

Quantitative Assessments of Environmental Factors Controlling Distribution of Oil –Degrading Bacteria in Shatt al-Arab and North-west Arabian Gulf

Tehran S. Zeiara and Adnan N. Abdual-Ritha

Department of Biology, College of science, University of Basrah.

Abstract:

Considerable numbers of physical, chemical and bacteriological parameters were measured at seven stations in Shatt Al-Arab and North- west Arabian gulf during the period extended from winter 1994 to autumn 1995.

The observed densities of oil- degrading bacteria were comparable to those reported for the same region. In general higher densities were observed in summer, while the lower densities were recorded in autumn.

Statistical treatments of the monitored parameters demonstrated the following:

- 1. Stations 4,6 and 7 revealed a different trend in their distribution functions of the measured environmental parameters varied significantly of the situations of other stations.
- 2. Summer season showed dissimilar mode of variations dynamic, different largely, of the rest seasons, while winter and autumn were coordinated- approximately, in the same patterns of responsibility and distribution functions.
- 3. Same groups of environment parameters were marked in highly percentages of representation in the principal components reflecting specific signification effects of these parameters on the studied circumstances and occurred fluctuations.

Key words: oil degrading bacteria (ODB), oil, pollution.

Introduction

Oil –pollution represent the dominant threat in the Arabian gulf, so the qualitative and quantitative studies of oil –degrading bacteria (ODB)- as bio-indicator of oil pollution in the aquatic environments- has been attracted the attenuations of environments.

Isolation and enumeration of ODB from aquatic environment are subjected to a number different factors.^(1,2,3,4)

The factors contribute to the distribution of ODB including: pH, temperature, dissolved oxygen, salinity, nutrients, hydrocarbons concentrations...etc^(2,5,6,7)

Many studies indicated the fluctuations and correlations between bacterial density and environmental factors, and these studies marked, clearly, the significant effects of the factors on the density and distribution of ODB $^{(8,9,10)}$

Studies concerned with the quantitative assessment of the aquatic environment, normally, require many qualitatively different types of measurements, included: estimation of the concentrations of what may be a considerable number of physical, chemical and biological components, each of which must be estimated separately, so that contribution of each to over all problems must be inferred ^(11, 12, 13)

If the environmental sampling collection for surface water is to yield adequate information, we must point which physical, chemical and biological measurements provide the most sensitive indices of :



- 1. Water quality and differences of water quality between different sampling sites.
- 2. Patterns of the fluctuations and correlations between various environmental factors at different sites and in different circumstances, to adapt the best choice of sampling sites and the frequency of reported measurements ^(14,15)

In the present study we try to make a clear declaration of quantitative assessment of the significant environmental factors, which play an important role in controlling the distribution of oil degrading bacteria in Shatt al-Arab and North-west Arabian gulf.

This assessment is done to demonstrate the relationships between studied parameters providing best clustering of the environmental factors which help in indicating the most effective ones that directed the abundance and distribution of oil-degrading bacteria.

Materials and Methods:

Seven stations were chosen to represent different sectors of Shatt Al-Arab estuary and North West Arabian gulf. Figure (1) for the study of the physical, chemical, and bacteriological parameters.

At each of the above station 24 samples of surface water (10cm deep) were collected in 500ml sterile glass bottles during the period of the study, this period was extended from winter 1994 to autumn 1995. Collected samples were freezing deeply and transferred in cool box to the laboratory.

Direct plating of ODB was carried out on agar medium B ⁽¹⁶⁾ for north –westen Arabian gulf samples, while for samples collected from Shatt Al-Arab estuary the medium described by ⁽¹⁷⁾ was used for enumeration ODB.

These above media were supplemented with 100M1/100ml of sterile weathered regular Basrah crude oil (API 33.9) as the sole of carbon and energy source .

Five milliliter of each water sample was filtered through sterile 0.45 μ m Millipore filter which was then placed on oil ager plates , triplicate plates were incubated at 20 ± 2 °C for up 28 days , after incubation period the numbers of ODB of each plate were recorded and the averages of bacterial densities were reported ,PH , water , temperature , dissolved oxygen ,and salinity were measured in situ using direct reading neater . Another liter of the collected surface water filtered through 0.45 μ m Millipore filter before measuring nitrite, nitrate, inorganic soluble phosphate and chlorophyll a concentration using the methods described by (18).

Determination of hydrocarbons concentration was done according to the procedures described by $^{(19,20)}$

Statistical procedures:

Two approaches were used in the analysis of the reporting data of the measured parameters:

Firstly: Cluster Analysis

Secondly: The principal component Analysis (PAC) .

Cluster analysis:

Several resemblance coefficients may be used in this analysis among these are two coefficients are very common and used in the present study:

1. Correlation coefficient which computed by the formula :



$$r_{xy} = \frac{n \sum_{i=1}^{n} X_{i} Y_{i} - (\sum_{i=1}^{n} X_{i}) (\sum_{i=1}^{n} Y_{i})}{\sqrt{\left[n \sum_{i=1}^{n} X_{i}^{2} - (\sum_{i=1}^{n} X_{i})^{2}\right] \left[n \sum_{i=1}^{n} Y_{i}^{2} - (\sum_{i=1}^{n} Y_{i})^{2}\right]}}$$

The equation is used to measure the correlation between each pair of factors. The range of each correlation coefficient should be between -1 and 1 where higher similarity and correlation when the value of this coefficient become closer to 1.

2. Standardized Euclidian which is computed by the formula :

$$d_{xy} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(S_{xi} - S_{yi}\right)^2}$$

Where:

 d_{xy} = distance between X and Y.

 S_{xi} = the standardized value of X_i was measured follow:

$$S_{xi} = \frac{x - x}{S}$$

Where:

 \mathbf{x} = the value of determined factor (variable)

- x^- =maen of x value
- *S* =standard of deviation
- n =number of variable

The distance coefficient given by the above equation is a dissimilarity measurement, therefore coefficients which are closer to zero indicate higher similarity ⁽²¹⁾ Principal Component Analysis (PCA):

Principal Component Analysis (PCA):

This constructing technique is used to reduce the variable into fewer orthogonal composite variables which account for the variance in the measured parameters ⁽²²⁾ in order to indicate the most effective factors.

The original variable may be rotated individually to be orthogonal with each other .Each principal component is calculated as follows:

Let: Z_1 be linear combination of original set variable $(x_{Li})^n i = 1$ for station L.

$$Z_{L1} = \sum_{i=1}^{n} b_{i1} S_{Li}$$

For Z_{L1} to be principal component, the vector $b_1 = (b_{11}, b_{21}, \dots, b_{n1})^T$ must be chosen so that the variance of Z_{L1} be maximum, This could be achieved if **b** is eigen vector of **S**. This eigen value will be equal to the variance of the first principal component $Z_{L1}^{(22)}$.

The second principal component \mathbb{Z}_{L2} may be found similarly by computing a corresponding eigen vector b_2 to the second largest eigen value of . Other principal component may be found similarly. Total variability of the fewer principal compound will be higher than 70%.

• Prepared computer program was used for cluster analysis and principal component analysis.



Results and discussion:

Averages of all measured environmental parameters at the studied station were summarized in table 1.

The determined levels of the densities of oil –degrading bacteria were comparable to those reported for the region previously ^{(7, 23, 24).} But they were lower than the densities recorded for other areas ^(17, 25, 26).

According to the seasons analysis, the dendogram of winter showed that station 1 & 5 and 3 &7 were closely related, these two groups were also related strongly, followed by stations 2 & 6. The above six station were correlated together representing main group. Finally the station 4 was linked to this main group.

The dendogram of spring indicated closely relation between station 3 & 5, This related group was closely related with station 1 making an group correlated with station 7. Other correlation was observed in this dendogram, this was between station 2 & 4. The main related groups were linked finally to station 6.

In the summer dendogram station 1 &7 were closely related followed by station 5 and 2 making an group correlated to station 3. The related group if the mentioned station was liked to station 4. The main produced related group was correlated to station 6.

The dendogram of autumn marked clearly two main group firstly was between station 1 &4 followed by station 5, secondly was between station 3 &6 followed by 2, these two major groups were correlated to station 7.

The above dendograms (Fig 2) were showed clearly the situations of the fluctuations and variation in measured parameters at the studied station during the period of the study, where greater variations were observed in summer and spring seasons.

The Euclidian distances for these two seasons were extended from zero to 20000 .0 and zero to 2000 respectively .while lower variations were indicated during autumn and winter .

The studied station were exhibited heterogeneity in the monitored circumstances during summer and spring reelecting the wide range of fluctuations in the determined parameter, while the variations were became lesser ,making the station closer to the homogeneity during autumn and winter seasons.

Correlation coefficient value (table 2) remarked strong correlation between hydrocarbon concentration and bacterial density during winter and spring. Also there was a strong correlation between chlorophyll a concentration and bacterial density in spring. In summer there were positive correlation between dissolved oxygen, Nitrite and bacterial density. Nitrite was the only parameter correlated strongly with the density in autumn.

The gained value of correlation coefficient were according to the season analysis where the measured parameters were analysis at each season . so it was clear that the environment –in winter –was slackly and the mobility of the environmental activities , relationships , reaction and other processes were done at low level of efficient functionality ,because of this ,the recovery of oil –degrading bacteria (as specific group) was depending at large degree –on the substrate source (hydrocarbon concentrations) which providing them with the energy and other essential requirements . The strong correlation between hydrocarbon concentrations and bacterial densities was observed previously (3, 25).

In summer –due to the high water temperature –the dissolved oxygen concentration became low depending on the reversible relationship between these two parameters .

Dissolved oxygen is one of the most effective factors on the aquatic life, because of the importance of this essential requirement for the continuation of the aquatic environment



activities (which seem in high levels of efficiency at summer) so this parameter was correlated to the bacterial density .

Oil –degrading bacteria in the aquatic environment –with large interference , and activities –required dissolved oxygen intensively to grow , multiply and dissolved oxygen and bacterial density was reported in the previous studies $^{(27\,,28\,)}$.

Three principal components were obtained with eigen values >1.0 foor three seasons (winter, spring and summer), in autumn. four principal components was more than 80 %, generally : dissolved oxygen, chlorophyll a ,salinity and nitrate were weighted higher in first component which explains more than 34% of the variability in all cases. This component represented the orientation and coordination of some chemical, biological and physical environment factors (table 4).group of acidity –nutrient factors was weighted highly in the second component during winter and summer, while group of organic source –nutrient factors was representing mainly during in the second component.

The group of organic matter –nutrient –temperature was appeared in high representation in the third principal component.

According to the station analysis , dendograms of all station (Fig3) were exhibited the same pattern of the correlation between seasons with in each station , where seasons 1&4 (winter &autumn) were closely related making an group related to the season 2 (spring) , the produced group was correlated to the season 3 (summer) .

Lower fluctuations were observed in station 6 followed by stations 1& 3 while other station (2,4,5 &7) were indicated higher fluctuations.

Correlation coefficient values (table 5) marked that the station (1, 2, 3, 4 & 5) showed highly positive correlations between bacterial density and temperature & salinity, strongly positive correlation between bacterial density and temperature was also continued in the station 6 & 7. This was in agreement with the studies of ^(4, 10). Other strong correlations were between nutrients and bacterial density and this was in agreement with the results obtained previously ^(8,9,29,30).

Form the above value of correlation coefficient it was clear that the temperature was the most effective environmental it was clear influenced the bacterial density .

Three principal compounds were obtained with eigen values > 1.0 for the stations (1,2,5,6 & 7) while two components were observed in the stations (3 & 4) (table 6).

The variability of these components was more than 91%, generally dissolved oxygen, temperature , hydrocarbon concentration , salinity and chlorophyll a concentration were represented highly in the first component – which explain more than 54% of the variability in all stations. While acidity –nutrient group was highly weighted in the second component (table 7).

Form the present results- according to the seasons and station analysis, one can conclude:

- 1. Stations 6, 4 and 7 revealed different trend in their distribution functions on the measured environmental parameters varied significantly of the situation of either stations.
- 2. Summer season show dissimilar mode of variations dynamic, different larely, of the rest seasons, while winter and autumn coordinated –approximately –in the same pattern of responsibility and distribution functions.
- 3. Same groups of environmental parameters were observed in highly percentages of representation in the principal components reflecting specific signification effects of these parameters in the studied circumstances and occurred fluctuations.



References:

[1] Floodgate, G.D. 1972. "Microbial degradation of oil. "Mar. pollute. Bull., 3(3):41-43.

[2] Atlas, R.M., and Bartha, R. 1973, ."Abundance, distribution and oil degradation potential of microorganisms in Raritan Bay," Environ .pollute .,4:291-300.

[3] Atlas, R.M., and Bartha, R . 1972. "Biodegradation of petroleum at low temperatures." can .J. Microbial ., 18:1851-1855.

[4] Atlas .R .M . 1975 , :Effects of temperature and crude oil composition on petroleum biodegradation ." APPI .Microbiol ., 30(3) :369 -403 .

[5] Bartha ,R. and Atlas , T. M .1973 ." Biodegradation of oil in saea water : Limiting factors and artificial stimulation ." In :Ahearn , D. G . and Meyer ,S. P. (Eds) . Microbial degradation of oil –pollutants .Louisiana state unvi. Pub . No . LSU-SG -37-01 , 322 , 147-152 .

[6] Cundell , A. M ., and Traxler , R.w. 1973 . "Microbiol degradation of petroleum at low temperature . "Mar .pollut . Bull ., 4:125 -127.

[7] Shamshoom , S. M. , Zeiara , T. S. , Abdual- Ritha ,A. N ., and Yacoub . A. E. 1990 , "Distribution of oil –degradation bacteria in the north- west Arabian gulf . "Mar . pollute .Bull., 21 (1):38-40 .

[8] Hpoener .T., Felzmann , H ., and atruck , H.1993 , "the nature and extent of oi;concentration of saudia Arabia gulf beaches: Examination of beaches of Dawhat, Dafi and Musallamiya in summer 1991 and winter 1991/1992 ". The Arabian. J. For sci. and Engineering., 18: 243-255.

[9] Mulkins-phillips , G.J., and Stewart, J.e. 1974. "Effect of environmental parameters on oil and hydrocarbons ". Appl. Microbial., 28:915-922.

[10] Haines, J.R., and Atlas, R.M. 1982. "Insitu microbial degradation of Prudhoe bay crude oil in Beaufort sea sediments". Mar. Environ. Res. 7 :91-107.

[11] Meyer-Reil, L.A., Bolter, M., Dawson, R., Liebezert, G., Szweirinski, H., and wolter K. 1980. "Interrelationship between microbiological and chemical parameters of Sandy Beach sediments, a summer aspect". Appl. Environ. Microbial, 39(4) :552-557.

[12] Jannasch, H.W. 1982. "New approaches to assessment of microbial activity in polluted water". In: Mitchell, R. Water Pollution Microbiology. Wiley interscience, P. 292.

[13] Badge, U.S., Khan, M.A., and Varna , A.K. 1982. "Influence of physic-chemical factors on the coli form bacteria in a close –lake water system." Inter. J.Environ .Studies ,18 :237-241

[14] Faust , A.M., Aotaki ,A E ., and Margadom ,M.T . 1976. "Effect of physical parameters on the insitu survival of Escherichia coli in an estuarine." APPI. Environ .Microbial ., 30 (5) :800-806 .

[15] Ward , R.C ., Loftis ,J.C ., Nielsen ,K. S. and Anderson ,R.D. 1979. "statistical evaluation of sampling frequency in monitoring networks " .J. water . Pollute .Control .Fed .,51(9):2292-2300.

[16] Fujissawa ,H., Murakami ,M., and Manabe , T. 1977. "Ecological studies on hydrocarbon –oxidizing bacteria in Japanese coastal water , I :Some methods of enumeration hydrocarbon oxidizing bacteria ."Bull . of Japanese csoci of sci .Fisheries .43:659-668 .

[17] Gunkel, W. 1973. "Distribution and abundance of oil –oxidizing bacteria in the north sea "In :Ahearn, D.G. &Meyer, S.P (Eds). Microbial degradation of oil po;;utants. Louisuana state univ.publication No.LUS. DG 73-010seite. P: 127-139

[18] Stricland ,J.D.H. , and Parsons ,T.R. 1977. "A practical hand books of sea water analysis " 2th edition .Fisheries Research Board of Canada .



[19] Farrigton ,J.W., and Tripp ,W. 1975 .":Acomarisaon of analysis methods for hydrocarbons in surface sediments "In :Church ,T.M.(ed) .Acs symposium series No.18 marine chemistry in coastal environment .chapter 15 :267-281 .

[20] Milles , A.L., Breuil ,C.,and Colwell .R.R 1978 . "Enumeration of petroleum –degrading marine and estuarine microorganisms by the most proble number method ". Can .J. Microbiol .24:552-557 .

[21] Underberg .M.R. 1973."Cluster analysis for applications." Academic press .New York .P:359.

[22] Davis ,J.C.1973 ."Statistics and data analysis in geology ."chapter 7 :413-533 .

[23] Kadri ,M. H. ,Salem , A.A. and Salama ,M. 1986 . "oil –degrading bacteria in Kuwait bay " .Indian .J.Mar .sci ., 15:50-51 .

[24] Zeiara ,T.S., and Shamshoom , S. M. 1996 . "Bacterial indicators of fecal and oil pollution in Shatt al- Arab estuary ."Marina Mesopotamia .11(1):89-94 .

[25] Solanas ,A.M. ,Pares , R. ,Marfil ,C. and Albaiges , J. 1982 . "A comparative study of chemical and microbiological monitoring of pollutant hydrocarbons in unbans aqtie environments. "J. Ene Anal .chem. . 12:141-151 .

[26] Wenxing, W., Xingin, S., Fengao, L., and Jinxing, Z. 1986. "Ecological distribution of petroleum degrading microorganisms in Bahia sea. with an outline of marine environmental protection in fluxes of oil pollutants in the Arabian gulf .Marine science centre publication No.4 :405 -421.

[27] Bauer ,J.E., and Capone ,D.G. 1985. "Degradation and Mineralization of Polly cyclic aromatic hydrocarbons: Anthracites and Naphthalene in intertidal marine sediments." APPI. Environ. Microbial, 50:81-90.

[28] Gunkel , W., Pederson ,S. ,Dundas , I., and Eimhellen , K. 1985 ."Microbiological investigations after the Barov Blow out (EKO fisk oil field , north sea)" Highlander Meeresunters ., 39:21-32 .

[29] Zeiara , T. S. , Al-Timari , A.A., and shamshoom , S.M. 1999 . "Effect of nutrients on petroleum biodegradation :The degradation of pony aromatic hydrocarbons (PAHs) ." Basrah J. sciences .B. 17(1) : 87-94 .

[30] Atlas , R. M. 1975 . "Effects of temperature and crude oil composition on petroleum biodegradation ."APPI. Microbial. 30 (3):396-403.



Quantitative Assessments of Environmental Factors Controlling Distribution of Oil -Degrading Bacteria in Shatt al-Arab and North-west Arabian Gulf Tehran S. Zeiara and Adnan N. Abdual-Ritha التقديرات الكمية للعوامل البيئية المسيطرة على توزيع البكتريا المحللة للنفط في شط العرب وشمال غرب الخليج العربى

> عدنان نعمه عبد الرضا ظهران سيد زيارة قسم علوم الحياة كلية العلوم - جامعة البصرة

المستخلص: قيس عدد من العوامل الفيزيائية و الكيميائية و البكتريولوجية المهمة في سبعة محطات في شط العرب و شمال غرب الخليج العربي خلال الفترة الزمنية الممتدة من شتاء ١٩٩٤ إلى خريف عام ١٩٩٥.

كانت كثافة البكتريا المسكرة للنفط الملاحظة مقارنة مع تلك الكثافات المسجلة في نفس المنطقة و للعامل البكتيري المدروس. بصورة عامة لوحظت أعلى كثافة للبكتريا المحللة للنفط في فصل الصيف في حين سجلت ادنى كثافة في فصل الخريف

أوضحت المعاملات الإحصائية للعوامل المقاسة ما يلي:

١ أظهرت المحطات ٧،٦،٤ اتجاها مختلفا في الدوال التوزيعية للعوامل البيئية المقاسة يتغاير و بدرجة مهمة عن أحوال المحطات الأخرى.

٢ إظهر فصل الصيف نمطا متغايرا لحركية التغييرات يختلف و بدرجة كبيرة- عن بقية الفصول، بينما كان فصلى الشتاء و الخريف تقريبا- في نفس النمط من الاستجابة و التوزيع.

٣ لوحظت نفس المجاميع من العوامل البيئية متمثلة بنسب عالية في المكونات الرئيسية عاكسة أهمية خاصة لتأثير هذه العوامل في الظروف المدروسة و التذبذبات الحاصلة.

> الكلمات المفتاحية: - البكتريا المكسرة للزيت -الزيت - التلوث