EFFECTS OF THREE DETERGENTS ON THE METABOLIC ACTIVITY OF THE NILE PHYTOPLANKTERS SCENEDESMUS QUADRICAUDA, (TRUP) DE BREBISSON STAURASTRUM NATATOR W.WEST AND COELASTRUM MICROPORUM NAEGELI

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ABSTRACT

The effects of three commonly used anionic detergents: namely PERSIL automatic. OMO and ARIEL on growth and metabolic activities of the three Chlorophytes were investigated. The three green algae S. quadricauda, S. natator and C. microporum were isolated from the Nile River. The effective ingredient of these detergents is linear alkyl benzene sulphonate (LAS). The 96-h EC50 values of the three detergent indicated that C. microporum was the most susceptible alga, while S. quadricauda was the most tolerant species. The biochemical parameters varied greatly in their response to the tested detergents, where carbohydrate was the most susceptible parameter, while protein was the most resistant parameter. The protein levels were higher than the controls even at the highest detergent concentrations.

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INTRODUCTION

The available literatures dealing with effect of detergents or surfactants (surface active materials) on elements of aquatic food chain are scarce.

Tubbing *et al.*, (1993) investigated the sensitivity of planktonic photosynthesis to anionic detergent (tetrapropyl benzene sulphonate) in the River Rhine. They found that the effective concentrations of this detergent on the photosynthetic activity of Rhine phytoplankton ranged between 3 to 40 mgL⁻¹. *Chlamydomonas reinhardtii* cells were disrupted by mild treatment with detergents (Happe and Naber, 1993). Zachleder and Tukaj (1993) reported the effect of detergents on cell cycle and macromolecular synthesis in the green alga *Scenedesmus armatus*.

Ghatak and Konar (1993) found that the detergent Parnol reduced the zooplankton and phytoplankton populations in fresh water ecosystems. Saygideger (1992) studied the effects of detergent Linear Alkyl Benzen (LAB) on the green alga *Spirogyra fluviatilis*. He found that the cellular integrity of *S. fluviatilis* was broken in a short time exposure to 0.1 and 0.5% of the detergent. Drewa *et al.*, (1992) investigated the seasonal changes in the level of detergents and chlorophyll a in the Brda River. Lakshmi *et al.*, (1990) investigated the biodegradation of detergent phosphate by flocculating algal-bacterial system.

Nyberg (1985) studied the physiological effects of four detergents [Triton x-100, sodium desoxychlorate (SDC), Sodium dodecyl sulphate (SDS) and acetyl trimethylammonium bromide (CTAB)] on growth, membrane proteins, enzymes, biological membranes, membrane lipids, membrane permeability, chloroplasts and thylakoids of *Nitzschia actinastroides* (diatoms) and *Porphyridium purpureum* (red algae).

Privalle and Burris (1983) investigated the permeabilization of isolated heterocysts of AnabaenaNostoc muscorum sp. with detergent. In Egypt, little is known concerning the effect of local detergents on aquatic organisms. Heikal et al., (1986) reported the inhibition of nitrogen metabolism with continuous failure in protein synthesis of the blue-green algae Nostoc muscorum and Phormidium fragile under the influence of detergents Savo, Somatic and SDBS (Sodium dodeycl benzene sulphonate). Kobbia et al., (1986) studied the

diversity response of some enriched algal population augmented with some synthetic detergents. They recorded a remarkable reduction in the total algal counts and increased at the high doses of detergents. Kobbia *et al.*, (1985) studied also the effects of some detergents (Savo, Somatic and Sodium dodecyl benzene sulphonate SDBS) on growth, pigmentation and carbohydrate metabolism of the blue-green algae *Phormidium fragile* and *Nostoc muscorum*. They found that the detergents inhibited the total phosphorous accumulation while the nucleic acid synthesis was considerably favored at high concentration of detergents. Zaki (1983) investigated the biological responses of some freshwater algae *Nostoc muscorum* and *Phormidium fragile* isolated from the Nile to some detergents (Savo, Somatic and SDBS Sodium dodecyl benzene sulphonate). Abd-Allah (1995) measured linear alkyl benzene isomers (LABs) in sediment samples from the Alexandria coast.

MATERIALS AND METHODS

Isolation and purification of the tested algae:

The three tested algae Scenedesmus quadricauda (Turp, de Brebisson), *Coelastrum microporum* (Naegeli) and Staurastrum natator W. West were isolated from the Nile and classified according to Taft and Taft (1971) and Ljunggren and Oja (1961). Steps of isolation and purification reported by Pringsheim (1946) were followed.

Culture medium

The tested algae were cultured on an artificial medium that was reported by Staub (1961). Some modifications were introduced on this medium to obtain suitable growth for the experimental algae (Mayers *et al.*, 1961; Kuhl, 1962 and Werner, 1965). The clean dry 250ml flasks each containing 100ml of experimental medium was sterilized by autoclaving at 121 °C and 1.5 Kg/cm² for 15 minutes. After autoclaving, the culture flasks were left to cool for one day to allow for pH stabilization at 7.9-8.

Inoculation

Treatment and control flasks were inoculated with 9-10 μ g/100 ml *Chl. a*, for *Scenedesmus quadricauda* and with 84-96 μ g *Chl. a*/100 ml for *Staurastrum natator* and *Coelastrum microporum* in logarithmic growth phase. Stock and experimental cultures were incubated in a locally made

incubator with vigorous hand agitation once a day to prevent clumping of cells. The test duration was 96 h at temperature $25 \pm 1^{\circ}$ C and the day/night program in the incubator was 14 h light followed by 10h darkness (14L: 10D), with a mean light intensity around the flasks of 4000 lux.

Detergents

Three commonly used detergents, namely PERSIL automatic, OMO and ARIEL were used to elucidate their effects on some Nile phytoplankton.

The active component of these detergents was linear alkyl benzene sulphonate (LAS) which represents the effective ingredient of the detergents. This group of detergents is classified as ionic and anionic. In addition to the effective ingredient (LAS) in the three tested detergents, they contain substances (additives) e.g. activators and fillers. The concentration and types of these activators and varied from detergent to another. The chemical structure of LAS is illustrated as follows:

 $[RC_6H_4SO_3]$ Na⁻ Linear alkyl benzene sulphonate.

Measurements

Chlorophyll a

Chlorophyll a was measured specrophotometry according to the Golterman and Clymos method (1971).

Dry Weight

The dry weight of both control and treated cultures was measured using nucleopore filter paper 25 mm and 0.45 μ m pore diameter according to the technique reported by Ibrahim (1990).

Protein-N

Total nitrogen content was determined by the micro-Kijeldahl method A.P.H.A (1985) protein content was calculated by multiplying the value of total nitrogen by 6.25.

Total cellular Carbohydrate

The hydrolysis of carbohydrate was carried out according to Myklestad and Haug's method (1972) and determined by the phenol sulphoric acid method as reported by Dubois *et al.* (1956) using glucose as standard.

Total lipid

Total lipid contents were determined by the sulfophosphovanillin procedure (SPV) as reported by Chabrol and Castellano (1961).

RESULTS AND DISCUSSION

The impact of the organic detergents on aquatic life and water quality of Egyptian inland waters has raised much concern because of the environmental and health hazards of such pollutants.

The present study deals with effects of three commonly used anionic detergents in Egypt; namely PERSIL automatic, OMO and ARIEL on growth, metabolic activities and morphology of the three chlorophytes isolated from the Nile. The detergent concentrations were chosen on the basis of range finding test results and the 96 h EC₅₀ values (96 h EC₅₀: effective concentration of detergent that caused 50% reduction of growth and biochemical parameters relative to control after 96 h incubation). The results are illustrated in Tables (1,2,3) and Figures (1,2,3).

The results revealed obvious degradation in chlorophyll a content of each of the three chlorophytes under the influence of detergents. In this connection, Krasanovskii and Luganskaya (1976) realized this phenomenon to changes in pH that affect the physicochemical properties of cell membranes and the solubility of cellular pigments. There was a great difference in the response of chlorophyll a of the three tested algae to detergent concentrations. The chlorophyll a of Coelastrum microporum was the most susceptible to detergent concentrations with EC50 values of 11.49, 9.59 and 4.42 ppm for PERSIL, OMO and ARIEL. respectively. In the meantime, chlorophyll a of S. quadricauda was the most tolerant to detergents, its EC 50 values in ppm were 57.95, for PERSIL; 56.0, for OMO; and 72.73, for ARIEL. These results agree with the findings of Zaki (1983) who observed considerable decrease in chlorophyll a of the blue-green algae Phormidium fragile and Nostic muscorum when treated with anionic detergents (Sodium dodecyl benzene sulphonate, Somatic and Savo). She attributed the drop in chlorophyll a to differences in the reduction and oxidation efficiency of cytochrome "c" caused by detergents. Zachleder and Tukaj (1993) reported degradation of chlorophyll a. b and carotenoids of the Chlorococcal alga Scenedesmus armatus under the influence of the detergent DP 105.

Staurastrum natator and Coelastrum microporum after 96 incubation. Table (1): Effect of Persil on the metabolic activity of Scenedesmus quaricauda,

Tested algae	Conc of Persil	Chl		EC ₅₀	Protein	sin	Carboh		EC ₅₀	Lipids	EC50	D.W	Γ	EC ₅₀
	control	244	±5 34		8.23	±0.3	0.67	±0.06		0.15 ±0.003		30.7 ±2	±2.31	
	15	242	±2 67		83	±0.1	0.65	±0.05		0.15 ±0.001		29.3 ±2	±2.31	
	30	170	±5 34		3.76	±04	0 32	<u>+</u> 0 02		0.05 ±0.005		4	±2	
S.quadricauda	45	151	±5 34	58	6.13	±0 2	021	±0 03	293	0 04 ±0.003 2	26.4	113 ±1	±1.16	28.7
	60	117	±5 34		6 74	+0 3 .	011	±0 01	-	0.03 ±0 001		9.33 ±2	±2.31	
	75	57.1	±2.67		7.26	±0.2	0 09	±0.02		0.02 ±0.002	_	4.67 ±1	±1.16	
	control	355	±5.34		17 56	±0.4	60	±0 02	<u>.</u>	0.12 ±0.003		32.7 ±1	±1.16	
	16	234	±5 34		10.87	±0 3	0.45	±0 05		0.07 ±0.002		25.3 ±1	±1.16	
	24	173	±5 34		12 5	±0.3	031	±0 05		0.05 ±0.004		18.7 ±2	±2.31	
S natator	32	154	±5.34	23.4	15 03	±0 3	0 2	±0 04	16	0.03 ±0.002 1	18.5	14.7 ±2	±2.31	28 7
	40	140	±2.67		15 85	±0.2	0 13	±0 02	·	0 02 ±0.003		10.7 ±2	<u>±2</u> .31	
	48	91	±2.67		16.42	±0.4	0.08	±0.01		0.02 ±0.003		6.67 ±2	±2.31	
	control	484	±5 34		10 32	±01	1 26	±0.03		0.09 ±0.002		26	+2	
	4	458	±4.63		9.1	±0.2	1 21	±0.02		0.07 ±0.002		24.7 ±1	±1.16	
C.microporum	ω	284	±5.35	115	5 16	±0 1	0.69	±0 02	9.5	0.05 ±0.002 1	10	19.3 ±1	±1.16	16 7
	12	236	±4.63	_	6.6	±0.2	0.53	±0.05	_	0.04 ±0.001		16.7 ±1	±1.16	
	16	182	±5 34		7.28	±0.2	0.43	±0 02		0.03 ±0.003		14	÷	
	20	100	±2 67		8.8	±0.1	0 33	±0.02		0.02 ±0.001		8	±2	

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C microporum 4.5 242 ±2.67 4.42 6 153 ±4.63	4.5 242 ±267		3 273 ±4.63	1.5 497 ±5.35	Control 487 ±2.67	35 122 ±2.67	28 180 ±4.63	S natator 21 191 ±5.34 28.3	14 230 ±2.67	7 299 ±5.34	Control 356 ±4.63	90 41.6 ±4.63	80 98.7 ±2.67	S quadricauda 70 133 ±5.34 72 7	60 151 ±5.34	50 248 ±2.67	Control 247 ±5.34	Tested algae Conc of Ariel Chl. EC50
8 17 +0 1	7.76 ±0.1	7.19 ±0.1	5 11 ±0.2	11 2 ±0 1	10.5 ±0.3	17 ±0.1	15.7 ±0.2	15.2 ±0.1	14.8 ±0.2	12.2 ±0 1	17.7 ±0.1	6.94 ±0.1	6 65 ±0.2	5.66 ±0.2	5.25 ±0.2	8.52 ±0 1	8.29 ±0.3	u Protein
		6 17												2.77				EC ₅₀
0.13 ±0.02	0.3 ±0.02	0 38 ±0 02	0.57 ±0.02	1 26 ±0.05	1.25 ±0.05	0.06 ±0.02	0.13 ±0 02	0 19 ±0 01	0.37 ±0.01	0 53 ±0.05	0.89 ±0.01	0.11 ±0.01	0 21 ±0.01	0 31 ±0.01	0.47 ±0.02	0.71 ±0.01	0 69 ±0.02	Carboh.
		2.88						10.7						67.8				EC ₅₀
0.01 ±0.001	0.01 ±0.001	0.02 ±0.004	0.04 ±0 004	0.09 ±0.001	0.09 ±0.003	0 01 ±0.003	0 02 ±0 003	0.03 ±0.002	0.05 ±0.003	0.07 ±0.004	0.12 ±0.003	0.02 ±0.001	0 03 ±0.002	0.05 ±0.003	0.08 ±0.001	0.15 ±0.002	0.16 ±0.002	Lipids
		2 79					-	9 74						6 65				EC ₅₀
	10.7 ±2.3		18 ±2	28 ±2	26.7 ±2.31	667 ±1.1	13.3 ±2.3	18 ±2	20.7 ±1.1	23.3 ±1.1	30 ±2	7.33 ±1.1	10 7 ±2.3	13.3 ±2.31	17.3 ±2.3	34.7 ±2.3	31.3 ±1.16	D.W
6	<u>ت</u>	4.8			ī	6	Ē	25 5	б	б		6	1	81 64.2	ĩ	31	6	EC ₅₀

Table (2): Effect of Ariel on metabolic activity of Scenedesmus quadricauda,

Staurastrum natator Coelastrum nicroporum after 96 incubation.

Table (3): Effect of Omo on metabolic activity of Scenedesmus quadricauda,Staurastrum natator Coelastrum nicroporum after 96 incubation.

Conc of Omo Chl. EC ₅₀ Protein Carboh. EC ₅₀ Control 244 ±2.67 8.26 ±0.2 0.71 ±0.02 30 184 ±2.67 4.69 ±0.1 0.47 ±0.03
40 160 ±5.34 5.11 ±0.1 0.27 ±0.01 50 136 ±5.34 56 5.32 ±0.1 0.23 ±0.01
60 113 ±2.67 6.16 ±0.2 0.15 ±0.01
70 47.8 ±2.67 7.49 ±0.2 0.09
Control 358 ±5.34 17.5 ±0.2 0.89
10 247 ±5.34 11.3 ±0.1 0.57
15 177 ±2.67 13.5 ±0.3 0.38
20 [145 ±5.34 14.9 14.7 ±0.4 0.21
25 125 ±4.63 15.4 ±0.1 0.14
30 106 ±4.63 16.1 ±0.1 0.07
Control 486 ±4.63 11 ±0.1 1.27
450 ±5.35 895 ±0.1 1.17
251 ±2.67 6.26 ±0.1 0.61
208 ±4.63 959 7.43 ±0.1 0.46
5 170 ±5.35 8.84 ±0.2 0.29
18 81.7 ±2.67 9.63 ±0.1 0.21

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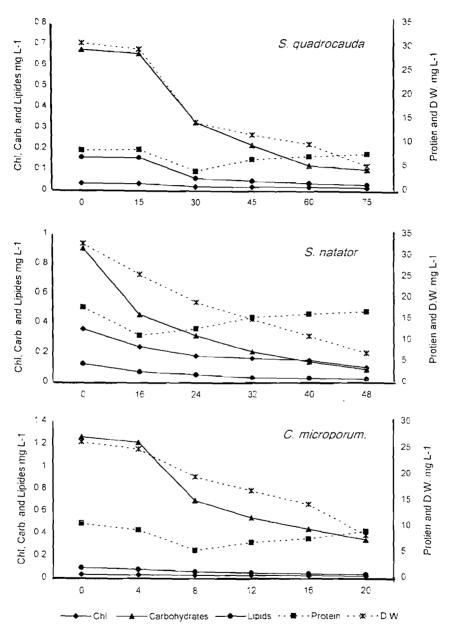


Fig. (1): Effect of Persil on chemical constitunts of S. guasdricauda, S. natator and C. microporum after 96 h incubation

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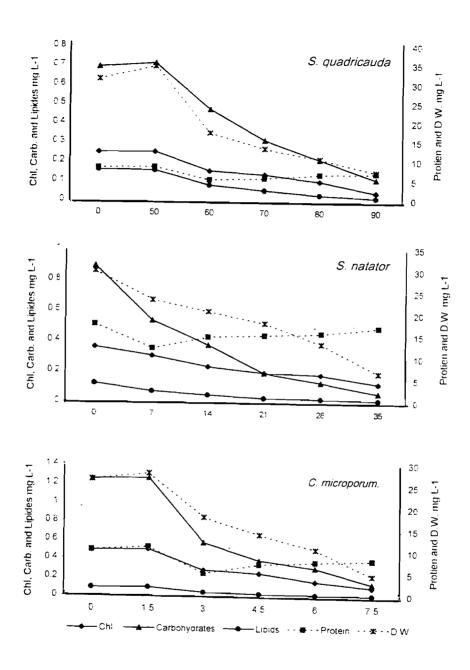


Fig. (2): Effect of Ariel on chemical constitunts of S. quasdricauda, S. natator and C. microporum after 96 h incubation

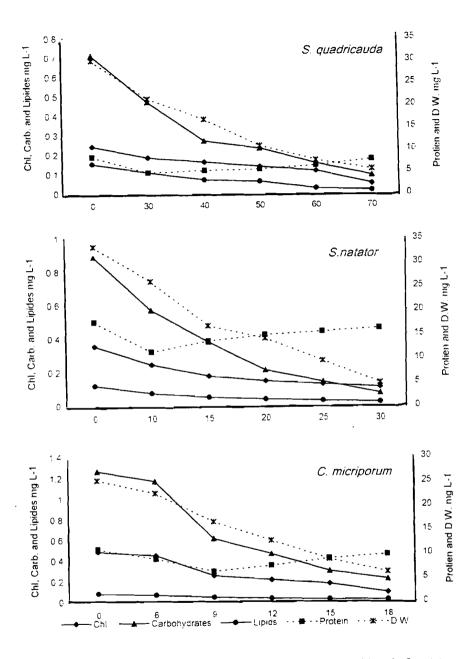


Fig. (3): Effect of OMO on chemical constitunts of S. quasdricauda, S. natator and C. microporum after 96 h incubation

They found that chlorophyll a was more sensitive to this detergent compared to chlorophyll b and the chlorophyll a:b ratio decreased with increasing detergent concentrations and the cells were bleached. The present data contradicts the finding of Kobbia *et al.*, (1985) who observed stimulatory effect of Savo (anionic detergent) on chlorophyll a content of the blue-green alga *Nostoc muscorum*. They didn't observe this phenomenon with the other tested anionic detergents, namely sodium dodecyl benzene sulphonate and Somatic.

The dry weight of the three tested algae had progressively decreased with increasing detergent concentrations, except for *S. quadricauda* and *C. microporum* where their dry weights were slightly increased at the least Ariel concentrations. The dry weight of *C. microporum* was the most sensitive to the detergents where their EC₅₀ values in ppm were 16.67, for PERSIL; 4.8, for ARIEL; and 12.004, for OMO, while *S.quadricauda* was the most tolerant (EC₅₀ 28.69, 64.16 and 42.79 ppm, for the three detergents, respectively.). The obvious drop in dry weights of the three chlorophytes is mainly realized to the inhibitory effects of detergents on their population growth. This view is supported by negative relation between detergent concentrations and *Chl. a* of the three algae.

The results indicated that detergents had peculiar effect on protein contents of the three chlorophytes. Although ARIEL and OMO suppressed the protein level of the tested algae, its values were still higher than 50% of the control even at the highest detergent concentrations. On the other hand, PERSIL was more effective for inhibiting the protein contents and C. microporum was more sensitive (EC₅₀ 5.16 ppm), while the protein-N of S. natator was still higher than 50% of the control even at the highest Persil concentration (30 ppm). The tolerance of protein to the tested detergents might be attributed to resistance to disruption of cells under our experimental conditions. The suppression of algal growth can be attributed to interference of surfactants with cytochrome synthesis and its activity through prophyrin synthesis. In this connection, Kobbia et al. (1985) attributed the disturbances in nitrogen metabolism of Nostic muscurum and Phormidium fragile by the anionic detergents Savo, Somatic and SDBS to lack of carbon skeleton as a result of depletion of chlorophyll synthesis and inhibition in photosynthesis. Moreover, this deficiency in carbon contents might impair peptide and protein synthesis. Zachleder and Tukaj (1993) realized the inhibition in protein synthesis of the Chlorococcal alga

Scenedesmus armatus by nonionic surfactants to photosynthetic impairment of the treated cells. On the other hand. Ibrahim (1975) reported the inhibitory effect of the anionic detergents Savo on the protein contents of three marine phytoplankton species.

The results revealed that the slight increase in chlorophyll a, dry weight and protein of S. quadricauda and C. microporum under the influence of lowest ARIEL concentrations was also associated with similar increase in carbohydrate This phenomenon was completely reversed at high detergent concentrations. In general, the detergents had deleterious inhibitory effect on carbohydrate contents of the three chlorophytes. The carbohydrate content of C. microporum was the most susceptible to the detergents with EC₅₀ values 2.88, 8.87 and 9.5 ppm for ARIEL, OMO and PERSIL respectively. The slight increase in carbohydrate content of the two algae at least doses of ARIEL might be realized to the increase in chlorophyll *a* that consequently activated their photosynthetic activities. The inhibition of carbohydrate synthesis by detergents is mainly attributed to activation of terminal oxidation that increased activity of cytochrome "c" oxidase and reduced NAD dehydrogenase. This is usually accompanied by inhibition of succinate-cytochrome "c" reductase and NAD-(Khan et al., 1969 and Fry and David, 1980). cvtochrome "c" reductase According to Zochleder and Tukaj (1993) the disturbances in carbohydrate metabolism of Scenedesmus armatus under the influence of nonionic detergents were mainly due to inhibition in starch synthesis. Moreover, Zaki (1983) reported an obvious decrease in carbohydrate contents of the blue-green algae Phormidium fragile and Nostic muscorum under the influence of the anionic detergents SDBS. Savo and Somatic, a phenomenon that was enhanced by realized degradation increasing detergent concentrations. She the in carbohydrate metabolism inspite of increasing chlorophyll a content to inhibition of photophosphorelation. On the other hand, Kofkina et al. (1977) attributed the disturbances in carbohydrate to the ability of detergents to disrupt the osmotic barrier of protoplasts and inhibition of NADH oxidase of membrane functions. This may lead to the conclusion that the disturbances of carbohydrate in green algae by detergents is due to suppression of the enzymes involved in metabolism of algal starch (chlorophycean phosphorylases). The importance of starch as a reserve product of green algae is due to its role for energy production and biosynthesis of protein (Muller, 1961 and Ruppel, 1962), chloroplast and pigment synthesis (Matsuka et al., 1966 and Ohad et al., 1967).

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Detergents also inhibited lipid metabolism of the three chlorophytes although a slight increase in lipid content of *C. microporum* was observed at lowest dose of ARIEL. The EC50 values of 26.4, 38.03 and 59.93 ppm indicated the tolerance of *S. quadricauda* lipid to PERSIL, OMO and ARIEL, respectively. In the meantime, *C microporum* lipid was the most sensitive to detergents with EC₅₀ values of 10, 9.11 and 2.79 for the three detergents, respectively. The slight increase in lipid contents of *C. microporum* at lowest dose of ARIEL agrees with Nyberg (1981) who observed similar increase in phospholipid of *Niteschia actinastroides* in the presence of SDBS (Sodium dodecyl benzene sulphonate), the phenomenon was associated with considerable decrease of soluble fractions of lipid phosphorus.

The obvious degradation of lipid contents of algae by detergents can be attributed to their effects on the enzyme system regulating unsaturated fatty acids and lipid synthesis. This changes the membrane fatty acid composition in the tested algal cells and consequently affects the function of cellular membranes.

All data obtained so far for the fatty acid synthesis, pointed to the fact that the chloroplast is the sole site for the biosynthesis of both saturated and unsaturated fatty acids (Douce & Joyard, 1980; Maziliak *et al.*, 1982; and Stumpf, 1982). These acids are transported to the cytoplasmic compartment, where they are incorporated in lipid or further desaturated (Stumpf & Shimakata, 1983). The degradation of lipids by anionic detergents was also reported by Nyberg (1985) who observed an increase in the degree of saturation and changes of acid contents of glyco- and phospholipids and disappearance of arachidoniic acid from the lipids of the diatom *Nitzschia actiastroides* and *Porphyridium purpureum*.

It is generally believed that the lipids exist in chloroplasts only in the form of fluid bilayer However, intact chloroplast membranes contain also other lipids (e.g. sulpho- and phospholipids) and the relatively massive chlorophyll/protein light harvesting complexes, which means that interactions in chloroplast membranes (lipid-lipid, lipid-protein, lipid-detergents, protein- detergent) must be very complex (Quinn & Williams, 1983).

The results revealed morphological aberrations of S. quadricauda and C. microporum cells under the influence of PERSIL and OMO (Plates from 1-4). On the other hand, ARIEL didn't cause any morphological distortion to the three tested algal cells. In the meantime, S. natator cells didn't show any abnormalities at all doses of the detergents. S. auadricauda and C. microporum cells were more or less similar in their morphological response to PERSIL and OMO. These detergents increased cell multiplication in the coenobium, swelling, grainy cytoplasm and destruction of cell contents. In this connection, Savgideger (1992) reported morphological abnormalities of Spirogyra fluviatilis cells when treated with the anionic detergent linear alkyl benzene sulphonate (LAS). The cellular integrity of this alga was broken at 0.1 and 0.5% of the detergent. The stimulatory effect of detergents on cell multiplication agrees with the findings of earlier investigators (Guminska and Osmelak, 1980; and Zaki, 1983) who observed this phenomenon with the bluegreen algae Nostoc muscorum and Phormedium fragile when treated with Somatic (anionic detergent).

From the above discussion one may come to the conclusion that the three chlorophytes varied greatly in their response to the detergents where C. *microporum* was the most susceptible alga and S. *quadricauda* proved to be the most tolerant species. On the other hand, the biochemical parameters also showed considerable variations in their response to detergent concentrations. In general, carbohydrate was the most susceptible parameters, whereas the dry weight was the most resistant, except for S. *quadricauda* where chlorophyll a was the most tolerant to detergents.

The present investigation revealed the ecological threat of the three detergents on growth and productivity of the three chlorophytes that represent the base of the food chain in the River Nile on which hetrotrophic organisms depend. Therefore, detergents may have direct inhibitory effect on fish production and/or indirect effect through inhibiting the elements of food chain (phytoplankton, zooplankton and bottom fauna) which represent the main source of natural food for fish and fish larvae.

On the other hand, the use of non-biodegradable hard detergents in Egypt e.g. linear alkyl benzene sulphonate (LAB) will cause serious water pollution problems. This finding agrees with Abd-Allah (1995) who stated that Linear

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Alkyl Benzene isomer LAB_s has high surface activity or resistance to biodegradation and consequently causes harmful effects on fauna and flora inhabiting natural water and waste streams.

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