Physiological And Biochemical Studies On The Effect Of Crude Oil And Two Of Its Derivatives On Some Microalcae

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Summary

Aquatic algal communities are often affected by anthropogenic disturbances to the environment. The main pollutants that could be found in polluted water bodies may constitute heavy metals, insecticides, pestisides, nitrogen and phosohorus elements, ammonia and lastly oil pollution. In general oil pollution is the main serious problem along the coast of the seas and oceans.

Oil in the water bodies may occur in different forms and fractions in water. It is well known fact that ships are the only means for transportation of crude oil which may play a role in sea water pollution. So the main aim of this work was concentrated on the effect of different concentrations of the water soluble fractions of crude oil and two of its derivatives: benzene and kerosene and metabolism of two microscopic marine green algae Chlorella salina and Nannochloropsis salina which were considered to be within planktonic algae used mainly for fish feeding.

The results obtained could be summarized in the following:

1- Values of growth parameters of both C. salina and N. salina cultured in the basal medium (control) showed nearly linear relationships from the begening to the end of the experiment with a very short lag phase. However, both organisms reached nearly their maximum growth values at the 8th day of culturing

2- Taking into consideration the effect of different concentrations of the W.S.F. of crude oil, benzene and kerosene on growth of both C. salina and N. salina, the

results cleared that toxic effects of these 'soluble fractions depend mainly on:.

a) Type of oil extract used: kerosene was found to be more toxic than benzene on growth of both Nannochloropsis salina and Chlorella salina whereas crude oil was the least of them.
b) Concentration of the extract: the low concentration stimulated growth but high concentrations had an inhibitory effect. This phenomenon also varies according to the type of extract.
c) Length of culture period: a lag phase usually resulted prior to the onset of growth. The length of the lag phase depends on type of organism as well as type and concentration of the extract.
d) Type of the organism tested: where growth of Nannochloropsis salina was found to be more tolerant to toxicity of water extract of crude oil, benzene and kerosene than Chlorella salina.

3- Under normal conditions, the IR band assignments based on the studies of whole cell constituents at the region from 3650 to 700 cm' are nearly the same for both C. salina and N. salina.

4- The treated organisms with the water soluble extract of crude oil and its refined fractions used in this work showed another peaks (when compared to untreated cells) which might resulting either from new compound or the changes in the position of some side chains of the same compounds or disappearance of some others. The number of beaks in the

treated organisms usually in most cases higher than control and were more prominent at higher concentrations.

5- The toxicity of kerosene concentrations on the cellular constituents of Chlorella and Nannochloropsis was more prominent than that of crude oil and benzene.

6- A glimpse at values obtained for quantitative analyses of chlorophyll fractions in C. salina and N. salina under normal conditions, it could be ascertained that total chlorophylls increased gradually by increasing days of culturing till the 10" day where it began to decrease.

7- On the basis of results obtained for the effect of different concentrations of W.S.F. of crude oil, benzene and kerosene on chlorophyll fractions in both C. salina and N. salina, it is relevant to mention that both organisms gave different values at the three different extracts: Chlorella was more sensitive to crude oil and kerosene than Nannochloropsis, while for Nannochloropsis the toxic effect of benzene was significant at concentration 25%, but very highly significant at concentration 100% which indicates that Nannochloropsis is more sensitive to benzene than Chlorella.

8- Chlorophyll fractions in both organisms cleared that toxic effect of all the extracts was very highly significant at higher concentrations, but at lower concentrations the toxic effect varied from non-significant to significant depending on concentration, type of the extract and on the organism tested.

9- Content of protein fractions in C. salina and N. salina under normal conditions (control), (soluble, insoluble and total proteins) increased gradually by increasing days of

culturing till the 16ch day, then it decreased as the culture ages due to deficiency of nutrients specially at the last days of culturing.

10- Protein synthesis by C. salina and N. salina treated by W.S.F. of crude oil, benzene and kerosene revealed that by increasing either the concentration of the extract and period of culturing the content of insoluble proteins decreased while soluble ones increased and N. salina was found to be more sensitive to toxicity of all extracts than C. salina. However, the degrees of decrease or increase for both types of proteins differed according to the type and concentration of extract and the organism itself.

11- At normal state synthesis of carbohydrate fractions by both tested organisms increased gradually with increasing periods of culturing till the 12 h day, then decreased. These give nearly parallels similarities to the values of growth rate for the two algae.

12- Content of carbohydrate fractions in Chlorella and Nannochloropsis treated by W.S.F. of crude oil, benzene and kerosene greatly affected by the different concentrations of the extract and by the length of culturing periods. The lower concentrations of all types of extracts (25%) significantly increased the synthesis of carbohydrates. The reverse could be observed at higher concentrations where the toxic effects of extract were found to be highly significant. Also, C. salina was found to be more sensitive to extracts of crude oil and kerosene than N. salina, while in case of benzene extracts the reverse could be observed.

13- The experimental two alga shared a pattern with 17 different amino acids having different concentrations for each alga.

14- Under normal conditions, N. salina was found to be richer in the content of conjugated, free and total amino acids grown on basal medium than in C. salina (2.4 fold). Also, the content of essential amino acids in protein of N. salina was higher than in C. salina which may prove that protein of N. salina is more nutritive than C. salina.

15. With regard to the changes in amino acid composition in response to W.S.F. of crude oil, benzene and kerosene treatment, the obtained results proved that toxic effect of these extracts caused inhibition in the biosynthesis of all groups of amino acids in the two tested algae except proline. The degree of inhibition differed according to the type of alga, the type and concentration of the extract and the group of amino acids. The sharp decrease in the content of total amino acids was recorded at 100% water extract and the decrease was more prominent in kerosene than benzene and crude oil extracts.

16- At concentration 100% crude oil extract, the decrease in free, conjugated and total amino acids was very highly significant in Nannochloropsis while in Chlorella it was found to be only significant.

17- In case of water soluble fractions of benzene, the decrease in the total amino acids in Nannochloropsis was very highly significant, while for Chlorella it was highly significant.

18- The response of free, conjugated and total amino acids to concentration 100% kerosene extract in both Nannochloropsis and Chlorella cleared that this concentration in both organisms drastically decreased when compared to those results obtained in case of crude oil and benzene and, the toxic effect on Nannochloropsis was more effective than in Chlorella.

19- The total amino acid of krebs cycle family that contained most of the essential amino acids decreased by 50.8% and 37.17% in Nannochloropsis and Chlorella, respectively compared to control.

20- The effective agents could be arranged with respect to their drastic action on amino acid synthesis in the following sequence:

crude oil < benzene < kerosene.

21- On the contrary, an increase in the content of proline in the two algae cultured under the toxic effects of water extract of crude oil, benzene and kerosene was observed. The increase in proline content was more prominent in N. salina than in C. salina.

22- Under normal stage, the two tested genera (Nannochloropsis salina and Chlorella salina) contained nearly the same fractions of fatty acids (18 fractions in Chlorella and 17 fractions in Nannochloropsis), but differed in their quantities. Contents of the three groups of fatty acid fractions after 16 days of culturing reached nearly 2 fold of those obtained after 8 days of culturing. In both species the monounsaturated fatty acid C16:1 (n-7) and the

polyunsaturated fatty acid C22:6 (n-3) were the most dominant ones and N. salina was found to be richer in the content of total fatty acids than Chlorella salina.

23- The three groups of fatty acids content in the two tested organisms after 16 days of incubation in relation to the four concentrations of crude oil, benzene and kerosene, are greatly affected especially at higher concentrations of the extract. The toxic effect of the extract differed according to type and concentration of the extract, group of fatty acids and the organism itself.

24- Both organisms suffered greatly from the toxic effects of crude oil, benzene and kerosene extracts, but Nannochloropsis was more sensitive to these extracts than Chlorella. Also, kerosene was found to be more toxic than both benzene and crude oil.

25. Fatty acids belonging to the omega-3 type in both N. salina and C. salina, at higher concentrations of water extract of crude oil, benzene and kerosene were greatly reduced. The degree of reductions was found to be closely related to type and concentration of extract and type of algae where it was prominent at concentration 100% extract of kerosene than benzene and crude oil and in Nannochloropsis than Chlorella.

26- The above mentioned conclusion could prove that both organisms under the toxic effect of crude oil, benzene and kerosene extracts lost their nutritive value as a source for polyunsaturated fatty acid specially those of omega-3 family.

27- The profile of total soluble protein bands for both the treated C. salina and N. salina with concentration 100% crude oil, benzene and kerosene showed that some bands that appeared in control disappeared, while others appeared new still others remained in both control and the treated organisms.

28- The protein bands that disappeared in the treated lanes are at the region from 167 to 212 kd. i.e. at the region of high molecular weight while those that appeared new were of low molecular weight region. The appearance and disappearance of bands depend on the type of extract and the organism itself. Protein profile proved that C. salina is more tolerant to the toxic effect of the three water extracts than N. salina.

29- Plates of the electron microscope for Chlorella salina and Nannochloropsis salina under the toxic effect of 100% water soluble extract of crude oil, benzene and kerosene cleared that both organisms are greatly affected by the toxicity of the extract. The deleterious effect of kerosene was more prominent followed by benzene then crude oil. The most clear symptoms are dissolution of chloroplast lamellae, shrinking of cell contents away from the cell wall and the cell wall become irregular and more thicker.