^4 ml^{-1} = 25.3, chl "a" µg l^{-1} = 574.5

For LSD:

Alpha = 0.05 df = 10 Critical value of T = 2.23

Means in the same columns with the same letter are not significant.

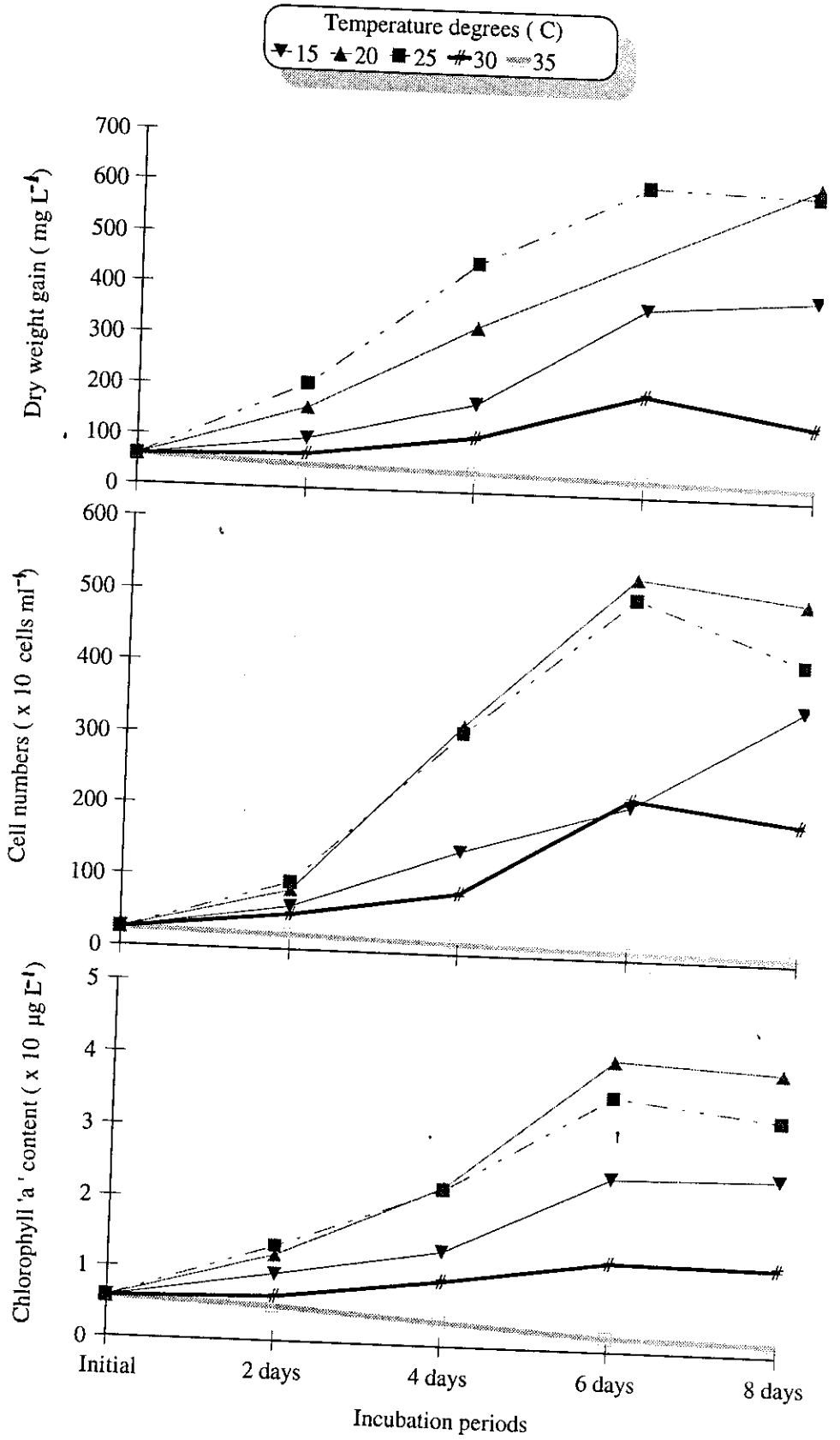


Fig (10) : Effect of different temperature degrees on dry weights, cell numbers and chlorophyll "a" contents of ***Chlorella vulgaris*** at 2 days interval for a period of 8 days incubation in indoor cultures .

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried and represented in Table (15). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

The dry weight gain was high positively correlated with the count of the organism at 2 days incubation periods ($r = 0.94$). Regression line: $y = 1.19\ x + 21.30$, $r^2 = 0.94$ for all culture age. In addition, very strong positive correlation was found between dry weight gain and chlorophyll "a" content ($r = 0.96$) at the same day during incubation periods.). Regression line: $y = 6.75\ x + 327.88$, $r^2 = 0.95$ for all incubation periods (Fig 11).

Also, the dry weight gain showed very strong positive correlation with cell count and the content of chlorophyll "a"(r= 0.93 and 0.92 respectively) at 4th days incubation periods.

The cell number of the organism showed very highly positive correlation with the amount of chlorophyll "a" !($r = 0.95$) at 2 days incubation periods. Regression line: $y = 0.17\ x - 34.22$, $r^2 = 0.98$ for all culture time (Fig 11). The other correlation of other variables at other incubation periods are shown in Table (15).

Table (15): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** at different temperatures at 2 days interval for a period of 8 days incubation in indoor cultures.
Data listed are only the coefficient of the significant correlations ($P< 0.05$).

Time days ⇓				d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
Day 2	Dry weight	d. wt. 2	:	1											
	Cell number	no. 2	:	0.94	1										
	Chlorophyll "a"	Chl. 2	:	0.96	0.95	1									
Day 4	Dry weight	d. wt. 4	:	0.98	0.93	0.92	1								
	Cell number	no. 4	:	0.93	0.95	0.93	0.93	1							
	Chlorophyll "a"	Chl. 4	:	0.93	0.94	0.94	0.92	0.92	1						
Day 6	Dry weight	d. wt. 6	:	0.97	0.96	0.96	0.97	0.96	0.95	1					
	Cell number	no. 6	:	0.92	0.96	0.96	0.9	0.94	0.98	0.95	1				
	Chlorophyll "a"	Chl. 6	:	0.9	0.93	0.94	0.88	0.91	0.98	0.94	0.98	1			
Day 8	Dry weight	d. wt. 8	:	0.96	0.98	0.96	0.96	0.96	0.97	0.99	0.97	0.97	1		
	Cell number	no. 8	:	0.82	0.92	0.9	0.8	0.9	0.93	0.88	0.97	0.96	0.92	1	
	Chlorophyll "a"	Chl. 8	:	0.862	0.91	0.94	0.85	0.92	0.97	0.92	0.98	0.99	0.96	0.98	1

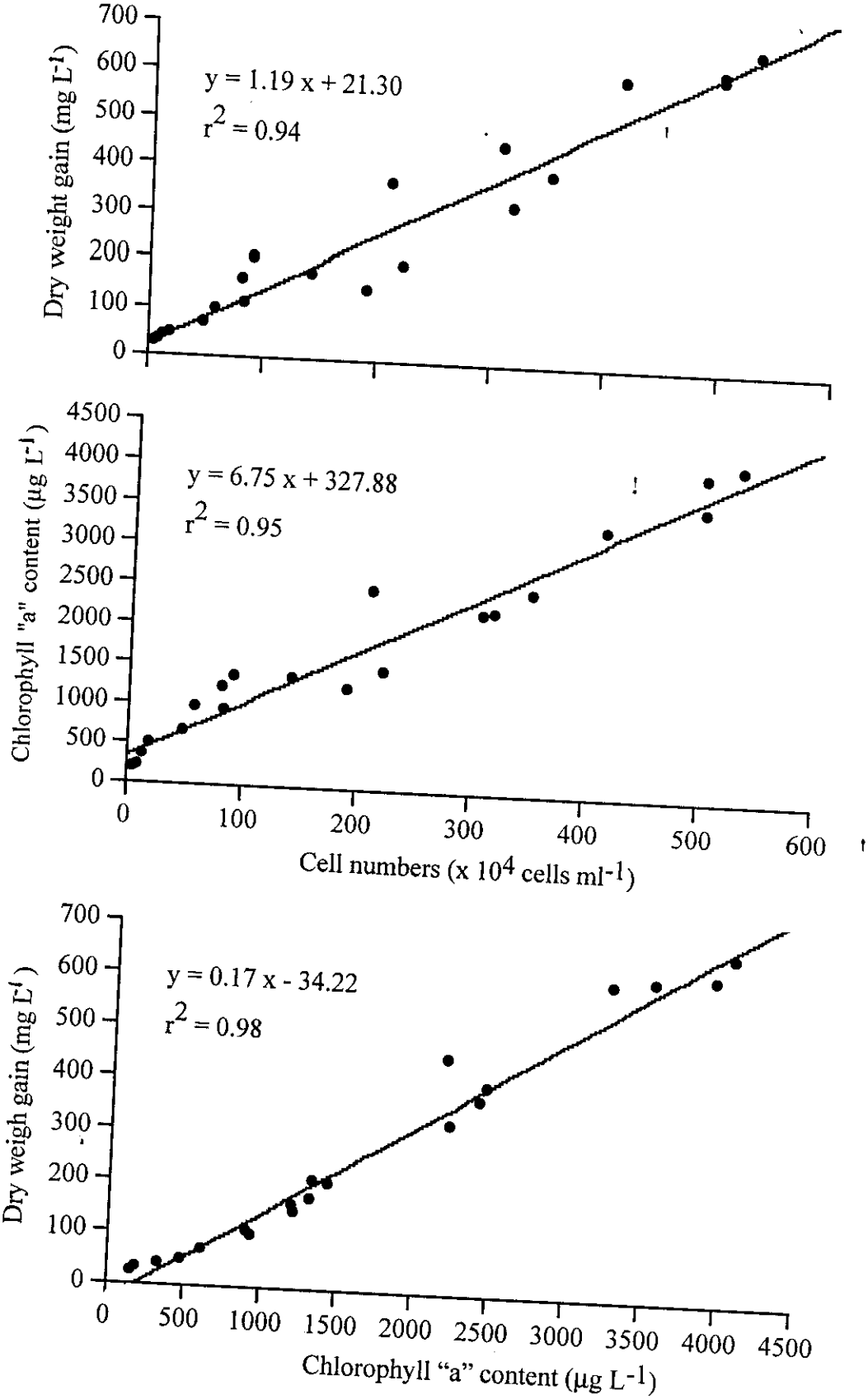


Fig. (11): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content of different temperature degrees for ***Chlorella vulgaris*** at all incubation periods in indoor cultures.

Part II

II-Algal Culture (Mass Production) of *Chlorella vulgaris* in Outdoor Cultivation:

Inoculum is prepared in the laboratory using single-cell technique as mentioned before. After reaching adequate concentration in indoor culture, algal suspension was then transferred to outdoor cultures using glass aquaria of 100 litres. In the aquarium normally a concentration of 0.2 g dry weight / litre (cell density about 1 x 105 cells ml-1) was started with and it was then transferred to the outdoor cultivation tank after a retention time of 7 days when it reached a concentration of 0.7 g dry weight / litre (cell density about 5 x 10 6 cells ml-1). The yield of the biomass of ***C. vulgaris*** obtained was 5.4 g m-2 day-1.

Harvesting:

Harvesting took place 5-6 days after inoculation. As the main aim was to study growth stages during different periods, harvesting was always carried out on the day of maximum growth. Harvesting was carried out by the method of gravity separation. Such technique was used as the first step towards harvesting the algal cells in order to reduce the total volume of the algal suspension required for subsequent centrifugation. In this study, a comparison of moisture and crude protein contents of the algal biomass dried by oven drying at 110 °C and air drying at about 25 °C was attempted (Table 16). The moisture content of the algal biomass dried at 110 °C was 5.1 % and that of air dried algal biomass was 10.3, while crude protein content of the algal biomass at 110 °C and of the air dried algal biomass were 45.6 and 46.5%, respectively. Oven drying at 110 °C was chosen for drying the harvested algal cells because a much shorter drying time was required. The dried algal pellets can be stored indefinitely when the moisture content is below 12%. The moisture contents of the algal biomass

dried by all two methods were found to be lower than 12%, which enabled the practice of storing the dried algal biomass in a desicator for long period of time. However, it was found that the algal biomass dried by oven drying at 110 °C formed harder clusters of algal flakes, which needed to be blended into smaller particles before use for the fish-feeding experiment.

Table (16): Effect of drying method on moisture and crude protein contents of ***Chlorella vulgaris*** under optimum growth conditions

Drying method	Moisture	Crude protein (%)
Oven dry (110 °C)	5.1±0.3	45.6±1.4
Air dry (25 °C±2)	10.3±0.5	46.5±0.9

Mean value ± standard deviation.

Chemical Analysis of the Dried Algal Biomass:

Oven drying at 110 °C was chosen for drying the harvested algal cells because a much shorter drying time was required.

The quantitative most important components of diets for humans and animals are protein, carbohydrates, nucleic acids, amino acids and lipids, the later typically consisting of fats and / or oils.

The crude protein content of ***Chlorella vulgaris*** was 45.6% and the crude fat content was 17.9% on a dry-weight basis (Table 17). The total nucleic acid content was 3% (RNA = 2.1% and DNA = 0.9%) on dry weight basis, the ash content of ***Chlorella*** was 18.87% while crude fiber was 8.2%. The total carbohydrate contents was 10.1%.

Table (17): Chemical composition of oven dry ***Chlorella vulgaris*** grown at optimum growth conditions.

Component (%)	g per 100 g dry weight
Crude protein	45.60±1.4
Crude fat	17.90±2.5
Total carbohydrate	10.10±1.0
Ash	18.87±2.15
Crude fiber	8.20±1.1
RNA	2.10±0.3
DNA	0.90±0.1

Mean value ± standard deviation.

Amino acid analysis of oven dry ***C. vulgaris*** indicated that the algal cells possessed a rather complete amino-acid profile Table (17). The total quantitative contents of amino acids was 87.2 g / 16 g N. The amino acid profile of the algal dry tissue indicated that there are 18 amino acids as represented in Table (18). The amino acid profile was well-balanced except that the level of the sulfur-containing amino acids, e.g. methionine and cystine (1.8 g / 16 g N) recording 2.06 % of total that could be considered low compared with the FAO pattern (6.4 g / 16 g N). However, sulfur-containing amino acids can be easily supplemented from artificial animal feeds. On the other hand, the essential amino acids phenylalanine + tyrosine recorded higher values (7 g / 16 g N) compared with (5.8 g / 16 g N) in the FAO pattern. Glycine recorded the largest values among all amino acids (13.88%) followed by Aspartic (9.75%) and Alanine (9.29%). Generally speaking, leucine, isoleucine, lysine, threonine, tryptophan and valine amino acids recorded in higher amounts than that of the FAO pattern.

Table (18): Combined amino acid content along with their percentage of participation in total amino acid content of ***Chlorella vulgaris*** grown at optimum growth conditions.(Data expressed as g/16 g N).

Amino acid	*C. vulgaris*	% of total	FAO pattern*
Alanine	8.1	9.29	
Arginine	5.3	6.08	
Aspartic acid	8.5	9.75	
Glutamic acid	5.1	5.85	
Glycine	12.1	13.88	
Histidine	1.5	1.72	
Isoleucine	4.3	4.93	4.2
Leucine	7.4	8.49	4.8
Lysine	5.0	5.73	4.2
Methionine+Cystine**	1.8	2.06	6.4
Phenylalanine+tryosine**	7.0	8.03	5.8
Proline	4.8	5.50	
Serine	4.3	4.93	
Threonine	4.2	4.82	2.8
Trypyophan	2.3	2.64	1.4
Valine	5.5	6.31	4.2
Total amino acids	87.2		

* Recommended essential amino-acid content for an ideal protein for animal consumption (WHO/FAO) (Bhumiratana, 1976).

** Essential amino acids' in ***C. vulgaris*** (g / 16 g N)

Part III

Outdoor Experiments:

Exp. 1- Growth response of *Chlorella vulgaris* at different fertilizers with tap water:

Studies carried out to determine the growth response of ***C. vulgaris*** when grow on some commercial fertilizers in tap water using urea-N (5 mg l^{-1}), super phosphate-P (0.78 mg l^{-1}), combination of urea-N (3 mg l^{-1}) + super phosphate-P (0.78 mg l^{-1}) and organic matter as chichen manure (0.5 g l^{-1}). The cultures were left in open system with adjusted pH 7, at temperature 20 to 25 °C and 13 light / 11 dark photoperiod. Growth parameters including dry weight gain, cell count and chlorophyll "a" content were estimated at two days intervals for a period of 8 days incubation. The collected data were compared with Oceanic Institute (OI) medium for outdoor culture as control, as referred in Dawah (1994).

Dry weight gain:

The data given in Table (19) and Fig (12) indicate that maximum growth expressed as the gain in dry weight (736.7 mg l^{-1}) was recorded at 6 days of incubation periods in cultures of OI medium. Cultures of urea-N had better growth than that of other fertilizers and was increasing during the culture time of experiment to reach 164.7 mg l^{-1} at the end of incubation period (8 days). Urea-N and super phosphate mixture was following to urea alone in dry weight gain which showed gradual increase with laps of time to reach maximum dry wt at 8 days of incubation (144.7 mg l^{-1}). However, in cultures supplemented with super phosphate and organic manure, the growth increased slowly and the stationary phase reached at 8 days incubation periods which amounted to 122 and 125.3 mg l^{-1} respectively. Table (19) and Fig (12).

Part III

Outdoor Experiments:

Exp. 1- Growth response of *Chlorella vulgaris* at different fertilizers with tap water:

Studies carried out to determine the growth response of ***C. vulgaris*** when grow on some commercial fertilizers in tap water using urea-N (5 mg l^{-1}), super phosphate-P (0.78 mg l^{-1}), combination of urea-N (3 mg l^{-1}) + super phosphate-P (0.78 mg l^{-1}) and organic matter as chichen manure (0.5 g l^{-1}). The cultures were left in open system with adjusted pH 7, at temperature 20 to 25 °C and 13 light / 11 dark photoperiod. Growth parameters including dry weight gain, cell count and chlorophyll "a" content were estimated at two days intervals for a period of 8 days incubation. The collected data were compared with Oceanic Institute (OI) medium for outdoor culture as control, as referred in Dawah (1994).

Dry weight gain:

The data given in Table (19) and Fig (12) indicate that maximum growth expressed as the gain in dry weight (736.7 mg l^{-1}) was recorded at 6 days of incubation periods in cultures of OI medium. Cultures of urea-N had better growth than that of other fertilizers and was increasing during the culture time of experiment to reach 164.7 mg l^{-1} at the end of incubation period (8 days). Urea-N and super phosphate mixture was following to urea alone in dry weight gain which showed gradual increase with laps of time to reach maximum dry wt at 8 days of incubation (144.7 mg l^{-1}). However, in cultures supplemented with super phosphate and organic manure, the growth increased slowly and the stationary phase reached at 8 days incubation periods which amounted to 122 and 125.3 mg l^{-1} respectively. Table (19) and Fig (12).

The dry weight gain of ***C. vulgaris*** cultures showed significantly differences between fertilizers and OI medium as control at 6 days incubation periods LSD = 16.9 ($\alpha = 0.05$). Table (19) and Fig (12).

Cell count:

Table (19) and Fig (12) show that the cell count of ***C. vulgaris*** treated cultures was statistically significantly increased using OI medium, urea-N and super phosphate-P + urea-N fertilizers. The maximum cell count was maintained in culture of OI medium at 6 days incubation periods which amounted 553.3 x 10^4 cells ml^{-1} and it being significantly different LSD = 75.9 (α= 0.05). While the cell count in case of combinations of urea-N + superphosphate-P and urea-N alone increased slightly during the culture age which amounted 286.7 & 273.3 x 10^4 cells ml^{-1} respectively at the end of incubation period (8 days). Organic manure was inhibitory for cell count since it recorded 156.7 x 10^4 cells ml^{-1} after 8 days growth.

Chlorophyll "a" content:

The results presented in Table (19) and illustrated in Fig (12) show clearly that, the OI medium significantly stimulated the biosynthesis of chlorophyll "a" of ***C. vulgaris*** cells. The maximum chlorophyll "a" content of ***C. vulgaris*** was maintained using OI medium at 6 days incubation periods which amounted 3382.5 µg l^{-1} and showed significantly differences between OI medium as control and other fertilizers, LSD = 344.4 (α= 0.05. Cultures of urea-N had better growth, being expressed as chlorophyll "a" content than that of other fertilizers, a phenomenon that was further increase during the culture age to reach 1952.4 µg l^{-1} at 6 days of incubation period. Urea-N and super phosphate mixture was following to urea alone in the biosynthesis of chlorophyll "a" since it recorded 2661.2 µg l^{-1} at 6 days of incubation. While in cultures of super phosphate, the chlorophyll "a"

content of ***Chlorella*** slightly increase to reach maximum content at 6 days incubation periods which amounted 1599.9 µg l^{-1}. On the other hand, the organic manure reached maximum growth at 8 days of culture age which amounted 1585.5 µg l^{-1}. Table (19) and Fig (12).

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for the experiment Table (20). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

The dry weight gain was high positively correlated with the count of the organism at 2 days incubation periods ($r = 0.89$). Regression line: $y = 1.12\ x + 2.33$, $r^2 = 0.58$ of all incubation periods. In addition, strong positive correlation was found between dry weight gain and chlorophyll "a" content ($r = 0.83$) at the same day of incubation periods which showed linear relationship regression line: $y = 5.18\ x + 722.44$, $r^2 = 0.82$ of all culture time (Fig 13).

Also, the dry weight gain showed very strong positive correlation with the cell count and the content of chlorophyll "a"(r= 0.92 & 0.90 respectively) at 4th days incubation periods.

The cell number of the organism showed positive correlation with the amount of chlorophyll "a" ($r = 0.74$) at 2 days incubation periods. Regression line: $y = 0.23\ x - 178.02$, $r^2 = 0.73$ of all experiment time (Fig 13). The other correlation of other variable at other incubation periods are shown in Table (20).

Table (19): Growth response of ***Chlorella vulgaris*** using different commercial fertilizers with tap water at 2 days interval for a period of 8 days incubation in outdoor cultures (pH = 7, temperature 25 °C±2 and 14 light / 10 dark photoperiod.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Fertilizers type ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
OI medium	206.7	49.0	1496.6	474.7	233.3	2602.0	736.7	553.3	3382.5	617.0	341.0	2862.6
Significance	A	A	A	A	A	A	A	B/A	A	A	A	A
Urea-N	86.0	41.7	918.5	99.3	183.3	1402.7	137.0	246.7	1952.4	164.7	273.3	1856.3
Significance	B	B	A	A	A	B	A	B	B	A	A	B
Superphosphate-P	78.7	35.0	724.7	86.0	126.7	1113.1	120.0	210.0	1599.9	122.0	253.3	1559.7
Significance	B	B	B	B	B	C	B	B	C	C	B	C
Urea-N+ Superph-P	95.7	50.7	997.7	109.3	230.0	1793.2	142.7	260.0	2661.2	144.7	286.7	2229.0
Significance	A	A	A	A	A	A	A	A	A	B	B/A	A
Organic manure	76.3	28.3	644.3	195.0	76.0	1050.3	110.0	133.3	1523.6	125.3	156.7	1585.5
Significance	C	C	D	D	C	D	D	D	D		D	D
LSD	7.4	8.6	137.7	11.1	54.9	216.8	16.9	75.9	344.4	13.6	110.0	170.2

Initial (0 day): Dry weight = 56.7 mg l^{-1}, algal count x 10^4 ml^{-1} = 18.3, chl "a" µg l^{-1} = 526.6

For LSD:

Alpha = 0.05 df = 10 Critical value of T = 2.23

Means in the same columns with the same letter are not significant.

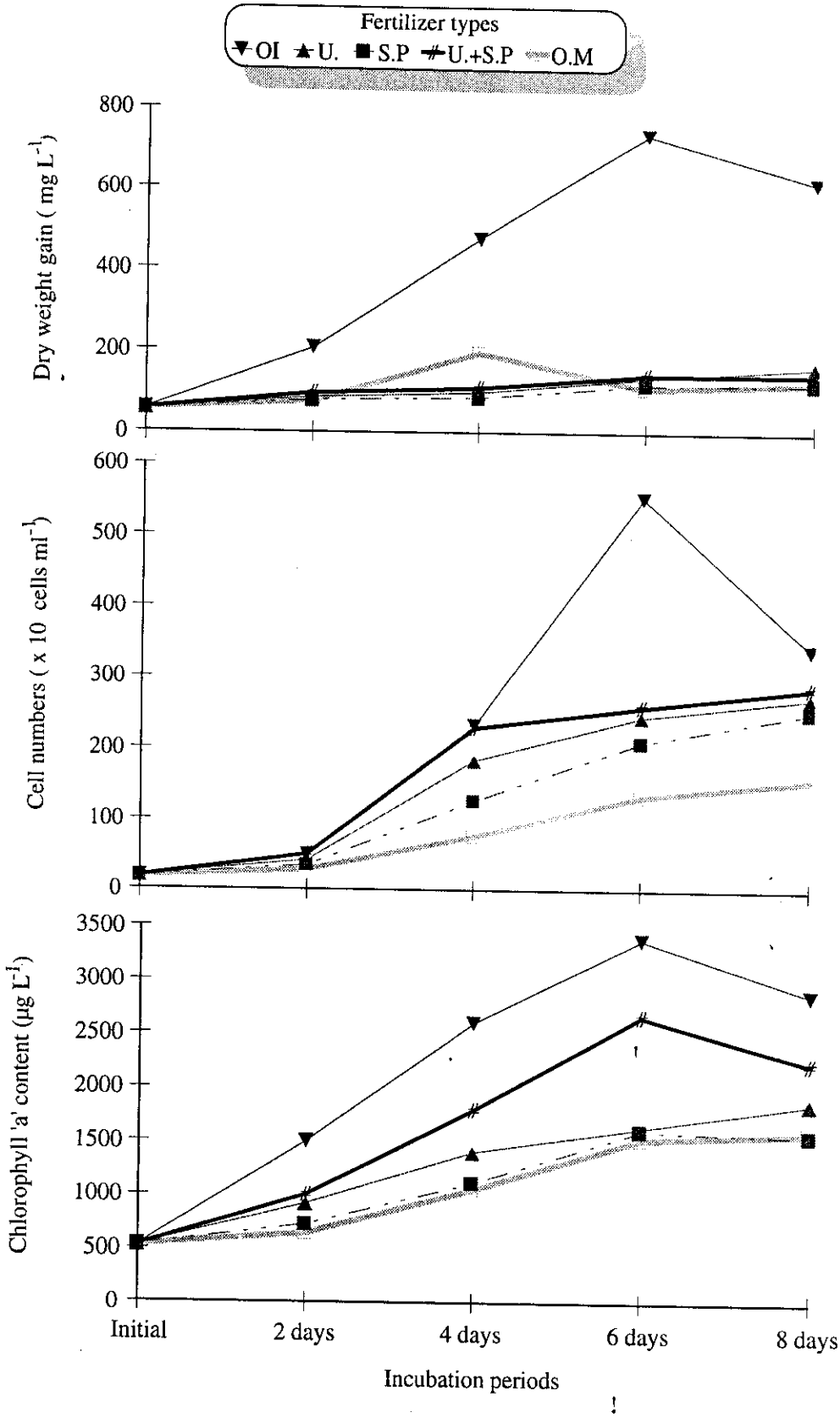


Fig (12) : Growth response of ***Chlorella vulgaris*** using different fertilizers with tap water at 2 days interval for a period of 8 days incubation in outdoor cultures .

Table (20): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** at different fertilizers with tap water at 2 days interval for a period of 8 days incubation in oudoor culture
Data listed are only the coefficient of the significant correlations ($P< 0.05$).

Time days ⇓				d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
	Dry weight	d. wt. 2	:	1											
Day	Cell number	no. 2	:	0.89	1										
2	Chlorophyll "a"	Chl. 2	:	0.83	0.74	1									
	Dry weight	d. wt. 4	:	0.97	0.87	0.89	1								
Day	Cell number	no. 4	:	0.9	0.89	0.78	0.92	1							
4	Chlorophyll "a"	Chl. 4	:	0.86	0.77	0.98	0.9	0.78	1						
	Dry weight	d. wt. 6	:	0.91	0.86	0.85	0.9	0.77	0.91	1					
Day	Cell number	no. 6	:	0.52	0.44	0.64	0.57	0.39	0.71	0.66	1				
6	Chlorophyll "a"	Chl. 6	:	0.85	0.79	0.95	0.92	0.83	0.96	0.9	0.69	1			
	Dry weight	d. wt. 8	:	0.9	0.83	0.84	0.88	0.77	0.91	0.98	0.62	0.88	1		
Day	Cell number	no. 8	:	0.83	0.73	0.9	0.89	0.85	0.91	0.83	0.56	0.94	0.84	1	
8	Chlorophyll "a"	Chl. 8	:	0.87	0.79	0.93	0.91	0.81	0.96	0.95	0.7	0.98	0.94	0.93	1

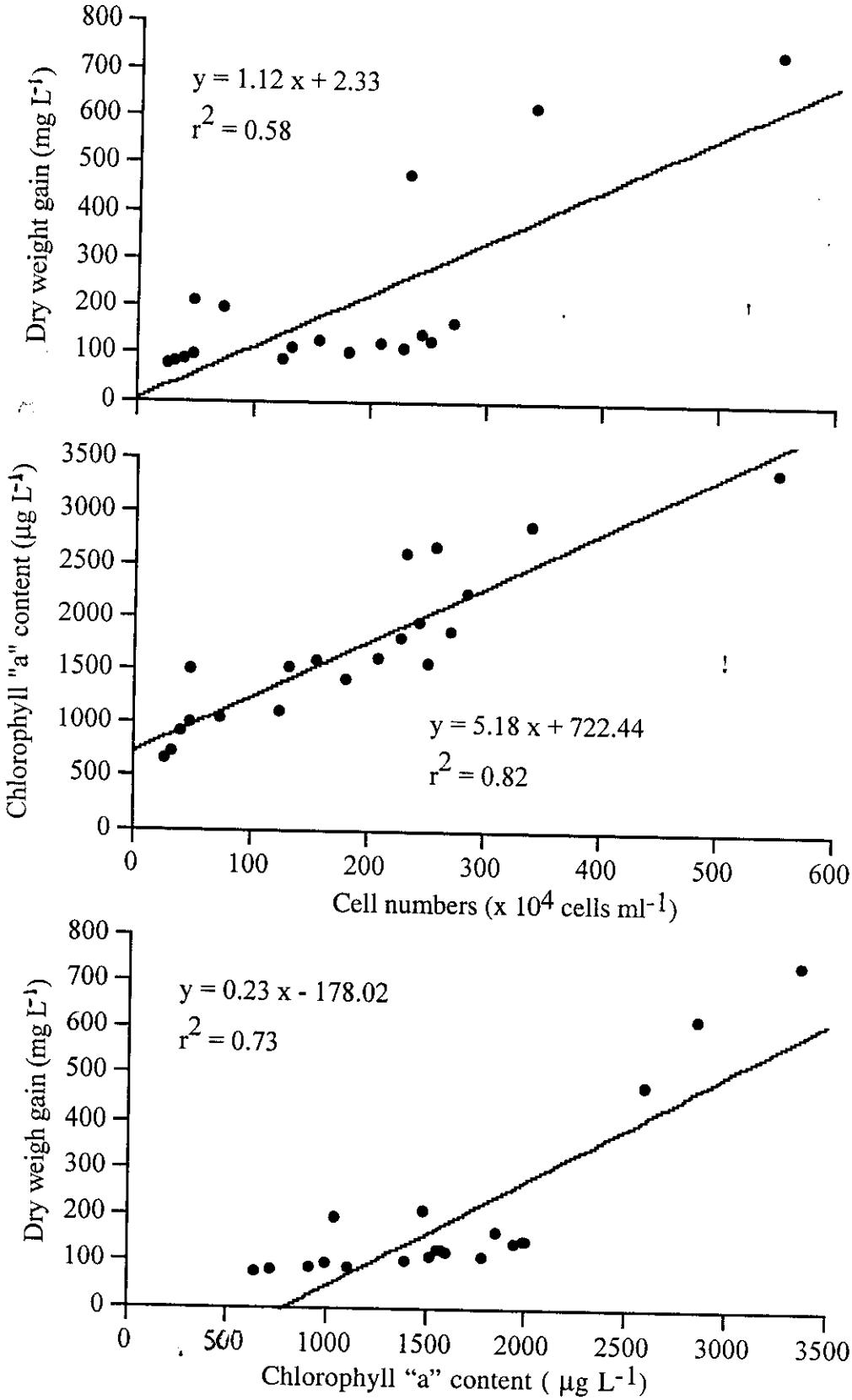


Fig. (13): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content using different fertilizer with tap water for ***Chlorella vulgaris*** at all incubation periods in indoor cultures.

Exp. 2- Growth response of *C. vulgaris* using different nitrogen sources of media of outdoor cultures:

Data indicating growth of ***C. vulgaris*** grown in media of OI medium for outdoor culture to which different sources of nitrogen were added according to the recommended media by Allen and Nelson (1910) are listed (Table 21, Fig 14). By using (Ammonium sulfate 100 g / 1000 L + urea-N 5g / 1000 L), and substituted by (Potassium nitrate 187g / 1000 L), or (sodium nitrate 127g / 1000 L) in open system which adjusted to be pH 7, at temperature 20 to 25 °C and 13 h light / 11 h dark photoperiod . Growth parameters were determined every two days until the end of incubation period (8 days).

By comparing the growth of ***C. vulgaris in*** cultures containing the different sources of nitrogen, it was more higher in case of ammonium sulfate + urea at 6th days incubation periods. It can be clearly seen that sodium nitrate supplemented to algal cultures induced a significant increase in the growth of ***C. vulgaris***, a trend that was continued up to the end of incubation period (8 days).

Dry weight gain:

The dry weight gain was progressively increased in medium containing ammonium sulfate + urea at 6 days incubation periods which amounted to 682.3 mg l-1 and showed significantly differences between media at the same day of incubation periods, LSD = 30.6 ($\alpha = 0.05$).

The dry weight gain in cultures containing sodium nitrate or potassium nitrate was progressively increased during culture time up to the end of incubation periods (8 days) which amounted to 664.7 and 596 mg l^{-1}

respectively and showed high significantly differences, LSD = 37.4 (α = 0.05). Table (21) and Fig (14).

Cell number:

Chlorella cell count registered highest amounts in medium containing ammonium sulfate + urea (380 x 10^4 cells ml^{-1}) at 6 days incubation periods, which was significantly increased relevant to other media, reaching maximum level, LSD = 207.8 (α = 0.05). (Table 20 and Fig 7). However, In case of sodium nitrate and potassium nitrate supplemented to the cultures media, the cell number of the organism was intensed and increased until the end of culture time which amounted 340 and 263.3 x 10^4 cells ml^{-1} respectively and showed significantly differences at end of incubation periods LSD = 97.3 (α = 0.05).

Chlorophyll "a" content:

The mean values of chlorophyll "a" content in ***C. vulgaris*** cells following nitrogen addition as fertilizers to the culture media are included in Table (21) and presented in Fig (14). The data clearly reveal that maximum accumulation of chlorophyll "a' was obtained at medium containing ammonium sulfate + urea fertilizers at 6 days incubation periods which amounted 2791.7 $\mu g\ l^{-1}$ and appear highly significantly difference LSD = 353.1 (α = 0.05). While in the cultures containing sodium nitrate or potassium nitrate, the amount of pigment, particulary as chlorophyll "a" was intensed and increased up to the end of incubation periods, which amounted to 2483.3 and 2152.4 $\mu g\ l^{-1}$ respectively and showed significantly differences at end of incubation periods LSD = 227.4 (α = 0.05).

Table (21): Growth response of ***Chlorella vulgaris*** grown in media of different nitrogen sources at 2 days interval for a period of 8 days incubation in outdoor cultures (pH = 7, temperature 25 °C±2 and 14 light / 10 dark photoperiod.

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Fertilizers type ⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
Amm. sulfate + urea	197.7	50.3	1227.3	429.3	196.7	2108.1	682.3	380.0	2791.7	635.3	370.0	2512.9
Significance	A	A	B	B	B	B	B	A	B	B	B	B
Potassium nitrate	75.7	30.7	985.9	225.7	86.3	1518.0	593.3	256.7	2006.2	596.0	263.3	2152.4
Significance	C	C	C	D	C	C	C	B/A	C	C	C	C
Sodium nitrate	138.7	39.7	1077.4	306.0	102.7	1976.2	656.0	330.0	2402.4	664.7	340.0	2483.3
Significance	B	B	C/B	C	C	B	B	B/A	C/B	B	C/B	B
LSD	7.4	8.6	189.5	32.6	35.9	337.0	30.6	207.8	353.1	37.4	97.3	227.4

Initial (0 day): Dry weight = 59.3 mg l^{-1}, algal count x 10^4 ml^{-1} = 14.3, chl "a" µg l^{-1} = 443.3

For LSD:

Alpha = 0.05 df = 6 Critical value of T = 2.45

Means in the same columns with the same letter are not significant.

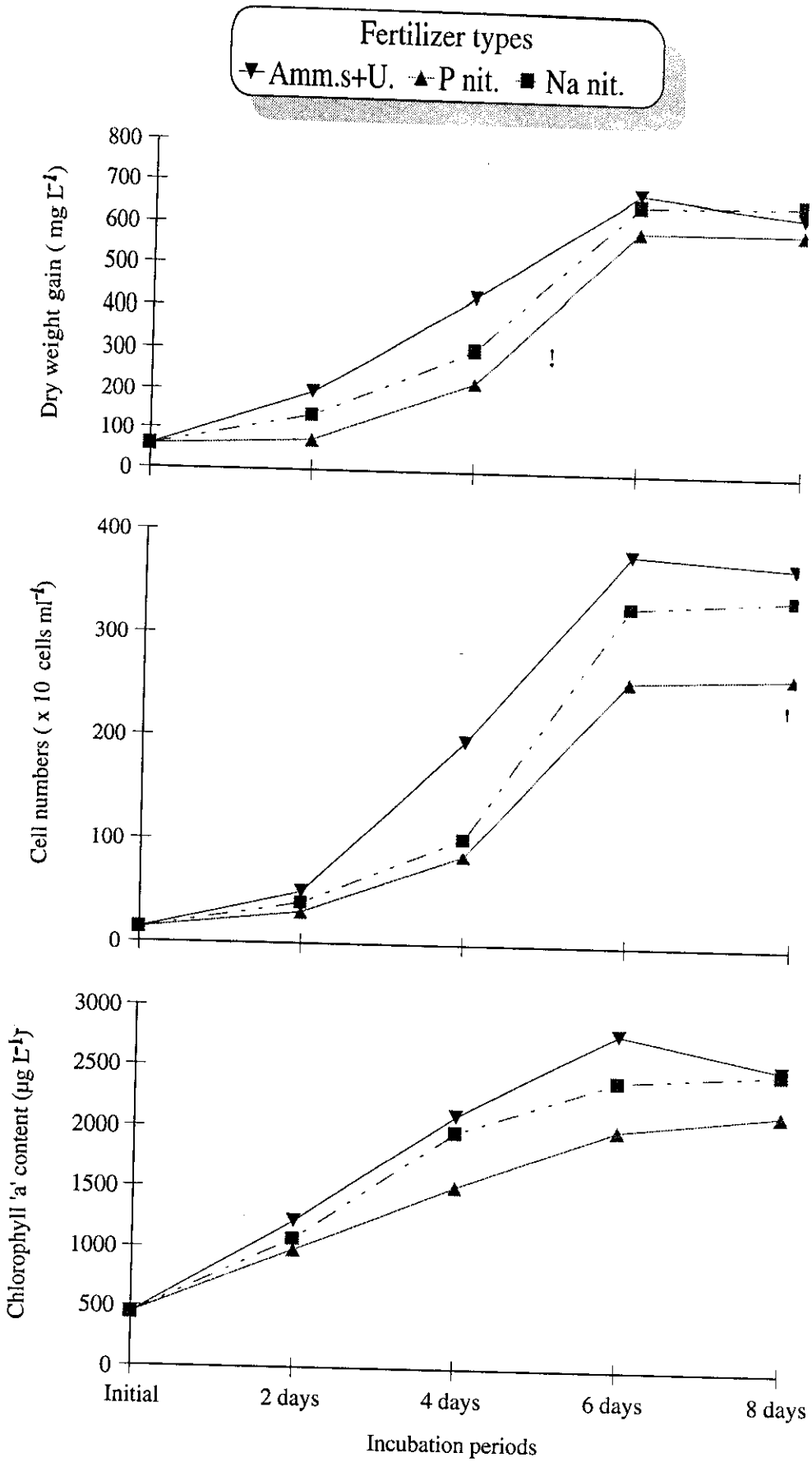


Fig (14) : Growth response of ***Chlorella vulgaris*** grown in different nitrogen sources with tap water at 2 days interval for a period of 8 days incubation outdoor cultures .

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for the experiment (Table 22). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

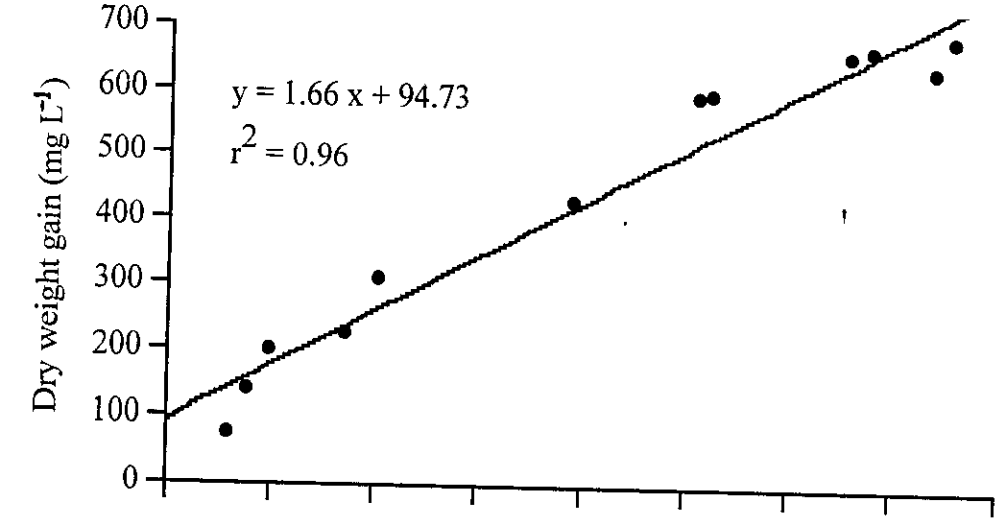
The dry weight gain was very high positively correlated with the count of the organism at 2 days incubation periods ($r = 0.92$). Regression line: $y = 1.66\ x + 94.73$, $r^2 = 0.96$ for all culture time. In addition, strong positive correlation was existed between dry weight gain and chlorophyll "a" content ($r = 0.86$) at the same day of incubation periods.). Regression line: $y = 4.19\ x + 1082.66$, $r^2 = 0.90$ for all incubation periods (Fig 15).

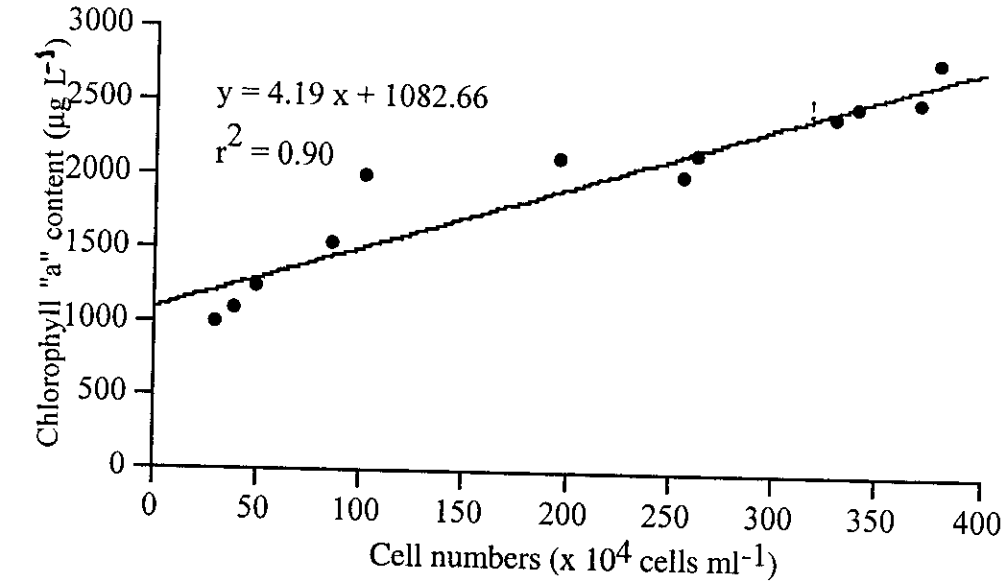
Also, the dry weight gain showed very strong positive correlation with the cell count and the content of chlorophyll "a"(r= 0.96 & 0.95 respectively) at 4th days incubation periods.

The cell number of the organism showed positive correlation with the amount of chlorophyll "a" ($r = 0.77$) at 2 days incubation periods. Regression line: $y = 0.36\ x - 270.37$, $r^2 = 0.90$ for all experiment time (Fig 15). The other correlation of other variable at other incubation periods are shown in Table (22).

Table (22): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** grown in media with different fertilizers at 2 days interval for a period of 8 days incubation in oudoor cultures.
Data listed are only the coefficient of the significant correlations ($P< 0.05$).

Time days ⇓				d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
	Dry weight	d. wt. 2	:	1											
Day	Cell number	no. 2	:	0.92	1										
2	Chlorophyll "a"	Chl. 2	:	0.86	0.77	1									
	Dry weight	d. wt. 4	:	0.81	0.86	0.84	1								
Day	Cell number	no. 4	:	0.88	0.92	0.81	0.96	1							
4	Chlorophyll "a"	Chl. 4	:	0.92	0.93	0.81	0.95	0.97	1						
	Dry weight	d. wt. 6	:	0.73	0.76	0.7	0.75	0.74	0.77	1					
Day	Cell number	no. 6	:	0.74	0.78	0.73	0.81	0.77	0.85	0.69	1				
6	Chlorophyll "a"	Chl. 6	:	0.87	0.74	0.79	0.78	0.79	0.88	0.59	0.81	1			
	Dry weight	d. wt. 8	:	0.46	0.4	0.67	0.5	0.5	0.43	0.69	0.26	0.33	1		
Day	Cell number	no. 8	:	0.48	0.37	0.63	0.47	0.51	0.42	0.66	0.17	0.33	0.96	1	
8	Chlorophyll "a"	Chl. 8	:	0.91	0.8	0.85	0.85	0.86	0.92	0.68	0.82	0.99	0.45	0.45	1





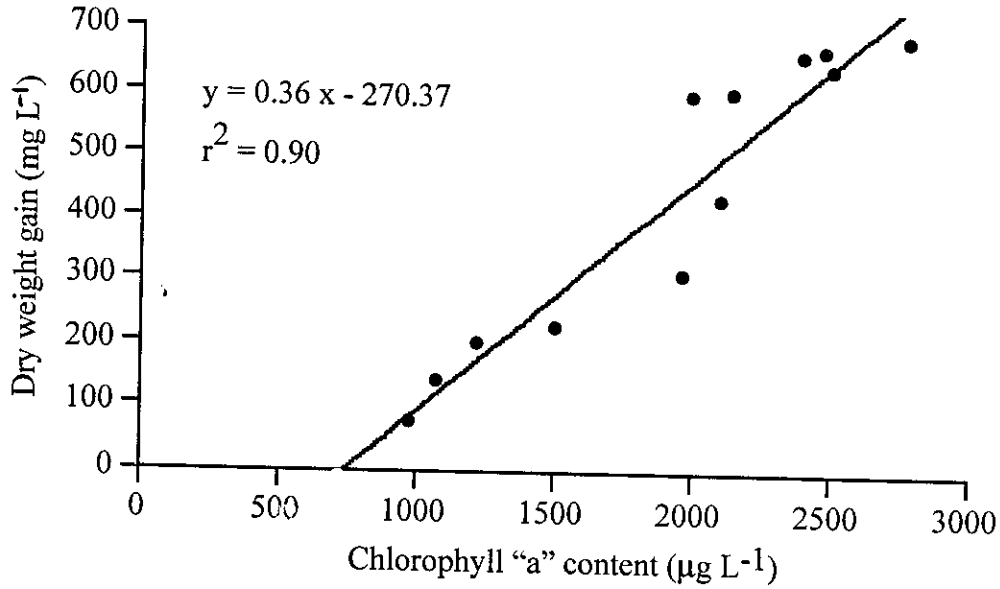


Fig. (15): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content for ***Chlorella vulgaris*** grown on different nitrogen sources at all incubation periods in indoor cultures.

Exp. 3- Growth response of *Chlorella vulgaris* at different lines of aeration:

The effect of using different air lines was extensively studied. One, two, three, four air lines on mass production of *Chlorella* cells grown in media of OI Algae Culture in open system which adjusted to be pH 7, temperature 20 to 25 °C and 14 light / 10 dark photoperiod (hrs). The diffused- air aeration system used in this study consisted of a 5-hp air blower of air compressor (which supplied aeration to all wet labs of Central Lab. of Aquaculture Research in Abbassa). The plankton lab (Algae Culture Room) was supplied with air line from the general air compressor unit. This line was branched to some 1/8 # ID polyethylene air delivery line, which conducted with glass tube of the same diameter. The air delivery tube entered the glass carboy at its bottom in the center of the carboy bottom. Growth parameters including dry weight gain, cell count and chlorophyll "a" content were estimated at two days intervals for a period of 8 days incubation.

Dry weight gain:

Results of these studies show that with high air flow rates (4 delivery air lines), the growth of *C. vulgaris* expressed as gain in dry weight was stimulated, a phenomenon that was furthered to reach maximum at 6 days of incubation periods. It amounted 239 mg l^{-1} which show significantly differences LSD = 5.9 (α = 0.05) Table (23) and Fig (16). Also, the dry weight gain in 3 delivery air lines attained maximum growth at 6 days of culture age which amounted to 124 mg l^{-1} . However, the growth of the organism represented as dry weight in both one & two air delivery lines favoured, a phenomenon that was furthered up to the end of culture time which amounted to 104.3 and 116 mg l^{-1} respectively.

Cell count:

Table (23) and Fig (16) demonestrate the growth of ***C. vulgaris*** in cultures with different air flow rates. It is to be pointed out that after 6 days of incubation, growth of ***Chlorella*** expressed as cell count reached maximum growth which amounted to 536.7 x 10^4 cells ml-1 in high flow rate (4 delivery air lines) and show highly significantly differences LSD = 220.8 (α = 0.05). But in three delivery air lines, the cell number of the organism amounted to 440 x 10^4 cells ml-1 at the same day of incubation periods. The growth in low flow rate (one and two delivery air lines) showed slight steady rise, an observation that was furthered up to the end of incubation periods (8 days) which amounted to 163 and 173.3 x 10^4 cells ml-1 receptively.

Chlorophyll "a" content:

The results presented in Table (23) and Fig (16) show clearly that, the high air flow rates (4 delivery air lines) in the culture of ***C. vulgaris*** significantly stimulated the synthesis of chlorophyll "a" which show highly significantly different at 6 days incubation periods which amounted to 2687.3 µg l-1, LSD = 282.5 (α = 0.05). The accumulated in algal cells as chlorophyll "a" in 3 delivery air lines reached maximum at 6 days of culture time which amounted 1948.0 µg l-1 . However, the chlorophyll "a" content in the cultures of the organism in low flow rates (one and two air delivery lines) were increasing up to the end of culture age which amounted 1575.0 and 1714.0 µg l-1 respectively.

Table (23): Growth response of ***Chlorella vulgaris*** exposed to different lines of aeration at 2 days interval for a period of 8 days incubation in outdoor cultures (on (OI medium), pH = 7, temperature 25 °C±2 and 14 light / 10 dark photoperiod).

Time (days)	2 Days			4 Days			6 Days			8 Days		
Parameter ⇒ Aeration lines⇓	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}	Dry wt. mg l^{-1}	Count x 10^4 ml^{-1}	Chl "a" µg l^{-1}
1 line	73.0	35.3	553.4	89.3	45.0	1112.4	102.0	105.0	1564.6	104.3	163.0	1575.0
Significance	A	D	C	C	C	C	D	C	C	D	D	C
2 lines	81.0	65.3	729.1	96.0	82.0	1275.5	112.0	253.3	1714.0	116.0	173.3	1708.1
Significance	B	C	B	B/C	B	C	C	C/B	C/B	C	C	C/B
3 lines	86.3	83.3	920.3	98.7	209.3	1456.5	162.0	440.0	1948.0	124.0	300.0	1871.9
Significance	B	B	A	B/A	B	B	B	B	B	B	B	B
4 lines	95.7	105.0	994.7	104.3	296.7	1820.0	236.3	536.7	2687.3	230.0	530.0	2229.7
Significance	A	A	A	A	A	A	A	A	A	A	A	A
LSD	7.1	14.5	116.2	7.4	35.0	171.4	5.9	220.8	282.5	7.5	56.8	174.7

Initial (0 day): Dry weight = 59.3 mg l^{-1}, algal count x 10^4 ml^{-1} = 14.3, chl "a" µg l^{-1} = 443.3

For LSD:

Alpha = 0.05 df = 6 Critical value of T = 2.45

Means in the same columns with the same letter are not significant.

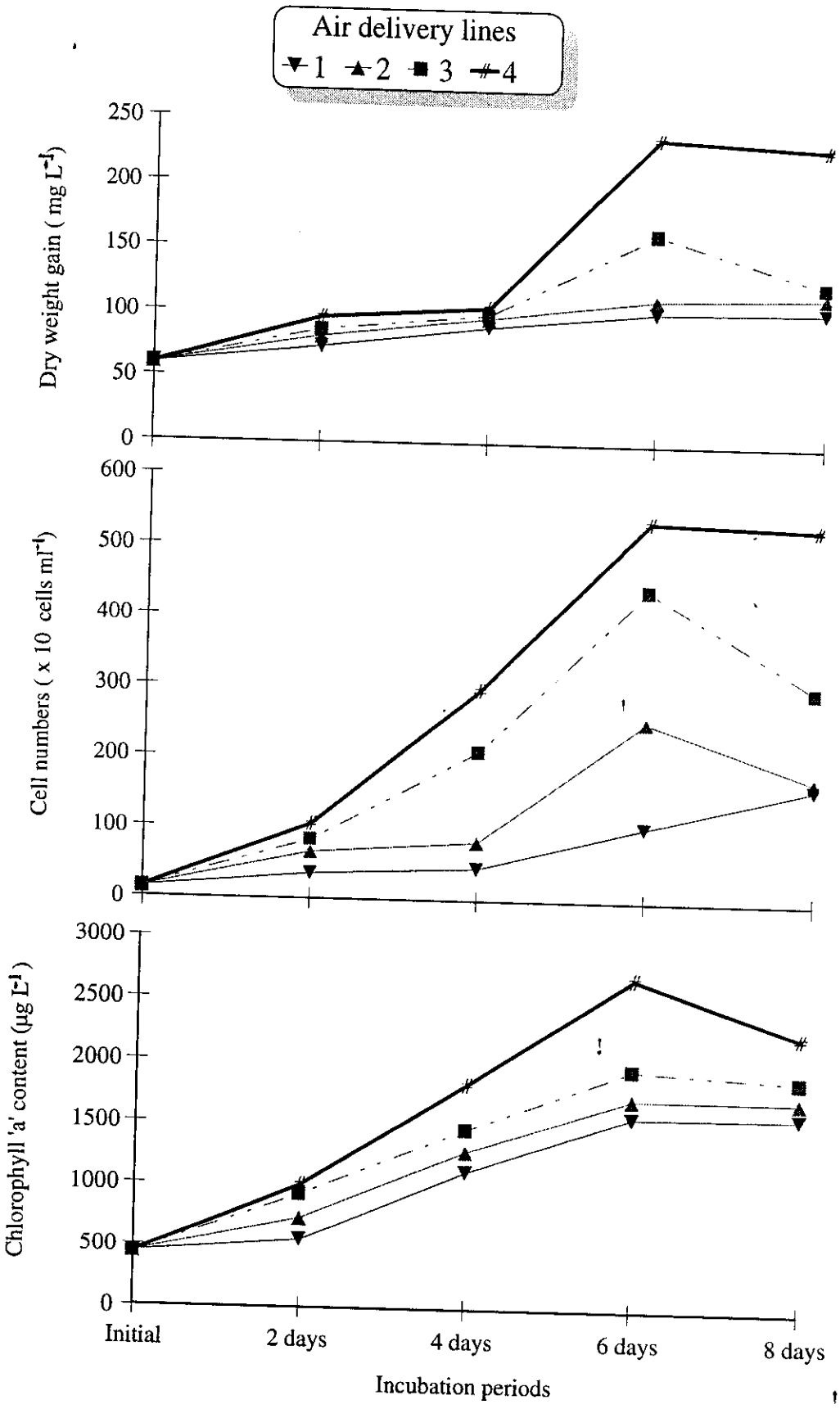


Fig (16) : Growth response of ***Chlorella vulgaris*** exposed to different lines of aeration at 2 days interval for a period of 8 days incubation in outdoor cultures .

Statistical analysis:

A multiple correlation analysis including three biological variables at two days intervals for a period of 8 days incubation, was carried out for the experiment Table (24). The correlation coefficient (r) of the significant relationships ($p < 0.05$) are only listed.

The dry weight gain was very strong positively correlated with the count of the organism at 2 days incubation periods ($r = 0.93$), whichshowed linear relationship, regression line: $y = 0.27\ x + 61.24$, $r^2 = 0.86$ for all culture time. In addition, strong positive correlation was found between dry weight gain and chlorophyll "a" content ($r = 0.86$) at the same day of incubation periods, regression line: $y = 3.06\ x + 85.56$, $r^2 = 0.82$ for all incubation periods (Fig 17).

Also, the dry weight gain showed high positive correlation with the cell count and the content of chlorophyll "a"(r= 0.82 and 0.82 respectively) at 4th days incubation periods.

The cell number of the organism showed very high positive correlation with the amount of chlorophyll "a" ($r = 0.95$) at 2 days incubation periods. Regression line: $y = 0.074\ x + 7.43$, $r^2 = 0.73$ for all culture age (Fig 17). The other correlation of other variable at other incubation periods are shown in Table (24).

Table (24): Correlation of biological analysis of growth response of ***Chlorella vulgaris*** exposed to different lines of aeration at 2 days interval for a period of 8 days incubation in outdoor cultures.
Data listed are only the coefficient of the significant correlations (P< 0.05).

Time days ⇓			:	d. wt. 2	no. 2	Chl. 2	d. wt. 4	no. 4	Chl. 4	d. wt. 6	no. 6	Chl. 6	d. wt. 8	no. 8	Chl. 8
	Dry weight	d. wt. 2	:	1											
Day	Cell number	no. 2	:	0.93	1										
2	Chlorophyll "a"	Chl. 2	:	0.86	0.95	1									
	Dry weight	d. wt. 4	:	0.84	0.85	0.81	1								
Day	Cell number	no. 4	:	0.9	0.94	0.84	0.82	1							
4	Chlorophyll "a"	Chl. 4	:	0.86	0.93	0.88	0.82	0.97	1						
	Dry weight	d. wt. 6	:	0.92	0.84	0.94	0.86	0.91	0.94	1					
Day	Cell number	no. 6	:	0.84	0.86	0.85	0.72	0.84	0.87	0.88	1				
6	Chlorophyll "a"	Chl. 6	:	0.9	0.91	0.91	0.86	0.89	0.91	0.95	0.82	1			
	Dry weight	d. wt. 8	:	0.89	0.97	0.97	0.83	0.87	0.9	0.99	0.87	0.95	1		
Day	Cell number	no. 8	:	0.87	0.86	0.86	0.78	0.88	0.91	0.94	0.83	0.82	0.92	1	
8	Chlorophyll "a"	Chl. 8	:	0.88	1	0.82	0.87	0.83	0.88	0.97	0.88	0.98	0.98	0.87	1

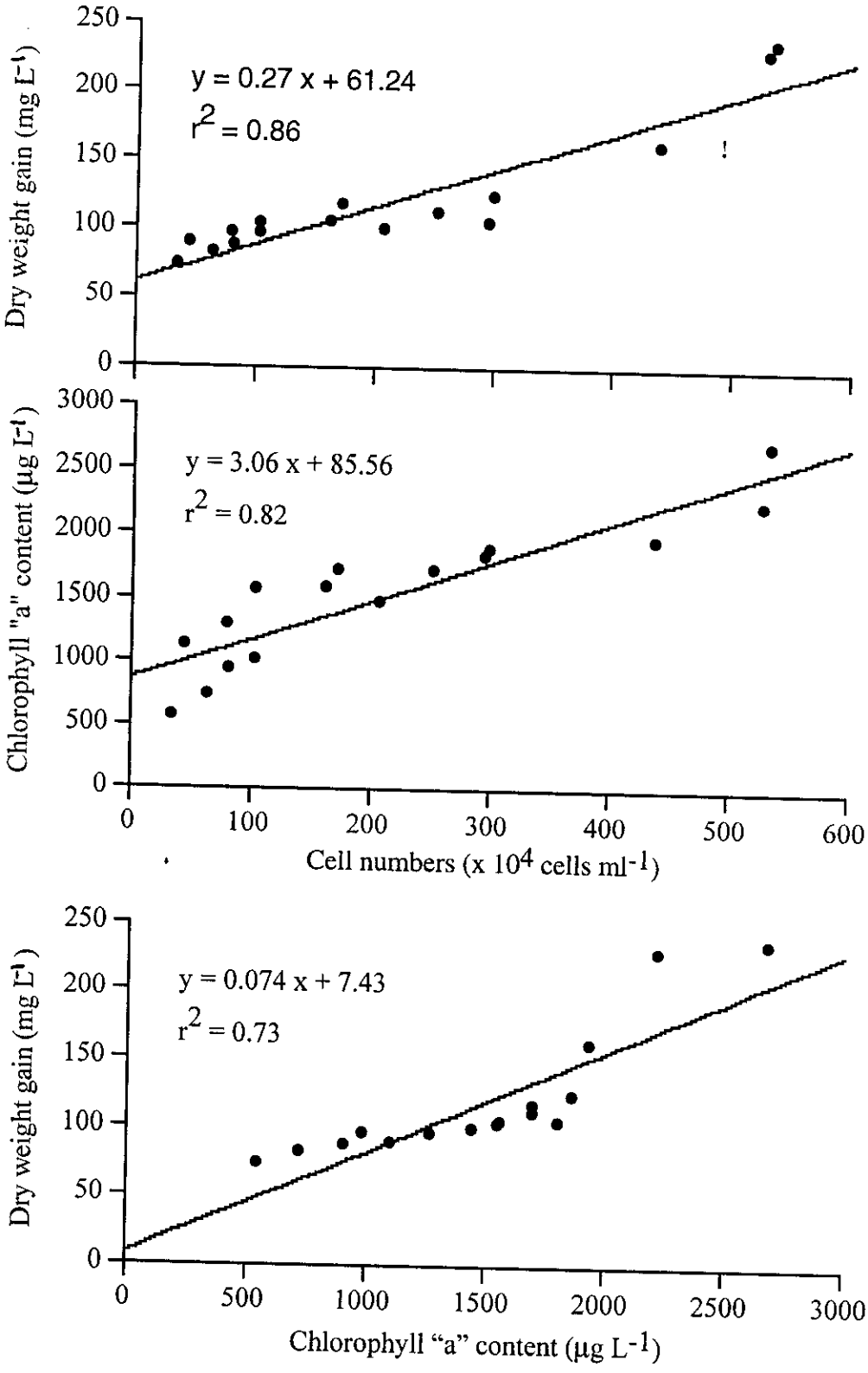


Fig. (17): Linear regression between cell numbers, dry weight gain and chlorophyll "a" content for ***Chlorella vulgaris*** exposed to different lines of airiation at all incubation periods in indoor cultures.

Part IV

Silver Carp Feeding Experiment:

Water quality:

The dissolved oxygen concentration was 7.5-8.4 mg L^{-1} with an overall mean of 7.2±0.11 mg L^{-1}. The pH range was 7.2-7.8 with a mean of 7.5±0.10. Temperature recorded 25.2 - 27.5 °C with an overall mean of 26.34± 1.5 °C. The dissolved oxygen level, pH and temperature were considered within desirable levels for optimum growth (Table 25).

The. ammonia level in the water was affected by the type of feed given. On day 14 significant differences ($P< 0.05$) between treatment means were observed. Significantly higher ammonia levels were observed when fish were feed with 100% dried algal (A100) or 75% dried algal biomass supplemented with artificial fish food (A75) compared with other treatments. The ammonia levels in fish diet with artificial feed alone AF and A5 of dried algal biomass supplemented artificial fish were significantly lower compared with the other treatments. On day 21, the ammonia level in water containing fish feed with 20% of dried algal biomass supplemented to artificial fish food (A20) was significantly lower compared with the other treatments ($P < 0.05$).

The concentration of ammonia on day 14 and 21, however, were within tolerable limits as shown by the insignificantly differences in the survival rates among different treatment means.

The mean concentration of ammonia µg L^{-1} during the experiment showed that the water in tanks contained fish fed on 20% of dried algal biomass supplemented artificial fish food had lower concentration of ammonia 11.13 µg L^{-1} compared with other treatments.

There were no significant differences between the mean concentration of ammonia in AF, A5, A10, A50, A (13.29, 13.66, 13.48, 14.94 and 13.9 μg L^{-1}) respectively. The highest concentration of ammonia was recorded in water tanks where fish fed on 100% dried algal biomass.($p < 0.05$) as a result of elevation the percentage of nitrogen as a result of increase in protein in diets. The ammonia concentration in water affected negatively individual fish weight and fish yield.

Table (25): Water quality measurements temperature (°C), pH, dissolved oxygen (mg L^{-1}) and ammonia content (μg L^{-1}) during feeding experiment (32 days).

Diet	Temperature (°C)	pH	Dissolved Oxygen (mg L^{-1})	Ammonia (μg L^{-1})		
				Day 14	Day 21	Mean
AF	26.31	7.59	7.28 AB	11.3 G	15.27 D	13.29
A5	26.40	7.61	7.23 CB	11.7 F	15.62 C	13.66
A10	26.37	7.58	7.22 A	11.75 F	15.21 D	13.48
A20	26.22	7.61	7.15 CD	12.3 E	9.96 G	11.13
A50	26.25	7.60	7.0 D	15.57 C	14.3 F	14.94
A75	26.51	7.58	7.24 B	37.9 B	16.45 B	27.18
A100	26.33	7.62	6.94 E	45.4 A	22.54 A	33.93
A	26.35	7.60	6.85F	13.1D	14.7E	13.9
Means	26.34	7.60	7.45	19.88	15.5	17.69

Means in the same columns with the same letter are not significant.

Tilapia fry were fed (average weight = 0.70 g day-1) after the egg yolk directly.

Growth parameters:

Body weight gain:

Table (26) showed the average body weight (±SE) of silver carp fish fed with different diets containing artificial fish food (AF) alone, on 5% (A5), 10% (A10), 20% (A20), 20% (A50), 75% (A75), 100% (A100) of dried algal biomass supplemented artificial fish food and on live algae. The data showed that there were no significant differences between 100% (A100) of dried algal biomass supplemented artificial fish food and on live algae ($P < 0.05$). The average body weight of fish fed artificial fish food supplemented with 20% dried algal cells (A20) was significantly the highest compared with the fish fed with other diets. The average body weight of silver carp fed with the living algal cells (A) was significantly lower than the average body weight of silver carp fed with (A20) diet.

The average body weight gain (±SE) of silver carp in this experiment are presented in Table (26). It could be noticed that there were significant differences between AF and A100 diets. However, the average of body weight gain of fish received (A20) diet was the highest than that fed the other diets.

Daily gain:

The daily gain in weight for fry fed with A20 diet gave gains which were significantly highest than the other diets (Table 26). Feeding with A5, A10, A50 and A100 diets gave daily weight gains which were showed no significantly differences inbetween. ($P < 0.05$).

Table (26): Mean individual weight and net daily growth of the silver carp (***Hypophthalmichthys molitrix***) fed on artificial fish food (AF), at 5% (A5), 10% (A10)), 20% (A20), 50% (A50), 75% (A75), 100% (A100) of dried algal biomass supplemented artificial fish food and on live grown algal cells (A) for 32 days growth.

Diet	Stock group	*Individual mean wt(g)			Net daily gain (g)	Fish survival %
		Initial±StD	Final±StD	**gain (g)		
AF	10	3.69 ± 0.16	4.47 ± 0.13 CD	0.78 E	0.0244 C	100
A5	10	3.52 ± 0.19	4.49 ± 0.04 C	0.973 D	0.0304 B	100
A10	10	3.38 ± 0.14	4.65 ± 0.17 B	1.270 A	0.0397 A	100
A20	10	3.75 ± 0.16	4.77 ± 0.20 A	1.017 BC	0.0318 B	100
A50	10	3.39 ± 0.16	4.42 ± 0.05 ECD	1.027 B	0.0321 B	100
A75	10	3.33 ± 0.09	4.40 ± 0.12 ED	1.007 C	0.0315 B	100
A100	10	3.57 ± 0.34	4.36 ± 0.27 EF	0.79 E	0.0247 C	100
A	10	3.64 ± 0.17	4.32 ± 0.15 F	0.683 F	0.0213 C	100

*Mean value±standard deviation; probability (P) from t-test for the value was significant at $P<0.05$.
**Gain = Final mean weight - Initial mean weight
Means in the same column with the same letter are not significant.

Survival:

In the present study Mortality was not recorded. The survival rate of all treatments was 100 %. The survival rates for all treatments could be considered satisfactory (100 %).

There were no significant effects on the survival rates of Tilapia fry fed on artificial fish food (AF), at 5% (A5),10% (A10)), 20% (A20),50% (A50), 75% (A75), 100% (A100) of dried algal biomass supplemented artificial fish food and on live grown algal cells (A) (Table 26).

Specific growth rate (SGR):

Results of average specific growth rate percentage when fry of silver carp fed on artificial fish food (AF) alone, at 5% (A5), 10% (A10), 20% (A20), 20% (A50), 75% (A75), 100% (A100) of dried algal biomass supplemented artificial fish food and on live algal are presented in Table (26). The data showed that, there were significant differences observed between treatments. Statistically, the means of specific growth rate showed significant differences at ($P > 0.05$) among treatments. The highest values (1.0) was observed following artificial fish food supplementation provided by 20 % dried algal cells (A_{20}) where the lowest values (0.54) was recorded for the living algal cells as fish died (A).

Data in Table (27) showed clearly that the percentage daily growth rate varied according to treatment. Artificial fish food supplemented with 50% (A_{50}) and 75% (A_{75}) dried algal cells showed more or less similar daily growth rate. Artificial fish food supplemented with 5% (A_5) and 10% (A_{10}) dried algal cells which recorded lower values than the above mentioned treatments.

Food conversion ratio:

The data presented in Table (27) show clearly that artificial fish food supplemented with 20% dried algal cells (A_{20}) was the best treatment for silver carp grown in mass culture, it supported much better growth and lower food conversion ratio (FCR = 1.70) than that of artificial fish food alone (AF) (3.04). In contrary, the living algal cells as fish died (A) was not suitable since it recorded the lowest growth and higher FCR of all treatments used (3.42%), which is followed by artificial fish food treatment

(3.04%). Artificial fish food treatments supplemented with 50% (A_{50}), 75% (A_{75}), 5% (A_5) 10% (A_{10}) of dried algal biomass and the use of dried algae (A_{100}) as fish diet alone respectively, were next to A_{20} in their FCR.

Yield:

The yield per treatment groups expressed in grams per (aquaria) for 32 days are presented in Table (27).

Fry fed with live grown algal cells (A) and 100 % (A100) of dried algal biomass recorded significantly lower yields than other treatment groups ($P < 0.05$). There were no significant differences between the yield of fish fed on artificial fish food (AF), at 5% (A5), 50% (A50) and 75% (A75) of dried algal biomass supplemented artificial fish food. Fish fed on 10% (A10)) and 20% (A20) showed significantly higher yields than other treatment groups. However there were no significant differences between their yields. Table (27) showed also that the fish yield was significantly higher in fish fed on diet A20 than the other treatments. The yield increased gradually from AF, A5, A10, till A20 then decreased after that with A50, A75, A100 and A diets.

Diet composition

Fish fed on 20% (A20) of dried algal biomass supplemented artificial fish food showed that the protein content in the diet was 29.2% the highest total yield (47.7 g/32 days) and the highest protein content in fish tissue Table (28). Increasing protein content in diets more than 29.2% had no effect in increasing fish yield. This means that, there was no significant effect on survival percentage or increasing protein content in fish tissue.

The percentage protein in various diets were significantly different. The artificial fish food and algal contain 25% and 46% protein respectively. The highest protein content was recorded in (A100) of dried algal biomass and live algal (A) 46.0%. Fish fed on 20% (A20) of dried algal biomass supplemented artificial fish food had the highest content of protein in fish, although the diet contained 29% protein (Table 28).

The differential content of protein in diets followed by differential percentage of nitrogen whereas, nitrogen represents 6.25% of protein.

The percentage of nitrogen increased gradually, being amounted to 0.945, 0.939, 1.041 respective with artificial fish food (AF), using 5% (A5) and 10% (A10) of dried algal biomass (Table 28).

Fish fed on 20% (A20) of dried algal biomass supplemented artificial fish food had the lower percentage of nitrogen compared with the rest treatments A50, A75, A100 of dried and live (A) algal biomass.

Increasing nitrogen content in A, A100, A75 and A50 caused increasing of ammonia concentration in water affect fish growth.

Decreasing protein content in fish tissue with increasing protein content in diets may be explained in the light of that fish from 4 g initial individual fish weight at the beginning of this experiment till 32 days age fish could not digest the diets which contain more than 29% protein.

Table (27): Specific growth rate, food conversion and yield of the silver carp (***Hypophthalmichthys molitrix***) fed on different for 32 days growth.

Diet	Growth rate		Specific Growth Rate (SGR)	***Food Conversion Ratio (FCR)	Total yield (g/10 fish)
	mg day^{-1}	% day			
AF	24.4	21.14	0.60 E	3.04 B	44.7
A5	30.4	27.64	0.76 BC	2.37 D	44.9
A10	31.8	27.12	0.75 C	2.38 D	46.5
A20	39.7	37.57	1.00 A	1.70 G	47.7
A50	32.1	30.29	0.83 BC	2.03 F	44.2
A75	31.5	30.24	0.87 B	2.21 E	44.0
A100	24.7	22.13	0.63 D	2.93 C	43.6
A	21.3	18.76	0.54 F	3.42 A	43.2

***Food conversion ratio (FCR) = total feed (g) / total gain (g).
Means in the same columns with the same letter are not significant.

Table (28): Total feed (g) and composition of diets consumed by silver carp (***Hypophthalmichthys molitrix***) after 32 days growth.

Diet	Stock group	Total feed(g)/ 32	Feed (g)/fish/ days	Feed (g) fish/ day	Diet composition (g)/ fish/ 32 day					
					artificial	algae	%protein	%	%Nitrogen	%
AF	10	236. 8	23.86	0.74	23.68	00	5.2	22	0.945	4.0
A5	10	224.0	22.4	0.70	21.28	1.12	5.85	26.05	0.939	6
A10	10	240.00	24.000	0.75	20.0	2.40	6.128	27.1	1.041	4
A20	10	217.6	21.76	0.68	17.41	4.35	6.397	29.2	1.009	4
A50	10	208.0	20.8	0.65	10.4	10.4	7.488	35.5	1.174	8
A75	10	208. 0	20.8	0.65	5.2	15.6	8.632	40.7	1.363	1
A100	10	227.2	22.72	0.71	00	22.72	10.678	46	1.68	6
A	10	233.6	23.36	0.73	00	23.36	10.98	46	1.713	

Biochemical composition of the fish:

Data presented in Table (29) and Fig (19) showed the biochemical composition of experimental fishes. The protein content of experimental fishes grown in artificial fish food supplemented with 20% dried algal cells (A_{20}) represented the highest value (64.88%). On the other hand, the lowest protein value of silver carp fish was observed in artificial food (AF) and living algal cell (A) treatments. These results indicated the suitability diet A20 as a source rich with crude protein instead of traditional diet (AF). The composition of fish fed on artificial fish food (AF), on 5% (A5),10% (A10)), 20% (A20), 50% (A50), 75% (A75), 100% (A100) of dried algal biomass supplemented artificial fish food and on live grown algal cells (A) (Table 28) showed the same trend as well as total yield. The percentage of protein in fish tissue increased gradually from fish fed on AF, A5, A10, till A20 then decreased in fish fed on A75 and A100 diets.

The percentage of protein fish fed on live algal cells (A), was significant lower than other treatment ($p < 0.06$).

The lowest fat content of the silver carp was observed in 20% (A20) of dried algal biomass supplemented artificial fish food (16.56%). The highest fat percentage was observed in (A) treatment with living cells (19.42%).

The lowest ash content was observed in A_{20}, A_{50} and A_{100} treatments while the highest content was recorded in artificial food (AF) treatment followed by A_5 treatment.

Table (29): Biochemical composition of fishes fed on different diets after 32 days growth.

Diet	% Ash	% Fat	% Protein
AF	17.66	18.09	61.08
A5	17.32	17.48	62.42
A10	16.33	17.04	63.72
A20	15.46	16.56	64.88
A50	15.36	17.44	64.08
A75	16.46	18.86	63.48
A100	15.58	18.98	63.06
A	17.23	19.42	61.82

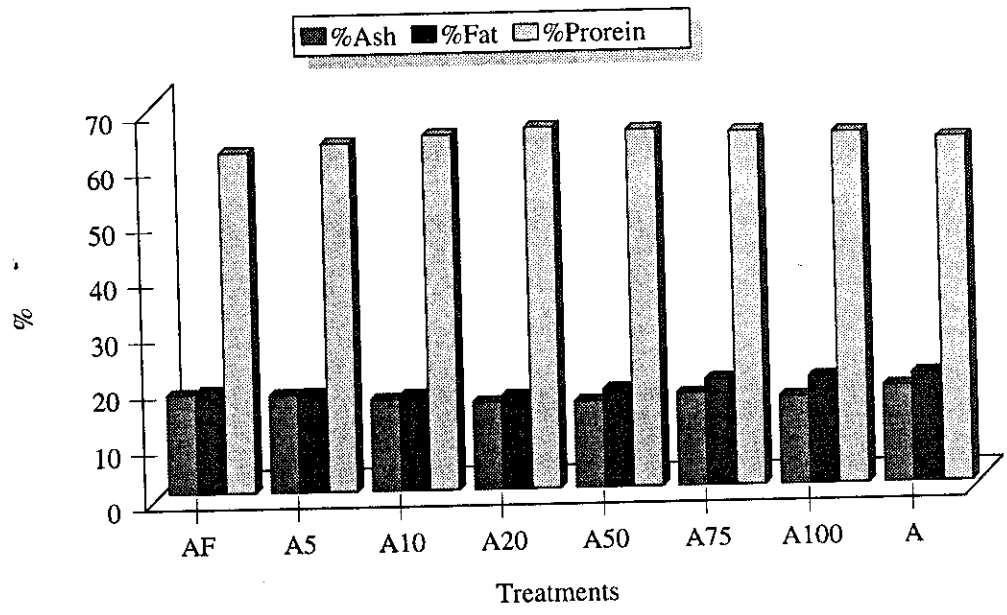


Fig (10): Biochemical composition of fishes fed on different diets after 32 days growth