EFFECT OF CRUDE OIL ON THE BIOSYNTHESIS OF CHLOROPHYLL-*A* AND PHAEOPIGMENT BY ESTUARINE MICROALGAL BIOFILMS IN THE WETLAND

LOVET T. KIGIGHA*[1] AND GRAHAM J. C. UNDERWOOD [2]

* CORRESPONDING AUTHOR: DR. LOVET T. KIGIGHA,
[1]DEPTMENT OF BIOLOGICAL SCIENCES,
NIGER DELTA UNIVERSITY
P. O. BOX 578, YENAGOA,
BAYELSA STATE, NIGERIA.
Email: lovet_k@yahoo.com
[2] DEPARTMENT OF BIOLOGICAL SCIENCES,
UNIVERSITY OF ESSEX,
WIVENHOE PARK,
COLCHESTER, CO4 3SQ, UK.

ABSTRACT

Four separate mesocosm-based Experiments (A-D) were carried out over a period of four months involving impactation of microalgal mats in cores at two levels of PHC concentrations and nutrient enrichment with nitrate and phosphate at three levels of concentrations. Two sets of experiments were set up; in the light and in the dark. The effect of maximum enrichment in increasing CHL-a biosynthesis in the dark was observed at low and high oil impactation from the effect of periodic variation (P = <0.001). Values ranged from 5.9 – 59.5 µg g^{-1} of sediment at low and high oil-spiking. After correction to percent baseline value, at low oil spiking, CHL-a was better synthesized in the light than in the dark. At higher oil impactation it appears CHL-a was better synthesized in the oil-spiked controls in the dark especially and also and in the light which were respectively 240 and 180% higher than the baseline values. The typical biofilm response to contaminants in the environment appear to have been exhibited in which they were sequestered and made non-available for biodegradation in the absence or reduced mineral enrichment. In this case oil

was sequestered resulting in the observed increase in CHL-a biosynthesis. PHAEOP production was observed to be significantly increased at maximum nutrient enrichment treatments or in the non-enriched control treatments in the light and in the dark from the effect of periodic variation. This also appears to be the typical biofilm response to contaminants in the environment in which nutrient enrichment appear to have improved PHAEOP biosynthesis. After correction to percent baseline, indication was that at low oil spiking, minimal nutrient enrichment accounted for 105 -110% baseline value in the light and in the dark respectively. At higher oil-spiking, with increased nutrient enrichment, in the light especially and also in the dark, values ranged from 110 – 280% baseline value. The effect of PHC on PHAEOP biosynthesis showed some departure from that of CHL-a. PHAEOP in the oil-spiked but non- nutrient enriched biofilms appear to be more negatively impacted both in the light and in the dark from oil than CHL-a biosynthesis. At higher oil impactation, CHL-a was higher in the oil-spiked but non-enriched controls; while for the PHAEOP it was higher at moderate to high enrichment in the light and in the dark.

KEY WORDS: Sediment, photosynthesis, petroleum hydrocarbon, phaeopigment, biofilms, Chlorophyll.

{**Citation:** Lovet T. Kigigha, Graham J. C. Underwood. Effect of crude oil on the biosynthesis of chlorophyll-A and phaeopigment by estuarine microalgal biofilms in the wetland. American Journal of Research Communication, 2013, 1(6): 199-124} www.usa-journals.com, ISSN: 2325-4076.

1.0 MATERIALS AND METHODS

1.1 Fluorescence materials in the marine environment

There are a number of fluorescence materials in the marine environment such as chlorophyll-*a*, dissolved organic materials (DOM) and dissolved organic compounds (DOC); poly

aromatic hydrocarbons (PAH) etc. DOM and DOC may be of autochthonous origin (i.e. produced *in situ* from degrading biogenic materials); DOM occurs allochthonously arising from terrestrial or riverine origin (Aiken, 2001). Chlorophyll-*a* is ubiquitous in the phytoplankton and all green plants. Interest in its study in the last three decades revolves around its importance as an indicator of photosynthetic biomass and productivity especially in the phytoplankton.

1.2 Chlorophyll-a and phaeopigment assessment

For chlorophyll-*a* determination, 0.1g of freeze-dried sediment was extracted overnight at 40 ^{0}C in 100% methanol and $MgCO_3$ added to saturation point. This was centrifuged at 4000 rpm for 15 min and the absorbance read at 665 and 750 nm. After acidification by addition of a drop of 10% HCl and leaving for 5 min, the absorbance was again read at the same wavelengths.

Chlorophyll-*a* (µg g^{-1} of sediment) = K x [(665-750)-(665_a-750_a)] x Vs x 1000/ (Ac x wt (g))

Where K= 1.32; Vs is volume of solvent used; Ac= 74.5

Phaeopigment (µg g^{-1} of sediment) = K x F x [(4.14 x (665_a – 750_a)) – (665 – 750)] x Vs/ (Ac x wt (g)). Where K = 1.34; F = 0.974 and AC = 74.5 (Stal *et al.* 1984).

2.0 RESULT

2.1 Chlorophyll-a and phaeopigment

Chlorophyll-*a* and phaeopigment are important physiological compounds of the estuarine microalgal biofilms that have direct effect on primary production of the ecosystem. They were assessed to determine the effect of PHC and nutrient enrichment on their biosynthesis. The chlorophyll-*a* and phaeopigment baseline status of each mesocosm is shown in Fig 1; the effect of periodic variation on enrichment or absence of enrichment on oil spiked biofilms chlorophyll-*a* and phaeopigment biosynthesis in the light and in the dark is as shown in Fig 2 and 3 respectively, while the percent correction to the baseline for each mesocosm chlorophyll-*a* and phaeopigment assessment is as shown in Fig 4 and 5 respectively.

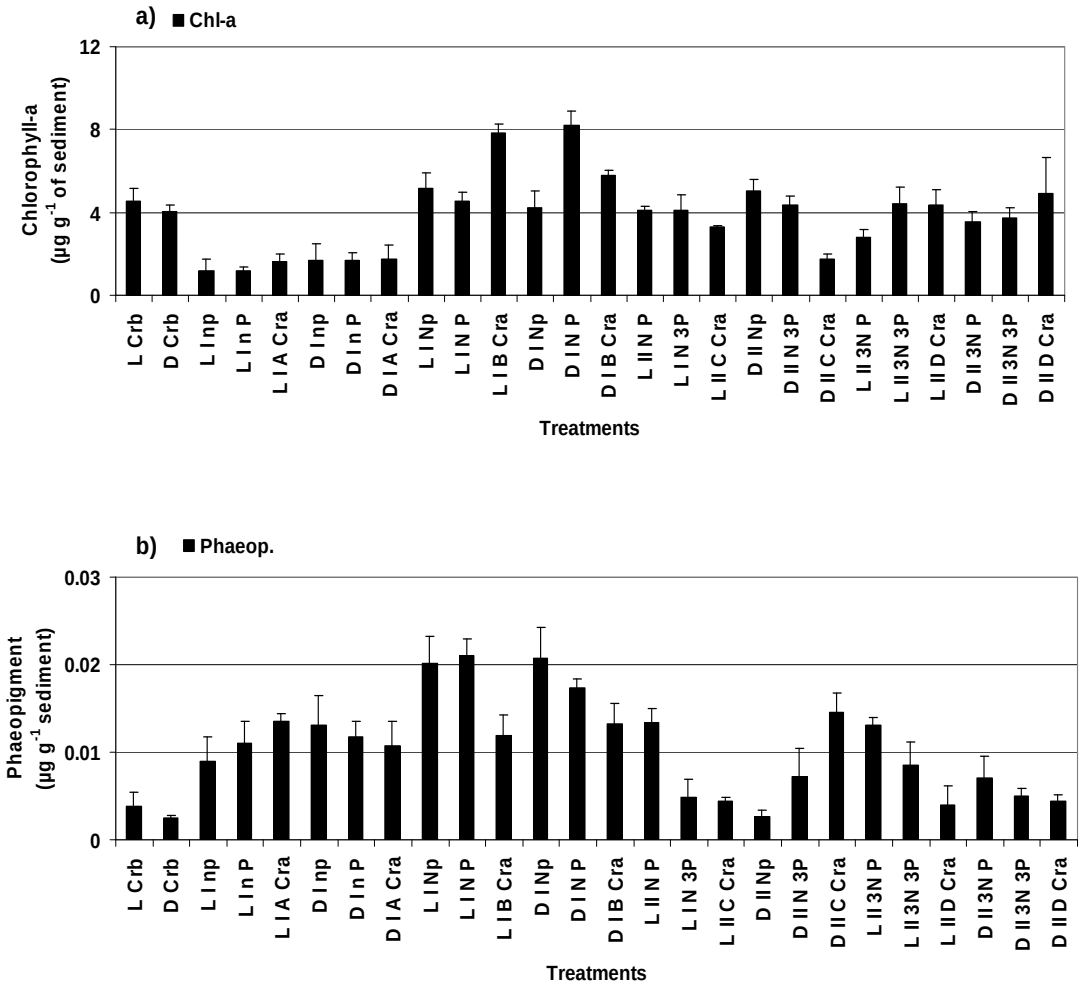

Fig. 1. Baseline a) Chlorophyll-*a* and b) Phaeopigment status of mesocosms.

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;*

L Crb and *D Crb*: Baseline status of CHL-a and PHAEOP of mesocosms (i.e. control without oil or nutrient added in the light and dark respectively) Mean ± SE, n = 3. Baseline status of mesocosms indicated significant differences in background CHL-a and PHAEOP concentrations in all the mesocosms (A-D, P = <0.001) before treatment was started.

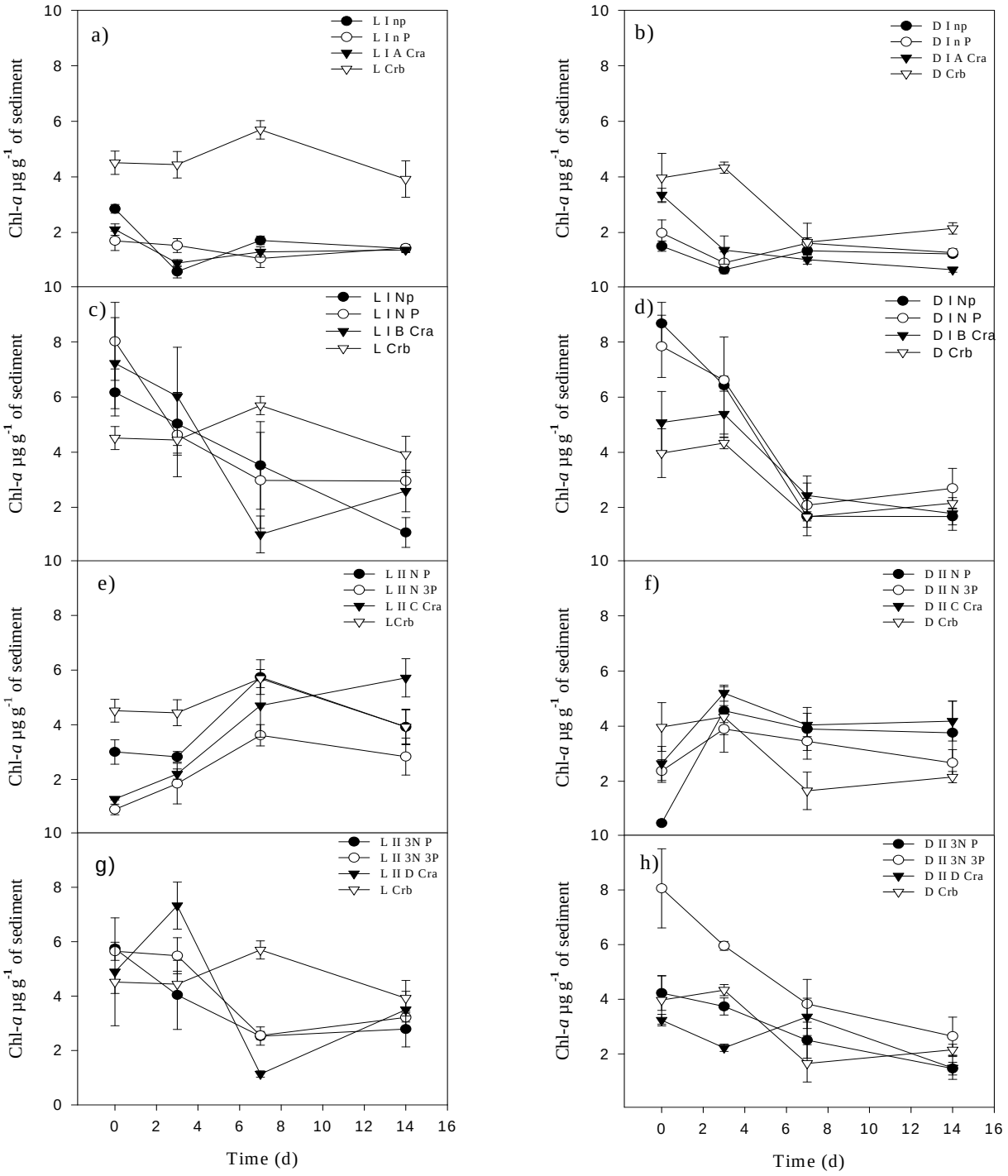

Fig. 2. Effect of periodic variation on PHC interaction with biofilm Chlorophyll-*a*.

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;* Mean (± S.E; n = 3).

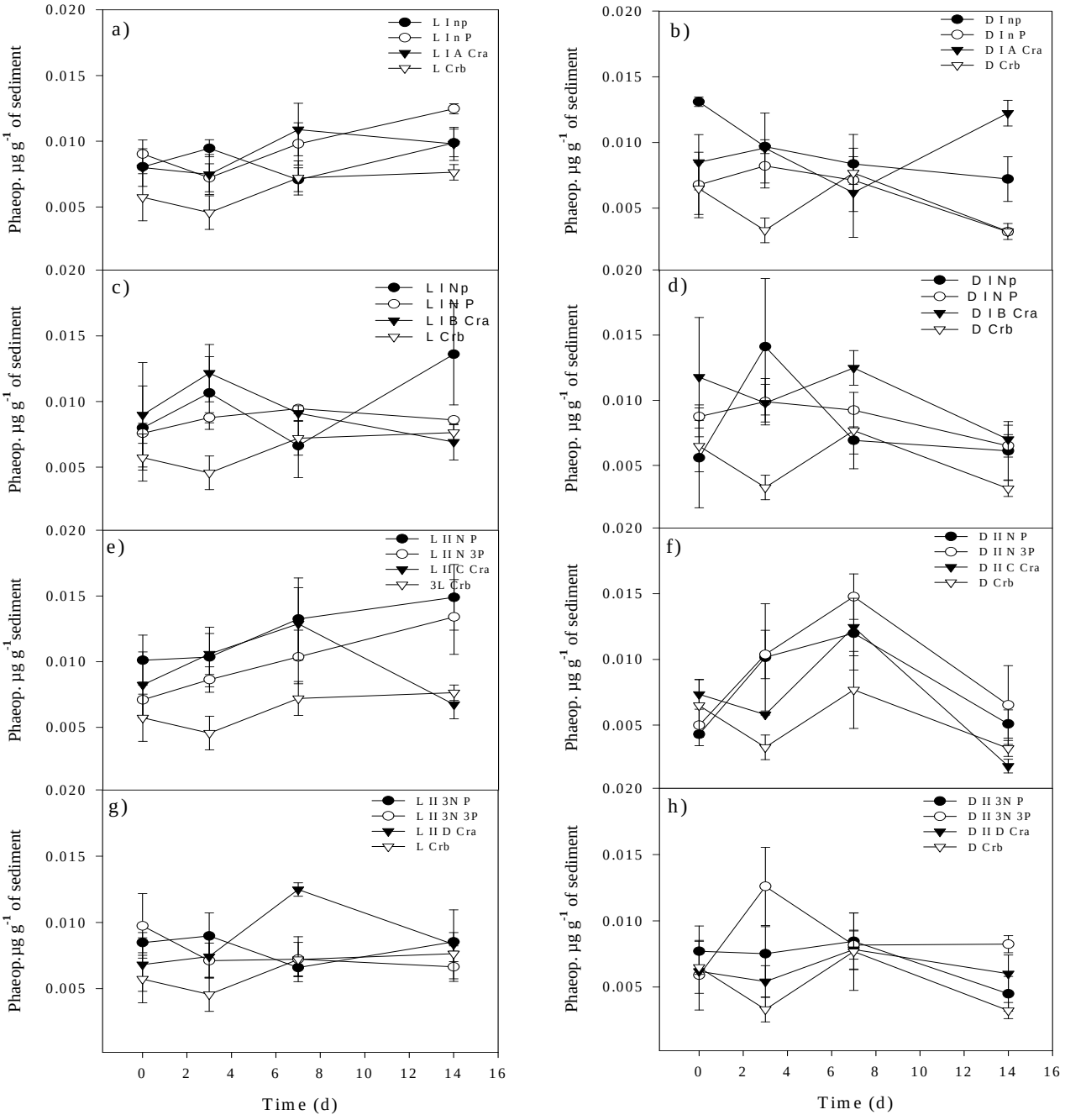

Fig.3. Effect of periodic variation on PHC interaction with biofilm phaeopigment .

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;* Mean (± S.E; n = 3).

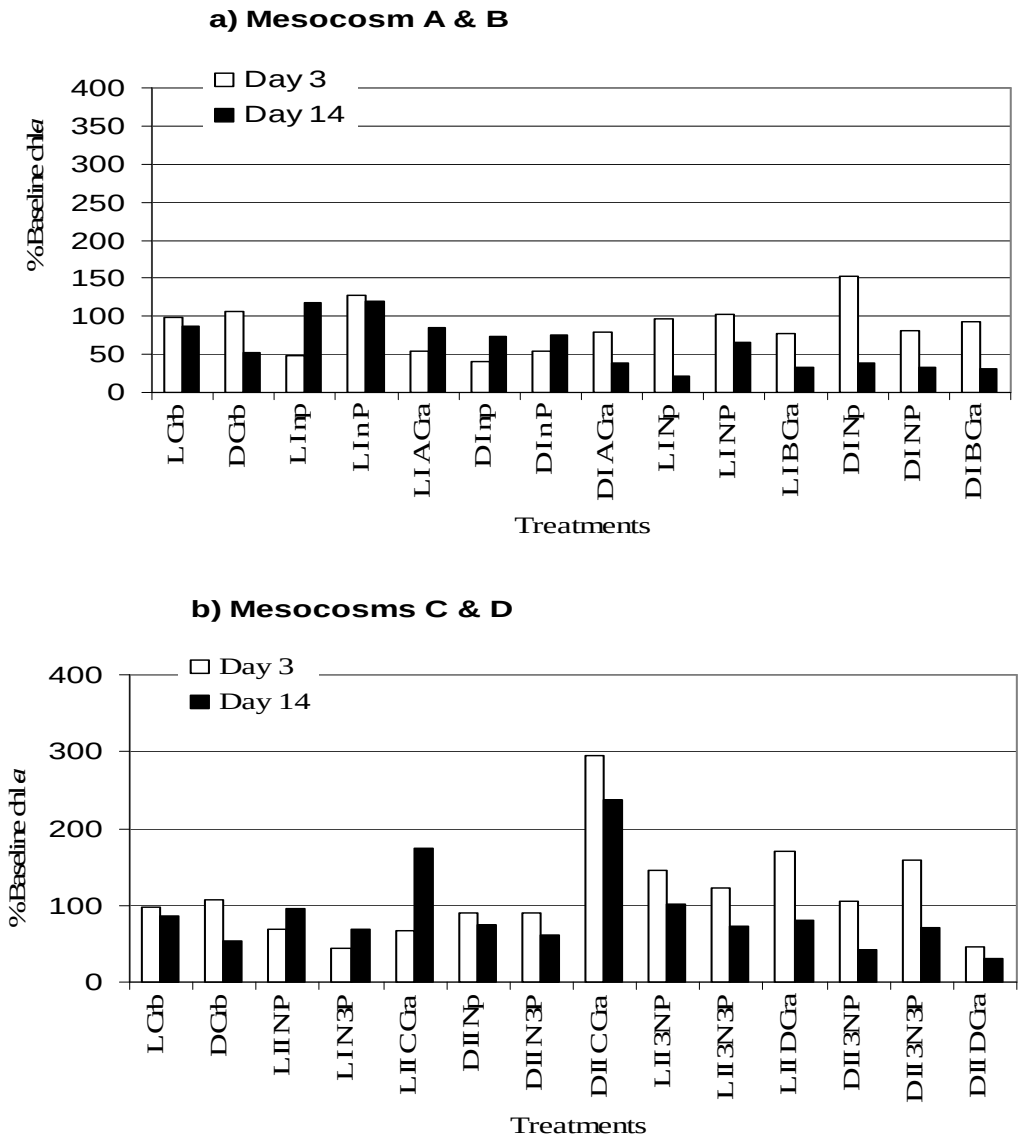

Fig 4. % Baseline corrected chlorophyll-*a* *at* a) low oil and b) high oil spiking:

Control treatments: L Crb; D Crb.

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;* Mean (± S.E; n = 3).

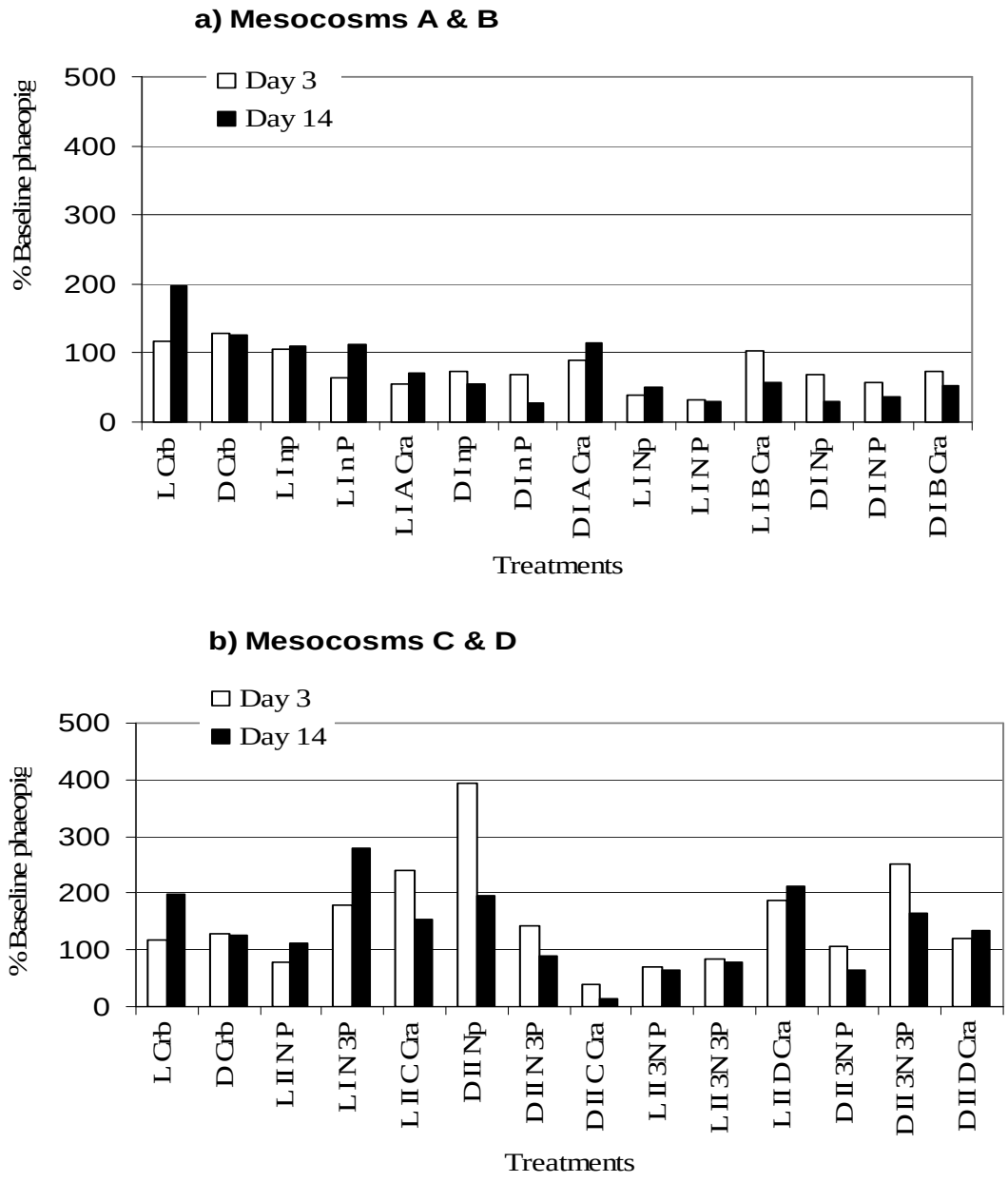

Fig 5: Percent Baseline corrected phaeopigment at a) low oil and b) high oil spiking:

Control treatments: L Crb; D Crb.

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;* Mean (± S.E; n = 3).

The baseline status of the mesocosms indicated significant differences in background CHL-*a* and PHAEOP in all the experiments (A-D; P = <0.001) before treatment was started (Fig 1). The effect of periodic variation on the interaction between PHC and Chlorophyll-*a* / phaeopigment in the mesocosm experiments were shown in Fig 2 and 3, while their correction as percentages of the baseline values are shown in Fig 4 & 5.

Chlorophyll-a assessment

Biofilms Response I & II (Crb & Cra)

From the effect of periodic variation, there were indications of decreasing chlorophyll-*a* (CHL-*a*) in the light and in the dark at low oil spiking (Fig 2 a-d) in the enriched treatments and in the oil spiked control *Cra* compared to the baseline control *Crb* on the 14th day (P = 0.002 and P = <0.001 in the light and dark respectively) except for the enrichment treatment *DINP*. Values ranging from 5.9 - 47.1 and 13.2 – 44.6 µg g^{-1} of sediment respectively in the light and dark treatments.

At higher oil impactation at moderate enrichment in Experiment C (Fig 2 e and f), the effect of periodic variation indicated that CHL-*a* was higher in the oil spiked control treatments *LIICCra* and *DIICCra* in the light and dark while in the baseline control CHL-*a* was lower on the 14th day. At maximum nutrient enrichment in Experiment D (Fig 2 g & h) the effect of periodic variation indicated that in the light it was the control *Crb* that was highest while in the dark it was the treatment *DII3N3P* (P = <0.001); ranging from 16.5 – 59.5µg g^{-1} of sediment.

Biofilms Response III

From the effect of periodic variation the treatment *DINP* at low oil-spiking and *DII3N3P* at high oil-spiking in the dark which were maximum enrichments at each level of oil-spiking appear to be higher than all the other treatments. Nevertheless after correction to percent baseline value, it was the treatments *LInp* and *LInP* that were about 120% higher than the baseline value. This appears to indicate that at low oil spiking, CHL-*a* was better synthesized in the light than in the dark.

After correction to percent baseline, at higher oil impactation at moderate enrichment in Experiment C (Fig 2 e and f), it appears CHL-*a* was better conserved in the oil-spiked controls in the dark than in the light as indicated in the controls *DIICCra and LIICCra* which were respectively 240 and 180% higher than the baseline values. Since these were non-enriched treatments these probably depended on the composition of the biofilms or appears to indicate that in the non-enriched and oil-spiked biofilms there was better CHL-*a* production or probably, there may be the probable effect of greater fluorescence of CHL-*a* from oil percolated biofilms or probably, PHC was much sequestered thereby allowing for the increased CHL-*a* biosynthesis especially in the dark. Also after correction to baseline it was the treatment *LII3NP* among the enriched treatments that was 100% baseline value. The trend for CHL-*a* biosynthesis in the oil-impacted estuarine biofilms, appear to suggest that at low and high oil-spiking there was a compensatory effect in the light; there appear to be better conservation of CHL-*a* accompanied by an increased requirement for more nutrient enrichment with increasing oil impactation especially in the light as shown by the increase in CHL-*a* on the 14th day.

Phaeopigment

Biofilms Response I & II (Crb & Cra)

The effect of periodic variation at low oil spiking in the light at minimal enrichment (Fig 3 a) indicated that the controls *Crb* and *Cra* indicated lower PHAEOP production than the enriched treatments on the 14th day in the light. In the dark at low oil-spiking at minimal enrichment (Fig 3 b), indication was that PHAEOP production was higher in the control *Cra* than in the enrichment treatments and the baseline control *Crb*. The effect of periodic variation at low oil spiking in the light at moderate enrichment (Fig 3 c) also indicated that the controls *Crb* and *Cra* indicated lower PHAEOP production than the enriched treatments on the 14th day in the light. Similarly, in the dark at low oil-spiking at moderate enrichment (Fig 3 d), indication was that PHAEOP production was higher in the control *Cra* than in the enrichment treatments and the baseline control *Crb*. The effect of the dark phase on the oil-spiked but non-enriched control was shown.

At higher oil spiking, the effect of periodic variation indicated that at moderate nutrient enrichment in the light and in the dark, Fig 5 (e & f) the enriched treatments were significantly higher in the light than the control treatments especially on the 14th and 7th day respectively. At higher oil spiking, the effect of periodic variation indicated that at maximum nutrient enrichment in the light on the 7th day, all the enrichment treatments were lower, the oil-spiked non-enriched control indicated increase in PHAEOP biosynthesis Fig 5 (g). In the dark, there was apparent sequestration in all the treatments on the 7th day, while on the 14 day, enriched treatments were significantly higher than the baseline control treatment on the 14th day.

After correction to baseline value at low oil-spiking (Fig 5), in the light, the indication was that there was generally, increase in PHAEOP biosynthesis from day 3 to 14 for all the enrichment treatments including the baseline control *Crb* in which the treatments *LInP* and *LInP* were 105% of the baseline value. The control *Cra* indicated a decrease from the 7th to the 14th day. After correction to baseline value indication was that control treatments were higher than the enriched treatments *LCrb* was 200%, *LIICCra* 155%, and *LIIDCra* 205%, while in the dark *DIICra* was 125% and *DIIDCra* was 130% baseline values. This would imply light compensatory effect on PHAEOP biosynthesis.

Biofilms Response III

PHAEOP production was significantly increased at moderate nutrient enrichment in the light and in the dark in Experiment C (Fig 3 e & f) on the 14th day. At maximum enrichment and higher oil impactation in the light in Experiment D (Fig 3 g & h) it was the treatment *LII3N3P* and the control *LIIDCra* that indicated higher PHAEOP production. In the dark it was also the reciprocal treatments i.e. treatment *DII3N3P* and the control *DIIDCra* that indicated higher PHAEOP production. After correction to baseline (Fig 5 a & b), indication was that *LInp* and *LInP* (were

105% baseline value) the control, *DIACra* was about 110% baseline value *LIIN3P* was 280%, and *LIINP* 110%, *DIINP* 198% and *DII3N3P* 170%.

The response of phaeopigment was not similar to that of chlorophyll-*a* with respect to requirement for nutrient enrichment especially in phosphate requirement. The trend indicated that at low and high oil-spiking in the light phase in all the mesocosms (Fig 3), there were inclinations towards increasing production of phaeopigment as a result of the enrichment treatments in the light on the 14th day. Significant increases in concentration of phaeopigment at day 14 were shown in the treatment *LInP* in Experiment A, in comparison to the controls (*Crb* and *Cra*) in the light. At higher oil spiking also the treatments *LIIN3P*, in comparison to the controls (*Crb* and *Cra*) was higher on the 14th day.

The effect of PHC on phaeopigment biosynthesis showed departures from that of chlorophyll-*a*. In CHL-*a* there was apparent higher biosynthesis in the baseline control *Crb*. The contrary effect was shown in PHAEOP biosynthesis in which there was decrease in the controls *Crb* and *Cra*, than in the enrichment treatments in the dark at the 14th day. Tentatively, phaeopigment in the innate estuarine biofilms (i.e. in the control *Cra*) appear to be more negatively impacted both in the light and in the dark from oil than in CHL-*a* biosynthesis. At higher oil impactation, CHL-a was higher in the oil-spiked non-enriched controls; while for the PHAEOP it was higher at moderate enrichment in the light and in the dark.

For CHL-*a*, after correction to baseline values the treatments *LInP* and *LInP* were 105% of the baseline value. The response of phaeopigment was similar to that of chlorophyll-*a* with respect to requirement for nutrient enrichment especially in phosphate requirement. The trend indicated that at low and high oil-spiking in the light in all the mesocosms (Fig 3), there were inclinations towards increasing production of phaeopigment at moderate nutrient enrichment in the light on the 14th day while for CHL-a, there was decreasing production in the light and dark. After correction to baseline values increases in concentration of phaeopigment at day 14 were shown in the treatment *LInP* in

comparison to the controls (*Crb* and *Cra*) in the light. At higher oil spiking also the treatment *LIIN3P*, in comparison to the controls (*Crb* and *Cra*) was higher on the 14th day. This was similar to the CHL-*a* response on the 14th day.

D. DISCUSION

Chlorophyll-a and phaeopigment

At low oil spiking (with its corresponding low nutrient enrichment) chlorophyll-*a* was higher in the light in the baseline control treatment compared to the oil-spiked and enriched treatments. This appears to indicated some inhibition on biosynthesis of chlorophyll-*a* in the light as a result of the added oil. The effect of PHC on primary production could be stimulatory, inhibitory or neutral with respect to the type and amount of oil, the ecosystem and organisms involved (Miller et al., 1978a).

For both chlorophyll-*a* and phaeopigment there were increases from nutrient enrichment at low and high oil-spiking; they differ in that while chlorophyll-*a* indicated positive response with increasing concentration on the 14th day in the dark, the phaeopigment was more enhanced in the light. As photosynthetic pigments, this could suggest that phaeopigment may be less negatively impacted than chlorophyll-*a* from the spiked oil. Also it is an indication of the importance of nutrients (especially of phosphate) in the bioremediation process in the dark and in the light (Lessard et al., 1995).

Armstrong and Calder (1978) have suggested that the primary effect of petroleum interaction with the microalgae is on the electron transport system. It is not clear if the same is true about bacterial forms. A probable explanation therefore appears to point to the vaguely and poorly understood finding that oil-smothering effect on microalgae (and probably bacteria) could be at the point of oxidative phosphorylation. Especially as it has been documented that nitrates, phosphates and sulphates serve not only as biosynthetic molecules but also as final electron acceptors in place of oxygen. (Caldwell *et al*., 1998). The effect of low oil spiking appear to cause an increase in

chlorophyll-*a* and especially in phaeopigment biosynthesis in particular in the light but in the dark there appear to occur a greater dependence on added nutrients (Gordon and Prouse, 1973; Karydis, 1979). The widely held view about the effect of PHC on chlorophyll-*a* appear to indicate divergence in opinion: The suggestions are that first, there was no significant effect, secondly, that the effect was innocuous and thirdly that it was destructive on chlorophyll-*a* biosynthesis (O'Brien and Dixon, 1978; Ellis, 1976; Cerniglia et al; 1980; Walker et al, 1975c); there were no records on effect of PHC on phaeopigment. With a greater understanding of the biology, chemistry and interactions of biofilms with contaminants in recent times; there is the probability that what was thus variously observed could be the result of interplay of three separate effects; that of a probable 'shock' effect in the control treatment in which oil was spiked without adding nutrients (as could be seen on day 3 in both light and dark of the unaided oil-spiked biosynthesis of chlorophyll-*a*); the effect of light compensation especially for phaeopigment in the light and the enhancement in the dark phase in the presence of added nutrients. From the summary of the effect of low and high oil spiking there are indications that phaeopigment appear to be less adversely affected from the spiked oil than chlorophyll-*a*. These effects appear to be dependent on the amount of added oil and level of nutrient enrichment (especially of phosphate).

From the baseline corrected values (Fig 5 a and b), for chlorophyll-*a*, both at low and high oil-spiking there were no particularly better treatments of choice; the treatments, *LInp; LInP; and LII3NP* were found to be slightly above 100 % in comparison to their baseline values; for chlorophyll-*a*; while the treatment *LIIN3P* was the treatment of choice for phaeopigment, indicating increases of over 270 % higher than the baseline value; a further indication of the relative deleterious effect of PHC to chlorophyll-*a*. More studies would be needed to elucidate the pathway and mechanism of nutrient enrichment and the compensatory effect of light on oil threatened primary producers (the estuarine biofilms) and how these effects were to some degree ameliorated by the addition of nutrients.

A summary of the effect of oil on biofilms photosynthetic pigments biosynthesis appear to suggest the following:

1. *That the inhibition on biosynthesis of chlorophyll-a and phaeopigment as a result of the added oil was demonstrated*
2. *That phaeopigment appear to be less negatively impacted than chlorophyll-a from the spiked oil.*
3. *That for chlorophyll-a, both at low and high oil-spiking, nutrient enrichment caused 100% enhancement; for phaeopigment this was over 200%.*

REFERENCES

AIKEN, J. (2001) Fluorometry for biological sensing. In: *Encyclopaedia of ocean science*. P 1073- 1081.

ARMSTRONG J. E. AND J. A. CALDER (1978). Inhibition of light induced pH increase and O_2 evolution of marine microalgae by water-soluble components of crude and refined oils. *Appl. Environ. Microbiol.* **35**:858-862.

CADWELL, M.E., R.M. GARRET, R.C. PRINCE, AND J.M. SULFLITA. 1998 Anaerobic biodegradation of long-chain n-alkanes under sulphate-reducing conditions. *Environ. Sci. Technol*. 32:2191-2195.

CERNIGLIA C.E., D.T. GIBSON AND C. VAN BAALEN (1980). Oxidation naphthalene by cyanobacteria and microalgae. *J. Gen. Microbiol*. **116**: 495-500.

ELLIS R.E. (1977). Degradation of phenolic compounds by freshwater algae. *Plant Sci. Lett.* **8**:213-216.

GORDON D.C. AND N.J PROUSE (1973). The effect of three oils on marine phytoplankton photosynthesis. *Marine Biol*. **22**:329-333.

KARYDIS, M (1979). Short term effects of hydrocarbons on the photosynthesis and respiration of some phytoplankton species. *Bot. Marine*. **22**: 281-285.

LESSARD, P.E., J.B. WILKINSON, R.C. PRINCE, J.R. BRAGG, J.R. CLARK, AND R.M. ATLAS. (1995). Bioremediation application in the cleanup of the 1989.

MILLER M.C. ALEXANDER, V. AND R.J. BARSDATE (1978a). The effects of oil spills on phytoplankton in an Arctic lake and ponds. *Arctic* **31**: 192-218.

O'BRIEN, P.Y. AND P.S. DIXON (1976). The effects of oils and oil components on algae: *A review. Br. Phycol. J.* **11**: 115-142.

STAL, L.J., H. VAN GEMERDENB AND W.E. KRUMBEINA (1984). The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. *Journal of Microbiol. Methods* Volume 2, Issue 6, August 1984, Pg 295-306

WALKER J.D. COLWELL R.R. VAITUZIS, Z AND S.A. (1975). Degradation of petroleum by alga Prototheca zopfi *Appl. Microbiol*. **30:** 79-81.