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FLUORANTHENE INDUCED CHANGES IN PHOTOSYNTHETIC PIGMENTS, BIOCHEMICAL COMPOUNDS AND ENZYMATIC ACTIVITIES IN TWO MICROALGAL SPECIES: *CHLORELLA VULGARIS* BEIJERINCK AND *DESMODESMUS SUBSPICATUS* CHODAT

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Abstract

The photosynthetic pigments, biochemical and enzymatic activities in two freshwater microalgal species, *Chlorella vulgaris* and *Desmodesmus subspicatus* at different fluoranthene concentrations were compared with the control conditions. During 16-days of incubation period when treated with fluoranthene, both microalgal species exhibited variable amount of photosynthetic pigment, biochemical compounds and enzymatic activities. The addition of fluoranthene at concentrations ranged from 1.5 mg l^{-1} to 10 mg l^{-1} to microalgal cultures led to changes in all different metabolites but the patterns varied from species to species. Among the two species tested, pigment, biochemical and enzymatic contents were remarkably declined from 7 % to 95% in *C. vulgaris*. Moreover, all metabolites in *D. subspicatus* also diminishing significantly by 3% to 88% of fluoranthene doses (10ppm). These results suggest that fluoranthene-induced changes of pigments, biochemical and enzymatic variations in test microalgae, *D. subspicatus* and *C. vulgaris*, might reveal its resistance and ability to metabolize PAHs. At the same time, the PAH impact changes on different metabolic activities were higher at 12 and 16 days than at 4 and 8 days in treated microalgae.

Keywords: Fluorathene, microalgae, pigments, biochemical compounds, enzymes.

Introduction

Diversity of indigenous microorganisms capable of degrading pollutants such as crude oil, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in different environments (Kanaly et al., 2003; Kasai et al., 2001) was taken into consideration recently. PAHs are acting as carcinogens, mutagens, and endocrine disruptors to a variety of aquatic organisms, (Pittinger et al., 1987; Hellou et al., 2006). Identification of the key organisms that play a role in pollutant degradation processes is relevant to the development of optimal *in situ* bioremediation strategies. Contamination of PAHs in the environment is a serious public health problem and is of increasing concern. Most PAHs in aquatic systems are associated with natural particulate and dissolved organic matter (Wakeham and Farrington, 1980). The major sources of PAHs are derived anthropogenically from pyrolysis and combustion processes and spillage of petroleum hydrocarbons (Kennish, 1998).

Microalgae are essential constituents of aquatic ecosystem since they are the first trophic level in the food chains and the major organism providing oxygen and organic substances to other life forms. Algae are essential components of the aquatic ecosystems and their contribution to the degradation and detoxification of recalcitrant PAHs and their potential mutagenic intermediates must be considered. The remediation of PAHs contaminated water using microalgae has received great attention in last few decades. During the degradation processes of PAHs , the response of photosynthetic pigment, biochemical and enzymatic activities of microalgal species towards PAHs treatments have not been investigated yet, therefore, in the present study , authors focused light on Flouranthene induced changes on photosynthetic pigments, biochemical and enzymatic activities of two microalgal species: *Chlorella vulgaris* Beijerinck and *Desmodesmus subspicatus* Chodat which are dominant and widely distributed algal species in freshwater environments.

Material and Method

Strain Selection

The axenic cultures of microalgae *Chlorella vulgaris* Beijerinck and *Desmodesmus subspicatus* Chodat were obtained from the Central Marine and Salt Research Institute, Bhavnagar, India. The growth media selected for microalgae *C. vulgaris* was develop in Zarrouks (1996) medium and for *D. subspicatus* slight modified BG 11 media (Rippika,1988) under controlled illumination of 40µEm-2s-1 at 27±1°C in aerobic and static conditions. As this species is non-heterocyst forming algae, nitrogen source was supplemented artificially to BG 11 media. All inoculations were carried out under aseptic conditions and the cultures were periodically checked for any contamination.

Polycyclic Aromatic Hydrocarbon-Fluoranthene

Fluoranthene is a polycyclic aromatic hydrocarbon (PAH) consisting of naphthalene and a benzene unit connected by a five-membered ring. Its name is derived from its fluorescence under UV light. Fluoranthene is found in many combustion products, along with other PAHs. Its presence is an indicator of less efficient or lower-temperature combustion, since non-alternant PAHs are less preferred in formation than alternant PAHs. Fluoranthene has been classified by the International Agency for Research on Cancer as a group 3 carcinogen, "not classifiable as to its carcinogenicity to humans, however it was found to possess carcinogenic

properties in case of newborn mice according to short-term lung tumor assay (Busby et al., 1989).

Dose Selection and inoculation

On the basis of a series of experiments for LC50, the effective doses were resulted in Table I.

Table I: LC50 values

Species	Lower LC50	LC50	Higher LC50
C. vulgaris	1.25ppm	2.5ppm	5.0ppm
D. subspicatus	2.5ppm	5.0ppm	10ppm

Fluoranthene stock solution of 200 µg ml^{-1} was prepared in HPLC grade acetone. Exponentially growing 2.0ml of the culture was inoculated to each effective dose and made up to 20.0 ml. For each experiment, the solution of Fluoranthene was prepared fresh. All solvents and chemical used in this experiment were Hi-Media and Merck made. Milli-Q water was used throughout the whole experiment. All the spectrophotometer readings were taken in UV-VIS spectrophotometer, made Perkin Elmer, model Lambda 19. Samples were taken at every four days interval up to 16 days with the set of three replicates.

Photosynthetic pigments measurement: Chlorophyll *a, b* and carotenoids were extracted in 80% acetone and determined spectrophotometrically by measuring the absorbance at 663, 645, 630 and 480 nm, respectively (Jeffrey and Humphrey, 1975; Parsons and Strickland, 1963). Phycobillin protein content was extracted in 50mM potassium phosphate buffer (pH 7.0) after repeated freezing and thawing and measured at 562, 615 and 652 nm, respectively (Bennett and Bogorad, 1973).

Biochemical compounds estimation: The total carbohydrates, proteins, amino acids and phenols were extracted in 80% ethanol. Carbohydrates were estimated by the method of Hedge and Hofreitte (1991), protein estimation was done by Lowry *et al* (1951), the total amino acids was performed by measured by Lee and Takahanshin, (1966) and phenols estimation was carried out as per Malick and Singh (1980).

Enzyme assays: Nitrate reductase enzyme was extracted using cysteine buffer (pH 8.8). The enzyme activity was assayed spectrophotometrically given by Sempruch et al, (2008). Glutamine synthetase enzyme was extracted in Tris HCl buffer (pH 7.5). The enzyme assay was determined by a slight modification of the method described by Yuan et al, (2001) and spectrophotometrically read at 540nm. The estimation of *in-vivo* succinate dehydrogenase (SDH) activity was measured by the method of Kun and Abood (1949).

Results

The ability to reduce the high molecular weight hydrocarbon by microalgae, chlorophyll has been identified as key compound in order to evaluate the growth capability in presence of PAHs (Fluoranthene). Total chlorophyll concentrations (Chlorophyll a and b) reduction showed a little effect in all treatments over the early days of experiment (fig.1). A loss of chlorophyll-a in *C. vulgaris* was observed on 8^{th} day by 15%, 22% and 25% for 1.5ppm, 3ppm and 6ppm respectively and was consistently decreased upto 16^{th} day by 78%, 81% and 86%. Whereas Chlorophyll b followed the similar trend which shown that the inhibitory rates were reached the maximum value on day 16^{th} day to all three doses of the high molecular weight fluoranthrene.

Moreover, inhibitory effect of *D.subspicatus* by fluoranthene increased from the first and then continued through the time of experiment. The dose- response on chlorophyll-a content declined by 18% at 2.5ppm followed by 27% at 5ppm and ceased to by 48% at 10 ppm on 8th Day as compared to control. Chlorophyll-b content in *D.subspicatus* also reduced by 0.0069±0.00075 mg ml^{-20} on 4th day to 0.0049±0.00056 mg ml^{-20} on 16th day. The utmost drop in chlorophyll-a content was recorded in *D.subspicatus* by 78% at 10 ppm, pursued by *C.vulgari* by 86% at 6 ppm at the end of 16th day.

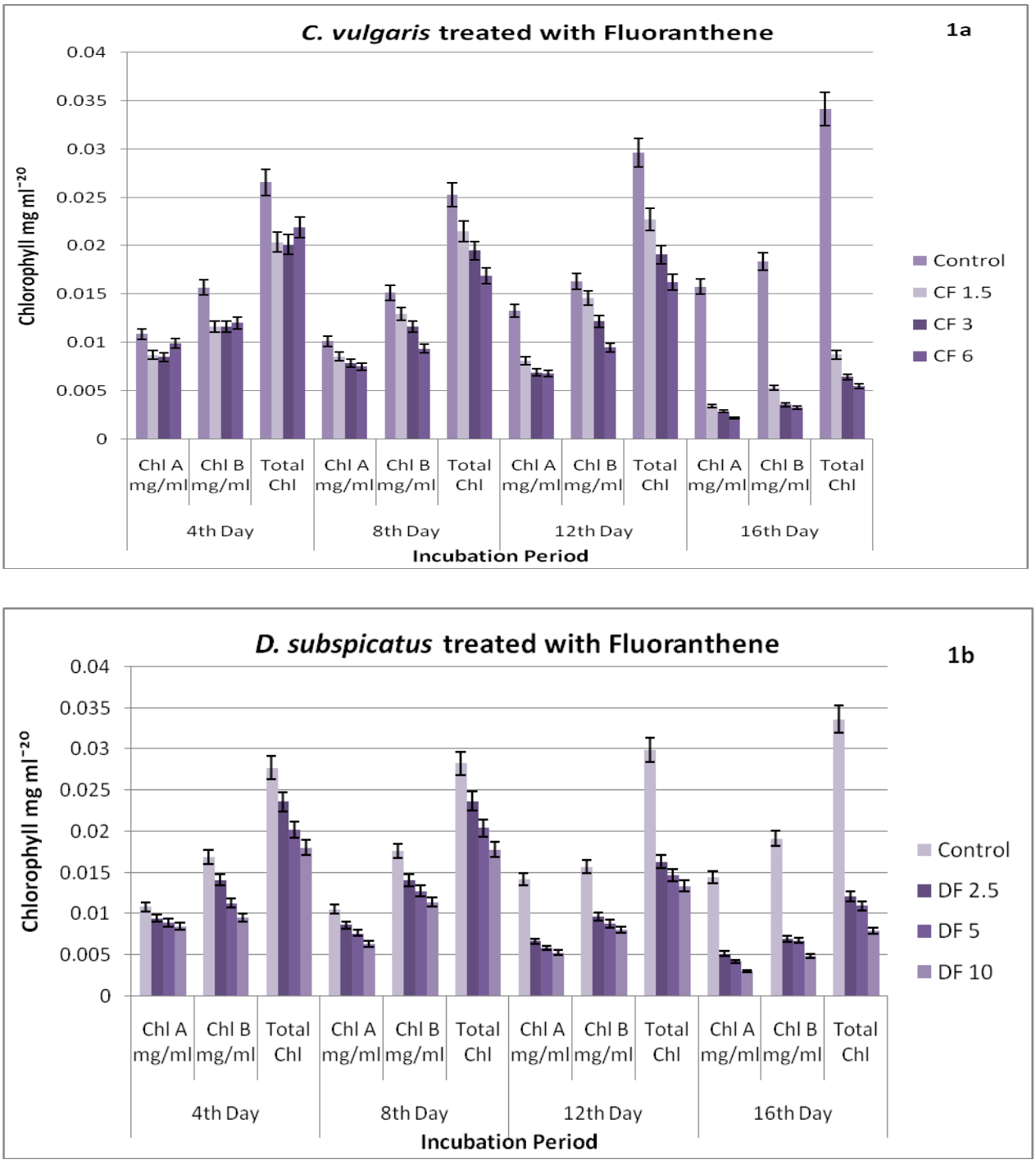

Fig. 1 (a and b) Response on Chlorophyll (a,b and total) content of *C. vulgaris* and *D. subspicatus* to Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

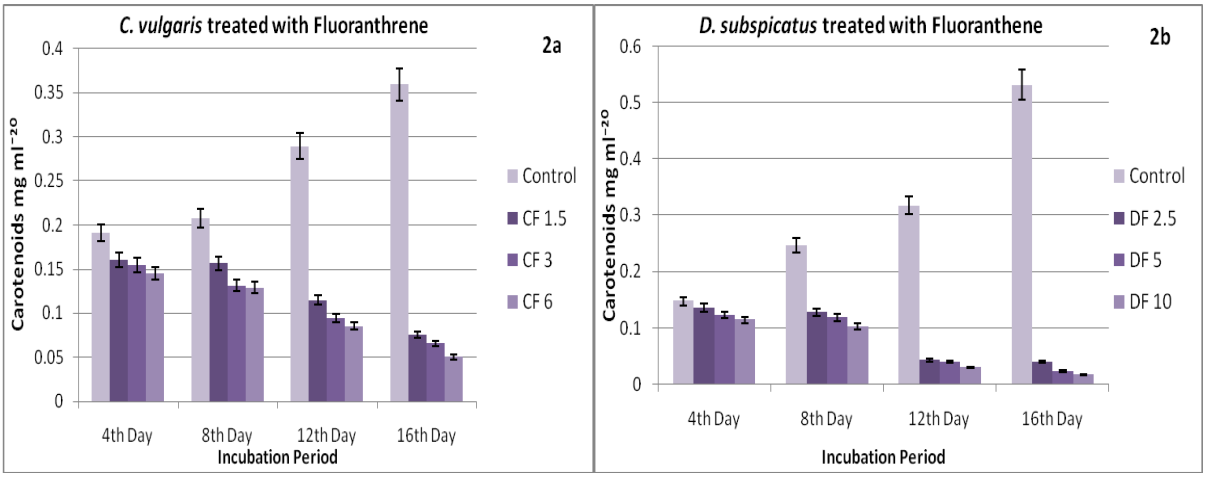

Fig. 2 (a and b) Response on carotenoids content of *C. vulgaris* and *D. subspicatus* to Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

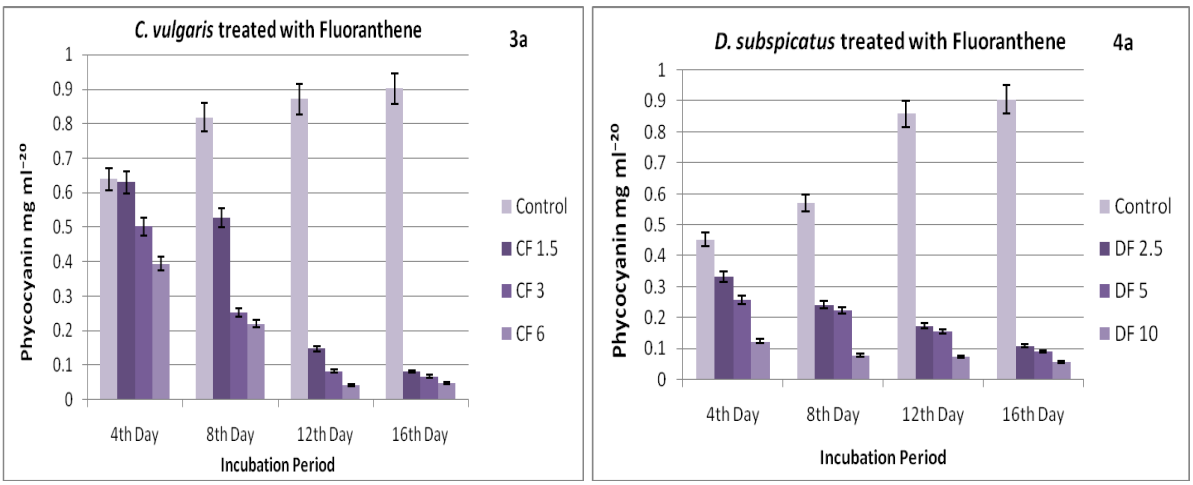

Fig. 3 and 4 (a) Response on phycocyanin content of *C. vulgaris* and *D. subspicatus* to Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

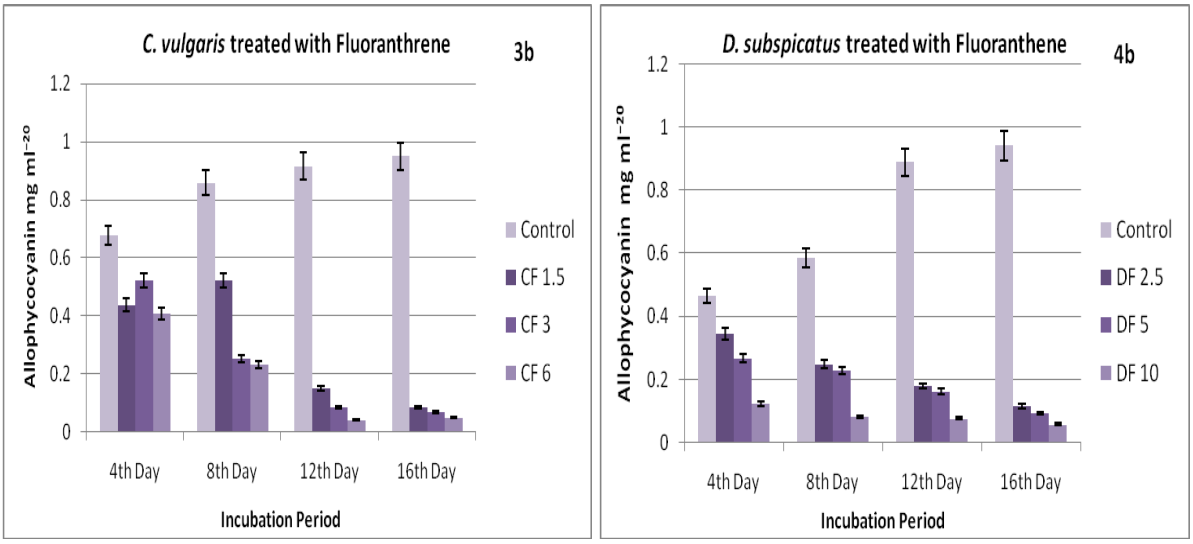

Fig. 3 and 4 (b) Response on allophycocyanin content of *C. vulgaris* and *D. subspicatus* to Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

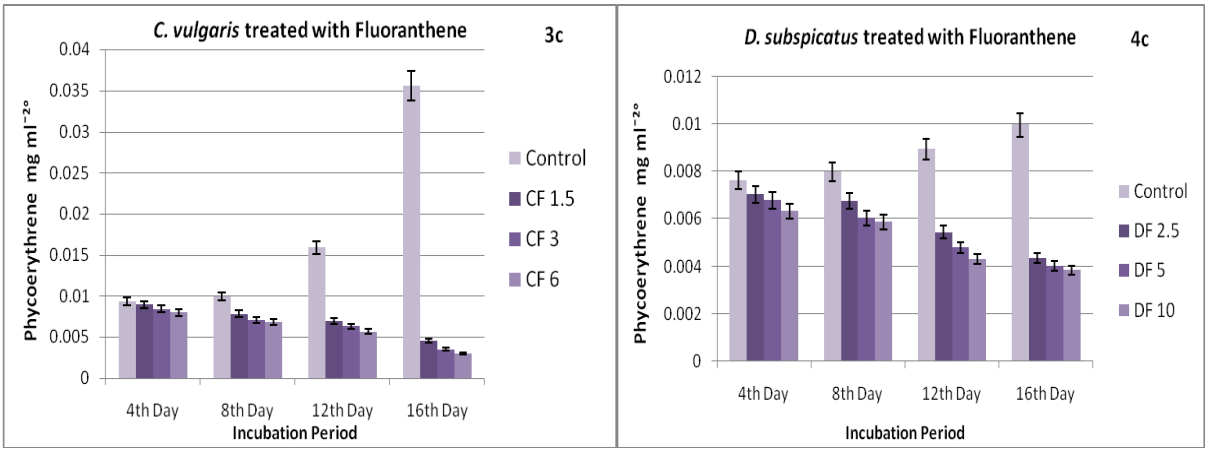

Fig. 3 and 4 (c) Response on phycoerythrene content of *C. vulgaris* and *D. subspicatus* to Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

Carotenoids decreased significantly over 16 days of the experiment in PAHs treatments (fig.2a and 2b) The Carotenoids reduced by 16%, 19% and 24% in the treatments of 1.5, 3 and 6 ppm on 4th day in *C. vulgaris*, whereas it declined by 8%, 16%, and 22% at the doses of 2.5 ppm, 5 ppm and 10 ppm in *D. subspicatus*. The *C. vulgaris* continued to reduced and reached maximum value of 0.050±0.0040 mg ml^{-20} on 16th day. While in *D. subspicatus* the fall of carotenoids reached to 0.016±0.005 mg ml^{-20} at 16th day by 98% at 10ppm than percentage decrease in *C. vulgaris* by 86% in 6ppm. The decline in the phycobilliprotein in response to increasing exposure periods (days) was more conspicuous and highly significant at higher doses as compared with lower doses. The phycobiliproteins (phycocyanin, allophycocyanin, and phycoerythrin) of the organism dropped continuously with increasing PAHS concentrations. The percentage reductions at the highest fluoranthene concentration (10 ppm) in *C. vulgaris* (6 ppm) were 93%, 95% and 91% by the end of 16 days (fig.3a,3b and 3c), on the other hand, in D. subspicatus (fig 4a, 4b and 4c) percentage

reductions were reclined by 92%, 93% and 61% in phycocyanin, allophycocyanin, and phycoerythrin, respectively.

The total carbohydrate contents of *D. subspicatus* cultures were gradually started to decrease, when the algal cultures subjected to lower level (2.5 and 5 µg ml^{-1}) to a higher dose of the PAHS (Fig 5a and 5b). Under high PAHS concentration, in 4^{th} day *C. vulgaris* at 6 ppm reduced the carbohydrate content to 0.74±0.055 mg/ml^{-20} whereas D. *subpicatus* showed declined value of 0.48±0.062 mg ml^{-20} at 10 ppm. The values recorder after 16 days showed a great reduction in carbohydrate content. The carbohydrate contents were depressed by 86% at 10 ppm in *D. subspicatus* followed by 91% in *C. vulgaris* at 6 ppm. The protein contents showed significant effect with the escalation of PAHs levels in the medium cultures at initial stage among both the algal species (fig 6a and 6b). Between 4 and 8 days, percentage decline of protein from *C. vulgaris* was 61% to 83% at 6ppm and for *D. subspicatus*, reduction was 33% to 66% at 10 ppm respectively. At later stage of the experiment, the protein content was 0.0434± 0.0059 mg ml^{-20} at 6 ppm in *C. vulgaris*, where *D. subpicatus* showed reduction value of 0.0284±0.0041 mg ml^{-20} at 10ppm.

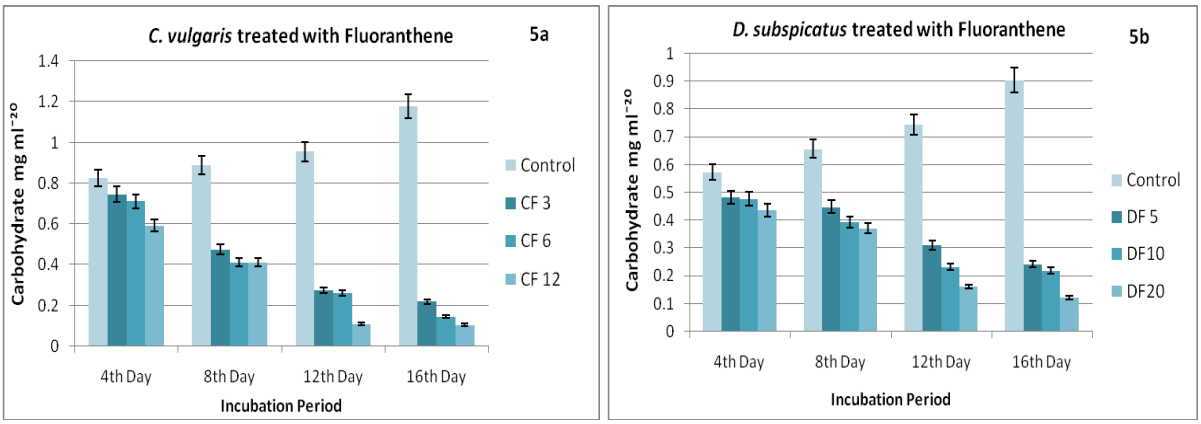

Fig. 5 a and b Response on Carbohydrate content of *C. vulgaris* and *D. subspicatus* by Fluoranthene. (Data were the mean values and standard deviations of three replicates).

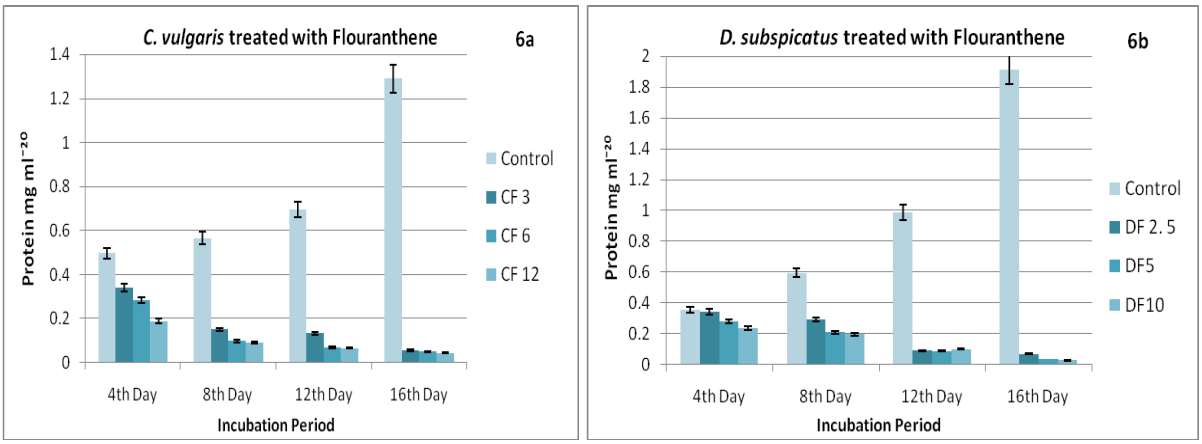

Fig. 6 a and b Response on Protein content of *C. vulgaris* and *D. subspicatus* by Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

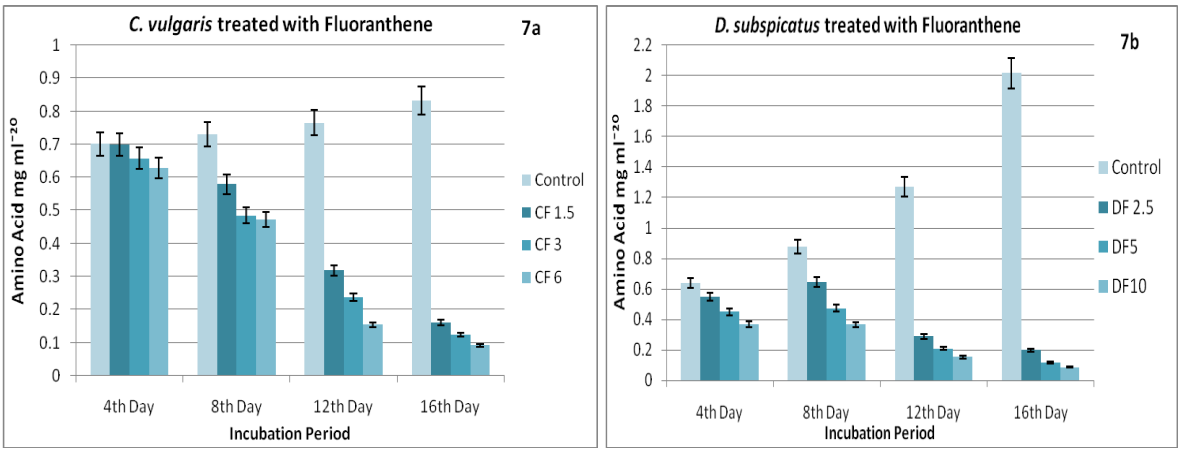

Fig.7a and b Response on Amino Acid content of *C. vulgaris* and *D. subspicatus* by Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

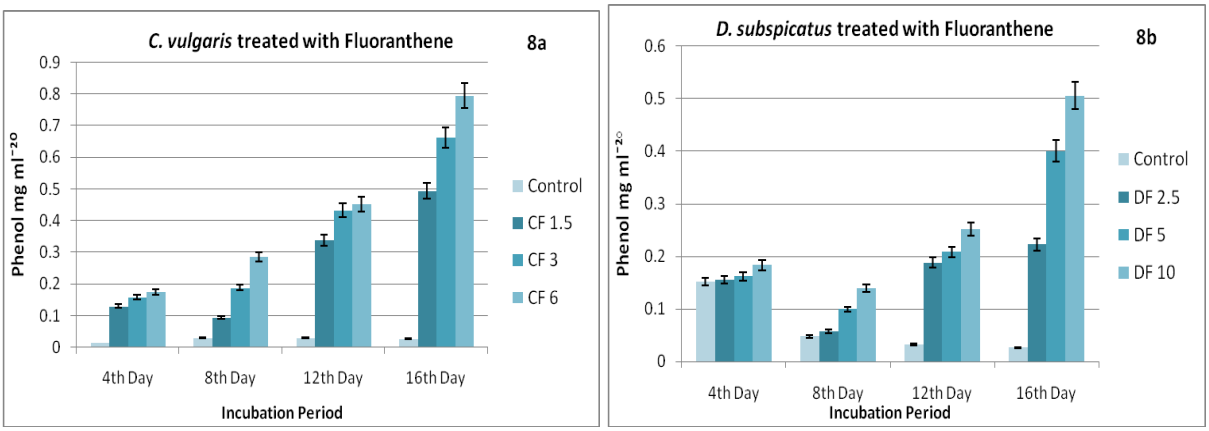

Fig. 8 a and b Response on Phenol content of *C. vulgaris* and *D. subspicatus* by Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

The total amino acid reduction from both algae was determined by the difference between the control and the different PAHS doses. *C. vulgaris* showed no such significant amount of reduction in amino acid content. The amino acid value at initial stage in *C. vulgaris* was 0.690±0.060, 0.657±0.037 and 0.627±0.047 mg/ml^{-20} (day 4) with different LC50 values, while in *D. subspicatus* , it showed lightly raised by 0.54±0.047, 0.44±0.046 and 0.36±0.055 mg ml^{-20} respectively. At 12th day, *C. vulgaris* showed immense decrease in amino acid by 58%, 68% and 79% and continued the suppressed at 16th day with 80%, 85% and 89% (fig. 8a and 8b). Same as in *D. subspicatus*, amino acid reduction at 16th day by 90%, 94% and 95% with respective doses.

Phenol content in respective to treated cells of *C. vulgaris* and *D. subspicatus as* compared to the control increased from 8th day onwards with respect to higher concentrations of the fluoranthene as well as exposure periods (fig. 8a and 8b). Phenol content in the two species, *C. vulgaris* (95%) and *D. subspicatus* (98%) exhibited a relative increase at 16th day at 6 µg ml^{-1} and 1.5 µg ml^{-1} concentrations of the PAHS.

An incubation period of up to 16 days, the enzymatic assay was constant showing decreasing linearity. At the preliminary stage nitrate reductase of *C. vulgaris* showed 0.70±0.071 mg ml^{-20} by 7% decrease and 0.34±0.047 mg ml^{-20} by 3% in *D. subspicatus* at 1.5ppm and 2.5ppm respectively compared to other doses. Maximal NR reduction was observed at 16^{th} day by 88% in *D. subspicatus* and 93% in *C. vulgaris* at 6 µg ml^{-1} and 10 µg ml^{-1} respectively (fig 9a and 9b). Glutamine Synthetase (GS) enzyme showed a sharp reduction by 38% and 68% in *C. vulgaris* and *D. subspicatus* respectively after 4th day. The reduction percentage increased by 87%, 88%, and 89% at the end of 16^{th} day in *D. subspicatus* on the other hand C. *vulgaris* showed fall of GS avtivity by 83%, 91% and 92% at 1.5, 3.0 and 6.0 ppm respectively. The treatment of these PAHs lowered the SDH activities of *D. subspicatus* by 59, 67, and 75 % at 16 days while in disparity in *C. vulgaris* showed a reduction of 70%, 76% and 83% at 1.5, 3.0 and 6 ppm after 16days (fig 11a and 11b).

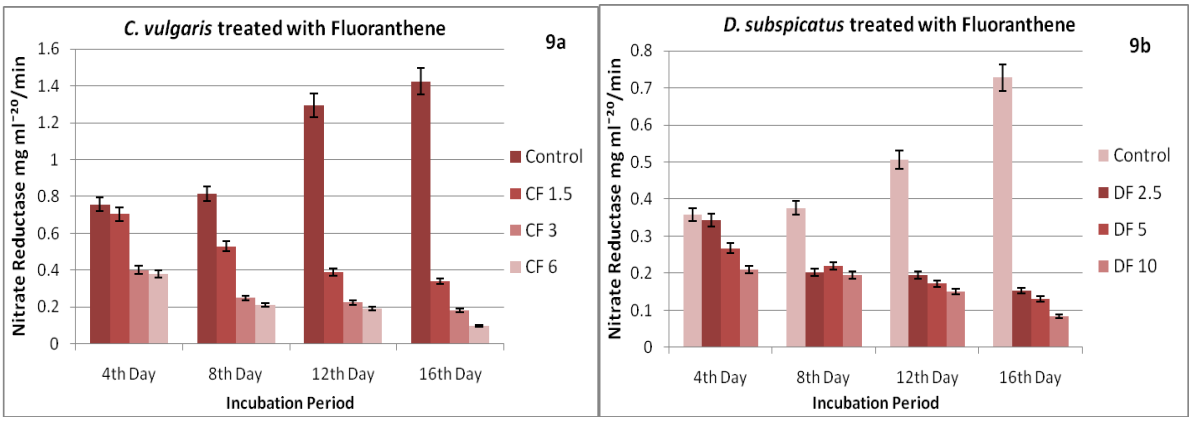

Fig. 9 a and b Response on Nitrate Reductase activity of *C. vulgaris* and *D. subspicatus* by Fluoranthene. (Data were the mean values and standard deviations of three replicates.)

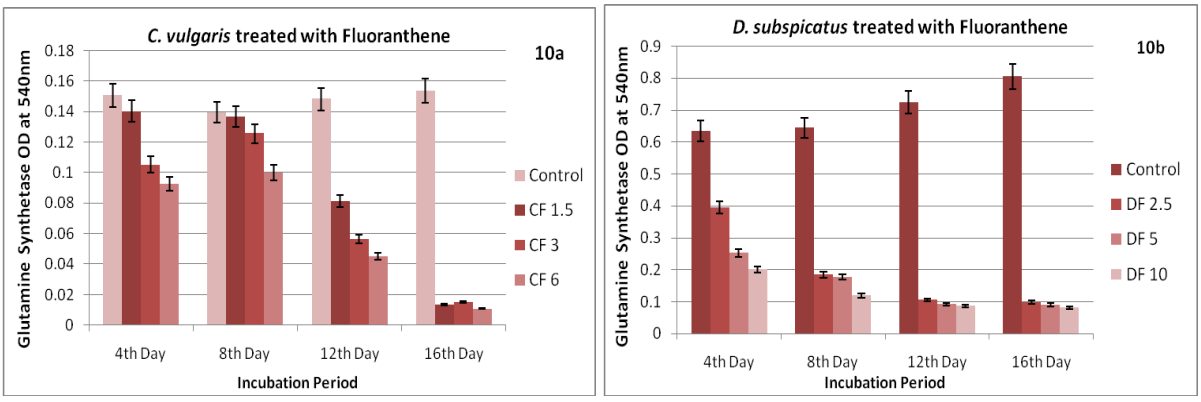

Fig. 10 a and b Response on Glutamine Synthetase activity of *C. vulgaris* and *D. subspicatus* by Fluoranthene (Data were the mean values and standard deviations of three replicates.)

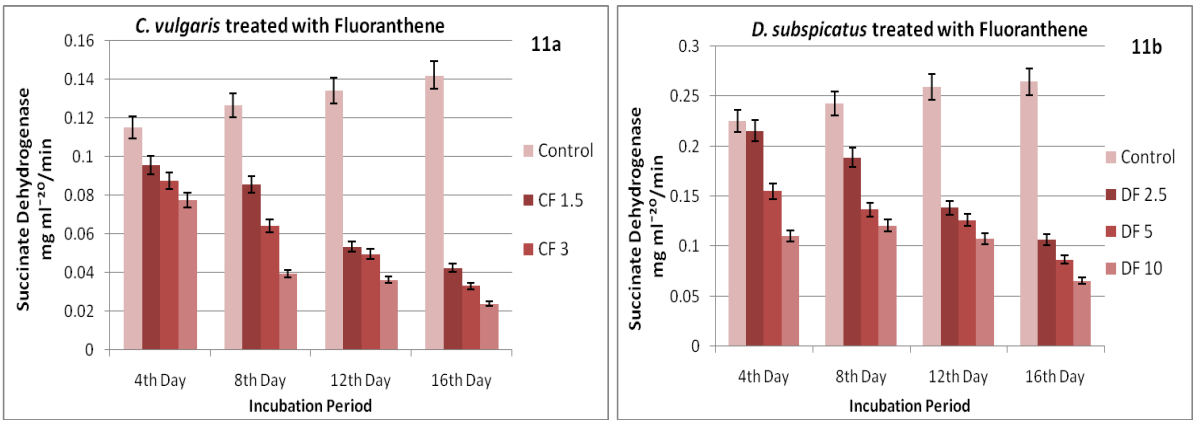

Fig. 11 a and b Response on Succinate Dehydrogenas activity of *C. vulgaris* and *D. subspicatus* by Fluoranthene (Data were the mean values and standard deviations of three replicates.)

Discussion

The effect of two PAHS on microalgal populations has been considered to be inhibitory at high doses (Panigrahi et al., 2003). Data obtained in the present investigation revealed that both the strains were more susceptible to PAHS. In present study, the effect has been considered to be inhibitory at higher doses to both the microlagal species. Soto et al. (1975) reported that high percentages of *Chlamydomonas angulosa*, a green microalgal species, inoculated in a medium saturated with naphthalene were killed but the remaining live cells could restore their growth when naphthalene in the medium gradually reduced through evaporation. According to Moradi and Ismail (2007), reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress. Bricker and Newman, (1982) also stated the decline in chlorophyll might be attributed to the earlier structural loss of photosystem I as compared to photosystem II). Bidwell (1979) reported that chlorophyll-b is formed due to oxidation of the methyl group ring II to the aldehydes from chlorophyll-a, this could be the reason that Chlorophyll b content more than Chlorophyll a. The results are also in consonance with the deleterious effects of clotrimazole fungicides on chlrophyll-a, carotenoids, and phycobiliproteins within marine microalgal communities (Porsbring et al., 2009). The phycocyanin content in fluoranthene treated cultures was more adversely affected than chlorophyll. Mostafa and Helling, (2002) suggested that decrease in chlorophyll-a, carotenoid and phycobiliprotein contents might be ascribed to the inhibition of pigment synthesis directly by the insecticide or accelerated degradation of pigments due to increased Active Oxygen Species (AOS) formation at various sites of the photosynthetic electron transport chain during stress. The retardation in the carbohydrate content might be due to interference of chemicals with photosynthesis process (Padhy, 1985). Nirmal Kumar (1991) reported the inhibition of sugar contents of the algae by accelerating doses of substituted urea herbicide N-(4-isopopylphenyl) N, N-dimethyl urea and stated that this retardation might be due to the interference of chemicals during photosynthetic process, which ultimately lapse the production of net gain of carbohydrates. Thiel (1990) emphasized that decrease in protein content in *Anabaena variabilis* was observed in starved cells. It could be suggested that accumulation of protein at low PAHS

concentrations may be one of the ways through which the algae can abolish their toxic effects, or to increase respiration leading to the utilization of carbohydrate in favor of protein accumulation. Similar observations were recorded by Nirmal Kumar *et al.* (2008) on treatment of *Tolypothrix tenuis* with fertilizer industrial effluents. Mallick and Rai (1994) stated that increase in phenols could be due to the possible conversion of primary metabolites into phenols as well as accumulation of detoxicants of fungicide during stress conditions. Similar observations were also made by Nirmal Kumar et al (2010) using fungicide.

The decrease in the nitrate reductase enzyme also simultaneously indicated a fall in the nitrogen fixing ability of *Anabaena sp*. (Nirmal Kumar et al., 2009). Glutamine synthetase (GS) activity also expressed a concentration dependent inhibition when treated with the fluoranthene (Fig 10a and 10b) which has also been further supported by Rajendran et al. (2007) expressing a remarkable decrease in the GS activity to different pesticide concentrarions. Succinate dehydrogenase (SDH) enzyme is a major respiratory enzyme responsible for conversion of succinate to fumarate in the tricarboxylic acid cycle (TCA). Similar inhibition of the enzyme succinate dehydrogenase activity was observed in the cultures of four Gram(+) bacteria, *Rhodococcus sp. AK 1*, *Bacillus cereus* Frankland & Frankland, *Bacillus subtilis* (Ehrenberg) Cohn, *Nocardia asteroides* and a Gram(-) bacterium, *Rhizobium leguminosarum* when treated with the fungicide tridenmorph by Kalam and Banerjee (1995).

Statistical Analysis

ANOVA: All the parameters found to be non- significant (P>0.05) between the pigments, metabolites, enzymatic activities and concentrations applied (Table 1). Correlation Matrix: A noteworthy positive correlation between pigments (r =0.711 to 0.988), metabolites [r = (-562) to 0.972] and enzymes [r = (-0.735) to r = 0.981] were recorded in *C. vulgaris*, moreover, in case of *D. subspicatus* also a positive correlation was recorded between pigments(r = 0.876 to r = 0.981), metabolites[r = (-0.572) to r = 0.992)] and enzymes [r = (-0.623) to r = 0.971] except with phenol content after 16th day exposure to Fluoranthene (Table 2 and 3).

Table II: One Way Analysis of variance (ANOVA) of *C. vulgaris* and *D. subspicatus with* reference to biochemical parameters (pigments, metabolites and enzymes) of control and three graded concentrations of fluoranthene after 16 days of incubation

Parameters	*Chlorella vulgaris*			*Desmodesmus subspicatus*		
	F(cal)	P(F<=F(cal))	F(0.05)	F(cal)	P(F<=F(cal))	F(0.05)
Total Chlorophyll	1.071	N.S. (P>0.05)	0.397	0.655	N.S. (P>0.05)	0.595
Carotenoids	0.059	N.S. (P>0.05)	0.980	0.079	N.S. (P>0.05)	0.969
Phycobilliproteins	6.328	*** (P<=0.001)	1.073	1.331	N.S. (P>0.05)	0.247
Carbohydrate	0.756	N.S. (P>0.05)	0.539	0.321	N.S. (P>0.05)	0.809
Protein	0.124	N.S. (P>0.05)	0.943	0.145	N.S. (P>0.05)	0.930
Amino Acid	2.179	N.S. (P>0.05)	0.1433	0.050	N.S. (P>0.05)	0.984
Phenol	2.760	N.S. (P>0.05)	0.088	2.199	N.S. (P>0.05)	0.140
Nitrate Reductase	0.042	N.S. (P>0.05)	0.987	0.051	N.S. (P>0.05)	0.983

Succinate Dehydrogenase	0.510	N.S. (P>0.05)	0.682	0.359	N.S. (P>0.05)	0.783
Glutamine Synthetase	2.519	N.S. (P>0.05)	0.107	0.138	N.S. (P>0.05)	0.935

Table III Corellation Matrix of *Chlorella vulgaris* with defferent compounds *Chlo=Total Chlorophyll, Car=Carotenoids, PC=Phycocyanin, APC=Allophycocyanin, PE=Phyceorythrene, CHO=Carbohydrate, AA=Amino Acid, NR=Nitrate Reductase, SD=Succinate Dehydrogenase, GS=Glutamine Synthetase

	Chlo	Car	PC	APC	PE	CHO	Protein	AA	Phenol	NR	SD	GS
Chlo	1											
Car	0.892612	1										
PC	0.826677	0.916496	1									
APC	0.839677	0.933013	0.985596	1								
PE	0.752849	0.907148	0.711557	0.732952	1							
CHO	0.856418	0.938338	0.972132	0.965088	0.777127	1						
Protein	0.792245	0.953932	0.855616	0.873669	0.944586	0.893836	1					
AA	0.860097	0.862236	0.942148	0.92117	0.645042	0.954895	0.74878	1				
Phenol	-0.90021	-0.80587	-0.86913	-0.85739	-0.56271	-0.87041	-0.65105	-0.94663	1			
NR	0.821364	0.959203	0.912787	0.913544	0.85276	0.904061	0.943101	0.791296	-0.73526	1		
SD	0.877926	0.926813	0.981303	0.978719	0.734845	0.969741	0.874038	0.923815	-0.88461	0.927083	1	
GS	0.882959	0.812147	0.876034	0.849734	0.583623	0.864421	0.668184	0.944186	-0.95832	0.734058	0.86785	1

Table IV Corellation Matrix of *Desmodesmu subspicatus* with defferent compounds *Chlo=Total Chlorophyll, Car=Carotenoids, PC=Phycocyanin, APC=Allophycocyanin, PE=Phycoerythrene, CHO=Carbohydrate, AA=Amino Acid, NR=Nitrate Reductase, SD=Succinate Dehydrogenase, GS=Glutamine Synthetase

Parameters	Chlo	Car	PC	APC	PE	CHO	Protein	AA	Phenol	NR	SD	GS
Chlo	1											
Car	0.876753	1										
PC	0.897879	0.933408	1									
APC	0.896522	0.93435	0.999957	1								
PE	0.981346	0.919847	0.916782	0.915735	1							
CHO	0.967489	0.941449	0.930945	0.92996	0.991047	1						
Protein	0.803417	0.984423	0.908303	0.910361	0.855397	0.880401	1					
AA	0.887022	0.992979	0.944453	0.945649	0.9206	0.936847	0.983096	1				
Phenol	-0.84993	-0.66695	-0.66543	-0.6639	-0.81004	-0.78519	-0.572	-0.68233	1			
NR	0.901991	0.971545	0.957174	0.958232	0.933569	0.947587	0.959891	0.971853	-0.66815	1		
SD	0.967931	0.832488	0.917096	0.916289	0.938747	0.92369	0.770467	0.853995	-0.79495	0.888501	1	
GS	0.914563	0.884914	0.956369	0.955023	0.919934	0.931163	0.839193	0.881641	-0.62338	0.928818	0.927875	1

Conclusion

Experiments were conducted with a view to determining the deleterious and differential effects of fluoranthene on the photosynthetic pigments, metabolic and enzymatic activities of microalgea. The results suggest that *Desmodesmus subspicatus* was the most

susceptible organism to fluoranthene studied. The order of PAH tolerance of each organism towards the Fluoranthene can be described as *D. subspicatus* being more tolerant than *C. vulgaris*. The present results also suggest that pigments, metabolites and enzymes are positively correlated with each other but not with phenolic content of both the microalgae.

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