EFFECT OF CRUDE OIL ON THE BIOSYNTHESIS OF CARBOHYDRATES BY ESTUARINE MICROALGAL BIOFILMS IN THE WETLAND

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ABSTRACT

Four different 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. The experiments were carried out in two phases viz. in the light and in the dark. While there is much published work on the adverse effects of oil pollutants on the activity of biota, there is limited work on the possible beneficial effects of such pollutants on the ecosystems. In these manipulated mesocosm study of the interaction between estuarine microalgal biofilms and fractionated crude oil interesting variations in the concentration of carbohydrates produced by the biofilms were observed. Of particular interest was the over 200% increase in the different types of carbohydrates (Total carbohydrates, Colloidal-S and EPS) produced by the biofilms compared to their baseline values under varying nutrient and diurnal conditions. In view of the fact that the presence of Extracellular Polymeric Substances (EPS) had been associated with sediment stability by various researchers, could it be inferred that dispersed oil probably improves sediment stability? Discussion was on the effect of fractionated oil on

carbohydrate production under three responses of the biofilms viz. Biofilms Response I, (in which neither oil nor nutrients were added to the mesocosms); Biofilms Response II, (in which oil was added to the mesocosm without nutrient enrichment) and Biofilms Response III (in which oil and nutrients were added to the mesocosms).

KEY WORDS: Sediment, Total carbohydrates, colloidal-S, extracellular polymeric substances, biofilms, fractionated oil.

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1.0 INTRODUCTION

The effect of PHC on carbohydrates (total carbohydrates (TCHO), colloidal-S (COL-S) and extracellular polymeric substances (EPS)) were assessed. The carbohydrates, especially EPS are important variables required to assess the effect of PHC on the physiological state of organisms such as the photosynthetic bacteria and the microphytobenthos which are actively involved in their production in the ecosystem.

2.0 MATERIAL AND METHODS

2.1. Carbohydrates determination

Total carbohydrates, colloidal-S and Extracellular Polymeric Substances (EPS) were determined using freeze-dried sediment according to the method of Underwood *et al* (1995). Total carbohydrates from the different samples from the locations were determined using the

spectrophotometer phenol-sulphuric acid assay method using glucose standard. For the colloidal carbohydrate fraction, water-soluble (colloidal-S) carbohydrate was extracted from 0.1 g of sediment samples using 2.5 ml of (25 ppt) of saline; this was placed in a water-bath at 20 ºC for 15 min and centrifuged at 3620*g* for 15 min. It was followed by the removal of 0.5 ml of the resulting supernatant and analysed and spectrum measured for the calculation of the colloidal carbohydrate.

Another 1 ml of the supernatant was removed and made up to 70% concentration using 100% ethanol and kept at 4 ºC overnight to precipitate the polymeric fraction (extracellular polymeric substances (EPS). The precipitate was centrifuged at 3620*g* for 15 min; the fluorescence intensity of the re-suspended pellet in 1 ml of distilled water was measure for the calculation of the EPS.

3.0 RESULTS

3.1. Carbohydrates

Carbohydrates are important metabolic products of the estuarine biofilms. Their assessment was used to determine the effect of oil on the primary productivity of the biofilms. Carbohydrates baseline status of each mesocosm is shown in Fig 1, 2 and 3 (for Total carbohydrates, Colloidal-S and EPS respectively). The effect of periodic variation on the enrichment or its absence on oil spiked biofilms in the light and in the dark on carbohydrates biosynthesis were shown in Fig 4, 5 and 6. Percent correction to the baseline value for each mesocosm was as shown in Fig 7, 8 and 9, for Total carbohydrates, Colloidal-S and EPS respectively.

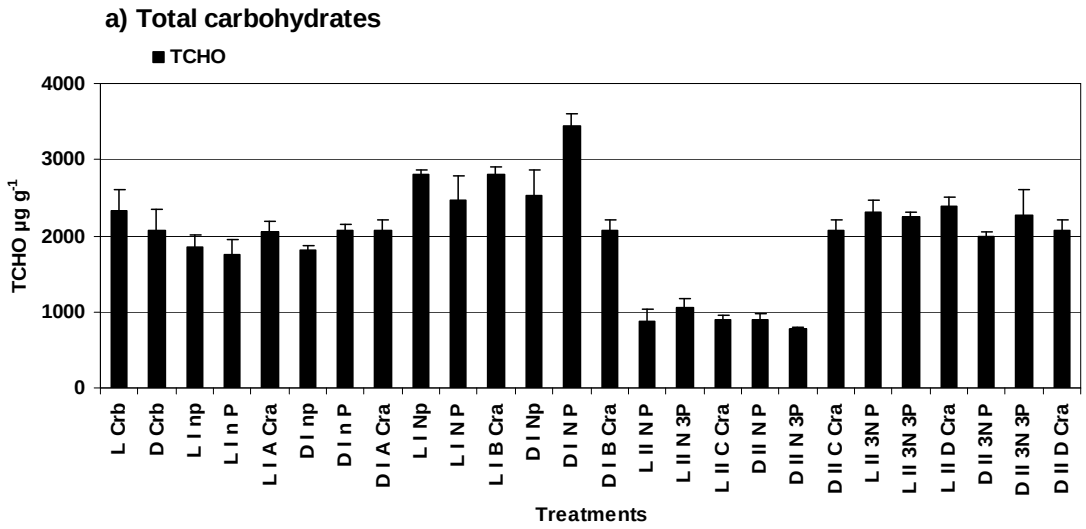

Fig. 1: Baseline status: Total carbohydrates (TCHO µg g^{-1} of sediment).

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). *LCrb* & *DCrb*: Controls (without added oil or nutrients) in the light and in the dark respectively. Baseline status of mesocosms indicated significant differences in background TCHO in all the Experiments (A, B, and C; P = <0.001; <0.05 for D) before treatment was started. Inter-mesocosm comparison of treatment effects were therefore expressed as a percentage of the background value (as shown in Fig 7 a & b).

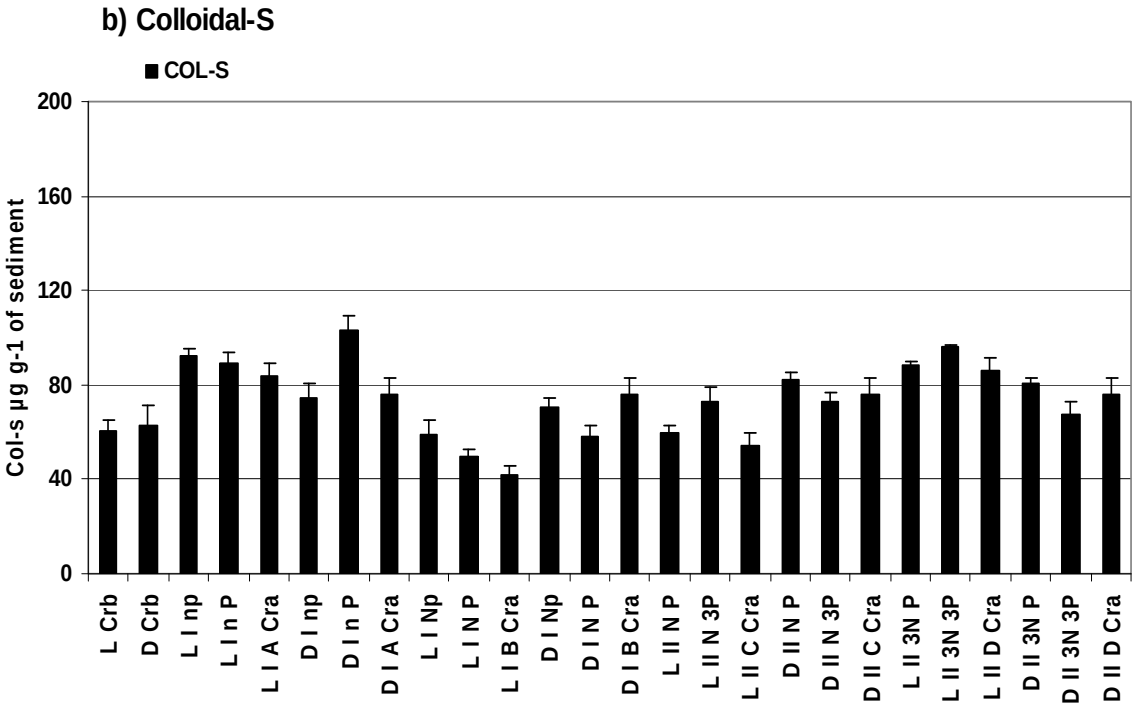

Fig. 2: Baseline status: Colloidal-S (COL-S µg g^{-1} of sediment).

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).

LCrb & *DCrb*: Controls in the light and in the dark respectively (without added oil or nutrients). Baseline status of mesocosms indicated significant differences in background COL-S concentration in all the Experiments (A, B; P = <0.001; <0.05 for C and D) in their background COL-S concentrations before treatment was started. Inter-mesocosm comparison of treatment effects were therefore expressed as a percentage of the background value (as shown in Fig 8 a & b).

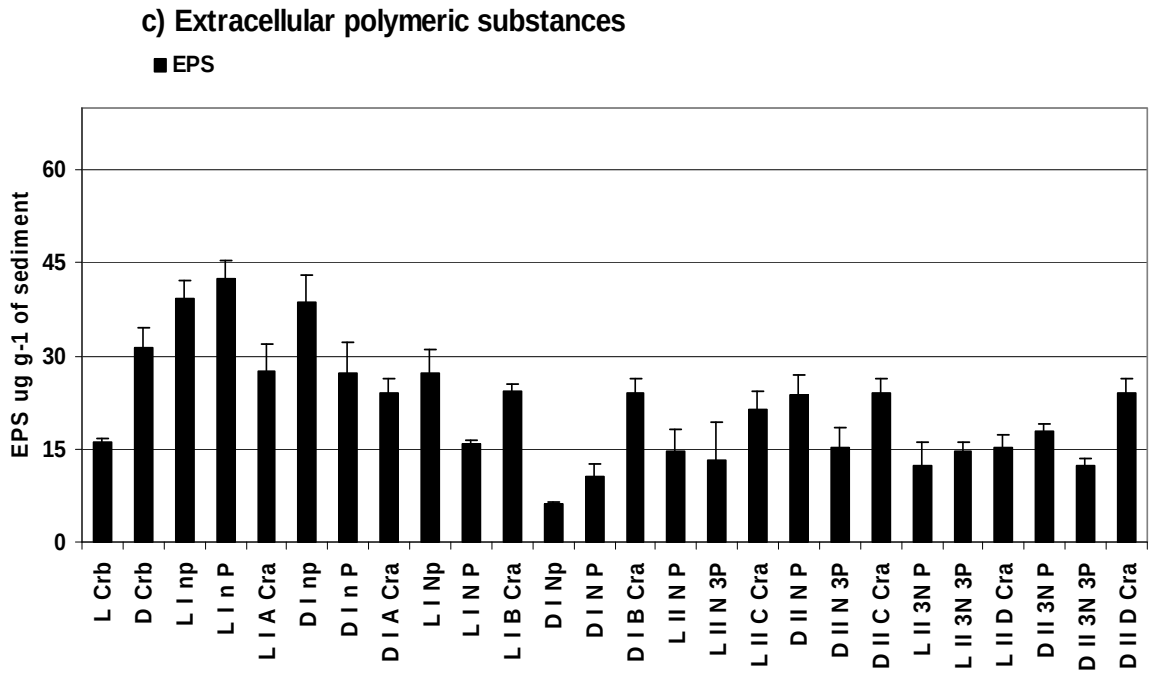

Fig. 3. Baseline status: Extracellular polymeric substances (EPS µg g^{-1} of sediment).

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).

LCrb & *DCrb*: Controls in the light and in the dark respectively (without added oil or nutrients). Baseline status of mesocosms indicated significant differences in background EPS concentrations in all the experiments (A, B and D P = <0.001; P = <0.05 for C) in their background EPS concentrations before treatment was started. Inter-mesocosm comparison of treatment effects were therefore expressed as a percentage of the background value (as shown in Fig 9 a & b).

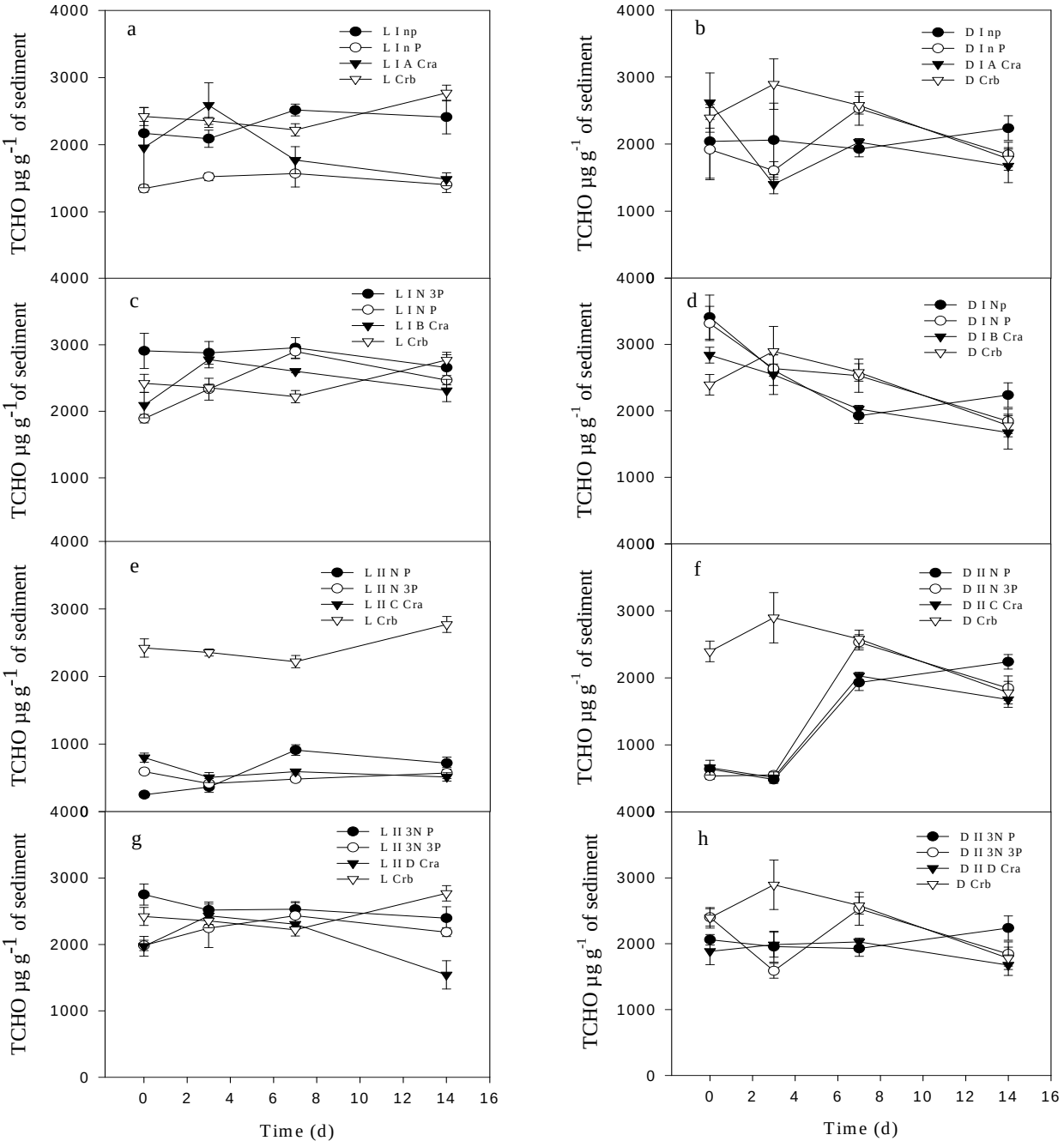

Fig 4. Effect of periodic variation on the interaction of PHC with Total Carbohydrates (TCHO).

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).

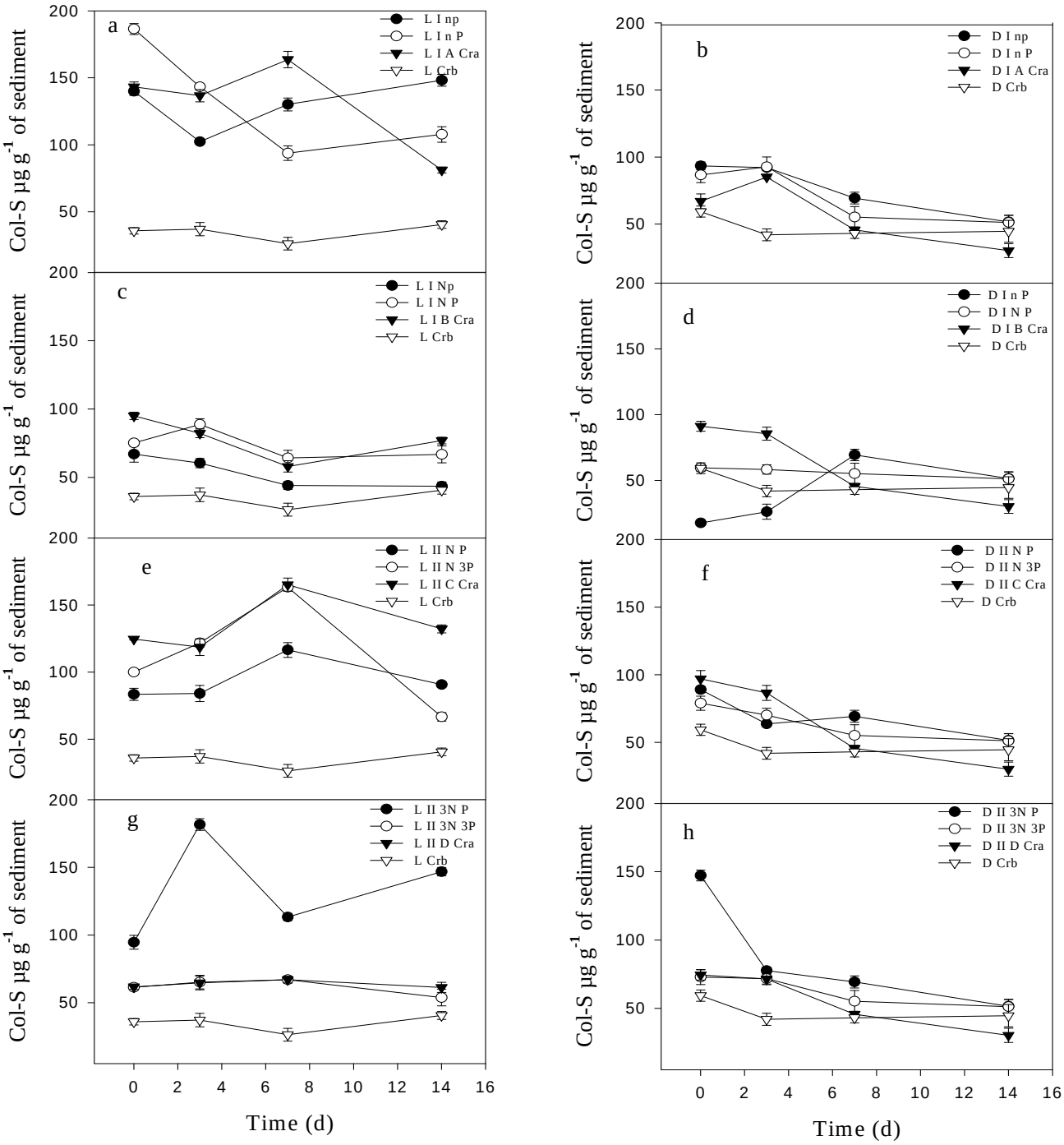

Fig 5. Effect of periodic variation on the interaction of PHC with Colloidal-S (Col-S).

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).

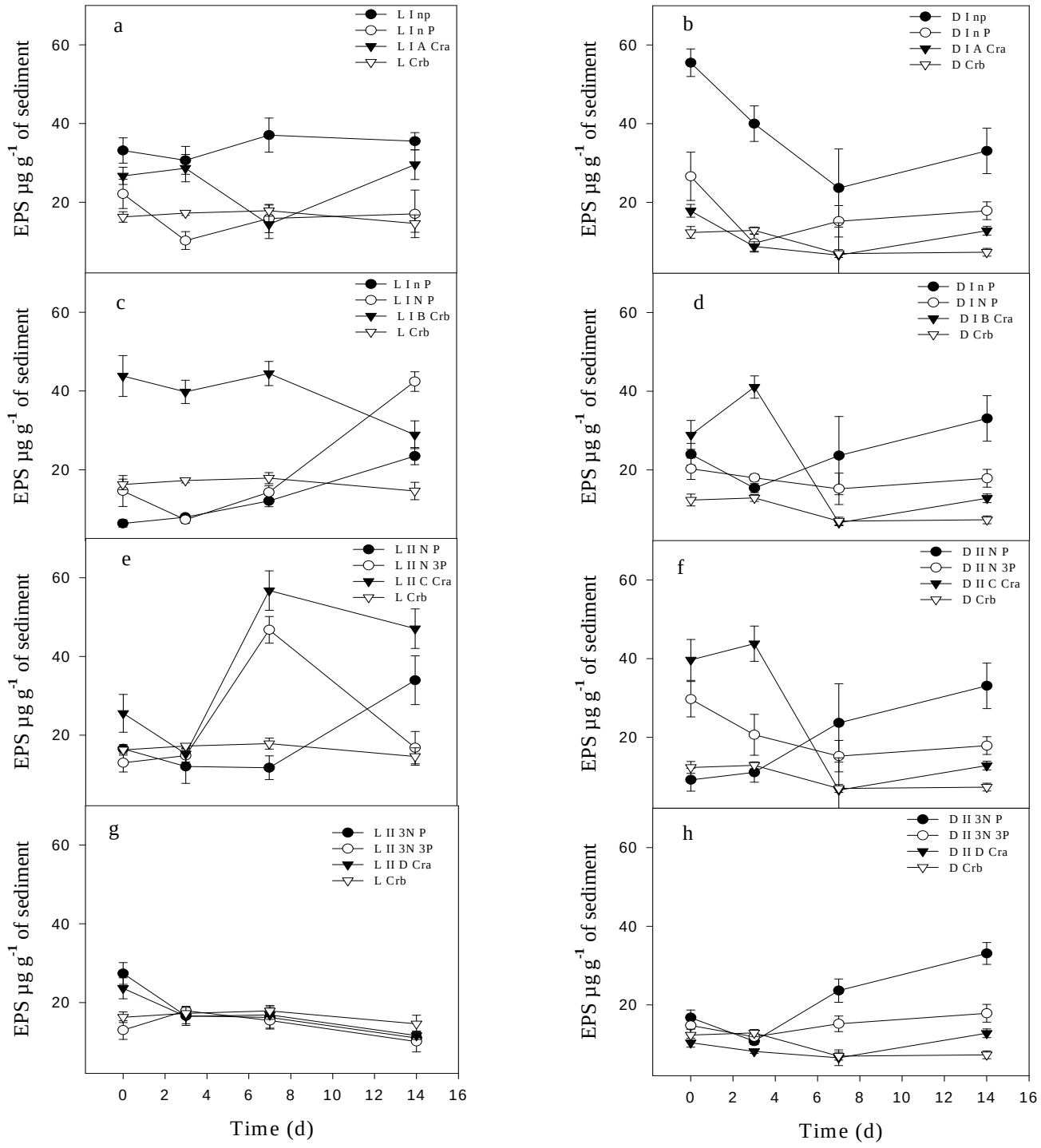

Fig 6. Effect of periodic variation on the interaction of PHC with Extracellular polymeric substances (EPS).

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).

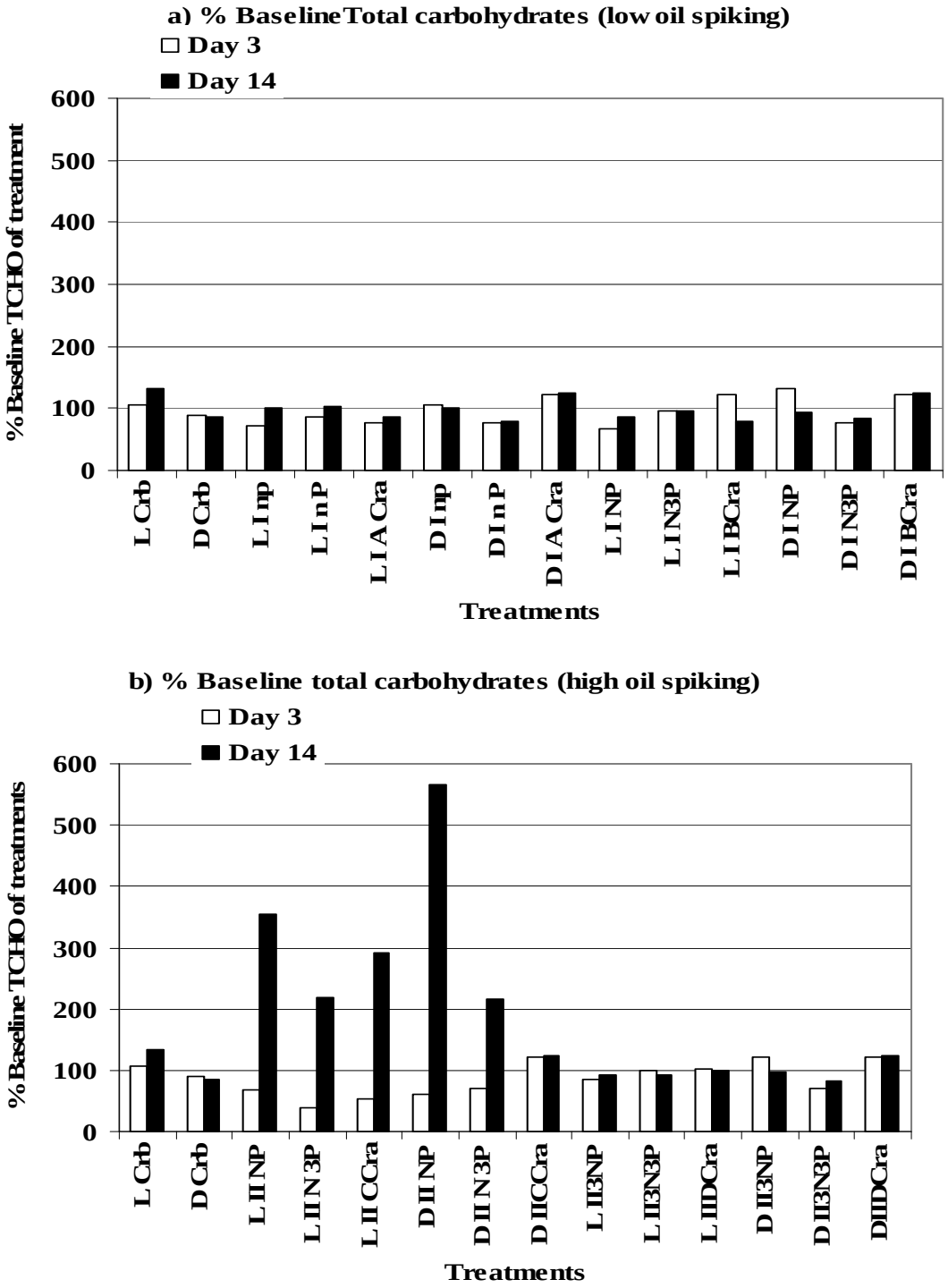

Figure 7: % TCHO corrected to the baseline values at a) low oil and b) high oil spiking:

Control treatments: *LCrb; DCrb.*

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).

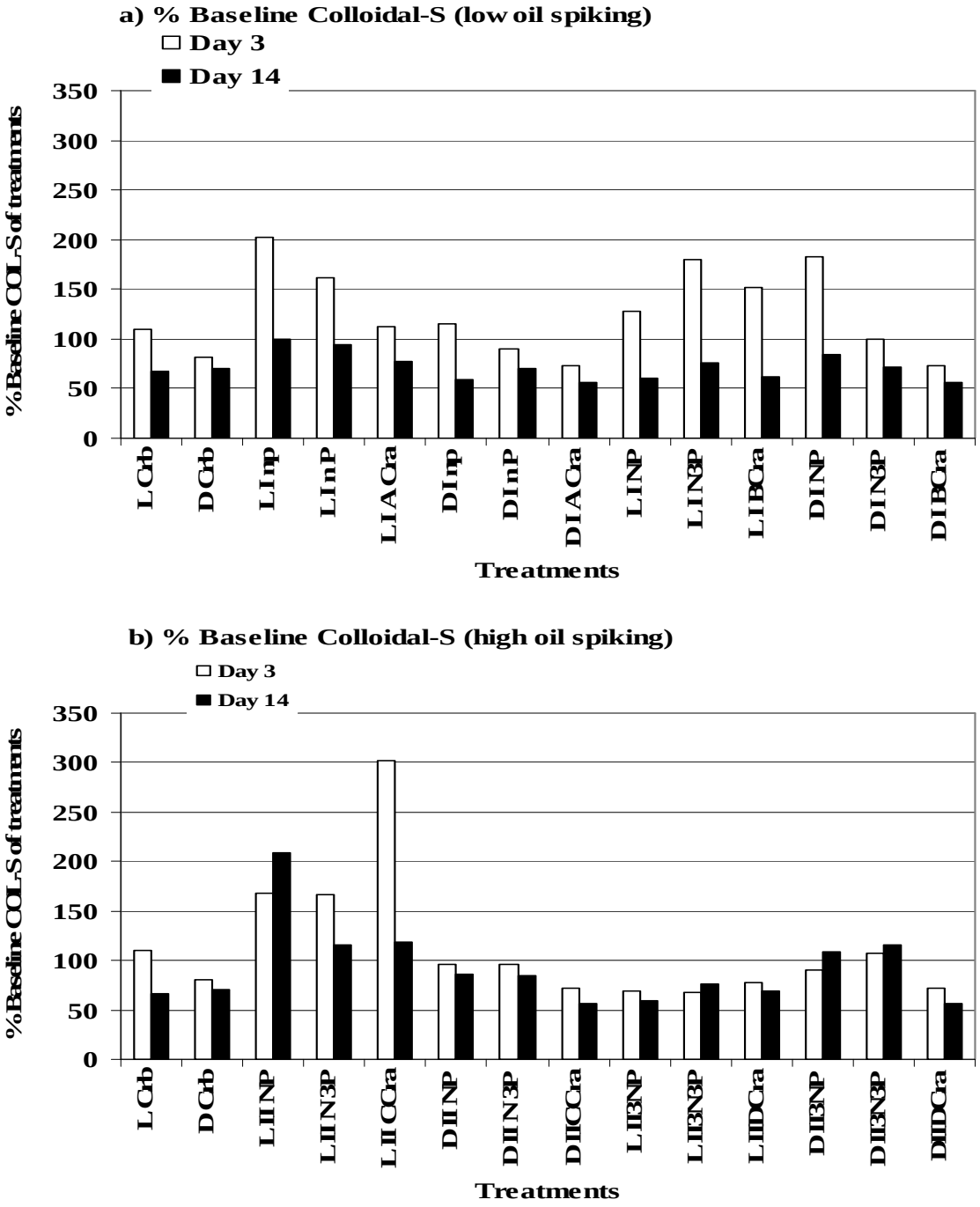

Figure 8: % COL-S corrected to the baseline value at a) low oil and b) high oil spiking:

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).

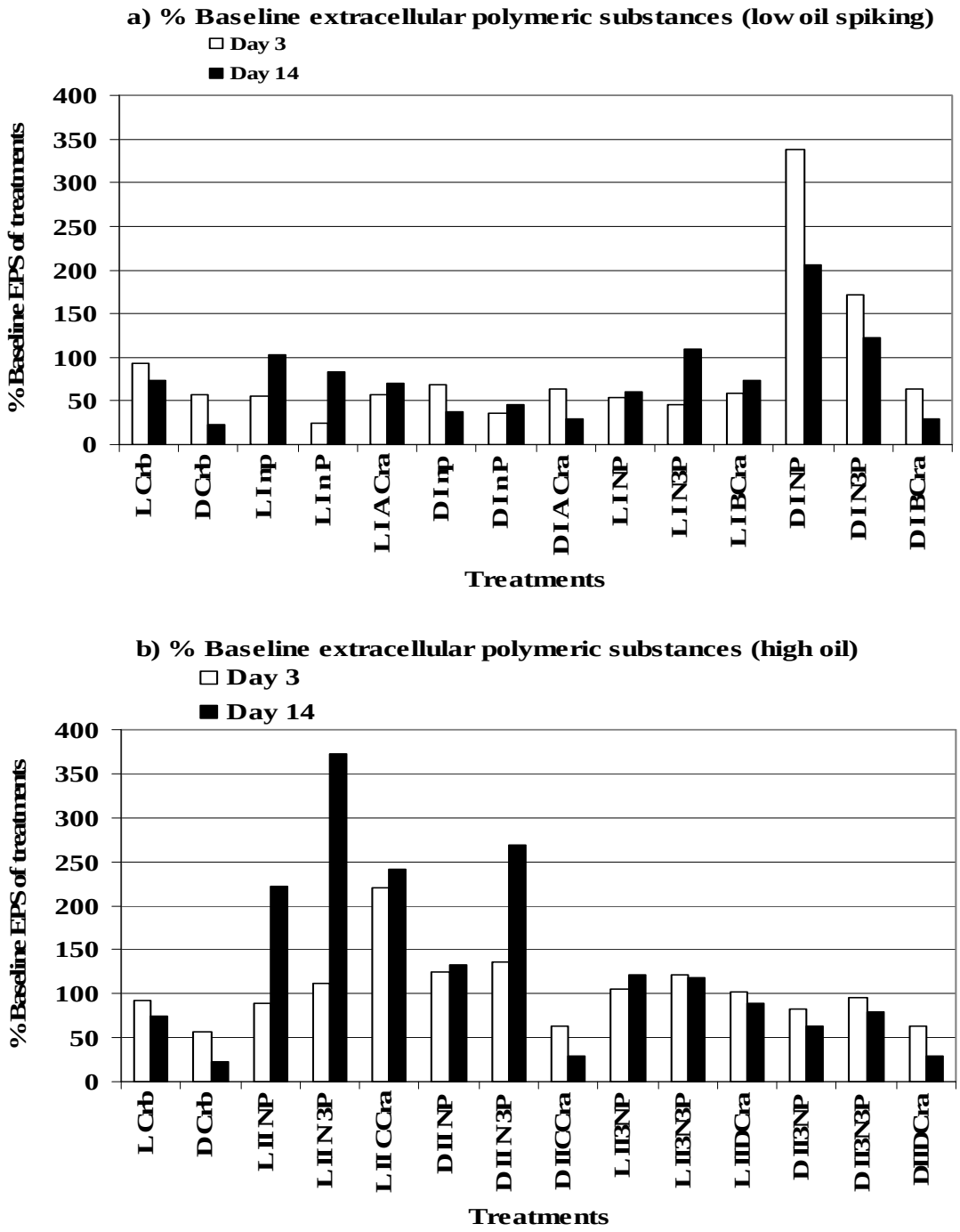

Figure 9: % EPS corrected to the baseline value at a) low oil and b) high oil spiking:

Control treatments: *LCrb; DCrb.*

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).

3.1 Total carbohydrates (TCHO).

The TCHO baseline status of the mesocosms indicated significant differences in all the Experiments (A, B, and C; P = <0.001; <0.05 for D) before treatment was started. Inter-mesocosm comparison of treatment effects were therefore expressed as percentages of the background values.

Biofilms Response I & II (Crb & Cra)

The effect of periodic variation at low oil spiking in the light in Experiment A & B (Fig 4 a & c) indicated that all the treatments on the 14th day were lower in TCHO than the baseline value (*LCrb*), (P = <0.05). Indicating the negative effect that oil-spiking could have on carbohydrate biosynthesis. The non-enriched oil-spiked control *Cra* was lowest. This could imply increased sequestration in this treatment. In the dark, indication was that all the enrichment treatments were higher in TCHO on the 14th day. This could result from the effect of nutrient enrichment and diurnal effect.

At higher oil impactation, the baseline TCHO was consistently and significantly higher than all the oil-impacted treatments in the light (P = <0.001). In the dark, all the control treatments were lower in TCHO biosynthesis than the enriched treatments. After correction to the baseline values (Fig 7 a & b; c & d), indication was that it was the control treatments *LCrb, DIACra*, *DIBCra* (130% each) and the treatment *DInp* (100%) that were higher than or equal to baseline TCHO biosynthesis.

Biofilms Response III

Among the enrichment treatments, *LInp* and *LINP* were significantly higher in which TCHO was above 2000 µg g^{-1} of sediment from the effect of periodic variation. In the dark indication was that there was lower TCHO at lower oil-spiking in the controls (Fig 4 b & d) in which the treatments *DInp* and *DINp* were higher than 2000 µg g^{-1} of sediment from the effect of periodic variation at day 14 (P = <0.05); ranging from 1015.3-2935.3 and 1515.3-2935.3 respectively in µg g^{-1} of sediment.

At higher oil spiking, a comparison of the treatments with respect to the control *Crb*, indicated increase in TCHO above 2000 µg g^{-1} of sediment in the light in the treatments *LCrb*, *LII3NP* and

LII3N3P (P = <0.001); ranging from 442.0-3495.3 µg g^{-1} of sediment as shown in Fig 4 e, f on the 14th day from the effect of periodic variation. In the dark, on the 14th day the treatments *DIINP* and *DII3NP*, were over 2000 µgg^{-1} of sediment.

After correction to the baseline values (Fig 7 a & b; c & d), the enriched treatments *LInp*, *LInP* and *DInp* were approximately 100% and were equal to the baseline values as shown (in Fig 4, a-d), in Experiments A and B. At higher oil-spiking, at moderate enrichment, after correction to the baseline value (Fig 7 b), the treatments that were over 100% baseline values were *DIINP* (570%) followed by *LIINP* (350%); *LIICCra* (295%). Also at higher oil spiking in Experiments C and D (Fig 4 f and h), it was the dark phase enrichments respectively *DIINP* and *DIIN3P; DCCra (130%)* at day 14 that indicated significantly increased TCHO; ranging respectively from 318.7-2935.3 and 1082.0-2942.0 µg $g^{-1.}$ The shift from light to the dark following nutrient enrichment from low to high oil-impacted treatments was a distinct departure from the defined pattern in the baseline control treatment *Crb* for the system under investigation. This appears to indicate a probable syntrophic relationship among the estuarine biofilms in which photo-autotrophs could cope better with low level of oil impactation in the light but at higher oil impactation, a switch was made to the dark phase in which some nutrient dependent heterotrophic forms were probably protected sufficiently in the dark (probably from photo-reactions), to cope with the increased level of toxicity of PHC. This effect was also corroborated by a similar shift to the dark phase at higher oil-impactation observed earlier in the response of the biofilms to PHC.

The inter-mesocosm comparison for the comparatively best treatment effects for TCHO biosynthesis during oil impactation, in Fig 7 a-h), also confirmed that the enrichment treatment *DIINP* (570%), in the dark was more than 5 fold higher in TCHO biosynthesis than the corresponding treatment *DINP* (100%) at lower oil spiking in enhancing TCHO biosynthesis. The implication of this appears to indicate that at day 14, the estuarine biofilms in the dark (with

increased nutrients and the corresponding higher spiked oil) were more efficient in enhancing TCHO biosynthesis.

3.2 Colloidal-S

The baseline status of mesocosms indicated significant differences in background COL-S concentration in all the Experiments (A, B; P = <0.001; <0.05 for C and D) in their background COL-S concentrations before treatment was started. Inter-mesocosm comparison of treatment effects were therefore expressed as a percentage of the background value.

Biofilms Response I & II (Crb & Cra)

The effect of periodic variation at low oil impactation in the light in Experiments A and B (Fig 5 a & b), indicated that all the enrichment treatments were higher than the control treatments *Crb* and *Cra*, ranging between 100 – 200 µg g^{-1} of sediment (P = <0.001) from the effect of periodic variation. For the control *Cra*, there was indication of decreasing COL-S on the 14th day. In the dark, indication was that all the enriched treatments were higher in COL-S than the control treatments. At higher oil impactation (Fig 5 e-h) in the light all the treatments were higher in COL-S than the baseline value. In the dark all the control treatments were lower in COL-S biosynthesis than the enriched treatments.

Biofilms Response III

At low oil impactation, the treatments *LInp* and *LInP* were on the increase on the 14th day. In the dark, in both Experiment A and B (Fig 5 b & d), all the treatments including the controls indicated decreasing COL-S on the 14th day. After correction to percentage baseline value however, it was only the treatment *LInp* that was100% baseline value with indication of decreasing COL-S, of 201 to 100% baseline from the 3rd to the 14th day respectively. Indicating that even at low oil impaction with limited enrichment, COL-S biosynthesis was adversely affected by the added oil.
At higher oil impactation with increased enrichment (Experiments C and D; Fig 5 e & g), the effect of light inducement was clearly shown in which all the treatments were higher than the control *Crb*

on the 14th day ranging between 50 – 150 µg g-1 of sediment from the effect of periodic variation. In the dark, as with the low oil impactation, COL-S was decreasing till the 14th day in all the treatments and was lowest with the control *LIICCra* in which the control *DIICrb* was higher than the oil impacted treatments. This probably indicated that while nutrient enrichment improved COL-S production in the light, it probably negatively affected the estuarine biofilms in the dark at the end of the 14th day. After correction to baseline values, it was the treatments *LIINP* (220%), *LIIN3P* (120%) and *LIICCra* (125%) at high oil spiking that were higher than the baseline value on the 14th day in the light. In the dark the treatments that were higher than baseline value were *DII3NP* (110%) and *DII3N3P* (120%) on the 14th day.

3.3 *Extracellular polymeric substances (EPS)*

The baseline status of mesocosms indicated significant differences in background EPS concentrations in all the experiments (A, B and D P = <0.001; P = <0.05 for C) in their background EPS concentrations before treatment was started. Inter-mesocosm comparison of treatment effects were therefore expressed as a percentage of the background value.

Biofilms Response I & II (Crb & Cra)

The effect of periodic variation at low oil impactation in the light (Fig 6 a & c), indicated that all the treatments were higher in EPS production than the baseline control *Crb* on the 14th day. While the oil-spiked non-enriched control was variously higher than the enriched treatments *LInp* and *LInP* in the light on the 14th day. In the dark (Fig 6 b & d), the enriched treatments indicated higher EPS than the control treatments. While the effect of nutrient enrichment in enhancing EPS biosynthesis in the light and dark phases could be appreciated, the effect of PHC on EPS by the non-enriched control could not be easily explained. It could be suspected that it was due to increased sediment sequestration of oil, which provided a suitable environment for an increased EPS biosynthesis.

At high oil-spiking, in the presence of moderate nutrient enrichment in the light (Fig 6e), indication was that the oil-spiked control *Cra* was significantly higher than all other treatments in

which the baseline control *Crb* was lowest. But at maximum nutrient enrichment in the light (Fig 6 g) there was sequestration or loss of response or state of 'shock' from day 7 – 14. It was not clear if this was the effect of light induced exacerbated oil breakdown accompanied by an increased PHC toxicity. In the dark at high oil impactation generally, the control treatments were lower in EPS biosynthesis than in the nutrient enriched treatments on the 14th day. This again points to the vaguely understood effect of dark phase in inducing increased EPS biosynthesis.

Biofilms Response III

At low oil-spiking, the effect of periodic variation indicated that the treatment *LINP* was higher than the control treatments *LICra* and *LCrb* indicating a positive effect as a result of the enrichment treatments on EPS production in the light. In the dark (Fig 6 c & d) at low oil spiking treatments *DInp* and *DINP* were higher than the controls *Crb* and *Cra* (P = <0.05); ranging from 5.7-47.6 and 4.5-53.0 respectively in Experiment A and B µg g^{-1} of sediment. This again indicated that enrichment was of greater importance in the dark for increased EPS production.

After correction to percent baseline value the indication was that the dark treatments (Fig 7 a) gave better EPS production viz. *DINP* (205%); *DIIN3P* (150%); while the following light treatments were also above 100% baseline value viz. *LIIN3P* (120%) and *LInp* (105%). At higher oil impactation in the light (Fig 6 e & g), indication was that at moderate enrichment in Experiment C (Fig 6 e) the control treatment *Cra* was higher in EPS production followed by the treatment *LIINP* and *DIINP* while the control *Crb* was least on the 14th day (P = <0.05) EPS ranging from 6.3-66.3 µg g^{-1} of sediment. Increasing nutrients in Experiment D appear to cause severe sequestration in all the treatments on the 14th day in the light. It was not clear what caused this effect. Again, it was not clear if this was the effect of light induced exacerbated oil breakdown accompanied by an increased PHC toxicity. But in the dark, some of the enrichment treatments (viz. *DIINP*, *DIIN3P*, *DII3NP* and *DII3N3P*) indicated significantly higher EPS production than the control treatments on the 14th day. After correction to baseline, the treatments *LIIN3P* (was 370%), *LIINP* (230%), *LIICCra* (240%),

DIIN3P (265%) and *DIINP* (130%) higher than the baseline value. This could imply that at increased oil spiking, there was light inducement of EPS production which contrasted with the observed effect at low oil spiking in which there was improvement in the dark.

3.4 Comparison of the effect of PHC on different Carbohydrates

At low oil-spiking, TCHO biosynthesis was higher in the baseline value than in the nutrient enriched treatments. For COL-S and EPS, their biosynthesis was higher in the oil-spiked and enriched treatments in the light than the baseline value. In the dark all the carbohydrates were higher in the enriched treatments than in the controls treatments.

At higher oil impactation, the baseline TCHO and COL-S were higher than all the oil-impacted treatments in the light. For EPS, at high oil-spiking, in the presence of moderate nutrient enrichment in the light (Fig 6e), indication was that the oil-spiked control *Cra* was significantly higher than all other treatments in which the baseline control *Crb* was lowest. This appears to point to **the** peculiar response of biofilms in exuding much EPS when challenged with high concentration of contaminants in the estuarine ecosystems. But at maximum nutrient enrichment in the light (Fig 6 g) there was apparent sequestration or loss of response or state of 'shock' from day 7 – 14 from the effect of periodic variation although there was actually overall improvement in EPS biosynthesis in the light when compared to the baseline value.

In the dark at high oil impactation the control treatments were lower in TCHO, COL-S and EPS biosynthesis than the nutrient enriched treatments on the 14th day. At higher oil impactation, the baseline TCHO and COL-S were higher than all the oil-impacted treatments in the light on the 14th day. For EPS indication was that the oil-spiked control which was not enriched was significantly higher than all other treatments in which the baseline control *Crb* was lowest. But at maximum nutrient enrichment in the light there was sequestration or loss of response or state of probable 'shock' from day 7 – 14. But in the dark, all the control treatments were lower in TCHO, COL-S and EPS biosynthesis than in the enriched treatments.

At low oil-spiking, after correction to the baseline values indication was that there were varied responses for TCHO, COL-S and EPS. For TCHO, it was the control treatments *LCrb, DIACra, DIBCra* (130% each) and DInp (100%) that were higher than baseline TCHO biosynthesis. For COL-S, after correction to percentage baseline value however, it was only the treatment *LInp* that was100% baseline value. For EPS the indication was that the dark treatment gave better EPS production viz. *DINP* (210%); *DIN3P* (125%); while the following light treatments were also above 100% baseline value viz. *LIIN3P* (110%) and *LInp* (105%).

At higher oil-spiking, for TCHO, after correction to the baseline value the treatments that were over 100% baseline values were *DIINP* (570%) followed by *LIINP* (350%); LIICCra (295%). For COL-S, it was the treatments *LIINP* (220%), *LIIN3P* (120%) and *LIICCra* (125%), *DII3NP* (110%) and *DII3N3P* (120%) that were higher than the baseline value. For EPS, the treatments *LIIN3P* (was 370%), *LIINP* (230%), *LIICCra* (240%), *DIIN3P* (265%) and *DIINP* (130%) higher than the baseline value.

4.0 DISCUSSION

4.1. Carbohydrates

An overview on the experimentation results on carbohydrates in the Mesocosms.

The general observation from the results in all the experiments for the carbohydrates showed that the total carbohydrates and colloidal-S fraction appear to be more attenuated (or stressed) in the dark with a steady decrease up to the 14th day than in the light from the effect of added oil. There was an actual enhancement in COL-S, in the light at moderate enrichment at high oil-spiking in which a steady increase was observed till the 14th day. On the contrary, the EPS showed some attenuation in the light with a steady increase in concentration in the dark till the 14th day in all the experiments.

The enhancement effect of the dark phase for EPS had been reported (Smith and Underwood, 2000); while the compensatory role of the light phase for the TCHO and COL-S had also been reported by Underwood and Paterson, 2003). There were indirect references from the review of Decho (1990) on the microbial extracellular polymeric substances secretions in ocean environments, to the effect that EPS was responsible for the sequestration, concentration, detoxification and biodegradation (by mediating enzymes) of not only detritus and protein substances but also of smaller particles like the amino acids (and probably contaminants like PHC). It was also important to note that the EPS in its biosynthesis (according to Decho 1990) exhibited much plasticity and could form 'customized' variants depending on available precursors, functionality of the cell and also with respect to the physical environmental conditions. Furthermore, that in its formation, lipoic lipid intermediates were involved. It could be inferred that EPS could be most likely incriminated in the active involvement in the sequestration, concentration, detoxification and enzymatically mediating the biodegradation of PHC in the mesocosms (and probably in the ecosystem). A more in-depth study is needed to confirm this observation.

In view of the fact that EPS was derived from COL-S and the later from TCHO, it was logical to adduce that probably there were other components of TCHO which were not assessed or captured in this report which may be responsible for the observed differences between TCHO and COL-S in their responses to PHC; similarly, that there may also exist other components of COL-S which were not assessed in this report which may be responsible for the observed differences between COL-S and EPS in their responses to PHC. There is need for more studies to elucidate the missing link.

4.2. Effect of petroleum hydrocarbon on estuarine biofilms carbohydrates biosynthesis.

The residual assessment of TCHO from periodic variation indicated that there was continuous increase in biosynthesis up till the 14th day in the non-oil impacted control *Crb*, in the light, but there was a steady decrease in the dark. For the oil-impacted control *Cra*, there was a

decrease in the biosynthesis of TCHO both in the light and in the dark but more so in the dark. For COL-S, there were some similar effects to the TCHO with increased concentration in the residual COL-S over time while for the oil-impacted control; there was decrease in the concentration in the dark. A close observation of the oil-impacted control *Cra*, showed higher concentration of COL-S than in the non-oil impacted control *Crb*, but then, while there was indication of increasing COL-S concentration in the later at the 14th day, the concentration in the former was in a state of steady decrease till the 14th day. This appears to indicate 'shock' effect in which stressed biofilms in the oil-spiked control *Cra* might have copiously exuded COL-S as a protective mechanism from the deleterious effect of PHC; the presence of 'shock proteins' which are mediated by EPS, by which biofilms non-specifically defend themselves had been reported (Allison et al., 2000).

The effect of oil on EPS biosynthesis appear to even add a further dimension to the above; as contrary to the observation of the effect of PHC on TCHO and COL-S biosynthesis, there was a steady decrease in the residual EPS (i.e. control *Crb* in the light) while in the dark there was a steady increase in concentration in all the Experiments on the 14th day. For the oil-impacted control *Cra*, there was largely the same effect as in the non-oil impacted control in which there was indication of some increase in EPS concentration in the dark on the 14th day (except at low oil-spiking in Experiment A in the light). By implication, therefore, as again shown by the increase in EPS concentration, the oil-impacted control could be described as being in a state of 'shock' from the impacting oil (Decho, 1990; Allison et al., 2000).

There are a number of reports on the importance of extracellular polymeric substances (EPS) in the biofilms. The whole concept of biofilm communal existence; resilience to chemicals, existence in extreme temperatures, resistance to desiccation; exchange of nutrients from the exterior of the cell to its interior, accumulation of organics, attenuation of minerals, quorum sensing etc. is almost synonymous to the nature and properties of EPS (Boult et al (1997; Decho 1999; Wolfaardt et al 1995; Allison et al., 2000).

4.3. Effect of nutrient enrichment on biofilm carbohydrates biosynthesis from oil-impactation.

A general overview of the effect of nutrient enrichment of TCHO biosynthesis for the oil-impacted mesocosms in the Experiments showed that there was enhancement only in the dark as all the treatment effects indicated decline in the concentration of TCHO in the light; the effect of adding nutrients appear to have enhanced a shift to the dark phase which state appears to indicate the *status quo* as observed for the control *Crb*. The treatment of choice for increase in TCHO concentration was *DIINP* (and this was over 500% compared to the percent baseline value). For COL-S, there were clear departures from what obtained for the TCHO with respect to the effect of nutrient enrichment. There were increases in COL-S in most of the Experiments in the light in which the treatment of choice was *LIINP* (this was 200% increase over the percent baseline value). The situation was also different from the TCHO and from COL-S for the effect of nutrient enrichment on EPS biosynthesis. While the assessed values were higher than those of the non-oil spiked control, there were indications that EPS concentration increased both in the light and in the dark from the percent baseline value; the treatments of choice being *LIIN3P* (over 350%) at high oil spiking and *DINP* (over 200%) at low oil-spiking. Yet the overall scenario clearly indicated that the dark phase was better than the light phase because at the 14^{th} day EPS concentration was on the decrease in the light (regardless of the higher determined values); but was on the increase in the dark in all the mesocosms. Viewed against the background effect of these nutrients being perceived not only as nutrients for biosynthesis but also as alternate terminal electron acceptors in place of oxygen it could be adduced that nutrient enrichment is of primary importance against smothering effect of aerobic forms in the oil-impacted systems (and probably in oil-polluted ecosystems). In other words, aerobic biodegradation of PHC (which is known to be faster than anaerobic biodegradation) could still be carried out by the biofilms *anerobically* in low oxygen gradient microenvironments (if there was sufficient nitrate and phosphates) as in the oil-in-water droplets. The effect of nutrient enrichment especially of nitrate and phosphate had been reported by a number of authors (Lessard et al., (1995).

The central question about how PHC affected the biofilm capability (*Cra*) showed that the effect on TCHO and COL-S indicated a decrease in their concentrations at the end of the 14th day in the light and in the dark (with the exception of the low oil-impactation in Experiment B). The estuarine biofilms appear to owe their survival when impacted with oil (at low or high concentrations) to the production of EPS especially in the dark (with possible enhancement in the light at low oil-spiking). The dark phase enhancement of estuarine biofilms to profusely synthesize EPS in the dark as earlier reported by Smith and Underwood, was not only corroborated but could be further extended to include that the fact that estuarine biofilms could concentrate, sequester, detoxify and enzymatically mediate the biodegradation of PHC (and probably other contaminants) in the dark (Smith and Underwood 2000; Decho 1990).

The whole episode appear to indicate that the biofilms could cope better with the toxic effects of PHC at higher concentrations in the dark, provided there was appropriately added nutrients (Lessard *et al*., 1995). With respect to the 'shock' effects on biofilms from toxic contaminants there is need therefore from recent research works and publications to review some earlier observations on the impact of PHC on the ecosystem which eluded that oil could impact positively or innocuously on the ecosystem or even on crops.

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

1. *That under natural conditions there was enhancement of TCHO and COL-S biosynthesis in the light while EPS was enhanced in the dark.*
2. *That while at low and high oil-impactation (without nutrient-enrichment) there was a mixed response of light and dark enhancement in TCHO biosynthesis, there was light enhancement for COL-S biosynthesis and dark enhancement for EPS biosynthesis.*

3. *That at low and high oil-impactation (with nutrient-enrichment) TCHO biosynthesis appears to shift to the dark phase while for COL-S and EPS there were mixed responses for the light and dark phases for better biosynthesis.*

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