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MEAN SEA LEVEL FLUCTUATIONS OFF ALEXANDRIA COAST

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ABSTRACT

The effect of atmospheric pressure on the fluctuations of monthly mean sea level (MSL) off Alexandria coast for the period from 1962 to 1978 is investigated by different ways. It is found that the sea level height is generally decreasing with increasing atmospheric pressure especially during summer. Also, the power and cross-power spectral densities of monthly MSL and atmospheric pressure are investigated for the frequency range between 0.014 and 0.50 cycles per month. It is found that most of the power is centered at low frequencies particularly at annual and semiannual cycles. There is some coherence between monthly MSL and atmospheric pressure at certain low frequencies, while it is moderate to poor at most high frequencies. Moreover, the pressure correction on mean sea level is calculated.

In addition, the effect of water density on sea level oscillations for the period from July 1977 to June 1978 is studied. The density changes off Alexandria coast showed a significant effect on the sea level fluctuations.

Finally the isostatic deperture in sea level is determined on the basis of the variation in atmospheric pressure and water density. The monthly MSL correspond in sign and relatively in magnitude with the isostatic departure except during winter when it strongly differs either in sign or in magnitude.

INTRODUCTION

Monthly mean sea level (MMSL) is obtained by averaging the recorded tide heights throughout one month to reduce the tidal components of astronomic origin as much as possible. In general, the monthly means show a marked seasonal effect. At Alexandria, for example, sea level is highest in August and lowest in April. The range of monthly sea level during the year is small, seldom exceeding 35 cm, and when averaged for many years is less than 20 cm.

Many investigators (Close, 1918; Nomitsu and Okamoto, 1927; La Fond, 1939; Rouch, 1944; Pattullo et al., 1955; Doniol, 1956; Lisitzin and Pattullo, 1961; Thomopson, 1980; Carrett and Toulany, 1982; Rebert et al., 1985; Pugh and Thompson, 1986) have found that the observed changes in MMSL are related to the variation in atmospheric pressure and the density of the upper layers of the ocean. The relation between sea level, atmospheric pressure and water density appears to be such that the total mean pressure at any fixed point on the bottom in sufficiently deep water remains constant. Thus sea level rises when the barometer falls and also when the mean density of a vertical column of sea water decreases.

Pattullo et al. (1955) examined the variations of MMSL on a global scale and found that general agreement between observed sea level and the sum of the density and atmospheric pressure effects was widespread. They suggested the term "steric sea level" for the sea level computed from the density (or specific volume) and that conditions be referred to as "isostatic" when the total pressure, due to ocean and atmosphere, at a point on the deep sea floor does not change with time.

The distribution of air masses over the oceans is not constant, it changes continuously and is, in addition, characterized by a marked seasonal variation. This change can not contribute materially to the variations in sea level and must therefore be eliminated from the data when examining sea level observations covering a time-span of one year or more. Pattullo et al. (1955) were probably the first oceanographers to carry out such an elimination.

The statistical removal of meteorological effects from MSL changes makes it possible to reduce the background noise to an extent which allows other cycles and secular changes to be extracted with greater confidence (Rossiter, 1962; Pugh and Faull, 1983).

The aim of the present work is to investigate the MMSL variations off Alexandria and to study the effect of atmospheric pressure and water density on it.

MATERIAL AND METHODS

The sea level data used in this work were taken from the tide-gauge located inside the innermost basin of the Western Harbour of Alexandria through the period from 1962 to 1978. The atmospheric pressure data were obtained from Ras El-Tin meteorological station for the period from 1962 to 1979. The hydrographic data (temperature and salinity) were taken from the work of Eid (1979). Three stations were chosen located on Kait Bey section at distances 1, 5 & 10 Km from the coast. Figure 1 shows the locations of the tide-gauge, the meteorological station and the hydrographic stations off Alexandria coast.

Pattullo et al. (1955) methods were used to eliminate the effect of atmospheric pressure on sea level fluctuations, and also to calculate the effect of water density on sea level (steric sea level). The effect of relatively long-term changes in annual MSL was allowed for by subtracting the long-term annual MSL from each monthly mean.

In addition, the methods of correlation and spectral analysis (Blackman and Tuky, 1958; Munk et al, 1959) are applied to investigate the relations between MMSL and monthly mean atmospheric pressure (MMAP). The auto-correlations and the cross-correlations between these two variables are computed and from them the power and cross-power spectral densities are obtained. To exploit all data available from 1962 to 1976 (180 data points), a 36

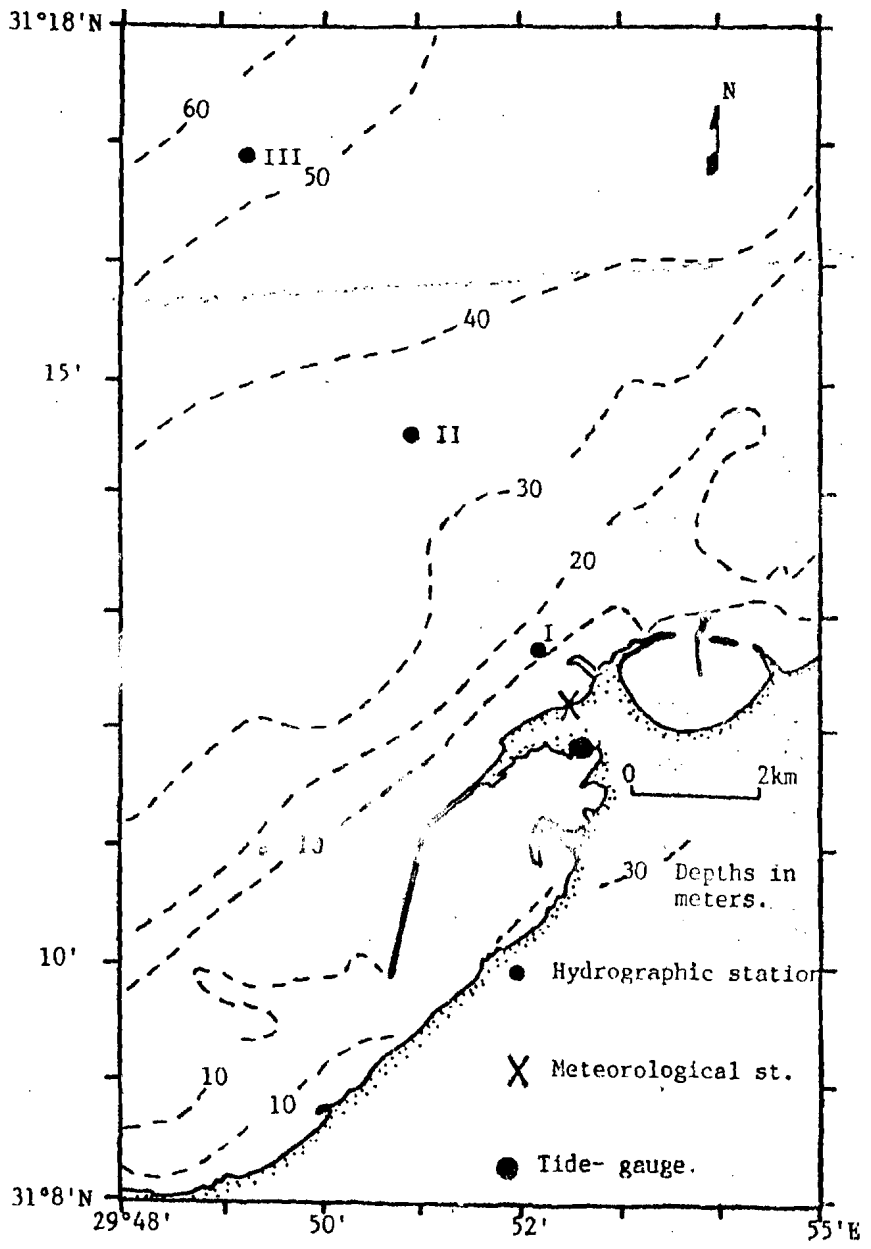

FIG. 1.
The location of the tide-gauge, the Meteorological and hydrographical stations off Alexandria.

numbers of lags are chosen. Table 1 shows the number of these data points (N), the number of lags (M), the number of degree of freedom (2N/M) and the confidence limits used in spectral analysis of both MMSL and MMAP.

TABLE 1
Number of data ponts (N), number of lags (M) and the confidence limit used in spectral analysis of data

N	M	No. of degress of freedom	Confidence limit (Munk et al., 1959)
180	36	10	0.49 - 3.10

RESULTS AND DISCUSSION

The Effect Of Atmospheric Pressure On Sea Level

a- The Relationship Between MMSL and MMAP

The relationship between MMSL and MMAP off Alexandria has been studied by different ways. In the first, the monthly mean values of sea level and atmospheric pressure for the period from 1962 to 1976 were plotted against lines as shown in Figure 2. Also the relationship between long-term monthly mean of both sea level and atmospheric pressure for the same period is shown in Figure 3a. Figure 3b shows the relationship between MMSL and MMAP for the period from July 1977 to June 1978. These three figures showed a slight decrease in sea level with increase in atmospheric pressure especially during summer months.

The second method of investigating the effect of atmospheric pressure on sea level was by plotting all the values for a particular month for the years 1962-1976 in one diagram to reduce the seasonal effects. Figure 4 shows the results in the form of scatter diagrams. for all months, for the years 1962-1976. In most cases there is evidence that sea level increases with decreasing atmospheric pressure. The highest correlation coefficient between sea level and atmospheric pressure (-0.94) is calculated during December, while the lowest correlation (-0.03) is found during May.

In general, it may be concluded that, there are other parameters which contribute, beside the atmospheric pressure, to the fluctuations of sea level off Alexandria coast.

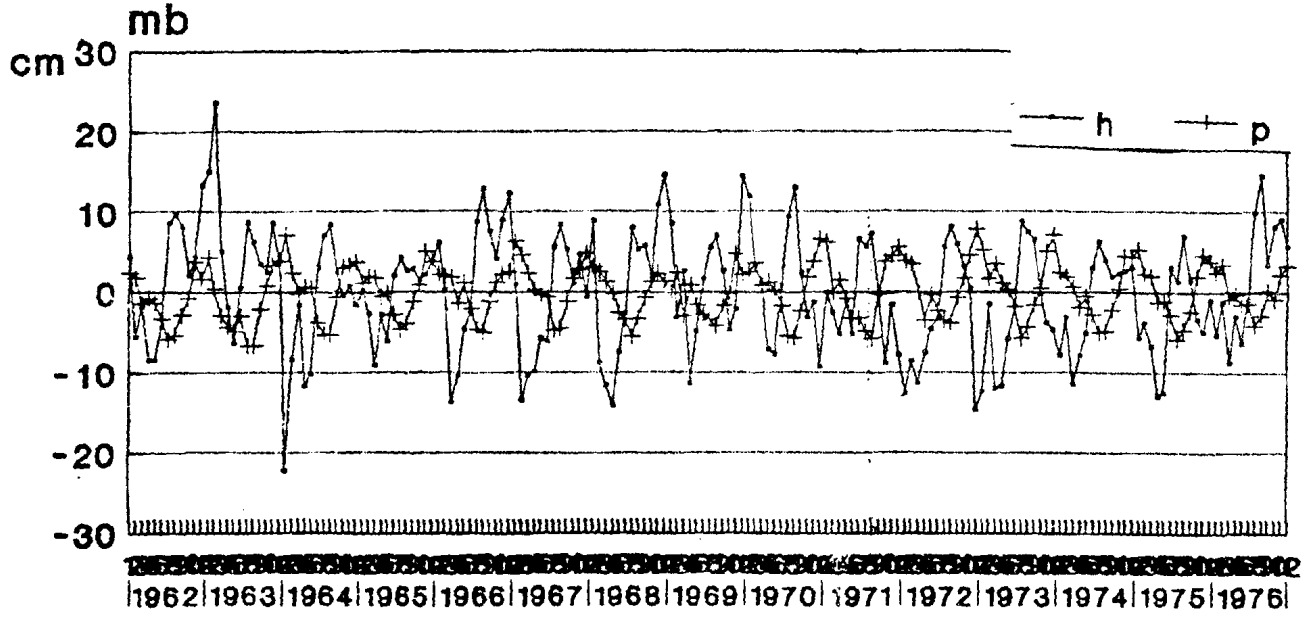

FIG. 2
The deviation of MMSL (h) and atmoshperic pressure (p)
from long-term annual mean (1962-1976).

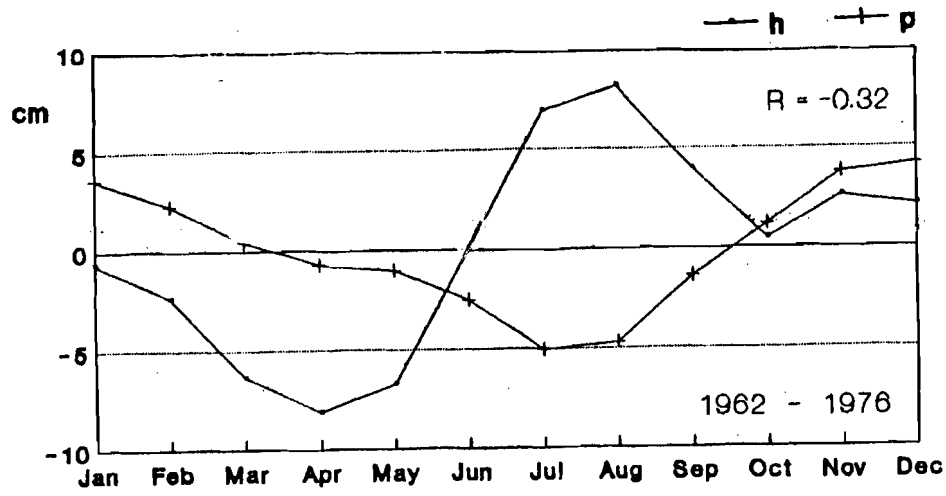

FIG. 3a
The deviation of long-term MMSL (h) and atmospheric pressure (p) from long-term annual mean (1962-1978).

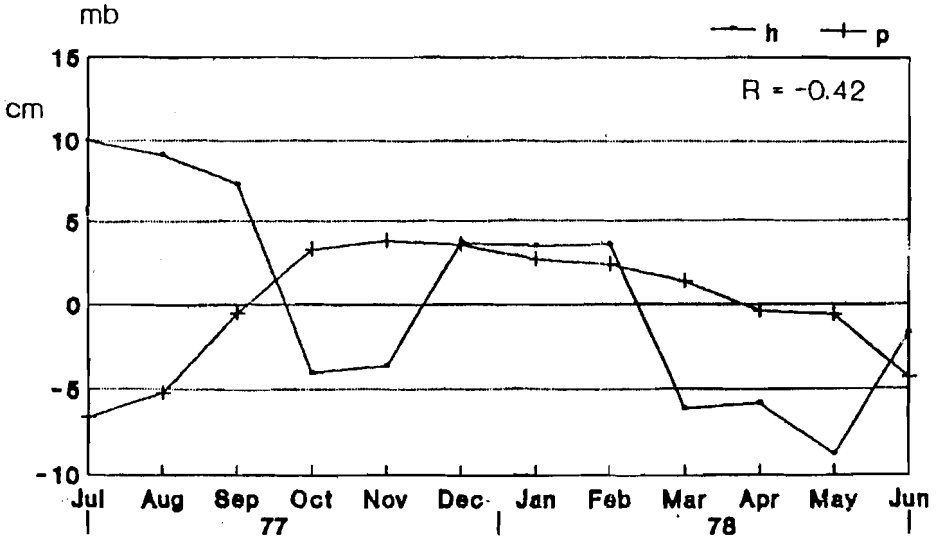

FIG. 3b
The deviation of MMSL (h) and atmospheric pressure (p) from the annual mean for the period July 77 to June 78.

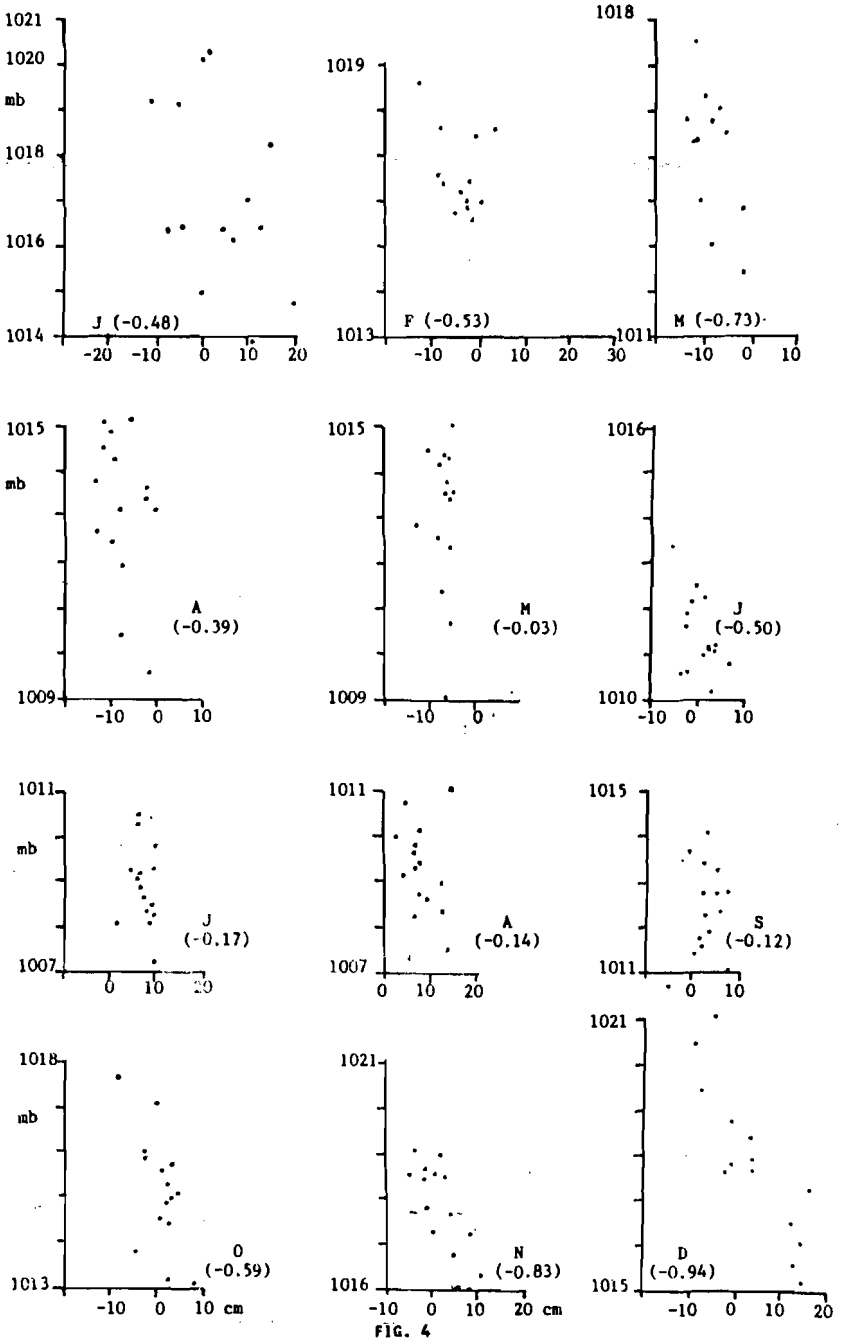

FIG. 4

The scatter diagrams between MMAP and the deviation of MMSL from long-term annual mean for all months for the years 1962-1976 (number between brackets is the correlation coeff. R).

b- The Power And Cross-Power Spectral Densities:

1- The sea level spectra:

The sea level spectra is shown in Figure 5a. Using the confidence limits for the degree of freedom (Table 1), the significant peaks were identified. The prominent peaks are centered at a frequency 0.083 cycle per month (cpm). This corresponds to a well pronounced annual sea level variation. There is also a high peak at 0.167 cpm, which indicates a large semiannual component. There are other significant peaks but with lower energy appeared at high frequencies with periods 4, 3.3 and 2.6 months.

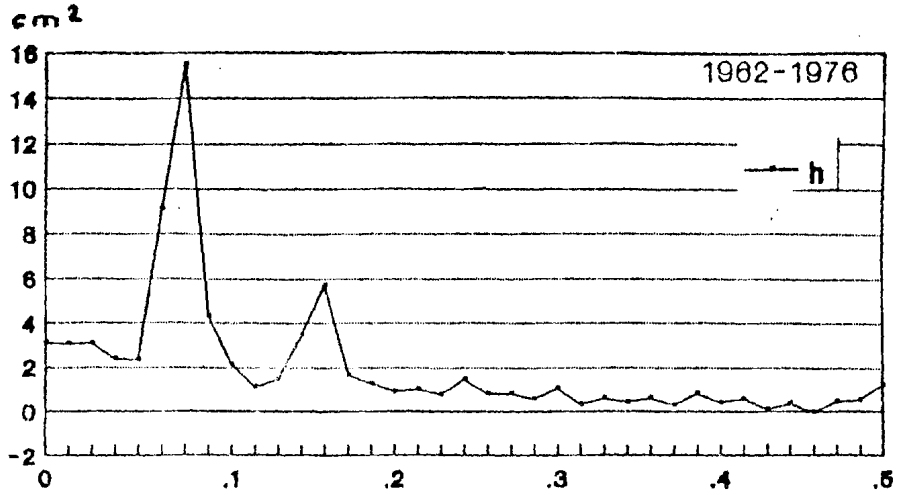

FIG. 5a
Power spectral density of monthly MSL.

The total energy contained in the sea level spectra amounts to only 15 cm^2.

2- The atmospheric pressure spectra:

The atmospheric pressure spectra is shown in Fig.. 5b. It is seen that, both the annual and semiannual cycles are significant, with the former dominating over the latter. Also, there are many other peaks with lower energy centered at many high frequencies with periods 4.5, 4.0, 3.6, 3.3, 3.0, 2.8, 2.6, 2.4, 2.3 and 2.1 months.

The total energy contained in the atmospheric pressure spectra amounts to about 31 mb^2.

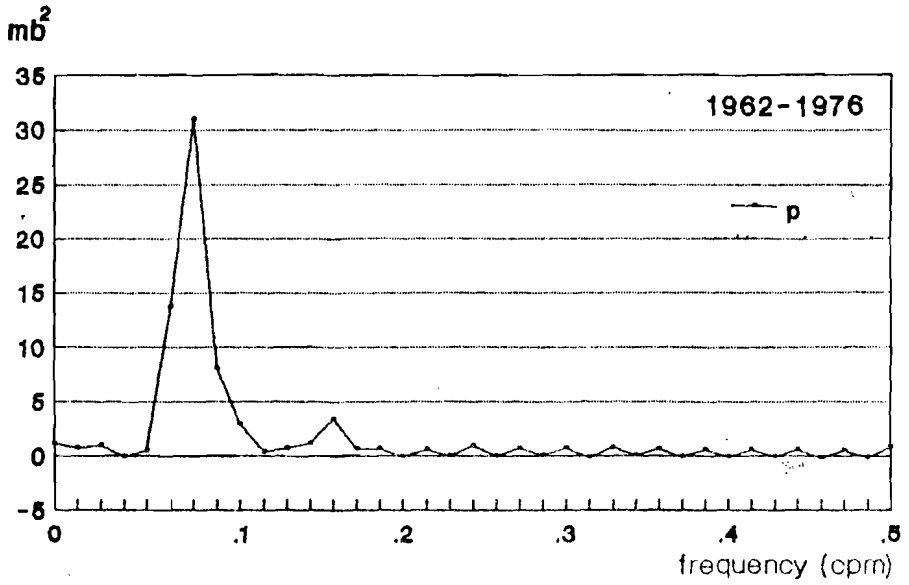

FIG. 5b
Power spectral density of monthly mean atmospheric pressure.

3- Coherence and phase between sea level and atmospheric pressure:

The relation between sea level and atmospheric pressure is now expressed in the coherence and phase difference as functions of frequency. The coherence between two time series at any frequency tends to be zero, if the components of the two series are not related at this frequency and tend to unity if they are fully related. The absence of coherence does not indicate that two records are independent, it merely states there exist no linear relation between them.

Figure 6 shows the coherence and phase between sea level and atmospheric pressure. The coherence between sea level and atmospheric pressure is relatively high (more than 0.5) at a certain low frequencies, while it is moderate to poor at high frequencies. It means that, there is a good linear relation between sea level and atmospheric pressure at some few frequencies, while at most frequencies the relation between them is not linear. This conclusion explains the scattering diagram shown in Fig. (4).

The phase between these two variables varies little with frequency and scatters mostly around 180^{o} (except at some frequencies it scatters so much). This indicates there is an inverse relationship between sea level and atmospheric pressure fluctuations at most frequencies.

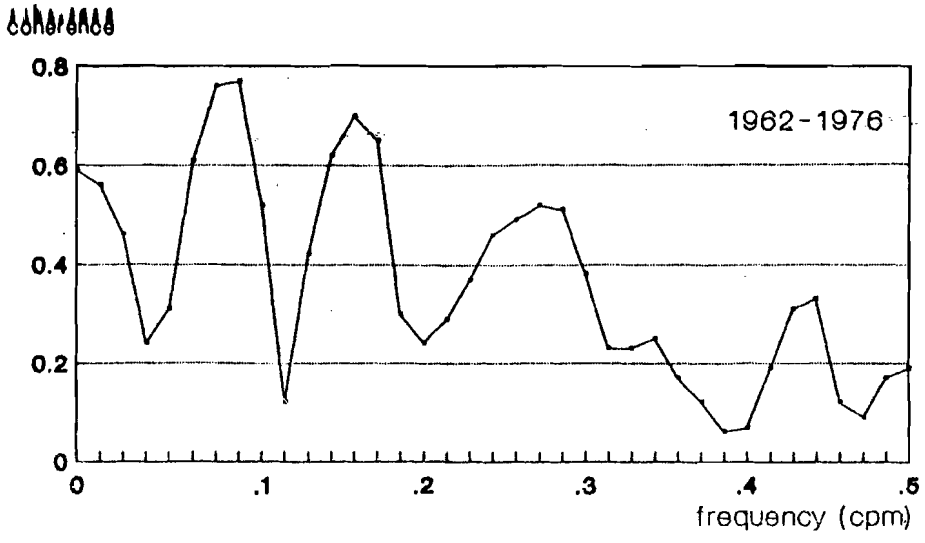

FIG. 6a
Coherence between monthly MSL & atmoshperic pressure

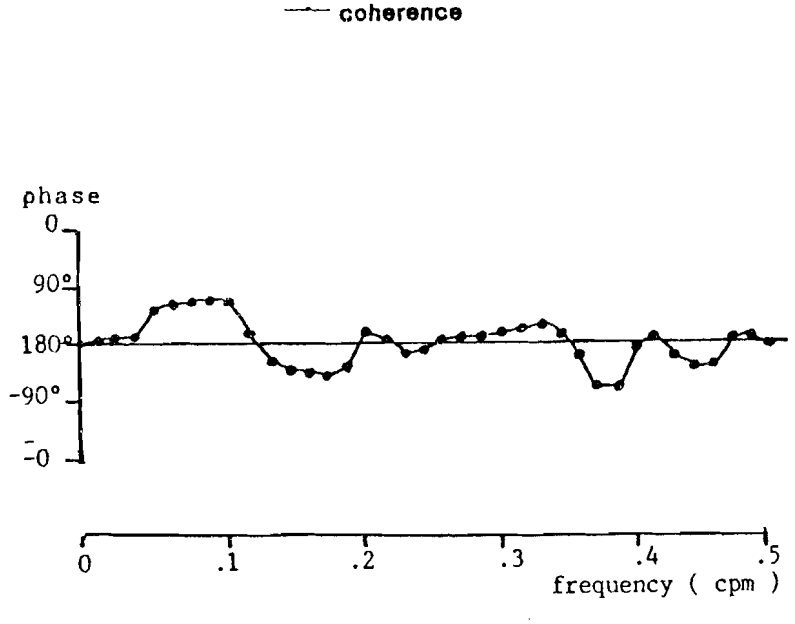

FIG. 6b
Phse between monthly MSL & atmospheric pressure.

TABLE 2

The values of pressure correction (c) off Alexandria

Year												
1962	4.2	3.5	0.0	-0.1	-0.5	-2.8	-6.0	-5.0	-2.3	0.1	5.3	3.5
1963	6.5	2.6	-1.1	-3.2	-3.5	-1.9	-6.4	-5.8	-1.1	2.2	5.3	6.3
1964	7.4	2.7	-0.2	-0.2	-0.1	-4.7	-6.2	-6.4	-1.5	2.5	3.4	4.1
1965	1.8	2.7	2.2	-1.0	-0.4	-3.4	-5.9	-4.7	-1.8	0.7	5.4	4.5
1966	3.1	2.9	2.4	-1.7	1.1	-2.4	-5.7	-5.6	-1.5	1.1	2.8	3.5
1967	6.1	4.4	1.8	-1.0	-1.4	-2.0	-6.9	-6.2	-2.6	0.6	2.4	4.7
1968	4.2	3.7	2.1	-0.2	-2.4	-3.5	-6.4	-3.6	-0.9	1.5	3.1	2.5
1969	1.2	3.7	-2.0	1.2	-1.1	-2.6	-4.1	-4.2	-1.6	0.3	5.8	3.5
1970	2.8	3.9	0.8	0.4	-0.4	-2.6	-7.1	-6.8	-3.3	1.3	3.9	7.0
1971	6.7	0.3	1.7	-1.4	-3.7	-3.9	-6.3	-6.7	-1.0	3.3	4.8	6.2
1972	4.0	3.7	-0.1	-4.5	-1.1	-3.4	-5.3	-5.1	-1.7	1.0	4.6	8.1
1973	5.0	1.4	3.0	-0.1	-0.7	-3.1	-7.8	-5.9	-3.0	-0.5	4.7	7.0
1974	3.4	2.9	1.3	-2.1	-0.3	-3.0	-6.0	-5.3	-2.4	0.6	5.3	5.5
1975	5.8	2.8	2.3	-1.7	-1.3	-3.4	-7.2	-5.4	-3.1	1.8	5.0	4.5
1976	3.3	4.2	-0.4	-0.5	-1.6	-1.8	-5.3	-3.5	0.3	-0.9	[illegible]	4.2
1977	4.9	5.1	5.2	-2.7	[illegible]	-4.5	-8.4	-6.4	-1.6	2.6	[illegible]	3.8
1978	[illegible]	2.8	1.5	-1.0	-1.0	-5.0	-6.9	-4.0	-0.8	0.3	6.9	4.0
1979	[illegible]	[illegible]	2.1	-0.6	-0.4	-3.2	-4.4	-5.6	-1.3	0.7	1.9	4.3

c- Elimination Of The Effect Of Atmospheric Pressure From MMSL:

1- Pressure correction (c):

Using Pattullo et al. (1955) method, the values of pressure correction (c) in cm off Alexandria are calculated. The result of these computations is shown in Table 2. The maximum effect at atmospheric pressure on sea level occurred during summer (negative effect) and winter (positive effect), because the atmospheric pressure has a minimum and maximum values during summer and winter, respectively. The highest positive effect of the pressure correction (c) was 8.1 cm occurred during December 1972. It corresponded to a maximum value of atmospheric pressure (1021.8 mb). It means that the sea level height must be arise by this amount of (c) to eliminate the effect of that pressure on it. The highest negative effect of (c) was -8.4 cm occurred during July 1977, i.e. the sea level data must be depressed by this amount to eliminate the effect of atmospheric pressure on it. The atmospheric pressure during that period had a minimum value (1007.2 mb).

The results of these computations are applied to correct the monthly MSL for the period from 1962 to 1976 as shown in Fig. 7. to correct the long-term MMSL as shown in Fig. 8a and finally to correct the MMSL for the period from July 1977 to June 1978 as shown in Fig. 8b.

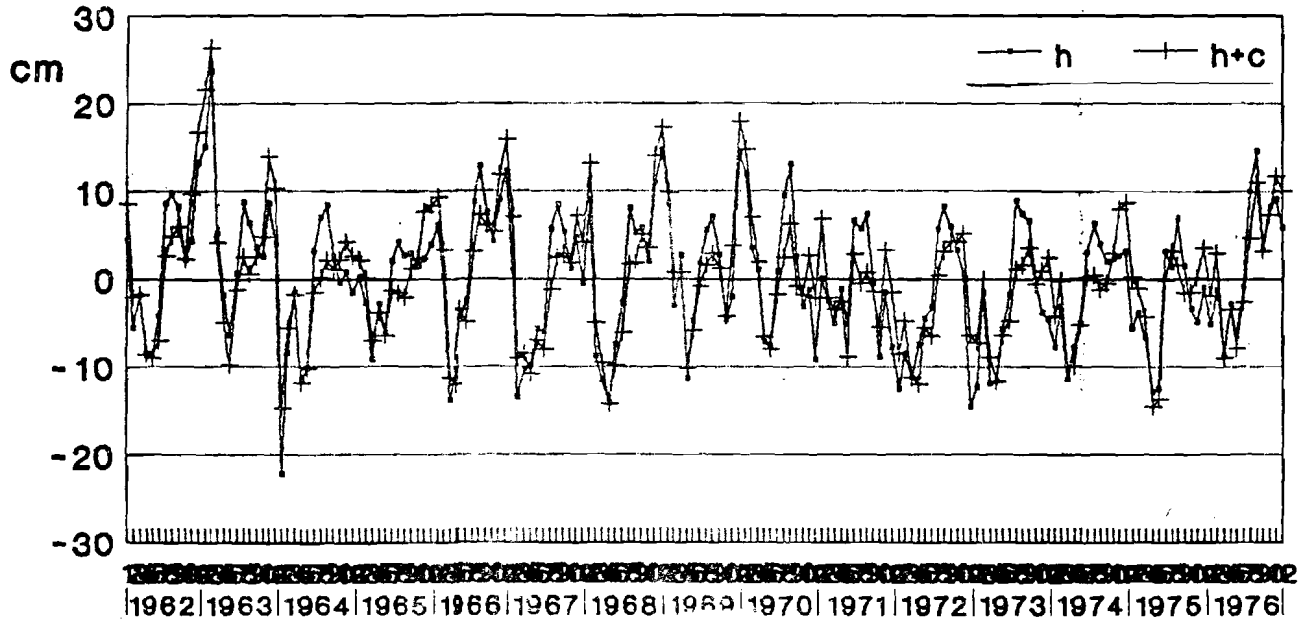

FIG. 7
The monthly MSL (h) and the corrected values (h+c)
to the atmospheric pressure for the period from
1962-1976.

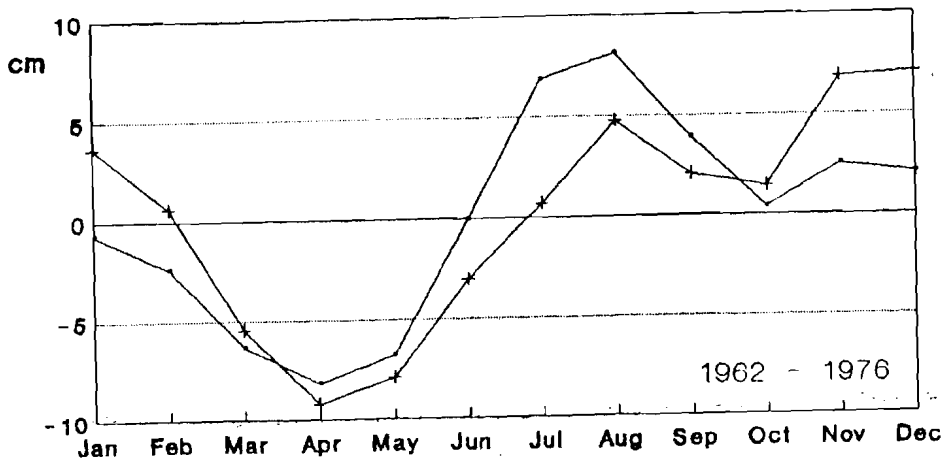

FIG. 8a
The average seasonal variations in MSL (h) and in its corrected value (h+c) to the atmospheric pressure.

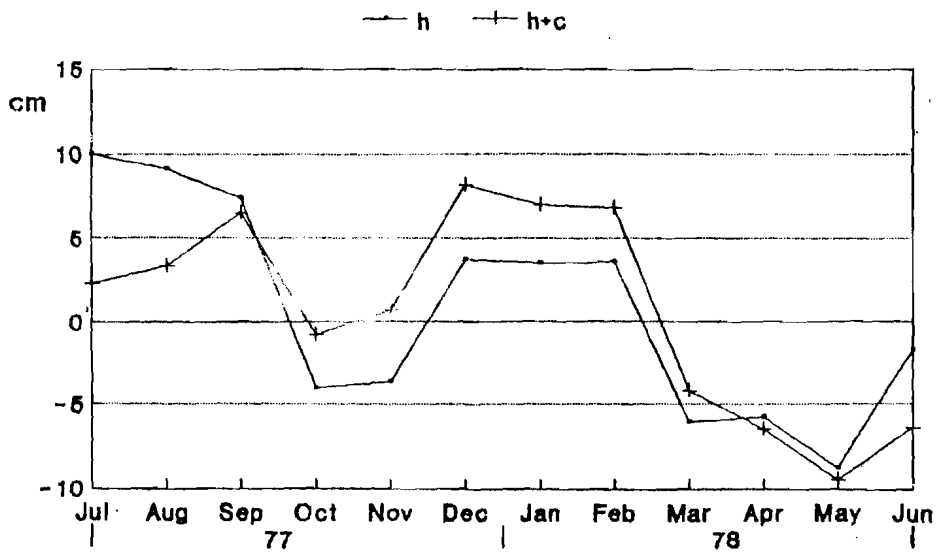

FIG. 8b
The monthly MSL (h) and its corrected value (h+c) to the atmospheric pressure for the period July 77-June 78.

2- Regression analysis:

The monthly values of pressure correction (c) and atmospheric pressure (p) were fitted using the least-square method. The results of these correlation were written in the form of a linear equation for each month. These equations give directly the values of pressure correction (c) for each month by knowing the corresponding values of atmospheric pressure (p). Table 3 shows these linear equations and the correlation coefficient (R) between the pressure correction (c) and the atmospheric pressure (p) at Alexandria.

TABLE 3
The monthly linear equations and the correlation coefficient (R) between the pressure correction (c) and atmospheric pressure (p).

Month	Linear equation	Corr. Coef. (R)
J	c = 0.85 p - 10.58 cm	0.95
F	c = 0.79 p - 9.79	0.87
M	c = 0.78 p - 10.41	0.97
A	c = 0.82 p - 11.99	0.91
M	c = 0.73 p - 10.64	0.91
J	c = 0.77 p - 11.82	0.77
J	c = 0.81 p - 13.51	0.80
A	c = 0.83 p - 13.08	0.80
S	c = 0.72 p - 10.90	0.74
O	c = 0.76 p - 10.53	0.87
N	c = 0.95 p - 12.64	0.89
D	c = 0.79 p - 9.55	0.97

where, p = (MMAP - 1000) mb.

3- Reference pressure (P_r):

Here, the reference pressure P_r, meaning that the values of the atmospheric pressure has a zero effect on sea level. So, the deviation of the monthly mean values of atmospheric pressure (p) from it directly gives the values of the pressure correction (c). If the values of (p) are large than those of reference pressure (P_r), then we have a positive pressure correction i.e. the sea level height must be arise by this amount of pressure correction to eliminate the effect of that atmospheric pressure on it, and vice versa.

The values of reference pressure (P_r) were calculated from the above results as follow:

1- From the above equations (Table 3), the values of atmospheric pressure (P_o) which give zero in pressure correction (c) were calculated for each month.

2- The deviation of MMAP (p) from (p_o) gives the positive and negative pressure corrections (c^*).

3- These values of pressure correction (c^*) were compared with those values of pressure correction (c) (Table 2). From this comparison, the magnitude of the error (dc^*) in the values of pressure correction (c^*) was calculated.

4- Adding this magnitude of the error (dc^*) to the values of (p_o) gives the values of the reference pressure (P_r).

Thus, if the MMAP at Alexandria are known, then the values of pressure correction (c) can directly be calculated by two ways: The first-by using the linear equations written in Table 3, let us denote this value by c (equation). The second way-by its deviation from the reference pressure (P_r) which is shown in Table 4, let us denote it by c (deviation).

TABLE 4
The values of reference pressure (p_r)
at Alexandria

Month	P_r (mb)	Month	P_r (mb)
January	1013.47	July	1015.38
February	1013.46	August	1014.83
March	1013.77	September	1014.68
April	1014.52	October	1014.38
May	1014.32	November	1013.68
June	1014.62	December	1013.35

4- Normal atmospheric pressure:

It is known that the normal atmospheric pressure is the pressure of atmospheric at mean sea level, at 45° latitude and at a standard temperature i.e. the pressure at normal condition, and it equals to 14.7 psi = 29.92 inches of Hg = 1013.3 mb.

The monthly MSL at Alexandria may be corrected to that normal atmospheric pressure. Let us denote the pressure correction due to the normal pressure by c (normal).

Figure 9 shows an example for these computations of pressure correction by different methods for the values of long-term MMAP (1962-1976). This figure shows there is a significant coherence between the values of pressure correction calculated by these different methods. Only the pressure correction calculated by using the normal pressure is slightly differ from that calculated by the other methods.

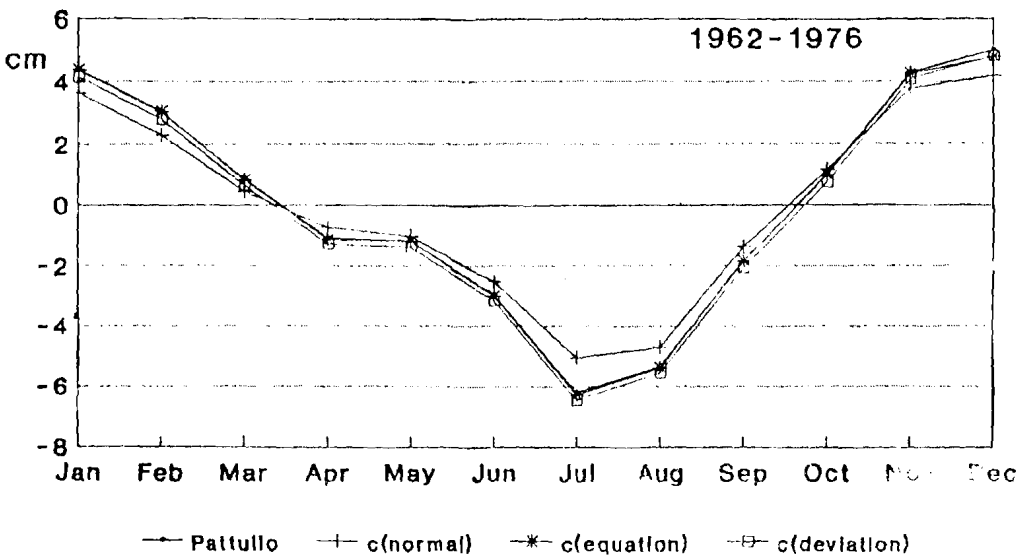

FIG. 9
The pressure correction (c) calculated by different methods for the long-term monthly mean atmospheric pressure.

Steric Effect On Sea Level

a- Steric sea level off Alexandria

Figure 10a shows the steric sea level at the three hydrographic stations located at distances of 1, 5 and 10 km off Alexandria coast with depths of 15, 35 and 55 m, respectively. The steric sea level is positive from June to November with maximum value during August. It means that the specific volume is high during these periods with maximum value during August, i.e. the water density is low during these periods. Hence, the observed sea level must be depressed by these positive values to be corrected to the water density. The steric sea level is negative from December to May with lowest value during February for the first two stations and during March for the third station. It means that the specific volume is low during these periods, and thus, the water density is high. Consequently, the observed sea level must be arise by these negative values to eliminate the effect of water density on it.

Also, it is seen that, from Fig. 10a, the value of steric level increases with increasing the water depth because the weight of water column becomes larger. The steric level at 5 Km offshore is nearly double of that at 1 Km, while those at 10 Km from the coast is almost 3-times of that at 1 Km. On an average, the second station located at 5 Km from the coast is considered to represent the steric sea level off Alexandria.

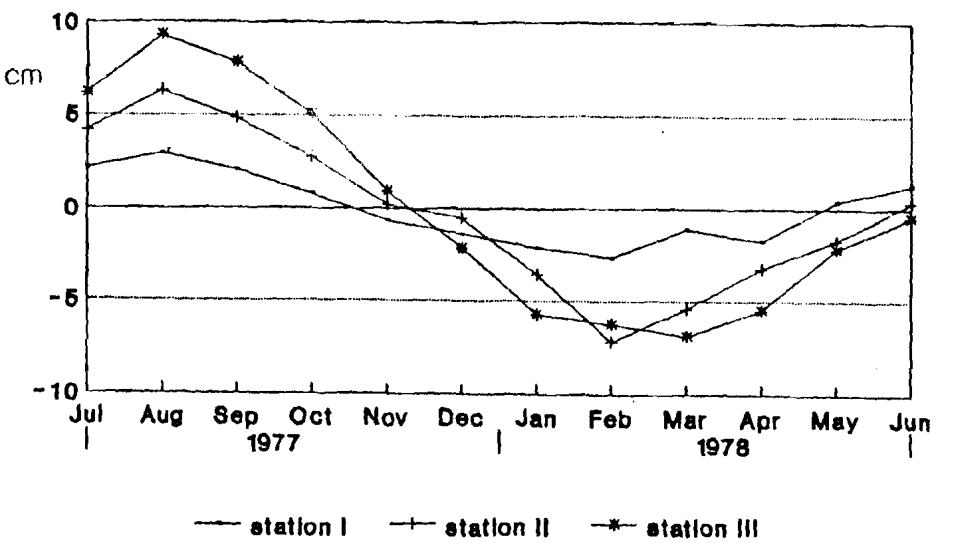

FIG. 10a
The steric sea level at 1,5 & 10 Km off Alexandria coast with depths 15, 35 & 55 m respectively.

Figure 10b shows the steric sea level at the mentioned three stations but only for the upper 15 m depth. The steric sea level at the first station (~ 1 km offshore) is slightly different from that at the other two stations due to the effect of diluted water from El-Mex and sewage pipeline at Kait Bey.

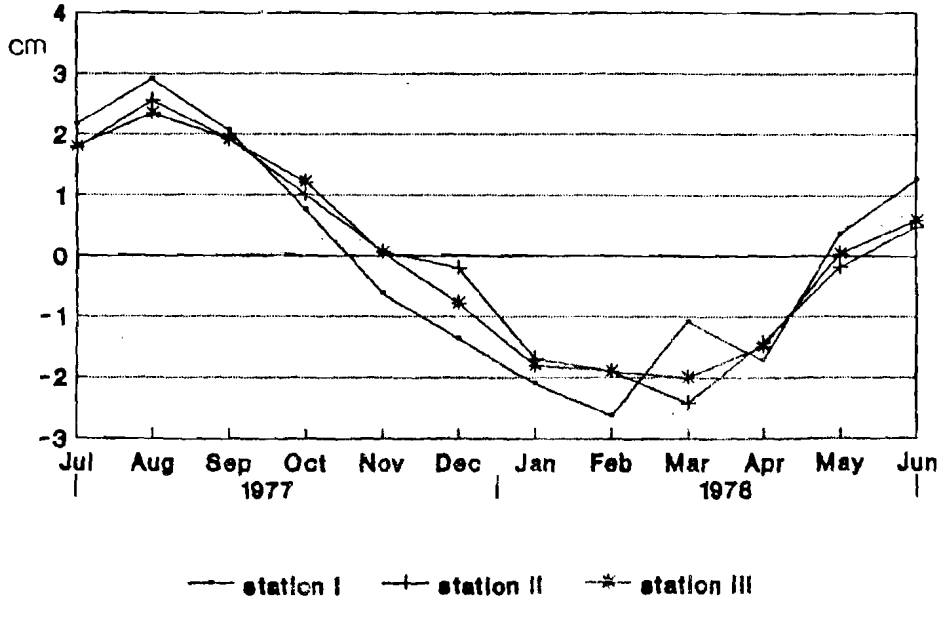

FIG. 10b
The steric sea level off Alexandria coast for the upper 15m depth.

b- Corrected Sea Level

Now, the observed monthly mean sea level off Alexandria is corrected to the water density for the water column from surface to 35 m depth as shown in Fig. 11. This figure shows that the density changes in the upper 35 m depth has a significant effect on monthly mean sea level especially during summer and winter.

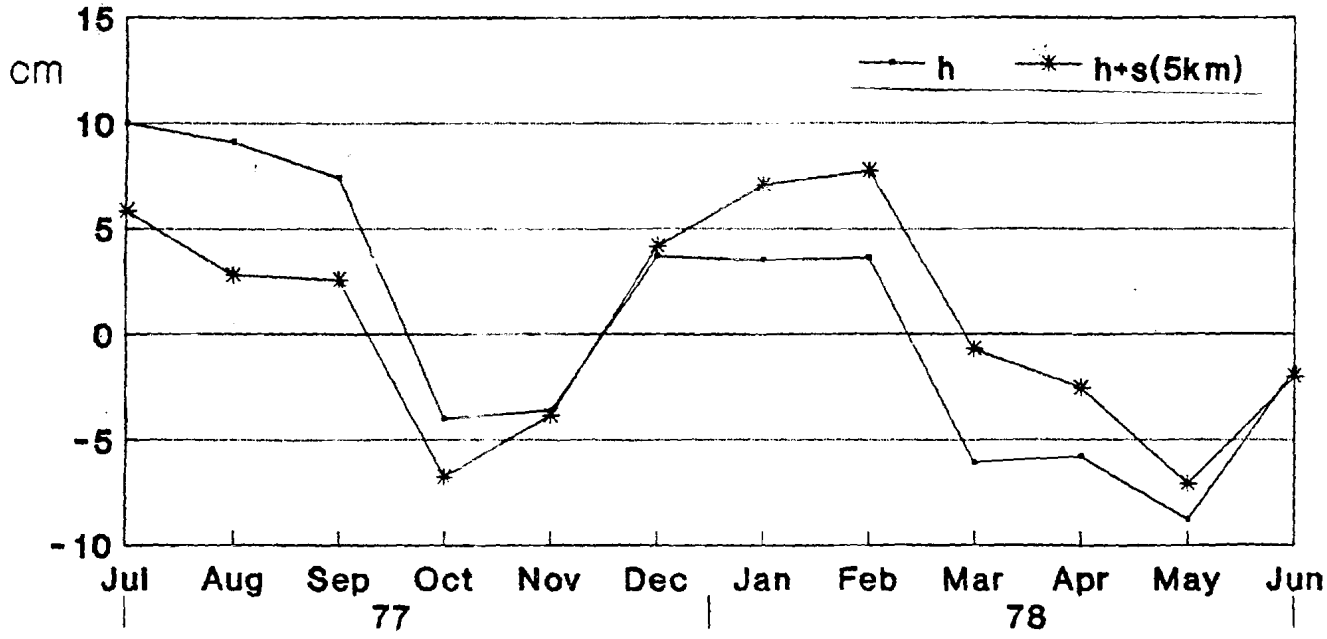

FIG. 11.

The monthly MSL (h) and its correction (h+s) to
the water density for the upper 35m depth (station II).

3- Isostatic departure:

The isostatic departure in sea level is determined on the basis of the variation in atmospheric pressure and water density, or specific volume. Figure 12 shows the values of isostatic departure (the pressure correction (c) plus the steric level (s)) after changing its sign to compare it with the observed monthly MSL. The monthly departure in sea level, except during winter, generally corresponds in sign and relatively in magnitude with the isostatic departure.

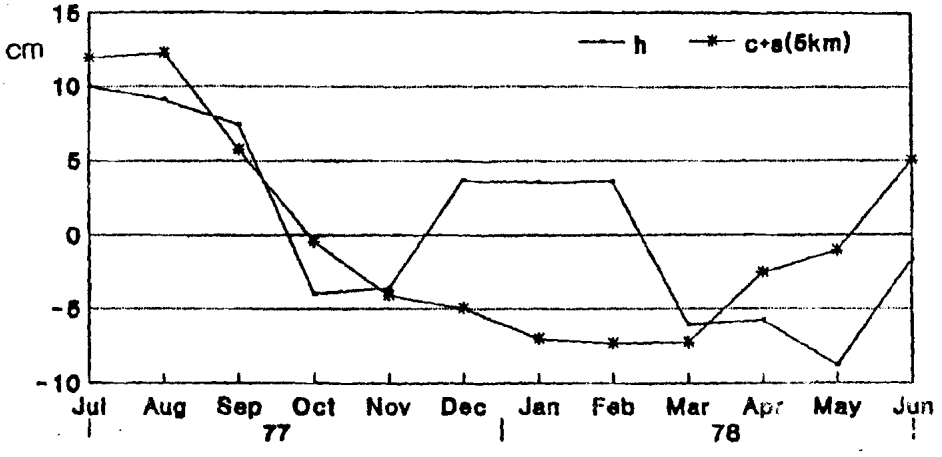

FIG. 12
The monthly MSL (h) and the negative effect of isostatic departure (c+s) at 5 Km off-shore.

Figure 13 shows the corrected sea level to the isostatic departure. The isostatic effect is large during summer and winter but with reverse effect. During summer its effect appears as a depression of sea level, while during winter its effect seen as a raising of sea level having a maximum corrected value during February.

Comparing Fig 8b and fig 11 with fig 13 it is concluded that, the atmospheric pressure and water density contribute equally to the fluctuations of monthly mean sea level off Alexandria.

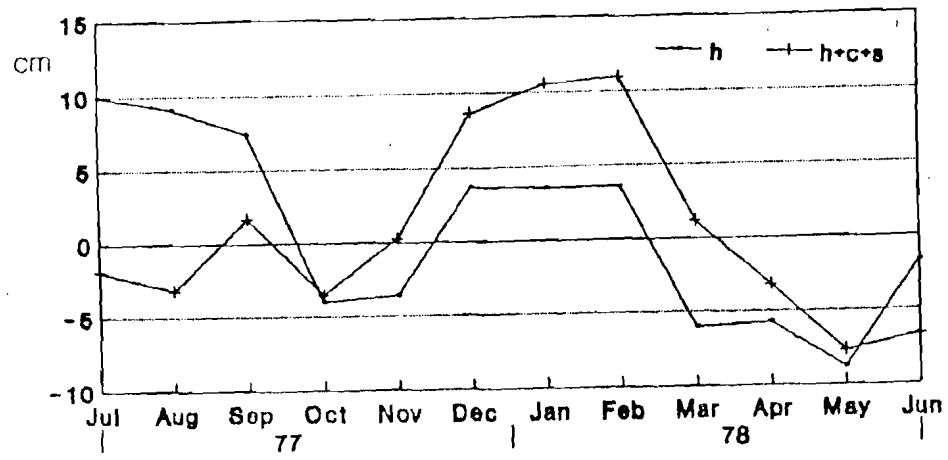

FIG. 13
The monthly MSL (h) and the corrected sea level to the isostatic departure (h+c+s).

SUMMARY AND CONCLUSION

The effect of atmospheric pressure on monthly MSL off Alexandria coast was studied in details during the period from 1962 to 1978 by different ways. The direct relationship between them showed that the sea level height slightly decreases with increasing atmospheric pressure especially during summer. The correlation coefficient between MMSL and MMAP for each month of several years (1962-1976) is calculated. The highest correlation coefficient (-0.94) is found during December, while the lowest one (-0.03) during May.

The power and cross-power spectral density of MMSL and MMAP are investigated. It is found that most of the power is concentrated at low frequencies with periods of 12 and 6 months. The coherence between these two variables is relatively high at certain low frequencies, while it is moderate to poor at high frequencies. The phase between sea level and atmospheric pressure is scattered very little around 180^{o} indicating an inverse relationship between them at most frequencies.

On the basis of the MMAP of several years (1962-1976) the pressure correction on the mean sea level was calculated. The results showed a maximum effect of atmospheric pressure on mean sea level appearing during winter and summer. The pressure correction had positive values during the cold months and negative ones during hot months. These results of pressure correction were fitted using the least squares method. The outputs were written in the form of 12 linear equations one for each month. If the MMAP is known at any time off Alexandria, then the values of the pressure correction can directly be obtained by using these linear equations. Also reference values of atmospheric pressure off Alexandria were calculated which give directly the values of pressure correction by subtracting from them the values of MMAP.

The effect of water density (specific volume) on sea level off Alexandria is also studied for the period from July 1977 to June 1978. The results showed that, the steric sea level is positive from June to November with maximum value during August, while it is negative from December to May with minimum value during February or March. Also, the density changes in the upper 35 m depth have a significant effect on monthly MSL especially during summer and winter.

Finally, the variations in atmospheric pressure and water density are combined to study the effect of isostatic departure in sea level off Alexandria. It is found that, the monthly MSL corresponds in sign and relatively in magnitude with the isostatic departure except during winter when it strongly differs either in sign or in magnitude.

REFERENCES

Blackman, R.B. and K.W. Tukey, 1958. The measurement of power spectra from the point of view of communications engineering. Bell System Tech. J., 57: 18-485 and 485-509.

Close, C., 1918. The fluctuations of mean sea level, with special reference to those caused by variations in barometric pressure. Geogr, J., 52: 51-58.

Doniol, R., 1956. Les variations saisonnieres du niveau moyen a Dakar. C.O.E.C. Bull. No. 5. 225 p.

Eid, F.M., 1979. Currents and water masses in the coastal area from Abu Kir area to Agamy. M.Sc. Thesis, Faculty of Science, Alexandria University.

Garret, C.J. and B. Toulany, 1982. Sea level variability due to meteorological forcing in the north east Gulf of St. Lawrence. J. of Geoph. Res., 87: 1968-1978.

La Fond, E.C., 1939. Variations of sea level on the Pacific coast of the United Stated. J. Mar. Res., 2: 17-29.

Lisitzin, E. and J.G. Pattullo, 1961. The Principal Factors Influencing the Seasonal Oscillation of Sea Level. J. of Geoph. Res, 66 (3): 845-852.

Munk, W.H.; F.E. Snodgrass and M.J. Tucker, 1959. Spectra of low frequency ocean waves. Bulletin of Scripps Institution of Oceanography, California Univ., Vol. 7 (4) p: 283-362.

Nomitsu, T. and M. Okamoto, 1927. The causes of the annual variations of the mean sea level along the Japanese coast. **Mem. Kyoto Univ. Coll. Sci., Ser. A.**, 10: 125.

Pattullo, J.; W. Munk; R. Revelle and E. Strong, 1955. The seasonal oscillation of sea level. **J. Mar. Res.**, 14: 88-154.

Pugh, D.T. and H.E. Faull, 1983. Tides, surges and mean sea level trends. pp. 59-69, in **Shoreline Protection**, London: Thomas Telford, 248 pp.

Pugh, D.T. and K.R. Thompson, 1986. The subtidal behavior of the Celtic Sea-1. Sea level and bottom pressures. **Continental Shelf Research**, 5: 293-319.

Rebert, J.P.; J.R. Donguy; G. Eldin and K. Wyrtki, 1985. Relations between sea level, thermocline depth, heat content and dynamic height in the tropical Pacific Ocea. **J. of Geoph. Res.**, 90: 11719-11725.

Rossiter, J.R., 1962. Long-term variations in sea level, pp. 590-610 in: Vol. 1, **The Sea** (ed. M.N. Hill). New York: Wiley Interscience. 864 pp.

Rouch, J., 1944. La variation du niveau de la mer en fonction de la pression atmospherique d'apres les observations du pourquoipas? dans l'Atlantique. **Bull. Inst. Oceanogr.** Monaco No. 870.

Thompson, K.R., 1980. An analysis of British monthly mean sea level. **Geophysical J. of the Royal Astronomical Society**, 87: 15-32.

ANIONIC DETERGENTS IN THE EASTERN HARBOUR, ALEXANDIRA, EGYPT.

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ABSTRACT

Surface and bottom sea water samples were collected from the Eastern Harbour during the period from May 1987 to May 1988. Detergents, salinity, total phosphorus and polyphosphates coccentrations were determined. Average surface contents of detergents at the surface was 0.92 mg eq. LAS/l and at the bottom was 0.43 mg eq. LAS/l. The frequency distribution of anionic detergents showed that 68% of the analysed samples lies in the range of 0.0-0.8 mg eq. LAS/l. Total phosphorus average concentration at the surface was 3.76 umol/l and at the bottom was 2.1 umol/l and polyphosphates content at the surface was 2.96 umol/l and at the bottom was 1.45 umol/l. Salinity concentration at the surface was 35.85%$_o$ while at the bottom was 38.2%$_o$ In June and July 1987 a significantly high negative correlation exists between booth salinity and detergents, total pgosphorus and polyphosphates. On the other hand detergents show high positive correlation with total phosphorus and polyphosphates in the same period. We aimed to study the distribution of anionic detergents in the Harbour and its effects on the total phosphorus content.

INTRODUCTION

Through great variety of chemicals which comprise detergents effective pollution problems may possibly arise from the discharge of laundering or cleaning processes. There are two main effects from these discharges and may be conveniently classified as the impact of dissolved inorganic compounds particularly nutrients leading towarsd enrichment. Phosphorus is conssidered as a limiting productivity factor. Therefore much attention has been paid to phosphorus control in municipal sewage. The principale sources of phosphorus are human excrement and detergents. The contribution of phosphorus from human excrement corresponding to about 1.0-1.2 lb of p/personal anualy but its contribution from detergents may be as high as 3.3 lb of p/person. It has been suggested that 30%-40% of all phosphorus entering the environment via detergents.

In the present work it is aimed to study the distribution of detergents in Eastern Harbour and its correlations with salinity, total phosphorus and polyphosphates and the impact of eleven outfalls which discharge untreated sewage into the Harbour.

MATERIAL AND METHODS

The Eastern Harbour is a semi-enclosed area with its mouth protected from the sea by an artificial break water leaving into two openings to the sea. The mean depth of the Harbour is about 3.5 m reaching a maximum depth of 9-11 m at some places.

Water samples were collected monthly during May 1987 to May 1988, except during October 1987 and April 1988. Surface and bottom water samples were collected from five stations inside the Harbour (Fig.1). Surface samples were collected by polyethylene backet attached to plastic line, while bottom ones were collected by Nesken bottle. Detergents samples were analysed using methylene blue method as described by standard Method for the examination of water and waste water (1981). The absorbance of the chloroform extract of anionic surfactant and methylene blue were measured at 650 nm using Schemadzu spectrophotometer UV-150-02 and results were expressed as mg eq. LAS/l. Total phosphorus and polyphosphates samples were collected during June 1987 to May 1988. They were analysed according to Grasshoff (1976), the absorbances were measured by the same instrument and the results were expressed as umol/l. Salinity samples were measured using salinometer "Beckman".

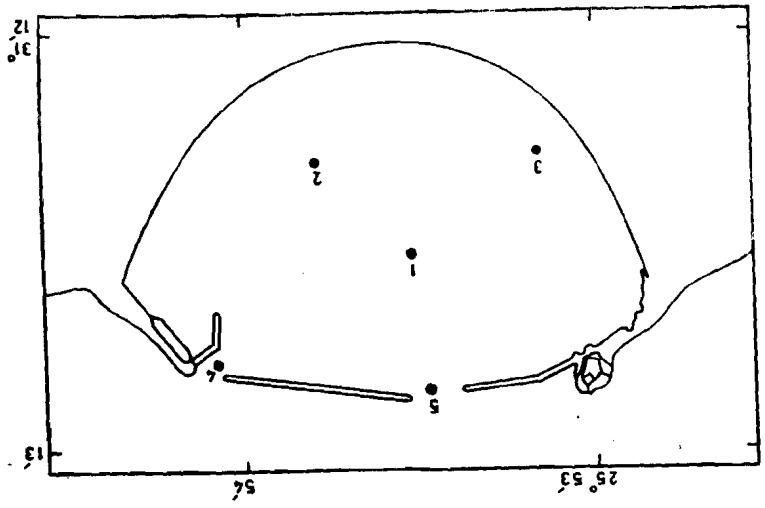

FIG. 1
Sampling stations

RESULTS AND DISCUSSION

The distribution of anionic surfactants in the Harbour is illustrated in Table 1. From this table it is observed that surface water is characterized by high concentrations of detergents, they ranged between 0.05-3.59 mg eq. LAS/l, while at the bottom they ranged between 0.00-1.57 mg eq LAS/l. This may be due to the discharge of sewage to the surface water.

Table 1

Surface and bottom distribution of anionic detergents in the Eastern Harbour area during May 1987 to May 1988,(mg eq. LAS/l).

Station No.		May 1987	June	July	Aug.	Sept.	Nov.	Dec.	Jan. 1988	Feb.	March	May
1	S	0.95	1.30	1.02	0.96	2.16	1.63	0.63	0.05	0.33	0.43	1.0
	B	0.42	0.47	0.11	0.63	1.57	0.96	0.23	0.00	0.17	0.31	0.43
2	S	1.23	2.48	3.59	1.16	0.60	1.02	0.43	0.15	0.58	0.15	0.56
	B	0.38	0.29	0.83	0.68	0.43	0.39	0.35	0.00	0.11	0.14	0.31
3	S	1.07	1.02	0.70	1.15	3.06	1.52	0.51	0.27	0.84	0.46	0.31
	B	0.90	0.25	0.55	0.84	1.33	0.00	0.26	0.09	0.46	0.26	0.32
4	S	0.66	1.16	2.02	0.69	0.53	1.04	0.54	0.96	0.22	0.33	0.38
	B	0.45	0.23	0.51	0.62	0.90	0.66	0.34	0.19	0.1	0.22	0.23
5	S	0.71	0.73	0.81	1.26	1.47	1.26	0.55	0.69	0.26	0.39	0.33
	B	0.23	0.30	0.09	1.17	0.67	0.99	0.41	0.18	0.15	0.34	0.21

The average distributions of detergents, total phosphous, polyphosphates and salinity are illustrated in Table 2 and Fig. 2. From the table it is noticed that the surfac water had higher average concentrations of total phosphorus and polyphosphates (3.76 and 2.96 umol, respectively) than the bottom (2.1 and 1.45 umol respectively). On the other hand surface average salinity was less than bottom one (35.85‰ and 38.2‰ respectively). From this table it is observed that stations 2 and 3 had higher content of detergents (1.09 and 0.99 mg eq. LAS/l), total phosphorus (8.51 and 3.02 umol/l) and polyphosphates (6.76 and 2.37 umol/l), and they had lower salinity 35‰ and 36.01‰. This may be due to the fact that these two stations are directly affected by the untreated sewage discharged to the Harbour. On the other hand stations 4 and 5 are situated at the interances of the Harbour and are affected by open sea water reflecting lower values.

Table 2

Average concentration of detergents, total phosphorus polyphosphates and salinity in the Eastern Harbour area during May 1987 - May 1988.

Station No.		Deterg.	T.p.	Poly.Phos	$S\%_o$
1	S	0.95	2.79	2.190	35.79
	B	0.48	1.57	1.22	38.20
2	S	1.09	8.51	6.76	35.0
	B	0.36	4.43	2.70	38.02
3	S	0.99	3.02	2.37	36.01
	B	0.47	2.02	1.58	37.96
4	S	0.78	2.53	1.97	35.75
	B	0.40	1.14	0.82	38.26
5	S	0.77	1.94	1.51	36.68
	B	0.43	1.33	0.91	38.54
avereages	S	0.92	3.76	2.96	35.85
	B	0.43	2.10	1.45	38.20

Seasonal variations of detergents in the Eastern Harbour during investigation period is shown in Table 3 and Fig. 3 From the table it is observed that higher concentration of detergents was recorded during summer and autumn; 1.34 and 1.43 mg eq. LAS/l respectively and lower one was recorded during winter 0.47 mg eq. LAS/l.

The detergents content during both summer and autumn was 6 times more than detergents content during winter and this is because more than million peoples visite Alexandria during summer.

The frequency distribution of the concentration of methylene blue active substances in the Eastern Harbour area during investigation period is represented in Fig. 4. The most abundant values are 0.0-0.4 mg eq. LAS/l which constitute about 40% of the total values while values between 0.4-0.8 mg eq. LAS/l constitute 28% of the total values. The means of detergents content in the Harbour ranged between 0.0 and 0.8 mg eq. LAS/l (68%). 18% of the total samples represents a concentrations of 0.8-1.2 mg eq. LAS. 8% of the total samples was ranged between 1.2 and 1.6 mg eq. LAS/l. While values ranged between 1.6 and 3.6 mg eq.

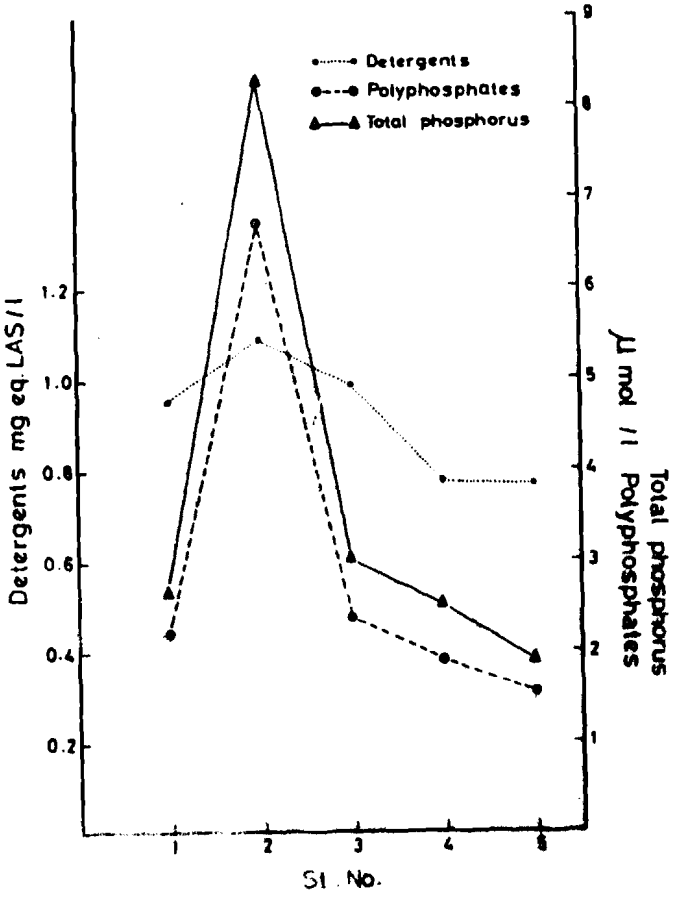

FIG. 2
Average concentrations of detergents, polyphosphates and total phosphorus in the Eastern Harbour at stations 1,2,3,4 and 5.

LAS/l constitute only 5% of the total samples. Mahmoud and Beltagy (1988) found that detergents content of Lake Borollos ranged between 0.0 and 0.3 mg eq. LAS/l, which is less than the values found at the Harbour. Albaster (1978) found that acute toxicity (LC_{50}) of the non ionic surfactant most commonly used in the detergents and cleaning agents in the range of 3-7 mg/l of active substances in static test with golden rofes. Kozarac et al. (1977) found that values above 0.01 mg/l are obtained in regions under the influence of urban pollutants. He found that values between 0.0 and 0.01 mg eq. LAS/l constitute more that 40% of the total

Table 3

Seasonal variations of detergents in the Eastern Harbour

	St.	Summer	Autumn	Winter	Spring
1	S	1.09	1.900	0.34	0.79
	B	0.40	1.26	0.13	0.39
2	S	2.41	0.81	0.39	0.65
	B	0.60	0.41	0.15	0.28
3	S	0.96	2.29	0.54	0.61
	B	0.55	0.67	0.27	0.46
4	S	1.29	0.79	0.57	0.46
	B	0.45	0.78	0.21	0.30
5	S	0.93	1.37	0.50	0.48
	B	0.52	0.83	0.25	0.26
	S	1.34	1.43	0.47	0.60
	B	0.50	0.79	0.20	0.37

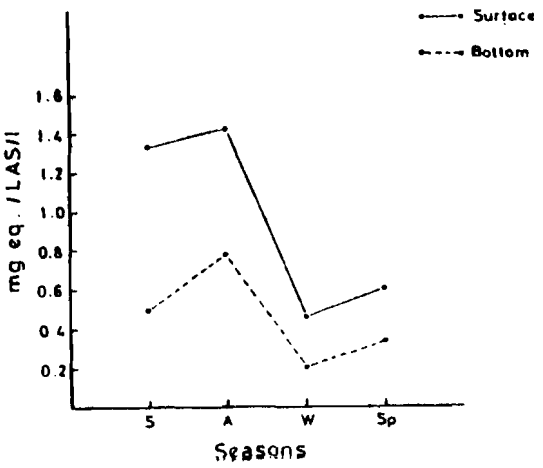

FIG. 3
Seasonal variations of detergents in the Eastern Harbour at surface and bottom water.

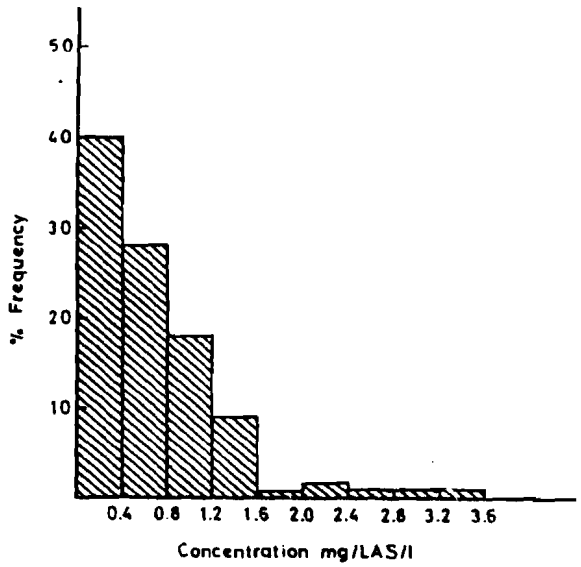

FIG. 4
Percent frequency distribution
of detergents in the Easter Harbour
during May 87-May 1988.

values at the North Adriatic. He concluded that the values below 0.01 mg/l correspond to unpolluted sea water. Cosovic et al. (1982) found that the concentrations of anionic detergents in the Adriatic Sea-off shore stations-were low and rarely exceed 0.05 mg/l, while the anionic detergents contents in Rovinj Harbour were 0.62, 0.01 and 0.08 mg/l, and its concentrations in Rejeke Harbour were 0.1 and .03 mg/l. He also found that the concentrations in Split plastics industry were 0.20-0.14 mg/l. Cosovic et al. (1979) concluded that the surface active substances modify the structure of inter boundary layers and affect the processes of mass and energy transfer. De Renzi et al. (1978) found that detergents content in coastal water infront of Italy ranged between 0.005 and 0.08 mg/l. Kozarac et al. (1975) found that the content of anionic detergents in samples of sea water ranged from 0.01 to 0.62 mg eq. SLS/l.

Correlation coefficients were calculated between detergents, total phosphorus, polyphosphates concentrations and salinity. Salinity as a conservative parameter is considered to be the most obvious indicator variable showing the extent of mixing of seawater in the Harbour with sewage discharged. During June and July (1987), there were a significantly high negative correlations between salinity, detergents, total phosphorus and polyphosphates They were r = -0.95, -0.93 and -0.92 during June (1987) and

r = -0.96, 0.7 and -0.7 during July (1987). This reversible correlations indicate the allochthonous origin of these parameters. During February (1988) there was a negative correlation between salinity and detergents r = -0.78. There were no correlations between salinity and the other parameters at the rest of the year. Also during June and July (1987) detergents gave a very high positive correlations with total phosphorus and polyphosphates r = 0.9 and 0.9 during June 1987 and r = 0.8 and 0.8 during July 1987.

This high significant correlations indicate that during these two months, detergents are the main factor that increases the concentrations of total phosphorus and polyphosphates. while in February (1988) there were no correlations between detergents and these two parameters indicating that detergents did not affect their content in the Harbour.

REFERENCES

Albaster, J.S., 1978. J. Am. Oil Chemists Soc., 55: 181.

Anonymous, 1981. Standard Methods for the examination of water and waste water. 15 edition. American Public Health Association, American water works association, water Pollution Control federation, New York.

Cosovic, B.; V. Zutic.; Z. Kozarac.; V. Vojodic and T. Novokovic, 1979. Investigation of surfactants in the North Adriatic in the period of 1974 to 1978. Evaluation of natural variations and pollution effects. Rapp. Comm. Int. Mer. Medit ., 25/26, 9: 55-60.

Cosovic, B.; V. Zutic; V. Vojvodic and T. Novarovic, 1982. Determination of surface active substances and anionic detergents in sea water and sea surface microlayer in the Mediterranean.

Vies Journees Etud. Pollutions, Cannes, C.I.E.S.M. 519-528.

De Renzi, G.; R. Palmerini Morelli.; P. Orlando.; S. Volta and C. Dardanelli, 1978. Research into the content of oil droplets, detergents and bacteria in the sea water and sea beds of the North Tyrrhenian Sea. IV^{es} Journees Etud. Pollutions, pp: 123-128, Antalya, C.I.E.S.M. 123-128.

Grasshoff, K., 1976. Methods of seawater Analysis. Verlag Chemie, Weinheim. New York. P: 300.

Kozarac, Z.; Z. Vonaric; T.; V. Zutic and B. Cosovic, 1977. Comparison of some methods for estimating surface active substances in sea water. Thalassia Jugosl., 13: 109-117.

Kozarac, Z; B. Cosovic, and M. Branica, 1975. Spectrophotometric determination of anionic surfactants in sea water. Marine science communication, 1 (2): 147-163.

Mahmoud, Th. and A. I. Beltagy, 1988. Detergents in Lake Borollos. Rupp. Comm. Int. Mer. Medit., 31, 2: P 72.

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1): 167 - 177.

GENETIC VARIABILITY AND SIMILARITY IN TWO FAMILIES OF CRABS

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ABSTRACT

Serum Proteins and esterase isozymes of crabs: Eriphia spinoforms (red); Eriphis spinoforms (green) Family xanthidae and Portunus pelagicus; portunus arcuatus, Carcinus mediterraneus and Charybdis helleri Family Portunidae were studied electrophoretically. SDS polyacrylamide gel was used to estimate the molecular weight of the variable protein between the two families. The genetic distance was calculated for the two families.

The results indicated that Eriphia spinoforms (red) and Eriphia spinoforms (Green) appeared as two species. Portunus pelagicus, Carcinus mediterraneus and Carybdis helleri were closely related to each other and constitute one group; while Portunus arcuatus constitutes another group, loosely related to the first. Esterase isozymes patterns showed genetic variability in the different species of Crabs.

The molecular weight of the variable proteins between the two families ranged between 140,000 D and 18,000 D.

The genetic distance indicated also that portunus pelagicus; Carcinus mediterraneus and Carybdis helleri were closely related to each other and Portunus arcuatus was loosely related to this group. Eriphia spinoforms (red) and Eriphia spinoforms (Green) were two related species.

INTRODUCTION

Crabs are the most commercially important edible Crustaceans. They are classified in Order decapoda, Suborder Reptania, Section Brachura. Its fishery has grown consideraply and are now commercially well exploited.

The study of protein variation enables us to evaluate the amounts of genetic variation in populations of economically important species that cannot yet be bred in culture and later to document the genetic changes brought by their domestication. Furtthermore, identification of biochemical genetic markers and their distribution in natural populations facilitate the development of quantitative genetics research and breeding programs as control over reproduction is gained.

The biochemical genetics, therefore, is being recognized as an immediate and efficient approach to generate much of the basic genetic information crucial to the development of aquaculture (Utter et al., 1974).

The study of gene protein variation is accomplished by straight forward of electrophoresis. All electrophoretic methods have proved useful to varying degrees in characterizing specific organisms. In particular, acrylamide gel electrophoresis has been extensive used because of its excellent resolving power (Brewer, 1970).

Few of these procedures were described adequately for use with crustaceans (Redified and salini, 1980).

Accordingly, the present study includes preliminary observation concerning the applicability of protein and esterase isozymes to evaluate the amount of genetic variation in six species of crabs collected from Mediterranean Sea at Alexandria.

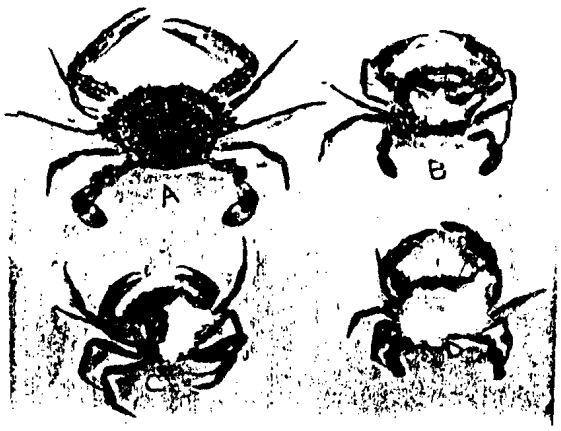
MATERIALS AND METHODS

The present investigation was carried out on crabs: portunus pelagicus (P.P), portunus arcuatus (P.a), Carcinus mediteraneus(C.m), Charybdis helleri (C.h) (Family Portunidae), Eriphia spiniforms red (ESR) and Eriphia spiniforms green (ESG) Family Xanthidae (Fig. 1). The samples were collected from Mediterranian Sea at Alexandria. Flesh was removed from alive samples and used for analysis immediately or after storage at 4°C. A crude aqueous extract of soluble proteins is obtained by homogenizing tissues and centrifuging for 20 minutes at 3000 rpm. The supernatants were vtilized to determine serum proteins by vertical polyacrylamide gel electrophoresis. The procedures were modified from Davis (1964).

Esterase isozymes were determined for the six species in the serium proteins by vertical polyacrylamide gel electrophoresis and the staining method was modified from Stordeur (1976) and Shaw and Prasad (1970).

SDS polyacrylamide gel electrophoresis was used to study serum proteins of Eriphia spinoforms red (Fam. Xanthidae) and Portunus pelagicus (Fam. Portunidae according to Stegemann et al. (1987). The molecular weight of the separated proteins was calculated from Table 1 which represents the molecular weight of the used marker proteins.

The genetic distances were calculated for each species of the two families from serum protein according to Sokal and Sneath (1963).



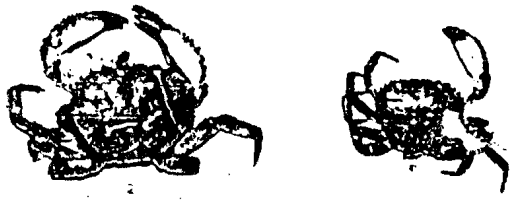


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FIG. 1
Family Portunidae
A= Portunus Pelagicus B= Portunus arcuatus
C= Carcinus mediterrneus D= Charybdis helleri

Family Xanthidae :
Eriphia spinoforms (red), Eriphia spinoforms (red)

TABLE 1
Marker proteins and their molecular weights.

Proteins as	Producer, Order number	Mol.Weight of Protomers
Immunoglobulin, Normal Human IgG, Purif.	Byk-Mallinokrodt, Nordic Immunology, D-6051 Dietzenbach-Steinberg	(150,000 D) (50,000 D 23,500 D
Phosphorylase b (rabbit muscle) lyophilized (40 mg= 5 mg protein)	Boehringer, 108 275	97,400 D
Albumin (bovine serum), dry puriss	Behringwerke, oRHD	67,000 D
Fumarase (pig heart) Crystal suspension	Boehringer, 104 957	49,000 D
Alcohol-dehydrogenase (yeast),(ADH) Lyophilized (50 mg=30 mg protein)	Boehringer, 102 709	37,000 D
Chymotrypsinogen A (bovine), 6 x Cryst., puriss.	Serva, 17 200	25,700 D
Lysozyme from egg white, puriss.	Serva, 28.260	14,300 D

RESULTS AND DISCUSSION

Since successful genetic improvement either through selection or hybridization depends on the amount of genetic variation within and between populations, this study has concerned with the quantification of genetic variation in natural populations by using protein and esterase electrophoresis in the species of crabs. Fig. 2 shows the serum protein patterns of Fam. Xanthidae: Eriphia spiniforms (red) and Eriphia spiniforms green. It is clear that 15 proteins migrated towards the anode. No apparent differences could be observed between the two species in major proteins except protein No. 13 and 14, but the differences between the two species appeared in the minor proteins at No. 1, 6, 9, 10, 14 and 15.

Figure 3 shows the serum proteins patterns of four species of Family Portunidae: Portunus pelagicus, portunus arcuatus, Carcinus mediterraneus and Charybdis helleri.It is clear that no two patterns are exactly alike in the four species. However, this family have been characterized by the presence of proteins 1, 2, 6, and 9 in the four species.

Shaw (1970) mentioned that closely related species which occur in the same genus, differ at about 50-80% of their genes. From Figs. 2 and 3, it is possible to state that in these samples the serum protein patterns are specific for any one of species and constitute a single by which these two families may be identified. The results also indicated that Eriphia spinoforms (red) and Eriphia spinoforms (green) are related to each other and appeared as two species.

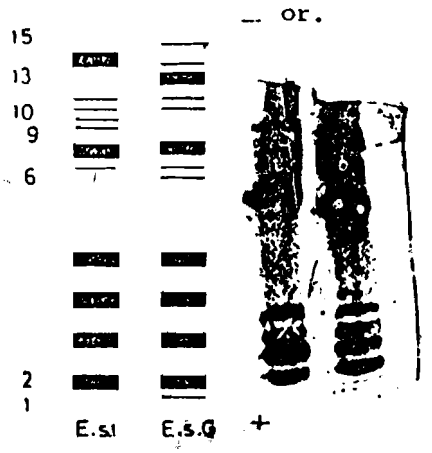

FIG. 2
Serum proteins of
Fam: Xanthidae

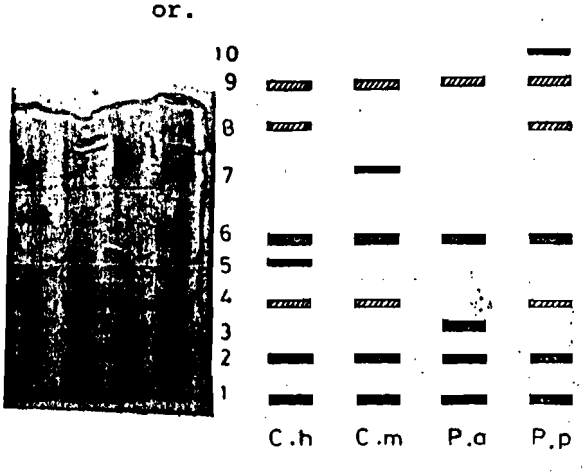

FIG. 3
Serum proteins
of Fam: Portunidae

Conventional systematics usually depend to a larger extent on mortphological characters. But morphological differences in congenetic animal species probably depend on relatively few genes. It can therefore not be ruled out that special selection pressure might have been exerted predominantly on this small fraction of the genome only, which mainly determines morphological characters, Morphological dissimilarities therefore may falsely imply in some cases considerable evolutionary divergence. Conversely it has already been shown that slight morphological and/or ecological dissimilarities exist between sibling species cannot be taken as evidence of little genetic differentiation. Sibling species of the Drosophila willistoni group are morphologically very similar but genetically very different (Ayala et al., 1971).

Figures 4 and 5 show the esterease isozymes of the different species of crabs. It is clear that four isozymes of esterases at Eriphia spp. and the differences between the two species appeared at E_2, E_3 and E_4. But in family Portunidae, it is appeared three isozymes of esterases and the differences between the four species in the three isozymes.

Kirpichnikov, (1973) said that esterases are inherited codominantly without formation of hybrid rings. As a rule, the esterase molecule is a homopolymer (probably a dimer) and therefore, the number of isozymes is not considerable for this enzymes. In some fishes, however, it is large (Holmes and Whitt, 1970).

Since electrophoresis examins only the structural genes which comprise approximately 1% of the genome. Hypothetically two proteins of different molecular weights may migrate toward the anode at the same rate if their size differences are balanced by compensating charge differences. For this reason acrylamide gel electrophoresis may not be used to gain information about molecular weight of a protein. A second restriction placed on electrophoretic techniques concerns the number of species observed on the gel molecules which are tightly but not covalently bound together and not usually separted from one another during electrophoresis.

Shapiro et al. (1967) attempted to surmount these problems by separate a mixture of proteins in the presence of sodium dodecyl sulfate (SDS), an anionic detergent. The binding of SDS introduces one negative charge per bound molecule of SDS on the protein molecule. At neutral pH the total charge of the protein SDS complex is almost entirely dependent upon the charge of the SDS molecules. It has been found that the charge per unit mass is approximately constant and therefore the electrophoretic mobility of the complex is mainly dependent on the molecular weight of the protein.

FIG. 4
Eeterase isozymes of
Fam. Xanthidae.

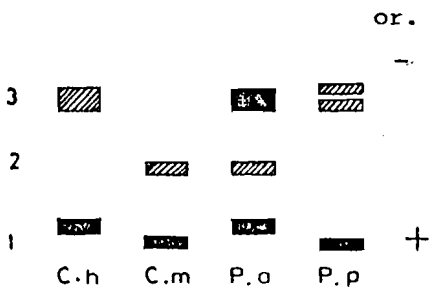

FIG. 5
Esterase isozymes of
Fam: Portunidae

To gain information about the molecular weight of proteins in the two families. Portunus pelagicus (Fam. Portunidae) edible performance species and Eriphia spiniforms red, the higher growth rates have been chosen to study the variation between the two species at the molecular weight (Fig. 6). The molecular weight of the variable proteins in the two species has been calculated from the standard curve (Fig. 7).

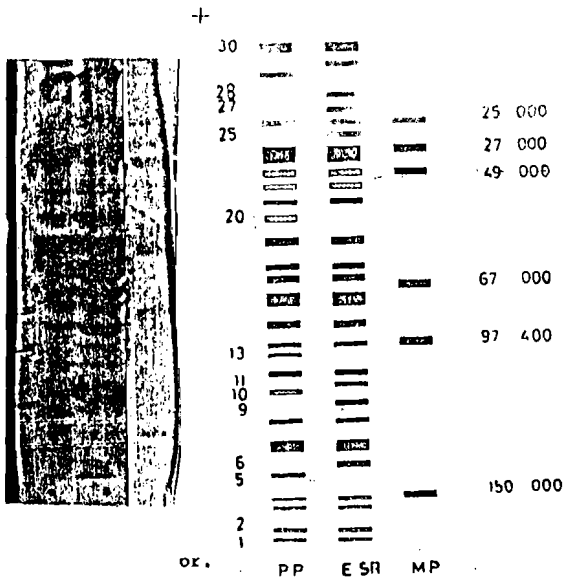

FIG. 6
SDS poly acrylamide gel
of P.P, ESR and marker
proteins.

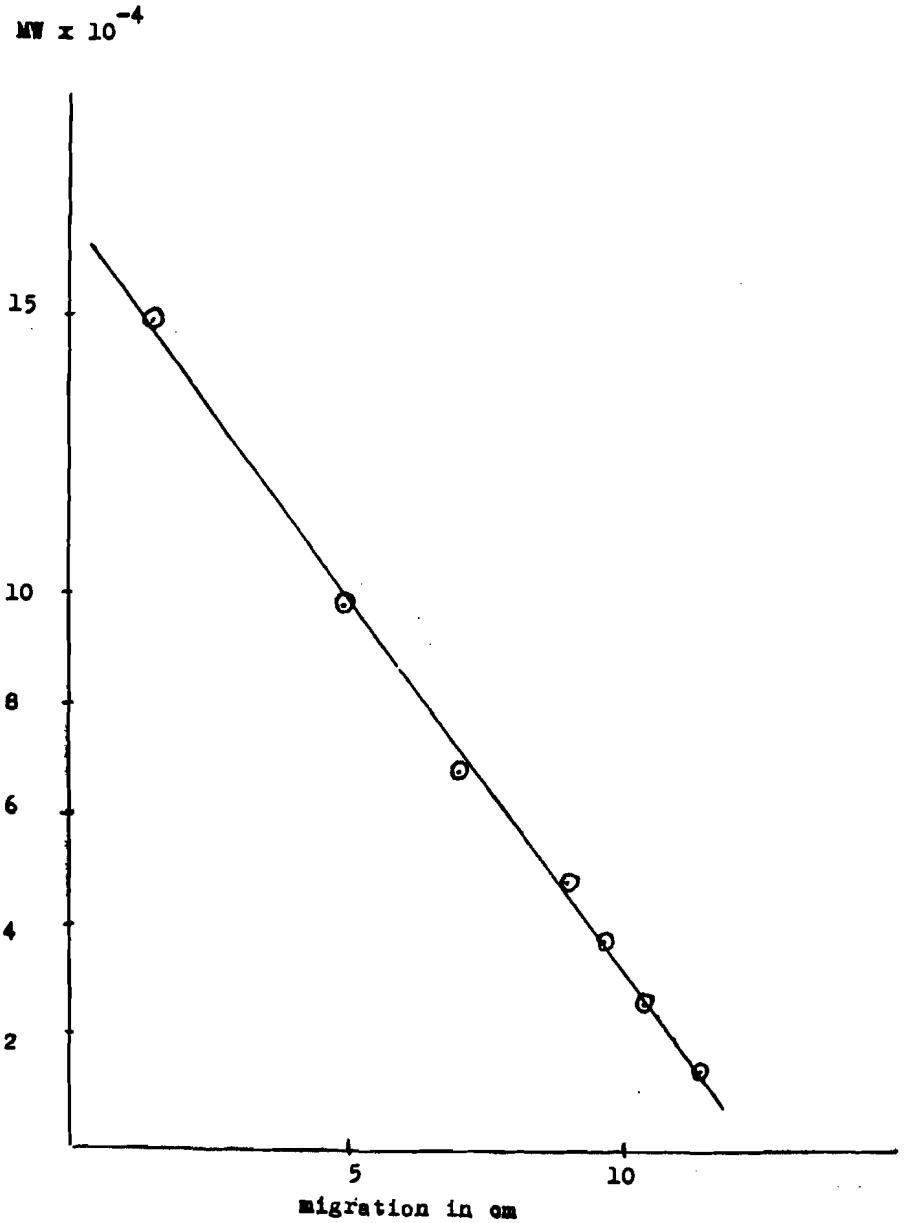

FIG. 7
Standard curve relating Protein molecular weight in Daltons and band migration in "Cm".

Table 2 represents the variable proteins in the two species and their molecular weights. It is clear that the variation between the two species is localized at 12 proteins and their molecular weight ranges between 140,000 D and 18.000 D.

For the development of inbreeding and cross breeding programs, biochemical genetics permits the evaluation of the degree of homozygosity and the genetic similarity of populations making designed crossing more likely to be productive.

TABLE 2
The molecular weight of the variable proteins in Portunus pelagicus and Eriphia spinoforms red.

Protein	Species		Mobility	Molecular weight MW 10^{-4}
	P.P	ESR		
5	+	-	2.1	14.0
6	-	+	2.3	13.7
9	-	+	4.0	11.5
10	+	-	4.2	11.2
11	-	+	4.4	11.0
13	+	-	4.9	10.1
20	+	-	8.0	6.0
25	-	+	9.9	4.5
27	-	+	10.4	2.5
28	-	+	10.6	2.4
29	+	-	10.8	2.1
30	-	+	11.0	1.8

The genetic distance was calculated from serum proteins of the two families at 12 genetic loci in Fam. Xanthidae and at 6 genetic loci in Family Portunidae. It is clear from Tables 3, 4 and Fig. 8 that the distance between ESR and ESG is 0.05 while the genetic distance in Family Portunidae range from 0.0054 to 0.125, and (P.P), (C.m) constitute one group closely related to each other while (P.a) constitutes another group loosely related to the previous group. The distance between the group (P.P), (C.m) and Eriphia spp. is nearly the same distance between (P.a) and the group (p.p, cm. c. h).

TABLE 3

Genetic distances between members of Family:Portunidae.

	P.P	P.a	C.m	C.h
P.P	—	0.078	0.0054	0.0062
P.a		—	0.1250	0.0890
C.m			—	0.0064
C.h.				—

Table 4

The genetic distances between ESr, ESr, ESG
Fam.: Xanthidae and C.h. Fam.: Portunidae.

	Ch	ESr	ESG
Ch	—	0.115	0.117
ESr		—	0.050
ESG			—

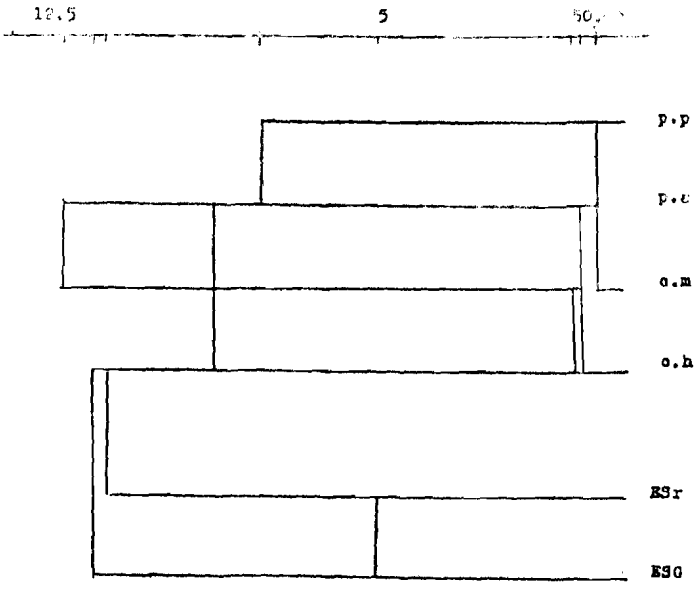

FIG. 8

Genetic distances of the Family: Portunidae and Family: Xanthidae.

Hedgecock et al.(1976) reported that genetic distance has enabled to measure the amount of genetic divergence between the American and European Lobsters (H. americanus and H. gammarus), Decapoda; Crustacia, using Nei's measure of genetic distance D. The statistic for this inter specific comparison D= 0.103 is 10 times that among different populations of American lobsters, D=0.006. Hybridization of the European and American species appears feasible and will result in highly heterozygous offspring. By measuring the performance of these lobsters, hybrid vigor can be correlated with heterozygosity and the practically of the hybridization program may by evaluated.

REFERENCE

Ayala, F.J.; J.R. Powell, and T. Dobzhansky, 1971. Polymorphism in continental and island populations of **Drosophila willistoni**. **Proc. Nat. Acad. Sci.** 68: 2480-2483.

Brewer, G.J., 1970. **An introduction to isozyme techniques.** New York; Academic Press.

Davis, B.J., 1964. Disc electrophoresis. II- Methods and application to human serum proteins. Ann. N.Y. Acad. Sci., 121: 404-407.

Hedgecock, D., R.A. Shleser and K. Nelson, 1976. Applications of Biochemical Genetics to Aquaculture. **J. Fish Res. Board Can.** 33: 1108-1119.

Holmes, R.S. and G.S. Whitt, 1970. Developmental genetics of the esterase isozymes of **Fundulus heteroclitus**. **Biochem. Genet.** 4 (4): 471-480.

Klyashtorin, V.S., 1973. **Biochemical polymorphism and microevolution processes in Fish.** Genetic and mutagenesis of fish. Edited by J.H. Schroder. Springerverlage.

Redfied, J.A.and J.P. Sellers, 1989. **Techniques of starch gel electrophoresis of penaeid prawn enzymes, *Penaeus esc.* and Metapenaeus spp.** Csiro Aust. Div. Fish Oceanogr. Rep. 116 (1989).

Shapiro, A.L.; E. Vinuela and J.V. Maizel, 1967. Molecular weight estimation of polypeptide chains py electrophoresis in SDS polyacrylamide Gels. **Biochem. Biophys. Res. Commun.**, 28: 815-820.

Shaw, C.R. (1970). how many genes evolve? **Biochem. Genet.** 4: 275-283.

Shaw, C.R. and R. Prasad, 1970 Starch gel electrophoresis of enzymes. A compilation of recipes. **Biochem. Gent.**, 4: 707- 718.

Sokal, R.R. and P.H. Sneath, 1963. **Principles of numerical taxonomy.** W.H. Freeman, San Francisco.

Stegemann, H.; W. Burgermeister and A. Shan, 1987. **Electro focusing in tubes.** Institut Fur Biochemie, Biologische, Bundesanstalt,Messweg, 11, D-330 Braunschweig (west Germany).

Stordeur, E., 1976. Esterase in the mosquito Culex Pipiens pipiens L: Formal genetic and polymorphism of adult esterase. **Biochem. Genet.**, 14: 481-493.

Utter, F.M.; H.O. Hodings and F.W. Allendrof. 1974. Biochemical genetics. Studies of Fishes: polentialities and limitations,P.213-238. In : J.K. Sargeant (ed.) **Biochemical and Biophysical Preapectives in Marine Biology.** Vol. 1. Academic Press,Inc., San Francisco Calif.

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989: 179 - 189

BIOMASS OF THE STANDING CROP OF PHYTOPLANKTON IN LAKE BUROLLUS (EGYPT)

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ABSTRACT

The phytoplankton biomass in Lake Burollus (Egypt) was estimated monthly during 1979. Results indicate that the biomass of the different classes was altered when compared with its numerical values. Thus, Bacillariophyceae constituted about 69 % of the total algal biomass, while Chlorophyceae and Cyanophyceae showed decreased frequencies to about 16 % and 15 % respectively. On the other hand, Chlorophyceae was numerically the most important plankters and comprised collectively 58.9 % of the total phytoplankton counts. This was followed by Bacillariophyceae (31.1 %) and Cyanophyceae (8.8 %).

The phytoplankton biomass showed a gradual increase from the eastern Lake towards the west particularly due to the increased biomass of Bacillariophyceae.

The more dominant species which contributed the bulk of the phytoplankton biomass comprised, Cyclotella meneghiniana Kutz., Nitzschia palea (Kutz) W. Sm., N. reversa W. Sm., Melosira varians Ag., Synedra ulna Ehr., Pleurosigma Sm. and Microcystis aeruginosa Kutz.

INTRODUCTION

Lake Burllus is a shallow brackish water lake lying at the North of the Nile Delta along the Mediterranean Coast of Egypt, with an area of about 50,000 hectares and an average water depth of 115 cm. The Lake receives most of its water from five main drains which open at the southern margin of the Lake, beside Burllus Drain which is located at the north-eastern side. The western extremity of the Lake is connected with Rashid Estuary through Brimbal Canal (Fig. 1). The amount of water discharged into the Lake amounts to about 2.6 milliard cubic meter per year. The surplus water flows constantly from the Lake into the sea through a small opening named as Boughaz El-Bourg. Sea water may on rare occasions invade the Boughaz region particularly during winter.

The phytoplankton community in Lake Burllus is rich, both in density and number of species. Its composition and numerical distribution were previously given by El-Sherif (1983). This paper deals with the quantitative estimation of the phytoplankton biomass in the Lake.

FIG. 1
Morphometry of Lake Burullus and position of stations.

MATERIAL AND METHODS

The phytoplankton biomass was determined by measuring the size of the different species (average volume of 30 specimens for each species) according to the formulae recommended by Edler (1979), taking in consideration that the specific gravity of phytoplankton cells is unity (cf. Strickland, 1960). The biomass is expressed in mg fresh weight/l. The rare plankters were excluded in the present estimation.

Eighteen stations were selected as representing the different habitats in the Lake, their locations are shown in Fig. 1. These stations were further grouped into three main sectors, namely; eastern Lake (Stations 1-6), middle Lake (Stations 7-12) and western Lake (Stations 13-18).

Sampling was carried out monthly from January to December, 1979.

RESULTS AND DISCUSSION

Cell Volume Measurements

Results of measurements of cell volumes of the different species and their standard deviation are illustrated in Table 1.

Composition And Distribution

While Chlorophyceae was numerically the dominant group as it formed about 58.9 % of the total phytoplankton counts during 1979 (El-Sherif, 1983), yet it contributed only 16.2 % of the total phytoplankton biomass. Similarly the biomass of Cyanophyceae was comparable to that of the green algae and it constituted about 14.8 % of the total phytoplankton biomass. On the other hand, Bacillariophyceae represented the major bulk of the phytoplankton and formed 69.0 % of its total biomass, although it ranked numerically as the second important group with 31.1 % of the total phytoplankton counts.

As shown in Table 2, the highest phytoplankton biomass appeared in the western sector particularly due to the increased values of diatoms and it decreased gradually eastwards.

Seasonal Variations

The monthly fluctuations of the total phytoplankton biomass is shown in Fig. 2. The eastern and middle Lake showed maximum persistence of phytoplankton biomass in early autumn (September). Relatively high values were also recorded during the winter in the eastern Lake and in March in the middle sector.

TABLE 1

Algal volumes in cubic microns of the different species of phytoplankton in Lake Burollus and their standard deviation.

Phytoplankton species	volume in cubic microns ($=mm^3 x10^{-9}$)
Bacillariophyceae	
Cells :	
- Nitzschia microcephala Grun	356.22 ± 27
- N. palea (Kutz) W. Sm.	824.12 ± 57
- N. reversa W. Sm.	1144.00 ± 119
- Cyclotella meneghiniana Kutz	3297.55 ± 235
- Melosira granulata (Ehr.) Ralfs.	1009.55 ± 81
- M. Varians Ag.	13766.13 ± 688
- Synedra ulna Ehr.	11726.39 ± 704
- S. tabulata Kutz.	1076.36 ± 54
- Cocconeis placentula Ehr.	2296.77 ± 207
- Mastogloia braunii Grun.	8522.95 ± 596
- M. Smithii Thw.	5174.45 ± 259
- Pleurosigma Sm.	56207.60 ± 1686
Chlorophyceae	
1. Cells :	
- Scenedesmus quadricauda (Turp.) Breb	159.03 ± 14
- Sc. diagonalis S. Fang.	32.71 ± 4
- Sc. bijugatus (Turp.) Kutz.	170.61 ± 15
- Sc. opaliensis Rich.	36.63 ± 4
- Dictyosphaerium pulchellum Wood.	35.09 ± 6
- Pediastrum duplex Meyen.	187.21 ± 15
- P. boryanum (Turp.) Menegh.	563.00 ± 45
- P. simplex Meyen.	6378.33 ± 127
- Ankistrodesmus falcatus var. mirabile W. & G.S. West	35.49 ± 7
- Ankistrodesmus falcatus var. spirilliformis G.S. west	47.74 ± 10
- Crucigenia quadrata Morren.	43.98 ± 4
- Oocystis borgei Snow.	2477.66 ± 148
- Tetraedron minimum (A. Braun) Hang.	490.74 ± 49
- Sphaerocystis schroeteri Chod.	220.78 ± 18

Table 1: Contiune

2. Filaments :	
- Geminella minor (Nag.) Hansg.	2532.24 ± 126
Cyanophyceae	
1. Cells :	
- Merismopedia minima Beck.	0.52 ± 0.08
- M. punctata Meyen.	47.42 ± 12
2. Filaments :	
- Lyngbya limnetica Lemm.	899.43 ± 33
- Oscillatoria limnetica Lemm.	1138.08 ± 155
- Anabaenopsis sp.	2474.45 ± 124
- Anabaena sp.	2664.29 ± 133
3. Caenobia :	
- Microcystis aeruginosa Kutz.	6198.00 ± 310

Table 2

Average biomass of the different groups of phytoplankton (mg/l) recorded in the three sectors of Lake Burollus and their percentage frequency for the whole Lake during 1979.

Section Phytoplankton	Eastern Lake	Middle Lake	Western Lake	Average	%
Bacillariophyceae	1.2726	1.1419	2.4398	1.6181	69.0%
Chlorophyceae	0.1251	0.5120	0.5052	0.3808	16.2%
Cyanophyceae	0.1292	0.3869	0.5258	0.3473	14.8%
Total	1.5269	2.0408	3.4708	2.3462	100%

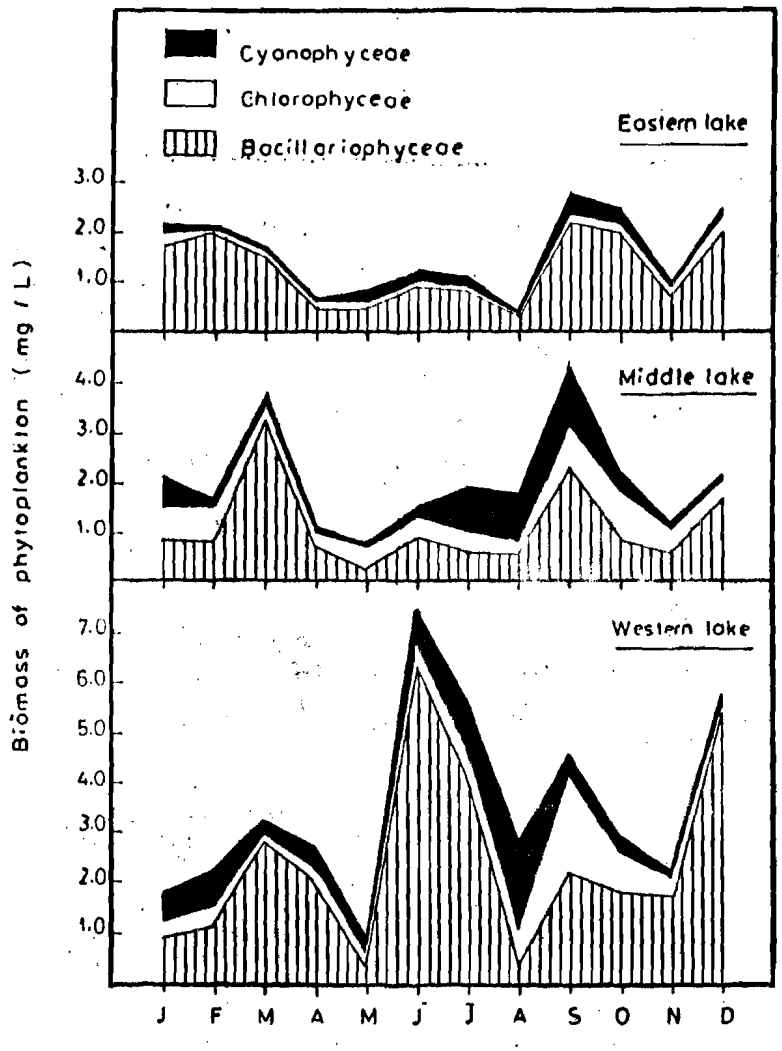

FIG. 2.
Seasonal variations of the phytoplankton biomass (mg\1) recorded in the three sectors of the lake during 1979.

The highest peak of abundance was observed in the western Lake during the summer (June & July) beside smaller ones in September and December. Most of these peaks were attributed to diatoms.

The following is a quantitative survey on the distribution of the different classes of phytoplankton recorded in the Lake.

a - Bacillariophyceae :

Diatoms contributed about 69 % by weight to the total phytoplankton (average 1.6181 mg/l). As shown in Table 3 and Fig. 3A the western sector sustained highest diatoms biomass, particularly due to Nitzschia palea, Nitzschia reversa, Cyclotella meneghiniana, Melosira varians, Pleurosigma sp. and Synedra ulna.

The other two sectors sustained more or less comparable values with the same diatom composition but showing different frequencies. The main diatoms there comprised Cyclotella meneghiniana and Synedra ulna.

Table 3

Average biomass (mg/l) and percentage frequency of the different species of diatoms to the total Bacillariophyceae in the three sectors of Lake Burollus during 1979.

Section	Eastern Lake		Middle Lake		Western Lake	
Diatoms	Biomass	%	Biomass	%	Biomass	%
- Nitzschia palea	0.0383	3.0	0.1558	13.6	0.6194	25.4
- N. reversa	0.0812	6.4	0.0490	4.3	0.2885	11.8
- N. microcephala	0.0440	3.5	0.0807	7.1	0.1316	5.4
- Cyclotella meneghiniana	0.3808	29.9	0.2267	19.9	0.5947	24.4
- Synedra ulna	0.2708	21.3	0.3640	31.9	0.1582	6.5
- S. tabulata	0.0133	1.0	0.0032	0.3	0.0026	0.1
- Melosira varians	0.0356	2.8	0.1704	14.9	0.3536	14.5
- M. granulata	0.0099	0.8	0.0199	1.7	0.0918	3.8
- Pleurosigma sp.	0.1246	9.8	0.0519	4.5	0.1869	7.6
- Cocconeis placentula	0.2046	16.1	0.0098	0.9	0.0029	0.1
- Mastogloia smithii	0.0411	3.2	0.0105	0.9	0.0096	0.4
- M. braunii	0.0284	2.2	---	---	---	---
Total	1.2726	100%	1.1419	100%	2.4398	100%

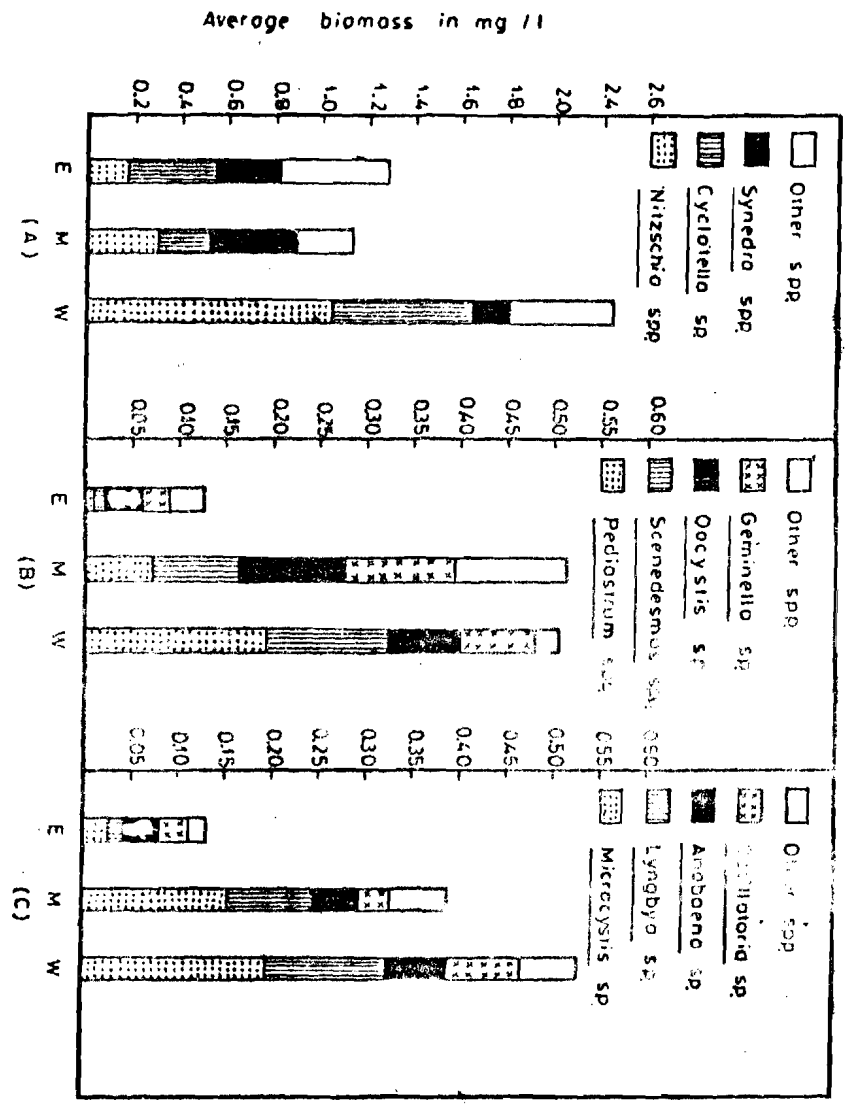

FIG. 3.
Average biomass of the different groups of phytoplankton (mg\1) recorded in three sectors of the lake during 1979.
(A) Bacillariophyceae (B) Chlorophyceae (C) Cyanophyceae

b - Chlorophyceae :

Members of chlorophytes contributed about 16.2 % by weight to the total phytoplankton biomass (average 0.3808 mg/l). The highest values appeared in the middle and western sectors, showing the same dominant species, namely; Pediastrum simplex, P. boryanum, Scenedesmus quadricauda, Sc. bijugatus, Oocystis borgei, Geminella minor and Dictyosphaerium pulchellum, but with different percentage frequencies (Table 4 & Fig. 3B). The eastern Lake harboured

Table 4

Average biomass (mg/1) and percentage frequency of the different species of green algae to the total Chlorophyceae in the three sectors of Lake Burollus during 1979.

Section	Eastern Lake		Middle Lake		Western Lake	
Green algae	Biomass	%	Biomass	%	Biomass	%
Pediastrum simplex	---	---	0.0283	5.5	0.1449	28.7
- P. boryanum	0.0067	5.3	0.0335	6.5	0.0291	5.8
- P. duplex	0.0017	1.3	0.0097	1.9	0.0168	3.3
- Scenedesmus quadricauda	0.0082	6.6	0.0581	11.4	0.0908	18.0
- Sc. diagonalis	0.0007	0.6	0.0058	1.1	0.0085	1.7
- Sc. bijugatus	0.0034	2.7	0.0283	5.5	0.0304	6.0
- Sc. opaliensis	0.0001	0.1	0.0003	0.1	0.0007	0.1
- Oocystis borgei	0.0403	32.2	0.1154	22.5	0.0769	15.2
- Geminella minor	0.0276	22.1	0.1169	22.8	0.0800	15.8
- Dictyosphaerium pulchellum	0.0224	17.9	0.0788	15.4	0.0012	0.2
- Tetraedron minimum	0.0081	6.5	0.0199	3.9	0.0086	1.7
- Sphaerocystis schroeteri	0.0024	1.9	0.0093	1.8	0.0113	2.2
- Crucigenia quadrata	0.0010	0.8	0.0062	1.2	0.0047	0.9
- Ankistrodesmus falcatus var. mirabile	0.0017	1.4	0.0013	0.3	0.0010	0.2
- Ankistrodesmus falcatus var. spirilliformis	0.0008	0.6	0.0002	0.04	0.0003	0.1
Total	0.1251	100%	0.5120	100%	0.5052	100%

low values of green algal biomass where Oocystis borgei, Geminella minor and Dictyosphaerium pulchellum formed the main bulk of chlorophytes there.

C - Cyanophyceae :

The blue green algae, as a whole constituted about 14.8 % by weight to the total phytoplankton biomass (average 0.3473 mg/l), while their numerical values dropped to 8.8 % of the total phytoplankton counts. The western sector harboured a high value of 0.5258 mg/l due to the increased weights of Microcystis aeruginosa, Lyngbay limnetica, Anabaena SP. and Oscillatoria limnetica. Their total biomass decreased gradually towards the eastern Lake but showing similar algal composition (Table 5 & Fig. 3C).

Table 5

Average biomass (mg/l) and percentage frequency of the different species of blue green algae to the total Cyanophyceae in the three sectors of Lake Burollus during 1979.

	Eastern Lake		Middle Lake		Western Lake	
Blue green algae	Biomass	%	Biomass	%	Biomass	%
Microcystis aeruginosa	0.0253	19.6	0.1512	39.1	0.1921	36.5
Lyngbya limnetica	0.0122	9.4	0.0896	23.2	0.1341	25.5
Anabaena sp.	0.0419	32.4	0.0492	12.7	0.0645	12.3
Oscillatoria limnetica	0.0316	24.5	0.0309	8.0	0.0761	14.5
Anabaenopsis sp.	0.0156	12.1	0.0485	12.5	0.0361	6.9
Merismopedia punctata	0.0023	1.8	0.0164	4.2	0.0217	4.1
Merismopedia minima	0.0003	0.2	0.0011	0.3	0.0012	0.2
Total	0.1292	100%	0.3869	100%	0.5258	100%

CONCLUSION

Results of the present investigation show that the biomass of the different classes of phytoplankton was altered when compared with its numerical distribution. Thus, while Chlorophyceae was numerically the most dominant group, this was shifted to Bacillariophyceae when discussing the algal biomass.

The phytoplankton biomass, in general, may give a more precise picture on the magnitude of its standing crop which represents the first trophic level in food cycle.

REFERENCES

Edler, L., 1979. Recommendations on methods for marine biological studies in the Baltic Sea. phytoplankton and Chlorophyll. The Baltic marine biologists, Working group publication No. 5, (9): 1-38.

El-Sherif, Z.M., 1983. Limnological investigation on the aquatic plant life in Lake Burollus in relation to the dominant environmental conditions. Ph. D. Thesis, Faculty of Science, Cairo University, 385 PP.

Strickland, J.D.H., 1960. Measuring the production of Marine phytoplankton. J. Fish. Res. Bd. Canada, 122: 172 PP.

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989: 191 - 195.

ROLE OF ANTIFOAMERS IN EXTRACTING OIL FROM DESALINATED SEA WATERS

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ABSTRACT

In the countries surrounding the Arabian Gulf, as in all similar arid areas, seawater desalination is the absolutely vital industry. Because of the concentration of enormous petroleum activities in this area, this industry is in continuous menace from all risks of marine oil pollution.

In the present work, an investigation is conducted for the possible role antifoaming agents in minimizing the load of dissolved petroleum products interfered within the distilled seawater during its desalination processes. By laboratory experiments, the results showed that the added antifoamers could extract considerable amounts of dissolved petroleum hydrocarbons. For two dominant used types of antifoamers (Antifroth and U-Con), equations for their capacities in extracting petroleum hydrocarbons from seawater were deduced.

INTRODUCTION

In the arid areas where potable and irrigation waters are rare, desalination of seawater is a convenient means of supplying the local water demand. In all countries surrounding the Arabian Gulf, seawater desalination represents a very vital industry for their survival and development. As this region is well known by its richness in various petroleum activities (production, transportation and processing), the oil pollution in the Gulf marine environment is normally existing in the same order of magnitude as the enormous petroleum activities. However, the coastal desalination plants in the area are usually in continuous menace by stopping their operational activities -when the pumped seawater become contaminated by oil. This was the case following the disaster of Nowruz in 1983 during the Iran-Iraq war (1982 -1988) when almost all desalination plants were closed for long duration. This is inspite of the fact that the regions of water intakes for most of the desalination plants in the area are usually protected by fence or curtain booms. In fact, this type of protection is useful for spreaded and to some extent for dispersed oil in the water, but it is useless for the dissolved or minute emulsified oil fractions. This later oil fraction could escape from the booms and interfere in the desalinated sea water causing unpleasant and harmful modifications in the final produced desalinated waters.

Multistage flash distillation (MSF) is the process used in almost all large sea water desalting production plants. The equipment is simple; the operation is relatively reliable and the manufacturing techniques and engineering design are sufficiently well established to allow dependable easily operated units to be produced (McIlhenny, 1975). This type of seawater desalting technique is in fact the dominant employed one in the area of Arabian Gulf. The method is composed of successive steps which could be summarized in the following lines :

- injection of the pumped saline water with concentrated H_2SO_4 for the prevention of scale formation of $CaCO_3$ and $Mg(OH)_2$. The used concentration is 120 ppm.

- injection with NaOH to nutralize the effect of the previous acidification step and for keeping the pH in the range of 7.4 - 7.7.

- treatment with the antifoam agent for preventing foams with saline waters during distillation step. Antifoam is used with concentration corresponding to 0.16 ppm.

- distillation of treated saline water in vacuum for reducing pressure to maintain the process at low temperature (about $40^{o}C$).

Before distillation step, there are two other steps which are not included in our design (treatment with hypochlorite as a source of free chlorine for killing bacteria and with lime water for preventing erosion in pipe network). According to the aim of our investigation, these two later steps are not practically significant.

Among these above operational steps, the present work is devoted to illustrate the possible role of using antifoams in extracting dissolved oil hydrocarbons from distilled sea water. The idea of this work is developed from the fact that the antifoaming agents are surface active in nature and composition. The physical action of these compounds is to diminish the surface tension of the air bubbles in the solution leading to burst them. Hence, the use of antifoamer is necessary to avoid both loss in equipment capacity (distillation chambers) and increase in processing time (distillation time). Indeed, in the seawater desalting plants, the use of antifoamers is necessary to avoid the contamination of condensed vapours with salt particles in the distillation chambers.

MATERIAL AND METHODS

The used materials in the present work are : two types antifoaming agents (Antifroth and U-Con), and two types of Arabian crude oils (light and heavy). The used two types of antifoamers were chosen because of their dominant use in the Saudian desalination plants which are the largest plants in the area. Also, the two selected oils are representing the most possible contaminant in the Gulf area.

The followed procedure for evaluating the efficiencies of the antifoamers in extracting dissolved hydrocarbon from sea water could be summarized in the following steps :

- working contaminated seawater by either of the two types of oil were prepared by shaking certain amount of oil in water for 2 hr; leaving for decantation overnight and withdrawing the clear water accommodated fraction (WAF) of oil. The percentage of oil in water was adopted to produce a working WAFs with a concentration of 4 ppm (0.3% and 0.2% oil in water for heavy and light oils respectively).

- for a fixed aliquot of the prepared oil WAFs (500 ml), increaments of antifoamers were added (2, 4, 6, 8 and 10 ml), shaked for 5 min, left overnight for phases separation.

- total hydrocarbon contents in the above solutions (pure WAFs and WAFs containing antifoamers) were measured following the standard procedure of IGOSS (Anon., 1976) : extraction by carbon tetrachloride and detection spectrofluorometrically using an excitation wave length at 360 nm and fluorescence wave lengthes at 371 and 380 nm for light and heavy Arabian crude oils respectively. The used instrument was Baird spectrofluorometer, model Ratiometric RC 200.

RESULTS AND DISCUSSION

Dispersion capacities for the considered antifoamers in seawater as well as their efficiencies in extracting hydrocarbons were investigated and results are included in Table 1 and represented in Fig. 1. From the results, it could be noted that by increasing the concentration of either antifroth or "-Co" antifoamers while their dispersion decrease parallely their efficiencies for eliminating dissolved/dispersed hydrocarbons increase. This phenomenon could be elucidated by the fact that when the concentration of an antifoaming agent increases in aqueous medium, its major amount tends to leave the medium and concentrate as a layer in the air/water interface as all surface active substances. However, it eliminates more hydrocarbons from the medium (by its surface activity property) and concentrates them on the water surface.

As shown in Figure 1, the relationship between the used concentration of antifoaming agent and the eliminated amount of dissolved/dispersed hydrocarbons from the medium is following a straight line relationship regardless of its type and source of existed hydrocarbons in the medium. The relationship could be expressed with the following deduced equations in which Y is the eliminated amount of hydrocarbons in mg/l and X is the used concentration of antifoaming agent expressed in ml/l :

a) Antifroth-like antifoamers

$$Y = 6.2 \times 10^{2} + 3.49 \times 10^{-3}X$$

(in the case of light Arabian-like crude oil spill)

TABLE 1

Efficiencies of used antifoaming agents in extracting hydrocarbons from seawaters.

conc. of antifoam ml l^{-1}	Antifroth			U-Con		
	% of dispersion	* LA	HA	% of dispersion	* LA	HA
4	9.8	640	250	6.8	170	340
8	7.0	690	305	4.3	270	460
12	6.1	733	355	3.5	320	610
16	4.7	770	413	3.0	370	686
20	3.8	850	470	2.8	420	760

* Amount of extracted petroleum hydrocarbons in mg l^{-1}

LA Light Arabian crude oil

HA Heavy Arabian crude oil

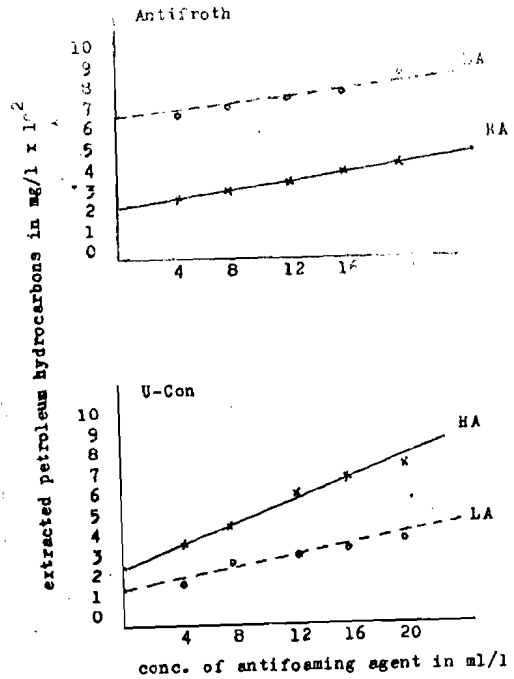

FIG. 1

Relationship between the amount of used antifoaming agents and extracted hydrocarbons from seawater.

LA : light Arabian crude oil

HA : heavy Arabian crude oil

$$Y = 2 \times 10^2 + 4.88 \times 10^{-3}X$$

(in the case of heavy Arabian-like crude oil spill)

b) U-Con-like antifoamers

$$Y = 1.3 \times 10^2 + 5.50 \times 10^{-3}X$$

(in the case of light Arabian-like crude oil spill)

$$Y = 2.85 \times 10^2 + 10.18 \times 10^{-3}X$$

(in the case of heavy Arabian-like crude oil spill)

From the above deduced equations, it could be concluded that the use of antifroth-like antifoaming agents is more suitable for eliminating interfered hydrocarbons coming from light crude oil-likes, while the U-Con-like antifoamers are more suitable for heavy crude oil-like sources.

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REFERENCES

Anon., 1976. Guide to operational procedure for the IGOSS pilot project on marine pollution (petroleum) monitoring. UNESCO, Manuals and Guides, N° 7.

Nelihaven, W.F., 1975. Extraction of Economic Inorganic Materials from Seawater. In: Chemical Oceanography, J.P. RILEY, and G. SKIRROW (EDS.). Academic Press, London, Vol. 4: 155-268.

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989:197 - 206.

HAEMOPIETIC ORGANS IN THE TELEOST CLARIAS LAZERA

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ABSTRACT

Histological examination of haemopoietic organs showed that the head kidney has the primary importance in blood cell formation of Clarias lazera. Haemopoiesis was found to be extravascular in the haemopoietic tissue, and shows highest activity in spring.

Spleen consists of highly vascular haemopoietic tissue. It is haemolymphatic in character. The blood forming tissue is scattered in the stroma of the spleen. It has open circulation and acts as blood destroying and storage organ. It shows lowest activity during summer.

Liver is less important as haemopoietic organ than either kidney or spleen. Seasonal haemopoietic activity is obscure.

The lamina propria of the intestine shows also its highest activity in summer.

The subendothelial areas of truncus arteriosus showed the presence of erythroblasts, erythrocytes, leucocytes as well as macrophages.

INTRODUCTION

Many authors considered that in teleosts, the mesonephric kidney, plays the most significant role in haemopoiesis (Jordan and Speidel, 1924; Yokoyama,1960; Nandi, 1965; Ogawa, 1962; Sabnis and Rangnekar 1962; Sharma, 1969 and 1972; Ward and Davis, 1975 and El-Feky, 1982). Others are of the openion that the primary site of haemopoiesis is the spleen (Walving, 1958 and Haider, 1967) or both kidney and spleen serve as haemopoietic centers (Duthie, 1939; Catton, 1951; McKnight, 1966; Haider, 1967 and Bielek, 1974).

Topf (1953); Yokoyama (1960) and Sabnis and Rangnekar (1962) reported that liver has a certain haemopoietic activity. Stem cells of hymphopoietic series, mature leucocytes were observed in liver and its sinusoid (Bielek, 1974). Kreutzmann (1976 and 1978) recorded the presence of cells of erythrocytes and leucocyte series in the liver.

A number of workers showed that the mucosa of the gut has the potency for haemopoiesis (Jordan and Speidel, 1924; Duthie, 1939, Al-Hussaini, 1949; Yokoyama, 1960; Sabnis and Rangnekar, 1962 and Kreutzmann, 1976). In addition, Yokoyama (1960) mentioned that heart may play a role in haemopoiesis of certain teleosts like the perch.

Except for the work of El-Feky (1982) no reports about haemopolesis or haemopoietic organs on Egyptian fish species are available. The present work is an attempt to study the structure of the major haemopoietic organs, namely kidney, spleen, liver, ileum and heart of the Egyptian catfish, Clarias lazera, in order to throw light on the role played by each of these organs in haemopoiesis.

MATERIAL AND METHODS

Fish were transported to the laboratory from Bab El-Abid zone, which is an unpolluted area of Lake Mariut near Alexandria, Egypt. Fish were left to acclimatize for 48 hr in aerated aquaria of 40 x 120 x 60 cm. Ten healthy fish were examined monthly. Prior to investigation, each fish was measured and weighed. Their body lengths ranged between 15 and 40 cm and weighed between 30 and 250 gm. Fish were dissected and various organs of haemopoietic importance were fixed in 10% neutral formalin. Sections were stained with Eosin-haemoxylin and Masson's Trichrome stain (Pearse, 1972).

RESULTS

Clarias lazera has a pair of red compact long kidneys lying dorsal to the coelom.

The head kidney (Fig. 1), consists of the haemopoietic tissue (HT), the adrenal gland (ag) embedded in the haemopoietic tissue, the kidney tubules (UT) and Malpighian bodies (MB). Blood formation in Clarias lazera is mostly extravascular in the haemopoietic tissue (Fig. 1), although some young stages undergo transformation in the venous sinusoids (Fig. 3, VS). All developing cells are present in groups surrounded by reticular fibres (Fig. 3, RC). In each the more mature cells are present in the center.

The haemopoietic tissue in the kidney appears to be very active in spring (Fig. 1), than in summer and fall (Figs. 2 & 3) producing large amounts of both lymphocytes and thrombocytes. Further, in spring, the uriniferous tubules have vacuolated cytoplasm (Fig.1).

Spleen is a small triangular dark red organ; it is highly vascular haemopoietic tissue and haemolymphatic in character, produces large nucleated erythrocytes and lymphocytes. But their number is considerably less than that produced by head kidney. SO, it comes after the kidney in importance as haemopoietic organ. It is covered by an outer Capsule, from which trabeculae pass into the substance of the spleen dividing it into compartments or lobules (Fig. 4 & 8 TR). The capsule and trabeculae are made connective tissue containing fibres (Fig. 4 RF).

The lobules have splenic cells of different sizes which may by pigmented dark brown (Fig. 7, P), it has also red (Fig. 5) and white pulps (Fig. 6). The red pulp, is made of diffuse lymphatic tissue (dLT) i.e. reticular cells (RC),


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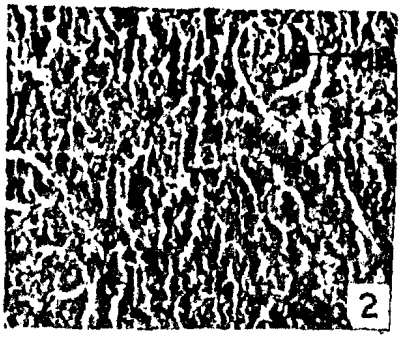
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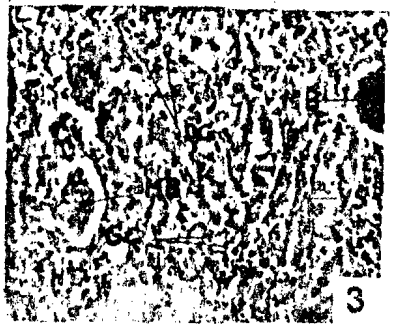
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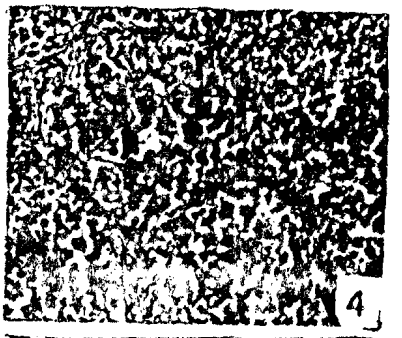
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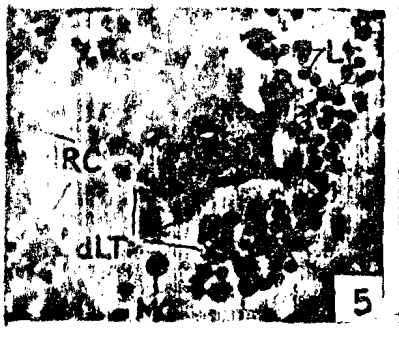
RC
dLT
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CLT

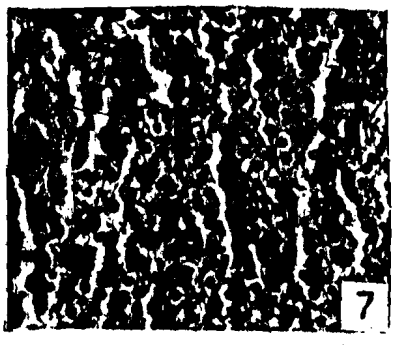
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FIG. 1.
Formalin-eosin haematoxylin.
T.S. of head kidney during spring
showing uriniferous tubules (UT),
malpighian body (MB). Haemopoietic tissues (HT),
including lymphocytes (L) and adrenal gland (ag). x 500.

FIG. 2.
Formalin-eosin haematoxylin.
T.S. of head kidney during summer showing
malpighian body (MB), haemopoietic tissues (HT),
and uriniferous tubule (UT) x 500.

FIG. 3.
Formalin-eosin haematoxylin.
T.S. of head kidney during fall,
showing increased number of developing cells (DC),
venous sinusoids (VS) and ghost cells (GC). x 500.

FIG. 4.
Formalin-Masson's trichrome.
T.S. of spleen during spring, showing
trabeculae (TR) formed of connective tissue,
blood vessel (BV) and reticular fibres (RF). x 500.

FIG. 5
Formalin-eosin haematoxylin.
T.S. of spleen during spring showing
red pulp. It consists of diffuse lymphatic tissue (dLT)and
reticular cells (RC), macrophage (MC) and lymphocyte (L). x1250.

FIG. 6
Formalin-eosin haematoxylin.
T.S. of spleen during spring showing white pulp.
It consists of compact lymphatic tissue (CLT) arround
small artery (SA) and large lymphocyte (LL). x 1250.

FIG. 7.
Formalin-eosin haematoxylin.
T.S. of spleen during summer.
Notice pigments (P). x 1250.

FIG. 8.
Formalin-eosin haematoxylin. T.S.
of spleen during fall, It shows higher activity
than in summer as revealed by the number of blood cells
on the tissue. Notice trabiculae (TR). x 500.

fibres, macrophages (Mc), lymphocytes (L) and other blood corpuscles. The white pulp, consists of compact lymphatic tissue (CLT) around a small artery (SA).

Blood supplies the organ through the splenic artery (Sa) and is collected by a splenic vein (SV) (Fig. 9). The artery is divided into arterioles (A) that are terminated by the sheathed artery (ShA). The latter opens directly into the reticular stroma through perforations in its wall (Fig. 10). The spleen of Clarias lazera has an open circulation like other teleost fish. The destroying function of the spleen can be demonstrated by the groups of macrophages and disintegrating blood cells (senile erythrocyte) (Fig. 11, SE).

During the different seasons it was noticed that spleen showed higher activities (i.e. forming large numbers of blood cells) in fall (Fig. 8 & 10), winter and spring (Fig. 11) than in summer (Fig. 7).

Liver of Clarias lazera is bilobed. Its tissue (Figs. 12, 13 & 14) consists of glandular cells or hepatic acini (HA), which contain the bile canaliculi (bc). Hepatic cells are polygonal in shape, with round nuclei, each nucleus contains one or more easily identifiable nucleoli. The hepatic cells are separated by light areas or sinusoids containing red blood cells (Fig. 14, S). No significant changes in liver tissue during different seasons were recorded. The only difference noted during the different seasons was the granular cytoplasm in hepatic cells in spring and summer as compared to granular ones during fall and winter (Figs. 12, 13 & 14).

In transverse section (Fig. 15), the ileum of Clarias lazera shows an outer serosa composed of simple squamous epithelium followed by a subserosa of connective tissue, muscularis consisting of circular muscle fibre then the submucosa and mucosa which is thrown into villi with columnar epithelium and goblet cells. The villi have a simple columnar epithelium cover and a core of highly reticular connective tissue, containing lamina propria (Lp). The villi are infiltrated by lymphocytes (L) and eosinophils (Es) indicating a haemopoietic function with higher activity in summer than in fall and winter (Figs. 15 & 16).

In the heart, the truncus arteriosus consists of a compact mass of tissue with small spaces lined by enlarged endothelial cells (Fig. 17 Edc). The larger cavities have flat cells differing from the normal endothelium in appearance, some of which protrude inside the cavity, hypertrophy and become detached forming blood cells., In the subendothelial areas there was loose connective tissue which has erythroblasts (Ebs), erythrocytes, leucocytes as well as macrophages (Figs. 17 & 18 Mc).

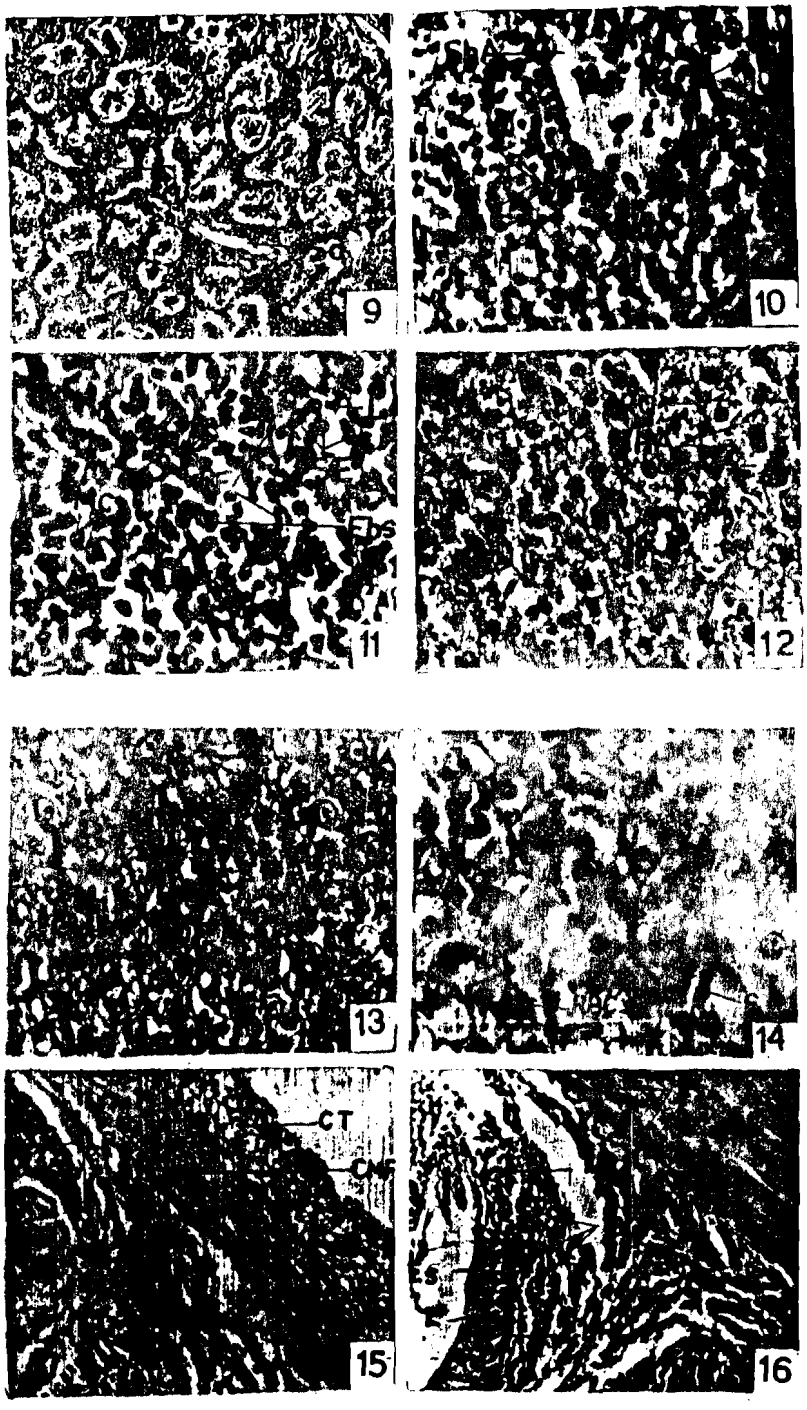
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FIG. 9.
Formalin-eosin haematoxylin. T.S. of spleen during spring, showing the splenic artery (Sa) and splenic vein (SV) traversing the centeral part of the organ. x 125.

FIG. 10.
Formalin-eosin haematoxylin. T.S. of spleen during fall showing arteriole (A), sheathed artery (SHA), groups of macrophages (Mc), reticular cells (RC) and thrombocytes (TH) x 1250.

FIG. 11.
Formalin-eosin haematoxylin. T.S. of spleen during spring, showing macrophages (Mc) insheathed with reticular fibre (RF), there are degenerating erythocyte (SE), lymphocytes (L) and erythroblast (Eba). x 1250

FIG. 12.
Formalin-eosin haematoxylin. T.S. of liver during spring showing hepatic acini (HA), bile canaliculi (bc), lymphocytes (L), and reticular cell (RC), x 1250.

FIG. 13.
Formalin-eosin haematoxylin. T.S. of liver during summer. Notice hepatic acini (HA), reticular cell (RC), thrombocyte (Th) and bile canaliculi (bc), x 1250.

FIG. 14.
Formalin-eosin haematoxylin. T.S. of liver during fall showing hepatic acini (HA) enclose sinusoids (S) which contain red blood cells (RBC's). x 1250.

FIG. 15.
Formalin-eosin haematoxylin. T.S. of ileum during summer, showing connective tissue (CT), circular muscule fiber (CMF) and lamina propria (LP), x 500.

FIG. 16.
Formalin-eosin haematoxylin. T.S. of ileum during fall. showing blood cells (BC) in lamina propria (LP), Tymphocytes (L) and eosinophils (Es), x 1250.

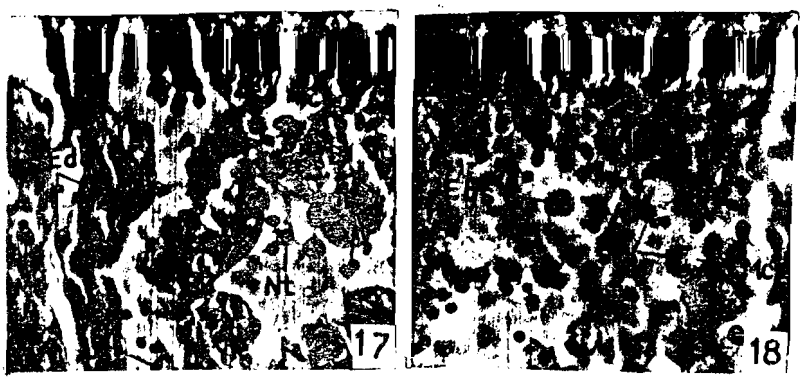

FIG. 17.
Formalin-eosin haematoxylin. T.S. of heart, showing endothelial cells (Edc), thrombocytes (Th), lymphocyte (L), neutro phil (Nt) and macrophage (Mc). x 1250.

FIG. 18.
Formalin-eosin haematoxylin. T.S. of truncus arteriosus showing erythroblast (Ebs) and macrophages (Mc) engulfed a senile lymphocyte (sL) x. 1250.

DISCUSSION

The interlobular connective tissue of the head kidney of **Clarias lazera** contains various developmental stages of both red and white blood corpuscles, beside their mature forms indicating that the kidney of this teleost plays a principal role in the blood forming process. This is in agreement with Jordan and Speidel (1924); Duthie (1939); Catton (1951), Yokoyama (1960), Radharkrishnan et aL. (1976) and El-Feky (1982).

The spleen seems to be mainly confined to erythropoiesis. Evidence gained from the work of Shabana and Khadre (Under publication) showed that the peak of erythropoietic activity in Clarias runs side by side with the apparent activity of haemopoietic tissues of the spleen. This result is in accordance with the work of Haider (1967) who pointed out that the spleen is the primary site of erythropoiesis in some fish. Fange and Mattisson (1981) reported that the white pulp of the spleen is lymphoid whereas the red pulp is mainly erythropoietic in the nurse shark. Mahajan and Dheer (1982) also proved that spleen plays an important role in both erythropoiesis and leucopoiesis except in the development of thrombocytes in Channa punctata.

In the present work, it is assumed that the spleen plays a role as blood destroying and storage organ. This is in complete accordance with results of Yoffrey (1929) on elasmobranches and Yokoyama (1960) on the perch.

Subsidiary haemopoietic organs in Clarias lazera are the liver, the lamina propria of mucosa of the gut and the heart. Many authors noticed that haemopoiesis in teleosts occur in other sites rather than kidney and spleen. Al-Hussaini (1949), Sabnis and Rangnekar (1962) and Kreutzmann (1976) reported the presence of eosinophils in the stomach and intestine of fish. Also, Yokoyama (1960) investigated the phenomenon of formation and passage of lymphocytes through the intestinal wall of the perch. El-Feky (1982) found that lymphocytes are scattered throughout the entire mucosa especially at the bases of the mucosal cells. Jordan (1938) reported the liver of trout as an erythropoietic organ. Topf (1953) mentioned that in the liver of fish there are stem blood cells and mature erythrocytes.

In Clarias, the subendothelial areas of the truncus arteriosus contains erythroblasts, erythrocytes, leucocytes as well as macrophages. This indicates blood cell forming and destroying capacities of the heart tissue. The present results agree with those of Yokoyama (1960).

In conclusion the present work reveals that , it is the Kidney rather than the spleen, where the primary haemopoietic activity is seen. The spleen serves as an accessory blood forming organ. Both organs show seasonal haemopoietic activity. The liver is less important as haemopoietic organ but with obscure activity. The lamina propria of the intestine also shows haemopoietic activity. The organ with the least haemopoietic importance is the endothelium of the truncus arteriosus of the heart.

REFERENCES

Al-Hussaini, A.H., 1949. On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits, cytology and physiology. Quart. Jour. Micr. Sci., 99: 323-354.

Bielek, E., 1974. Beitrag zur onthogenese der Blutbildug bei den teleostieren. I. Blutbilden-Twicklung bei der Asche Thymalus thymalus L. Zool. Jahrb. Anat., 93: 243-258.

Catton, W.Y., 1951. Blood cell formation in certain teleost fishes. Blood, 6: 39-60.

Duthie, E.S., 1939. The origin, development and function of the blood cells in certain Marine teleosts. Part I-Morphology. J. Anat., 73: 396-412.

El-Feky, N.K., 1982. Comparative studies on the blood picture and haemopoietic organs in some poikilothermic animals. M. Sc. Thesis. Faculty of Science, Tanta University, Egypt.

Fange, R. and A. Mattsson, 1981. The lymphomyeloid (Hemopoietic) system of the Atlantic nurse shark, Ginglymostoma cirratum. Biol. Bull., 160: 240-249.

Heider, G., 1967. Vergleichende Untersuchungen Zur Blutmorphologie und Hamatopoese einiger teleostier. Zool. Anz., 179: 355-384.

Jordan, H.E., 1938, Comparative hematology (Reptilia) In: H. Downey, Handbook of hematology, New York, Hoeber., pp.776-788.

Jordan, H.E. and C.C. Speidel, 1924. Studies on lymphocytes. II. The origin, function, and fate of the lymphocytes in fishes. Jour. Morpho., 38 (4): 529-549.

Kreutzmann, H.L., 1976. Untersuchungen Zur morphologie des Blutes Vom europaischen Aal Anguilla anguilla I. Die Erythrozyten und ihre En Ewichlungsstadien. Folia Haematol., 103: 226-235.

Kreutzmann, H.L., 1978. Studies on the morphology of the blood European eel Anguilla nguilla. Observation on monocytes and lymphocytes. Folia Haematol., (LETPZ), 104 (4): 538-557. Mahajan, C.L. and J.S. Dheer, 1982. Regenerative capacity of the spleen in splenectomized fish, Channa punctatus Bloch, with related investigations into changes in peripheral blood and haematopoiesis. Fish Biol., 20: 657-666.

McKnight, L.N., 1966. A hematological study on the mountain whitefish Prosopium williamsoni. J. fish. Res. Bd. Canada, 23: 45-64.

Nandi, J., 1965. Interrenal morphology of some Indian Cyprinid fishes. Proc. Zool. Soc., Calcutta, 18: 1-9.

Ogawa, M., 1962. Comparative study on the internal structure of the teleostean Kidneys. Sci. Rep. Saitama Univ., 4B: 107-129.

Pearse, A.G.E., 1972. Histochemistry. Theoretical and applied. Churchill Livingstone, Edinburgh, London andNewYork.

Radhakrishnan, S.; N. Stephan, and N. Balakrishnan, 1976. A study on the blood cells of a marine teleost fish, Diodon hystrix, together with a suggestion as to the origin of the lymphocytes. Proc. Indian Nat. Sci. Acad., 4213 (4,5) : 212-226.

Sabnis, P.B. and P.V. Rangnekar, 1962. Blood cell formation in the freshwater teleost, Ophiocephalus punctatus (Bloch). J. Animal Morpho. Physiol., 9: 126-130.

Shabana, M.B. and S.M.M. Khadre Haematological studies on the healthy catfish, Clarias lazera (under publication).

Sharma, B., 1969. The modes of the vascular supply in the kidneys of teleosts. Ann. Zool. Agra., 5: 85-109.

Sharma, 1972. Hemology of the so -called head kidney in certain Indian teleosts. Ibid., 7: 20-40.

Tepf, W., 1953. Die Blutbildung bei Cyprinus caprio. Zool. Anz., 150: 91-104.

Walving, P., 1958. Blood and parenchymal cells in the spleen of the Icefish Chaenocephalus aceratus (Lonnberg). Nytt Magasin F. Zool. Norway, 6: 112-120.

Ward, J.W. and H.S. Davis, 1975. Some aspects of the haematology of Clarias betrachus (Linn.). Folia. Sci., 37 (2): 91-94.

Yoffrey, J.M., 1929. A contribution to the study of the comparative histology and physiology of the spleen, with chiefly to its cellular constituents. I. In fishes. J. Anat., 63: 316-344.

Yokoyama, H.O., 1960. Studies on the origin, development, and seasonal variations in the blood cells of the perch, Perca flavescenes. Wildl. Disease., 6: 1-103.

Bull. Nat. Inst. Oceanogr. & Fish. ARE, 15 (1), 1989: 207 - 216.

THE INFLUENCE OF THE HERBICIDE PARAQUAT "GRAMAXON" ON GROWTH AND METABOLIC ACTIVITY OF THE CHLOROPHYTES SCENEDESMUS DIMORPHUS, SCENEDESMUS QUADRICAUDA AND ANKISTRODESMUS FALCATUS.

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ABSTRACT

The acute toxicity of the commercial herbicide paraquat was determined by 96-h static bioassay on the freshwater chlorophytes **Scenedesmus dimorphus** (Trup.) Kuetz., **Scenedesmus quadricauda** (Trup.) de Brebisson and **Ankistrodesmus falcatus** (Cord) Ralfs. The 96-h EC50 values of paraquat for reducing growth and metabolic products of the three algae were determined. In addition, the number of days required for division of control and all test cultures were calculated and these were seen to increase with increasing paraquat concentration. The three algae and their test parmeters respond differently to Paraquat. **Scenedesmus dimorphus** was the most susceptible alga and the chlorophll a was the most sensitive reponse parameter. On the other hand, the dry weight of the test algae was the most resistant parameter. It was observed that paraquat has a dangerous inhibitory effect on the primary producers.

INTRODUCTION

Paraquat (methyl viologen), 1,1-dimethyl-4,4-dipyridylium dichloride, is widely used herbicide effective against broad leaf weeds and grasses. In recent years, there has been an increase in the use of bipyridilium compounds, Paraquat and diaquat, for controlling aquatic weeds. The application of Paraquat to a fishing reservoir was successful in controlling the growth of macrophytic algae (Brook and Edwards, 1973).

The effects of Paraquat on growth and survival of aquatic organisms have been investigated (Benijts-Claus and Persoone 1975; Hendrich et al., 1976; Rao et al., 1980; Walsh 1972; Kapur and Yadav 1982; and Naqvi et al., 1981)

According to the available data, the toxicity of Paraquat and Diaquat is relatively low to man and to some fish species. Our knowledge, on the contrary, of the influence of these chemicals on the lowest levels of the aquatic food chain is extremely scarce.

In Egypt, Paraquat is widely used for controlling a variety of weeds associated with cotton, fruit crops, tomatoes and turf grasses. Recently, a trial will be made to use Paraquat in freshwater fish farms for controlling the macrophytes Potamogeton pectinatus, P. crispus and Ceratophyllum demersum.

The present study, however, was initiated in order to determine the effect of this herbicide on the freshwater green algae Scenedesmus dimorphus, Scenedesmus quadricauda and Ankistrodesmus falcatus representatives of the primary producers. The toxicity test experiments are urgently needed if we want to use Paraquat in safe concentrations which do not interfere with the more sensitive links of the aquatic ecosystem.

MATERIALS AND METHODS

Paraquat is dipyridylium derivative. It is a quaternary ammonium salt (double positive cation with chloride as anion). It is soluble in water and the commercial name is Gramaxon. The sample used in this study contains 40% paraquat and 10% of a mixture of the two detergents, Lissapol NX and DS 4392 or Ethomene S 25. The exact percentage of each of the detergent is a fabricate secret.

The chlorophytes Scenedesmus dimorphus, Scenedesmus quadricauda and Ankistrodesmus falcatus were isolated from the freshwater Lake Wadi El-Rayan.

The medium for stock algal cultures and tests with Paraquat was prepared by dissolving the following major salts in 1l glass distilled water: 35 mg $MgSO_4$, 31 mg $NaNO_3$, 18.5 mg $CaCl_2$, 31 mg K_2HPO_4, 10.5 mg $NaHCO_3$, and 56 mg Fe^{+3} as $FeSO_4$. To these major elements 1 ml of the following mixture of trace elements was added: 143 mg $CuSO_4$, 43 mg $ZnSO_4$, 13 mg Kl, 15 mg KBr, 18.4 mg $NaMoO_4$, 40 mg $MnSO_4$, 56 mg $CoCl_2$, 91 mg $Na_2B_4O_7$ and 16.7 mg Na_2WO_4. All were dissolved in 1l glass distilled water. The medium was sterilized by autoclaving for 15 min. at 1.5 kg/cm. The medium was prepared on the day before the test and began to allow the pH to stabilize at 8.1.

The bioassay was performed in 250 ml Erlenmeyer flasks containing 100 ml of the medium and sealed with cotton plugs. Treatment and control flasks were inoculated with 5000 algal cells ml^{-1} in logarithmic growth phase. The experiments were carried out at 25 ± 1^{o} C and 4000 lux from overhead "cool white" flourescent tubes, in cycles of 14 h light followed by 10 h darkness. The cultures were shaken once a day.

At the end of 96 h testing period the flasks were removed from the growth chamber, the cultures were thoroughly mixed to insure homogeneity for an accurate count, and the cells

were counted using brightline hemocytometer counting chamber. Replicate counts were made for each flask to insure counting accuracy. The dry weight of the control and treated cultures was measured gravimetrically after filtration onto millipore filter paper 0.45 um pore diameter (Ibrahim 1984).

Chlorophyll a & b and carotenoids were extracted with 90% acetone according to the method of Golterman and Clymo (1971). The chlorophyll a was calculated according to Lorenzen's equation (1967), while chlorophyll b and total carotenoids were calculated according to Parson's and Strickland equations (1963).

Cellular carbohydrate was extracted according to Myklestad and Haug method (1972). The total amount of carbohydrate in the extract was determined by the phenol sulphuric acid method using glucose as standard (Dubois et al., 1956).

Algal nitrogen was determined by the micro-Kjeldahl method (Hiller et al., 1948). Protein-N was calculated by multipling the value of total nitrogen by 6.25.

To construct dose response curves and calculate the Paraquat EC50 values for reducing growth, dryweight, chlorophyll a & b, carotenoids, carbohydrate and protein contents of the test chlorophytes, the pooled data were treated according to the statistical method of probit analysis (Bliss, 1952; Finney 1964a & 1964b). In addition to the EC50 values, the growth rate (k) and the number of days per division (Td) were calculated for each test concentration (Guillard, 1973 and Honig et al., 1980).

RESULTS

The regression lines representing the dose responses of Scenedesmus dimorphus, Scenedesmus quadricauda and Ankistrodesmus falcatus to the selected Paraquat concentrations are presented in Fig. 1 and the data from which these curves were drawn are given in Tables 1, 2 and 3. In addition Table 1 presents also the calculated days per division of the algae under the various test conditions. Since the percentage of control growth diminishes for each algal species as concentrations of paraquat increases, the respective Td values increase in a coinciding pattern. It can be observed that under their respective optimal control conditions S. dimorphus with Td value of 0.2 is a faster-growing alga than either S. quadricauda 0.62 and A. falcatus 0.53. The calculated EC50 values for reducing the growth indicate that S. dimorphus at 39.8 ppb (ug l^{-1}) is the most sensitive of the three algal species and A. falcatus with a value of 93.3 ppb being the least sensitive. On the other hand the dry weight gained by each of the test algal cells was less affected by Paraquat. The EC50 values of Paraquat for reducing the dry weight in ppb were: 73.9 for S. dimorphus; 132 for S. quadricauda and 114.2 for A. falcatus.

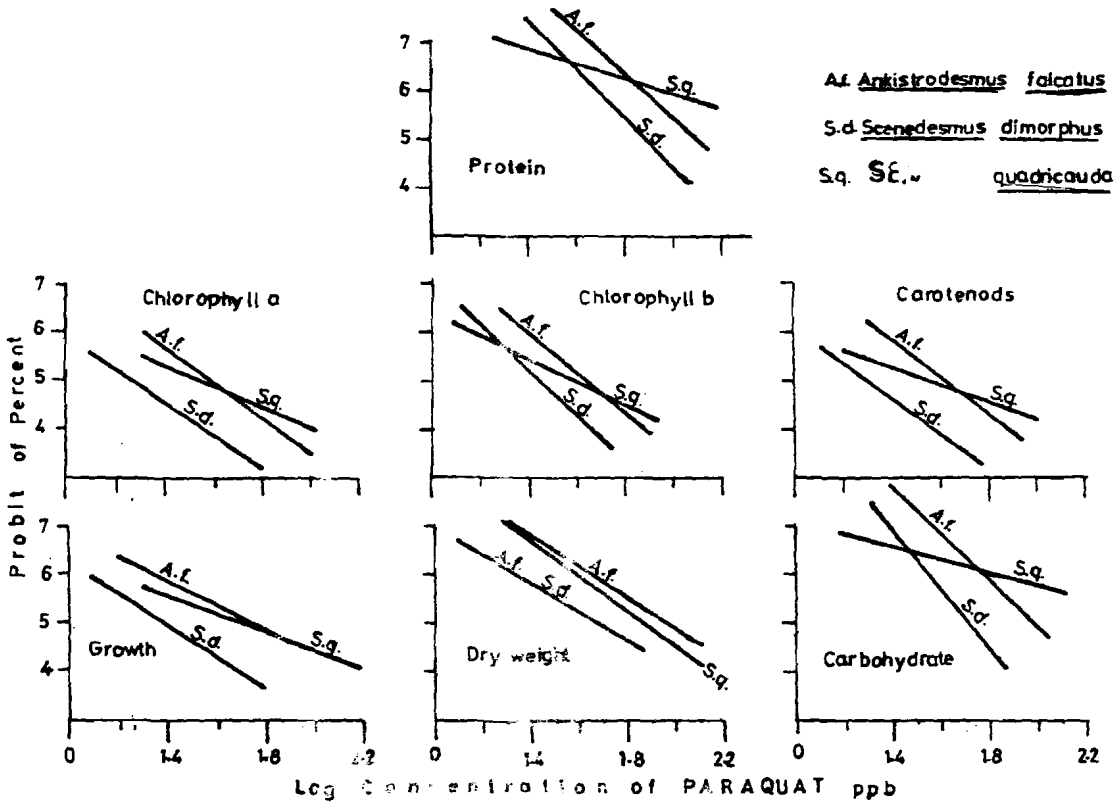

Fig. 1
The regression lines of the responses of the test parameters to the selected doses of Paraquat.

Table 1

Effect of Paraquat on growth of the three test chlorophytes

Test algae	Log dose (ppb)	Average count No.x 10^4/ml	% of control	Emperical probit	Td
	Control	170±2.94	----	----	0.20
	1.3	132.2±82.9	82.9	5.9502	0.5
	1.6	81.77±1.71	48.1	4.9529	0.55
S. dimorphus	1.8	52.5±1.2	30.9	4.5013	0.6
	1.9	26.33±0.68	15.5	3.9848	0.71
	2	16.43±0.36	9.7	3.7012	0.81
	b=-3.18, a=10.09, Y=-3.18x + 10.09 Log EC50=1.6, EC50=39.8 ppb				
	Control	44.4±1.3	----	----	0.62
	1.5	34.53±1.2	77.2	5.7655	0.66
	1.8	26.1±0.9	58.8	5.2224	0.71
S. quadricauda	1.95	20.45±0.4	46.1	4.9021	0.76
	2.08	16.96±0.30	38.2	4.6998	0.80
	2.18	13.72±0.3	30.9	4.5013	0.85
	b=-1.86, a=8.56, Y=-1.86x + 8.56, Log EC50=1.91 EC50= 81.6 ppb				
	Control	98.2±2.3	----	----	0.53
	1.5	85.93±2.0	87.5	6.1503	0.54
	1.8	64.5±1.4	65.7	5.4043	0.58
A. falcatus	1.95	50.08±1.6	51	5.0251	0.61
	2.08	39.1±1.2	39.8	4.7415	0.64
	2.18	30.34±0.50	30.9	4.5013	0.68
	b=-2.43, a=9.78, Y=-2,43x + 9.78, Log EC50=1.97 EC50= 93.3 ppb				

Table 2

Estimation of the EC50 for Paraquat from the percent response of Chlorophyll a & b and carotenoid contents of the three chlorophytes after 96 h exposure, with respective regression equations

Algal species	Log dose (ppb)	Chl. a ug l^{-1}	% of control	Probit of percent	Chl. b Ug l^{-1}	% of control	Probit of percent	Carotenoids ug l^{-1}	% of control	Probit percent
	Control	590±4.2	----	----	172±2.6	----	----	282±3.4	----	----
	1.3	418±3.8	70.9	5.5505	163±1.8	94.6	6.6072	214±2.2	75.9	5.7031
	1.6	182±2.2	30.9	4.5013	97±1.4	56.4	5.1611	96±1.6	34	4.5875
S. dimorphus	1.8	86±1.2	14.6	3.9463	36±0.8	20.9	4.1901	37±0.8	13.1	3.8783
	1.9	40±0.8	6.8	3.5091	15±0.4	8.9	3.6405	19±0.6	6.7	3.5015
	2.0	22±0.3	3.7	3.2134	8±0.2	4.7	3.3253	9±0.2	3.2	3.1478
	a=9.86,b=-3.33, Log EC50=1.46, EC50=29ppb Y=-3.33X+9.68				a=12.8,b=-4.78,Log EC50=1.63 Y=-4.78X +12.81 EC50=43 ppb			a=10.44,b=-3.65,Log EC50=1.49 Y=-3.65X +10.44, EC50=31 ppb		
	Control	197±3.6	----	----	61 ±1.4	----	----	104 ±3.4	----	----
	1.5	135±2.4	68.6	5.4845	45.3±0.8	74.2	5.6495	68.7±2.3	66.1	5.4152
	1.8	86±1.2	43.7	4.8414	30 ±0.6	49.2	4.6799	49 ±1.4	47.1	4.9272
S. quadricauda	1.95	67±1.4	34	4.5875	20 ±0.4	32.8	4.5546	35 ±0.8	33.7	4.5793
	2.08	48±0.6	24.4±	4.3065	13 ±0.4	24.6	4.3129	28 ±0.6	27.6	4.4082
	2.18	38±0.4	19.3	4.1331	11 ±0.2	18	4.0846	24 ±0.4	22.9	4.2579
	a=8.45, b=-1.99, Log EC50=1.74, EC50=54.5 Y=-1.99X +8*45 EC50=54.5 ppb				a=9.13,b=-2.32,Log EC50=1.78 Y=-2.32X +9.13, EC50=60.2ppb			a=8.02, b=-1.73, Log EC50=1.74 Y=-1.73X + 8.02, EC50=54.9 ppb		
	Control	512±5.2	----	----	125±2.6	----	----	248±4.4	----	----
	1.5	443±3.8	86.5	6.1031	114±1.8	91.2	6.3539	219±3.6	88.3	6.2004
	1.8	230±2.4	44.9	4.8718	66±2.2	52.8	5.0702	130±2.8	52.4	5.0604
A. falcatus	1.95	198±1.8	28.7	4.4378	37±1.4	29	4.4524	81±2.4	32.7	4.5518
	2.08	70±2.2	13.6	3.9015	18±0.6	14	3.9197	49±1.6	19.8	4.1512
	2.18	38±1.2	7.4	3.5534	10±0.4	8	3.5949	22±1.8	8.9	3.6531
	a=11.66, b=-3.72, Log EC50=1.79 Y=-3.72X + 11.66. EC50=61.3 ppb				a=12.5,b=-4.1,Log EC50=1.82 Y=-4.1X + 12.5, EC50=66.6ppb			a=11.68,b=-3.66,Log EC50=1.83 Y=-3.66X +11.68, EC50=67 ppb		

Table 3

Estimation of the EC50 for Paraquat from the percent response of dry weight, carbohydrate and protein contents of the test algal cells, with respective regression equations.

Algal species	Log dose µg l^{-1}	Dry weight mg l^{-1}	% of control	Probit of percent	Carbohydrate mg l^{-1}	% of control	Probit percent	Protein mg l^{-1}	% of control	Probit of percent
	Control	50.2±1.4	----	----	7.6±0.4	----	----	28.8±2.1	----	----
	1.3	48.4±2.2	96.4	6.7991	7.5±0.6	98.2	7.1015	27.2±1.2	97.2	6.9110
	1.6	40.3±2.6	80.3	5.8526	4.6±0.4	60.5	5.2663	18.4±1.4	65.6	5.4016
S. dimorphus	1.8	31 ±2.4	61.8	5.3002	1.4±0.2	18.4	4.0998	7.4±0.6	26.4	4.3689
	1.9	22.3±1.8	44.4	4.8592	0.5±0.04	5.9	3.4368	3.1±0.6	11.1	3.7241
	2.8	16.5±1.8	32.9	4.5570	0.1±0.02	1.4	2.8027	1.4±0.2	5	3.3551
	a=10.97, b=-3.19, Log EC50=1.87				a=15.06, b=-6.11, Log EC50=1.65			a=13.64, b=-5.17, Log EC50=1.67		
	Y=-3.19X + 10.97, EC50=73.9 ppb				Y=-6.11X + 15.06, EC50=44.2 ppb			Y=-5.17X+13.64, EC50=47 ppb		
	Contro	42.6±1.4	----	----	8.5±0.6	----	----	26.9±2.2	----	----
	1.5	42.5±1.4	99.7	7.7065	6.25±0.3	73.5	5.6280	18.8±1.4	75.5	5.6903
	1.8	39 ±0.8	91.5	6.3722	5.3±0.4	62.1	5.3081	15.7±1.4	63	5.3310
quadricauda	1.95	32 ±1.2	75.1	5.6776	4.14±0.08	48.7	4.9674	12 ±1.6	48.2	4.9549
	2.08	24 ±0.6	56.3	5.1586	3.74±0.4	44	4.8490	10.5±0.8	42	4.7981
	2.18	17.9±0.4	42.1	4.8007	3.26±0.06	38.3	4.7024	9.5±0.6	38.3	4.7024
	a=14.2, b=-4.32, Log EC50=2.12				a=7.74, b=-1.39, Log EC50=1.97			a=8, b=-1.53, Log EC50=1.97		
	Y=-4.32X + 14.2, EC50=132 ppb				Y=-1.39X + 7.74, EC50=92.7 ppb			Y=-1.53X + 8, EC50=92.2 ppb		
	Contro	27.2±1.6	----	----	6.5±0.4	----	----	15.1±1.4	----	----
	1.5	25 ±1.4	91.9	6.3984	6.2±0.6	95	6.7068	14.3±1.2	94.6	6.6072
	1.8	20.2±0.8	74.3	5.6526	4.0±0.7	61.8	5.3082	9.6±0.8	63.7	5.3505
A. falcatus	1.95	16.9±1.2	62	5.3055	2.29±0.2	35.2	4.6201	6 ±0.4	39.7	4.7389
	2.08	13.1±0.6	48	4.9498	1.22±0.08	18.8	4.1147	2.6±0.3	17.2	4.0537
	2.18	10 ±0.8	36.8	4.6628	0.44±0.04	6.8	3.5091	1.2±0.4	7.9	3.5882
	a=10.2, b=-2.33, Log EC50=2.06				a=13.62, b=4.61, Log EC50=1.87			a=13.3, b=-4.43, Log EC50=1.87		
	Y=-2.33X + 10.2, EC50=114.2 ppb				Y=-4.61X + 13.62, EC50=74 ppb			Y=-4.43X + 13.3, EC50=74.5 ppb		

Chlorophyll a & b and total carotenoids of the test algae progressively decreased with increasing Paraquat concentrations. The EC50 values of Paraquat for reducing chlorophyll a & b and carotenoids in ppb were: 29, 43, and 31 for S. dimorphus; 54.5, 60.2 and 54.9 for S. quadricauda; and 61.3, 66.6 and 67 for A. falcatus.

Carbohydrate and protein contents of each of the three test algal cells were more or less similar in their response to Paraquat. Their respective EC50 values in ppb were: 44.2 and 47 for S. dimorphus; 92.7 and 92.2 for S. quadricauda; and 74 and 74.5 for A. falcatus.

DISCUSSION

Previous studies on the effects of Paraquat have concentrated on its effects on growth of cultures of the primary producers. Whereas the present study provides a precise information not only on its effect on growth but also on the metabolic products of the three chlorophytes.

The results revealed the obvious inhibitory effect of Paraquat on growth and metabolic activity of the three test algal. The EC50 values of Paraquat for reducing growth of the chlorophytes indicate that S. dimorphus at 39.8 ppb was more susceptible than both S. quadricauda 81.6 ppb and A. falcatus 93.3 ppb. With EC50 for reducing growth of S. dimorphus taken as one, the relative tolerance of S. quadricauda 2.05 and A. falcatus 2.34 times as more tolerant as S. dimorphus. This agrees with the work of Thomas et al. (1973) who observed the remarkable decrease in growth of Chlorella pyrenoidosa, Chlorella vulgaris and Bacillus sp. when treated with Paraquat. Moreover, Hendrich et al. (1976) reported that Paraquat reduced cell size and caused morphological changes of Scenedesmus quadricauda cells. On the other hand, Benijts-Claus and Persoone (1975) reported that the cladocerun, as representative to the primary consumers was more tolerant to Paraquat as compared with the primary producers, while benthic meiofauna were the most sensitive.

The inhibitory effect of Paraquat on the dry weight of the test algae was mainly attributed to its depilatory effect on population growth that reduced the number of cells of the treated cultures as compared with the control. The dry weight, with EC50 values of 73.9 ppb for S. dimorphus, 132 ppb for S. quadricauda and 114.2 ppb for A. falcatus was more resistant to Paraquat than the growth.

Paraquat had the same sequence of inhibitory effect on chlorophyll a & b, carotenoids, carbohydrate and protein contents of the test chlorophytes as on growth and dry weight. The EC50 values indicated that chlorophyll a was the most sensitive response parameter whereas dry weight was the least sensitive.

The present results lead to the conclusion that the three chlorophytes varied greatly in their responses to Paraquat, S. dimorphus was more susceptible as compared with S. quadricauda and A. falcatus. These variations were also observed between the test parameters of the same species. This confirms the previous work of Naqvi et al (1981) and Ibrahim (1983) who reported that phytoplankton organisms and their metabolic products respond differently to the same insecticide.

Since the effective dose of Paraquat for controlling macrophytes is 1 mg l^{-1} (1 ppm), the application of this herbicide will cause a severe inhibitory effect on the primary producers.

The present study provided a predictive statement to the toxicity of paraquat based on its effects on unialgal cultures of the three chlorophytes under laboratory conditions. The precise and actual predictive statements should be made bearing on the problem of community response to Paraquat. This approach will be applied in future toxicological studies.

REFERENCES

Benijts-Claus, C. and G. Persoone, 1975. The influence of the formulation of the herbicide Paraquat on its toxicity for aquatic organisms. Med. Fac. Landbouww. Rijksuniv. Gent., 40(2):1161-1173.

Bliss, C. I., 1952. Statics of bioassay. Academic Press, N. Y. p.445-628.

Brooker, M.P. and R.W. Edward, 1973. Effect of the herbicide Paraquat on the ecology of reservoir. I. Botanical and chemical asbects. Freshwater Biol., 3:157-175.

Dubois, M.; K.A. Gilles; J.K. Hamilton; P.A. Repers and F. Smith, 1956. Colorimetric method of determination of sugars and related substances. Analyt. Chem., 18:350-356.

Finney, D.J., 1964 a. Statistical Method in Biological Assay. N.Y. Hafner 668 pp.

Finney, D.J, 1964 b. Probit Analysis. Cambridge Univ. Press, England. 318 pp.

Golterman, H.L. and R.S. Clymo, 1971. Methods for chemical analysis of freshwater. Blackwell Scientific publication Oxford. 166 pp.

Guillard, R.R.L., 1973. Division rates, P. 289-311. In Stein, Jr. (ed.) Handbook of Phycological Methods. Cultures Methods and growth Measurements. cambridge Univ. Press, London.

Hendrich, W.; Z. Kubiak; K. Jurajda and M. Pawlaczyk-Szpllow, 1976. Effect of herbicides on photosynthesis electron transport and on growth of the alga Scenedesmus quadricauda. Acta Societatis Botanicorum Poloniae., xLV: 101-110.

Hiller, J.A.; H. Plazin and D.D. Van Slyke, 1948. A study of conditions for Kjeldahl determination of nitrogen in protein. J. Biol. Chem., 176:1401-1420.

Honig, R.A.; M.J. McGinniss; A.L. Buikema, Jr and J, Cairns, Jr. 1980. Toxicity tests of Aquatic pollutants using Chilomonas paramecium Ehrenberg (Flagellata) populations. Bull. Environm. Contam. Toxicol., 25:169-175.

Ibrahim, E.A., 1983. Effects of some common pesticides on growth and metabolism of the unicellular algae **Skeletonema costatum, Amphiprora paludosa** and **Phaeodactylum tricornutum. Aquatic Toxicology**, 3:1-14.

Ibrahim, E.A, 1984. Experimental studies on the effect of sodium chloride, Dimethoate and Bayluscide on the growth and metabolism of the green alga **Staurastrum boreale** W. Weast. **Bull. Zool. Soc. Egypt**, 33: 87-97.

Kapur, K., and N.k. Yadav, 1982. The effect of some herbicides on the hatching of eggs in common carp, **Cyprinus carpio** var. communis. **Acta Hydrobiol.**, 24(1): 87-92.

Lorenzen, C.J., 1967. determination of chlorophyll and phaeo-pigments: spectrophotometric equations. **Limnol. Oceanogr.**, 12: 343-346.

Myklestad, S., and A. Haug, 1972. Production of carbohydrate by the marine diatom **Chaetoceros affinis** var. Willei (Gran) Hustedt. I-Effect of the concentration of nutrient in the culture medium. **J. Exp. Biol. Ecol.**, 9: 125-136.

Naqvi, S.M.; T.S. Leung and N. Z. Naqvi, 1981. Toxicities of Paraquat and Metribuzin (Sencor) herbicides to freshwater copepods **Eucyclops agilis** and **Diaptomus mississippiensis. Environmental pollution** (Series A). 26: 275-280.

Parson, T.R., and J.D.H. Strickland, 1963. Discussion of spectrophotometric determination of marine plant pigments with revised equations for ascertaining chlorophylls and carotenoids. **J. Mar. Res.**, 21:155-163.

Rao, I.; P.M. Madhusudona; V. Swahy and S. R. Das, 1980. Influence of Paraquat and 2, 4, 5-T on the leaf chlorophyll content of 6 shrub species. **Z. Pflanzenphysiol.**, 97(3): 189-196.

Thomas, V.M.; L.J. Buckley; J.D. Sullivan Jr. and N. Ikawa, 1973. Effect of herbicides on the growth of **Chlorella** and **Bacillus** using paper disc method. **Weed. Science**, 21(5): 449-451.

Walsh, G.E., 1972. Effect of herbicides on photosynthesis and growth of marine unicellular algae. **Hyacinth Control J.**, 10: 45-48.

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989: 217 -224

THE BENTHIC FAUNA OF LAKE BUROLLUS
1 - COMMUNITY COMPOSITION AND DISTRIBUTION OF THE TOTAL FAUNA

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ABSTRACT

Quantitative estimation of benthic macro fauna was carried out monthly in Lake Burollus during the period January, 1978 - December 1979. The community comprised eleven species and was dominated by Chaetogaster limnaei, Corophium volutator, Gammarus lacustris, Nesanthura sp. and Corbicula consobrina. The highest biomass of benthos appeared in the western sector of the Lake due to the increased weights of the bivalve Corbicula consobrina and it decreased gradually eastwards. Regarding the seasonal variations, the maximum persistence of benthos in the eastern and middle sectors was in spring of 1978, while this was shifted to the summer in the western Lake. The average annual values of the total bottom fauna amounted to 440 Organisms/m^2 with 13.7 gm fresh Wt/m^2 in 1978, decreased to 310 Organisms/m^2 and 6.1 gm fresh Wt/m^2 in 1979.

INTRODUCTION

Lake Burollus is a shallow slightly brackish water lake, situated at the north of the Nile Delta (Egypt), along the Mediterranean coast at longitudes 30^{o} 30/ and 31^{o} 10/ E and latitude 31^{o} 35/ N. It extends for about 70 Km, with a varying width between 6 and 16 Km and a total area of about 50,000 hectars.

The Lake receives most of its water from five main drains as shown in Fig (1). It opens into the Rosetta Estuary at its western extremity through Brimbal Canal. It is also connected to the Mediterranean Sea at its north eastern side through a narrow opening referred to as Boughaz El-Borg. The amount of the drain water discharged annually into the Lake fluctuates from one year to the other and it averages about 2.5 billion cubic meters per year. The surplus water flows constantly into the Sea through Boughaz El-Borg. Sea water may also enter the Lake during winter gales which are usually predominated by strong north wind.

The nature of the bottom sediments differs within the different regions. Thus, the sediments at the eastern and western sectors of the Lake as well as the southern margins are usually silty clay mixed with shell fragments. In the middle Lake it is either clayey sand or sandy silty clay.

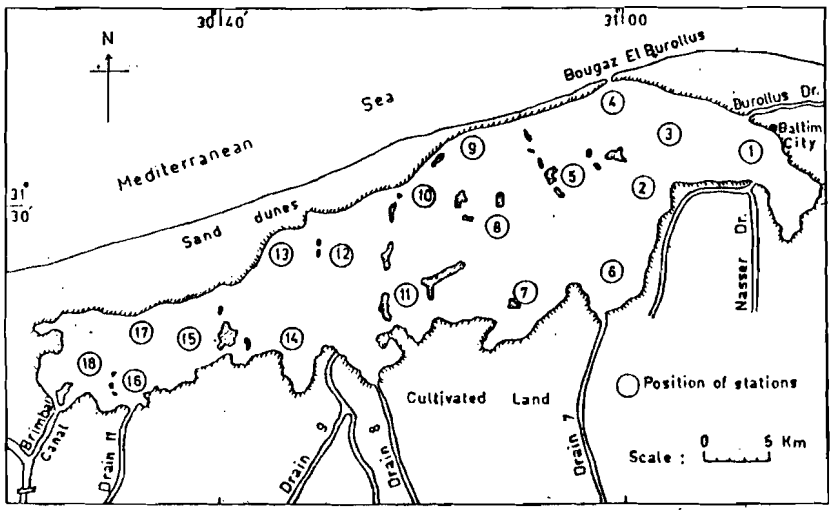

FIG. 1
Morphometry of Lake Burollus and position of stations.

Many islets are scattered in the Lake and these create semi-isolated basins named by fishermen as Berka or Houd. Due to the shallowness of the Lake the whole area is related to the literal zone where phanerogamic plants are widely distributed particularly at the eastern Lake as well as at the Lake margins (Samaan et al., 1988)

The composition of benthic fauna has long been considered as a good indicator of water quality because, unlike planktonic organisms, they form relatively stable communities in the sediments which integrate changes over long-time intervals, and reflect characteristics of both sediments and the upper water layer. However, the biologists have encountered many problems in obtaining base line information about the natural communities and comparing this accurately with altered associations because of the lack of good standardized sampling and sorting methods (Cook and Johnson, 1974). Investigations of benthos in the Egyptian Delta lakes are still few. These were mainly confined to that recorded in Nozha Hydrodrome (Elster and Jensen, 1960), Lake Mariut (Samaan and Aleem, 1972) Lake Edku (Samaan,1977) and Lake Menzalah (Guerguess, 1979). The present study deals with quantitative estimation of bottom macro fauna in Lake Burollus.

MATERIAL AND METHODS

The bottom fauna was hauled by using a modified Ekman bottom sampler. Two dredges were taken at each station which represent an area equivalent to 0.06 m^2 of the upper layer of bottom sediments containing benthos. The samples were

then washed directly in the field through a small hand net of bolting silk with 23 mesh/cm^2 and preserved in polyethelene jars after adding 10% formalin solution. The samples were washed again thoroughly in the laboratory with the same hand net to get rid of any silt that may remain within them. Sorting was carried by taking small portions of the sample under estimation in a petri dish. The animals were separated into groups and each group was counted and weighed separately after being left for five minutes on a filter paper to get rid of any external moisture.

Eighteen stations were selected as representing the different parts of the Lake (Fig.1). These were further grouped into three main sectors namely; the eastern Lake (stations 1-6), the middle Lake (stations 7-12) and the western Lake (stations 13-18).

Sampling of benthic fauna was carried out monthly at the different stations during the period from January, 1978 to December, 1979.

RESULTS

1- The benthic community

The benthic macrofauna of Lake Burollus comprised eleven species belonging to nine orders within three phyla as shown in the following list:-

Phylum Annelid
Class Clitellata
Order Oligochaeta
Family Naididae
Chaetogaster limnaei K. Von Baer

Class Polychaeta
Order Erranta
Family Nereiidae
Nereis limnicola (Johnson)

Phylum Arthropoda
Class Crustacea
Order Mysidacea
Family Mysidae
Mysis relicta (Loven)

Order Isopoda
Family Anthuridae
Mesanthura Sp.

Order Amphipoda
Family Gammaridae
Gammarus lacustris (Fabricius)
Corophium volutator (Pallas)

Class Insecta
Order Diptera
Family (Chironomidae) Tendipedidae
Tendipes (chironomus) tentans (Meigen)

Phylum Mollusca
Class Pelecypoda
Order Heterodonta
Family Corbiculidae
Corbicula consobrina (Cailliaud)

Order Cerastoderma
Family Cardiidae
Cerastoderma (Cardium) edule (L.)

Order Mesogastropoda
Family Melaniidae
Melanoides tuberculata (Muller)
Neritina nilotica (Reeve)

Five species predominated the bottom community namely; the oligochaete Chaetogaster limnaei, the amphipods Corophium volutator and Gammarus lacustris, the isopod Mesanthura sp. and the bivalve Corbicula consobrina.

2- Distribution and seasonal variations

Generally speaking, the distribution of the total benthos in Lake Burullus was subjected to pronounced variations within the two successive years of investigation (Fig. 2). Thus, in 1978, the highest numbers were observed around the southern and northern margins of the middle sector due to the increased numbers of Corophium volutator and less so to Corbicula consobrina, Gammarus lacustris and Chaetogaster limnaei. Other increase was also noticed in the western Lake near the outlet of Drain 11 with a main component of Chaetogaster limnaei. The eastern Lake sustained the lowest standing stock except at the surroundings of Drain 7 and the Boughaz region which harboured considerable numbers of Gammarus lacustris.

During 1979, the picture was much different as the highest density of benthos appeared in the western Lake between Drains 9 and 11 particularly due to the increased numbers of Chaetogaster. On the other hand, the total numbers of benthos in the middle sector dropped to lower values and this was accompanied by decreased counts of Corophium . The eastern Lake remained poor, showing further reduction in the numbers of Gammarus. The average annual values of the total benthic fauna in the Lake amounted respectively 440 and 310 organisms/m^2 during 1978 and 1979..

Concerning the total biomass, the heaviest bottom specimens were usually the molluscs Corbicula consobrina. Thus, whenever these animals increased quantitatively, there was always an increase in the benthos biomass. This relation

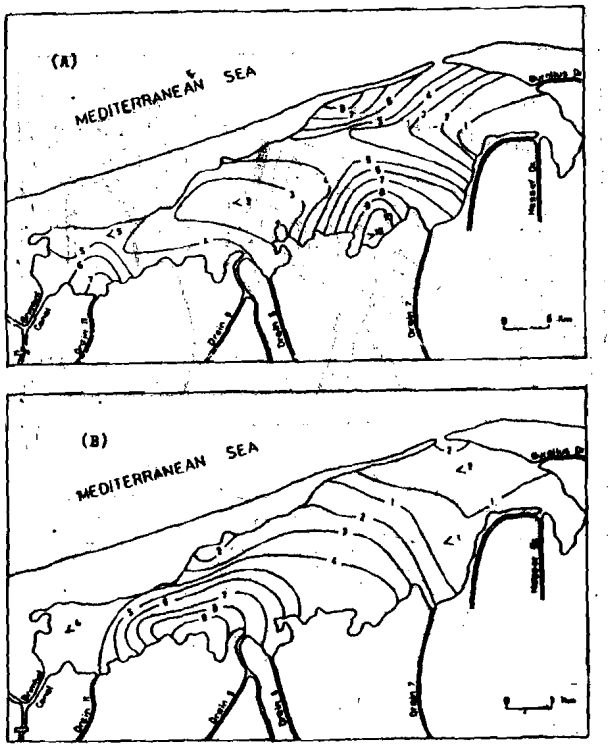

FIG. 2

Horizontal distribution of the total bottom faunin Lake Burollus (hundred organisms/m^2).
(A): Average of 1978. (B): Average of 1979.

was more clear in the western Lake which greatly exceeded the other two sectors in its total benthos biomass. The average annual biomass of benthos in the Lake amounted 13.7 gm fresh wt/m^2 during 1978 decreased to 6.1 gm fresh wt/m^2 in 1979. Such decrease is mainly attributed to the drop in the fresh weights of C. consobrina although their numbers increased slightly in the last mentioned year,(Fig.3).

Regarding the seasonal variations, the bottom fauna in the eastern sector showed a major peak of abundance during March-April 1978 and was dominated by Gammarus and to a less extent by Nereis and a smaller one in November, also due to Gammarus (Fig. 4). Its density remained low throughout 1979.

The middle sector harboured the highest counts of benthos in April and May, 1978, with the predominance of Corophium. Two other smaller peaks were recorded there in February and November, 1979 as produced respectively by Corophium and Corbicula.

In the western sector, the maximum persistence of benthos appeared between August and October, 1978 and consisted mostly of Chaetogaster and in may, 1979 which comprised both Chaetogaster and Mesanthura.

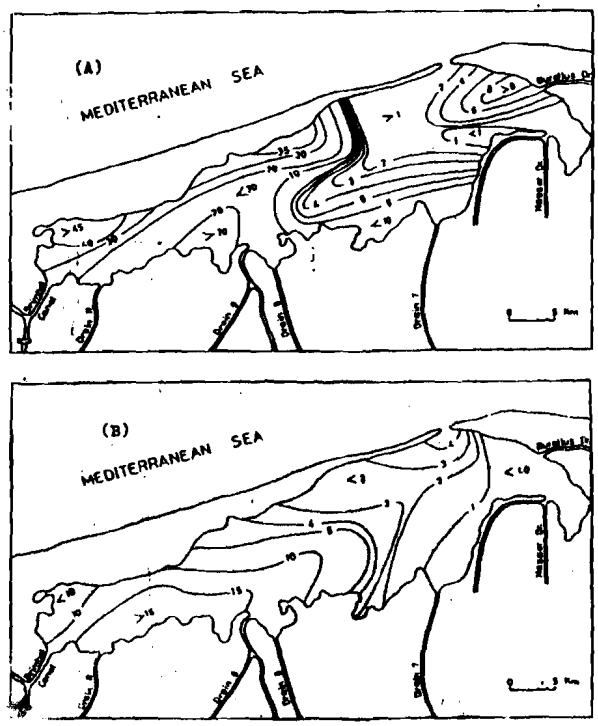

FIG. 3
Horizontal distribution of the total biomass of bottom fauna in Lake Burollus (gm fresh wt/m^2).
(A): Average of 1978. (B): Average of 1979.

DISCUSSION

The distribution of benthic fauna in the three sectors of Lake Burollus was subjected to pronounced seasonal as well as annual variations. The eastern sector which is mostly covered with the hydrophyte Potamogeton pectinatus sustained the lowest standing stock of benthos. This agrees with the observations previously recorded by Samaan and Aleem (1972) in Lake Mariut and Samaan (1977) in Lake Edku, where the plant belt harboured a poor standing stock of bottom fauna. The main bottom dwellers in this sector were Nereis limnicola, Gammarus lacustris and Tendipes (Chironomus) larvae. The two former species are considered as euryhaline forms and showed their maximum persistence around the Boughaz region (lake-sea connection). Chironomus larvae were mostly confined to the Potamogetom plant belt and are considered as good indicator of oxygen reduction at the bottom. The average annual counts of benthos in the eastern sector reached 237 organisms/m^2 with a biomass of 3.4 gm fresh wt/m^2 during 1978. These values dropped to 112 organisms/m^2 and 1.6 gm fresh wt/m^2 in 1979.

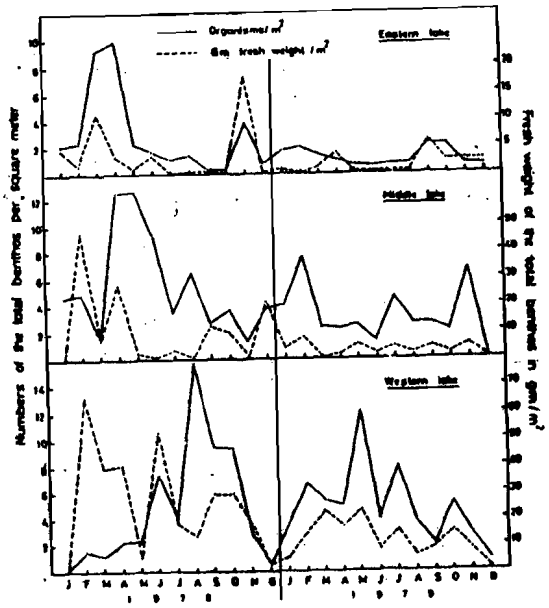

FIG. 4
Seasonal variations of the total bottom fauna at the three sectors during 1979 and 1979.

The middle Lake which is in its great part devoid of hydrophytes harboured a standing stock of benthos that consisted mainly of Chaetogaster limnaei, Corophium volutator, Corbicula consobrina and Mesanthura sp.

Corbicula formed the major bulk of benthos biomass there, while Chaetogaster was numerically the most abundant bottom dweller. Corophium appeared mainly in areas devoid of hydrophytes. Other species of infrequent distribution in the middle sector comprised Nereis limnicola, Melanoides tuberculata and Mysis relicta.

The standing crop of benthos in the middle Lake averaged 548 organisms/m^2 with 11.2 gm fresh wt/m^2 during 1978. It decreased to 321 organisms/^{2} but its average biomass increased slightly to 13.3 gm fresh wt/m^2 in 1979.

The western sector contributed the highest biomass, mostly due to Corbicula consobrina. On the other hand, the highest counts recorded there , were attributed to Chaetogaster limnaei followed respectively by Corbicula consobrina and Mesanthura sp., while Corophium volutator, Gammarus lacustris and Mysis relicta were rarely observed. The total counts of benthos in this sector amounted to 479 organisms/m^2 with 26.5 gm fresh wt/^{2} during 1978 and 498 organisms/m^2 weighed 13.3 gm fresh wt/m^2 in 1979.

The variations in the magnitude of the standing stock of benthos in the three sectors of the Lake were mostly related to the ecological conditions prevailing in these sectors, beside the nature of the bottom sediments and fertility of the Lake water. Thus, Lake Burollus sustained high densities of both phytoplankton and zooplankton with average of 2,745,364 cells/l and 111,154 organisms/m^3 respectively during 1978 and 3,429,582 cells/l and 45,255 organisms/m^3 in 1979 (El-Sherif, 1983 and Aboul Ezz, 1984). The western Lake and the surroundings of the outlets of the drains harboured highest density of both phytoplankton and zooplankton decreasing gradually towards the middle and eastern sector. These planktonic forms contribute the basic tool of food items for benthic fauna particularly in the form of organic debris accumulated at the bottom. The decreased biomass of benthos from 13.7 gm fresh wt/m^2 during 1978 to 6.1 gm fresh wt/m^2 in 1979 was accompanied with similar decline in the total zooplankton counts as previously mentioned.

The average biomass of benthos recorded during the whole investigation period amounted to 19.9 gm fresh wt/m^2 in the western sector, decreased to 7.3 and 2.5 gm fresh wt/m^2 respectively in the middle and eastern sectors. The annual biomass for the whole Lake averaged 9.9 gm fresh wt/m^2. This value is comparable to that previously recorded for benthic fauna in Lake Edku which reached 10.4 gm fresh wt/m^2 (Samaan, 1977) but slightly higher than that of the Nozha Hydrodrome which averaged 6.3 gm fresh wt/m^2 (Elster and Jensen, 1960). However, it was lower than the records of bottom fauna in the highly productive Lake Mariut which sustained an average annual of 76.6 gm fresh wt/m^2 (Samaan and Aleem, 1972).

REFERENCES

Aboul Ezz, S.M., 1984. Limnological investigations on zooplankton and benthos in Lake Burollus. Ph. D. Thesis, Faculty of Science, Mansoura Univ., 340 P.

Cook, D.G. and M.G. Johnson, 1974. Benthic macro invertebrate of the St. Lawrence Great Lakes. J. Fish. Res. Board Can., 31 : 763-782.

El-Sherif, Z.M., 1983. Limnological investigation on the aquatic plant life in Lake Burollus in relation to the dominant environmental conditions. Ph. D. Thesis, Faculty of Science, Cairo Univ., 385 P.

Elster, H.J. and W. Jensen, 1960. Limnological and fishery investigations of Nozha Hydrodrome near Alexandira. Notes and Mem., Alex. Inst. Hydrobiol., 43 : 1-99.

Guerguess, Sh. K., 1979. Ecological study of zooplankton and distribution of macrofauna in Lake Manzalah. Ph. D. Thesis, Faculty of Science Alexandria Univ., 361 P.

Samaan, A.A., 1977. Distribution of bottom fauna in Lake Edku. Bull. Inst. Oceanogr. and Fisheries, Egypt, 7 (1) : 59-90.

Samaan, A.A. and A.A. Aleem, 1972. Quantitative estimation of bottom fauna in Lake Mariut. Bull. Inst. Oceanogr. and Fisheries, Egypt, 2 : 377-397.

Samaan, A.A., Z.M. El-Sherif, E.Y. El-Ayouty, M.M. Abdalla, 1988. Distribution of Hydrophytes in Lake Burollus, Egypt. Bull. Inst. Oceanogr. and Fish. ARE, 14(2).

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989: 225 - 239.

GROWTH OF SOME GREEN ALGAE FROM RIVER NILE IN POLLUTED CULTURES AND THE POSSIBILITY OF THEIR USE AS WATER POLLUTION INDICATORS

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ABSTRACT

Four chlorophycean species namely: **Pandorina morum (Volvocales), chlorella vulgaris, Scendesmus quadricauda (Chlorococcales)** and **Cosmarium** sp. (Zygnematales) were isolated from water samples collected from Damietta Branch, River Nile. The four species have been grown in liqiud cultures under the presence of one pollution factor pH changes (acidic and alkaline), natural and autoclaved sewage concentrations, commercial detergent concentrations and nitrite concentrations, one at a time. The growth rates have been studied for all cultures. The polluting factors used proved to be quite different in their effects on the growth rates of every species, with acid pollution and natural sewage as the most drastic ones. The four test species also showed big fifferences in their response. **Cosmarium** was the most susceptible species; in general; and could be considered as a real oligotrophic one. On the other hand, **Scenedesmus quadricauda** was the strongest in withstanding pollution and could be considered as a real eutrophic species. **Pandorina morum** showed to be a kind of mesotrophic to oligotrophic species, while **Chlorella vulgaris** showed to be a mesotrophic with tendency towards the eutrophic species.

INTRODUCTION

It is well established that the environmental disturbances, such as pollution, induce changes in the structure and function of biological systems. As a result, many biologists have attempted to judge the degree and severity of pollution by analysing changes in biological systems (Kofoid, 1903; Forbes and Richardson, 1913; Forbes, 1928; Purdy, 1930 and Patrick, 1949).

Algae are perhaps the most suitable and convenient biological community for monitoring pollution effects. For a number of years, there has been a series of proposals indicating that one or more algae could be used as organism indicative of water quality.

Fjerdingstad (1964) and Williams (1964) considered diatoms to be the algal group that commonly used as indicative organisms for trophic state and saprobity of water. Palmer (1969) listed 80 species of fresh water algae according to their tolerance to pollutants. His list included species of Cyanophyceae, Euglenophyceae, Chlorophyceae, Cryptophyceae and Bacillariophyceae. The use of phytoplankton as biological indicators for water pollution was discussed by Fjerdingstad (1971) who pointed out that biological assessment for water quality is preferable, rapid and accurate. Patrick (1971) examined a number of streams in U.S.A. and suggested the use of a frequency of algae as an indicator for water quality. Phillips (1977) showed the significance of the use of algae as biological indicators to define areas of trace metal pollution. Round (1981) recommended the use of indicator species or indicator communities of algae for the assessment of water quality. Wu (1984) and Wu and Suen (1985), working on Hsin Dien River in Taiwan, have concluded that the change of the relative abundance of diatoms, green algae and Flagellates in general, was revealed to be a good indication to water pollution.

The use of phytoplankton as biological indicators of water quality is probably new to River Nile and other Egyptian water bodies. Based on a previous study (Zahran et al., 1988) on the water pollution of Damietta Branch of the River Nile through physico-chemical properties and their translation in algal populations; it was decided to isolate some species from water samples collected from our stations on Damietta Branch and check their tolerance against several pollution factors that the River is actually subjected to, like: sewage, detergent, nitrite, alkaline and acid pollutions; and the possibility of the use of such species as water pollution indicators.

EXPERIMENTAL

A- Culture media:

Two types of media were used for isolation and culturing the experiment algae. The first is Woods Hole MBL pH 7.2 medium (Nichols, 1973). The second one is Desmid Agar (Star, 1964).

B- Isolation and purification of test algae:

Water samples were collected from Damietta Branch of the River Nile (Egypt) at Mansoura and Farskour stations during fall 1984, centrifuged at 3000 rpm for 10 minutes, supernatant was then carefully decanted and the residual algal pellets were washed with sterile liquid media and recentrifuged. By means of a sterilized needle, algal pellets were streaked over sterilized agar plates of both above mentioned media; plates were then sealed by means of tape, kept in culture room at 25 $\pm$ 1^{o}C and light intensity approximately 3700 lux. Plates were examined microscopically

every 3 days. Restreaking into fresh plates was done every 10 days. Pure algal colonies that started to appear (1-1.5 month from the strait) were carefully picked up by means of sterile Pasteur pipettes, restreaked over agar plates and incubated as above mentioned. Finally, we were able to get unialgal cultures of Scenedesmus quadricauda, Chlorella vulgaris, Cosmarium sp. and Pandorina morum. The first two algae were found to grow best in Woods Hole MBL pH 7.2 medium, the third in desmid agar medium while the fourth one grew best in desmid agar supplemented with 50 ml/l soil extract.

To get axenic cultures, the test algae were first grown in liquid media for about 12 days to attain vigorous growth. 20 ml of culture medium were centrifuuged at 3000 rpm for about 10 minutes, algal pellets were then treated with an antibiotic solution prepared by dissolving 100 mg penicillin G (Na salt) and 50 mg streptomycin-SO_4 in 100 ml distilled water. After 30 minutes, algae were centrifuged and the excess antibiotic solution was decanted. The algal pellets were washed using sterile liquid media used for culturing. We were successful to get axenic cultures of the four test algae through streaking that was repeated every 10 days.

C- Treatments:

I- Sewage

Domestic sewage was collected from the main sewage station of Mansoura City at intervals of time, mixed thoroughly, filtered through Whatman No. 1 filter paper and volumes of filtrate were added to culture media to make them 0.01%, 0.1%, 1.0%, 20.0%, 40.0%, 60.0%, 80.0% and 100.0% in sewage. Same concentrations have been made up with another group of cultures using sewage that has been autoclaved for 30 minutes.

II- Detergents

One gm of a mixture (of equal amounts w:) of commercial detergents namely: Randy, Savo, Santo, Abeer, Nana and Fomo was dissolved in 1.0 liter of glass-distilled, deionized water, thus each 1.0 ml would contain 1.0 mg detergent. Culture media were supplemented with various volumes of detergent solution to make up the following concentrations: 1.0 mg/l, 2.0 mg/l, 3.0 mg/l, 4.0 mg/l and 5.0 mg/l.

III- Nitrite

Stock solution of $NaNO_2$ was prepared by dissolving 0.492 g $NaNO_2$ (AR) in one liter glass distilled water. 10.0 ml of the stock solution were, then, diluted to one liter, thus each one ml would contain 1.0 gm of NO_2-N. Volumes of the final solution were added to culture media to make the concentrations of 1.0 gm, 2.0 gm, 3.0 gm, 4.0 gm and 5.0 g/l in NO_2-N.

IV- pH changes

By means of dilute. solution of H_2SO_4, KOH and standardized PYE-UNICAM pH-meter, the pH of culture media was adjusted to obtain the desired pH (pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0). The pH of the control culture was 7.2. All cultures were readjusted daily for the exact pH. Before transferring the electrode from one culture to another, it was immersed in Lougl's solution (algal killing agent) for 2 minutes in addition to usual washing.

D- Counting:

Cell counting was made every couple of days uptil 16 days from the start, by means of a haemocytometer for three times after shaking, and an overall average was made up in every case. The growth rate was then estimated by calculating the log of cell number/ml.

RESULTS

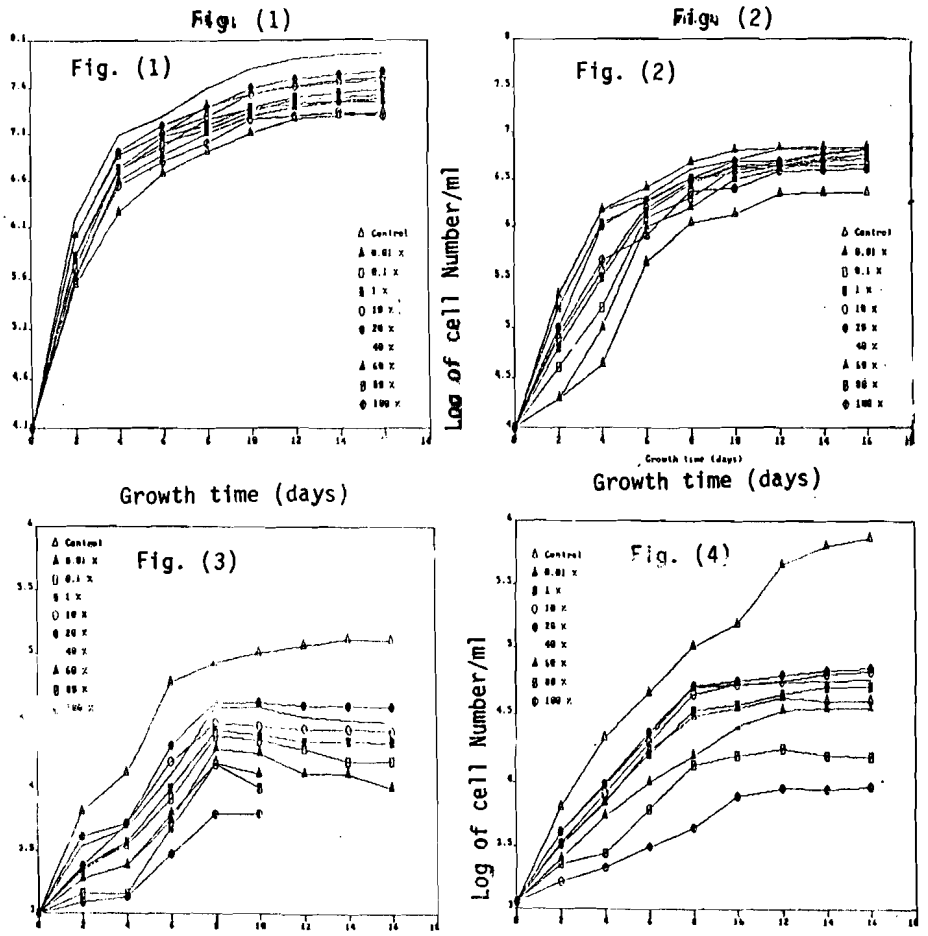
The growth curves representing the four test organisms treated with different concentrations of natural and autoclaved sewage are represented in Figs. 1-4 and 5-8, respectively. Those for treatments with different concentrations of detergent mixture, nitrite and acidic and alkaline cultures are represented in Figs. 9-12, 13-16 and 17-20, respectively.

Cosmarium had growth rates that were lower that of the control ones for all the treatments used except for that with autoclaved sewage. On the other hand, all the growth curves of Chlorella vulgaris with all pollutants showed to be higher than or similar to the control except for the acidic treatment. Scenedesmus quadricauda; another species of Chlorococcales showed to be quite similar to Chlorella vulgaris except for the cultures with detergent where it had growth curves lower than that of the control one. As for Pandorina morum, its growth curves for sewage treatment whether natural or autoclaved were very similar to those of Cosmarium, but quite different from it (with growth curves higher than the control) concerning the detergent and nitrite treatments. As for pH deviations from neutral, Pandorina morum had always lower growth rates whether for alkaline or acidic ones.

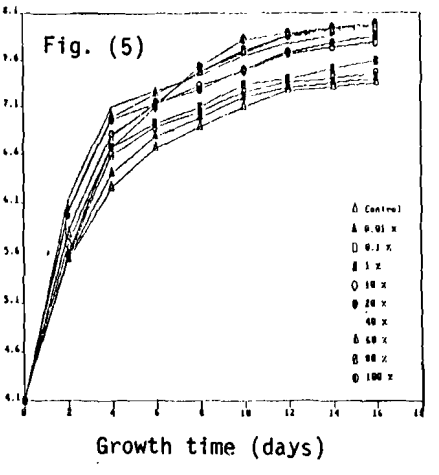
DISCUSSION

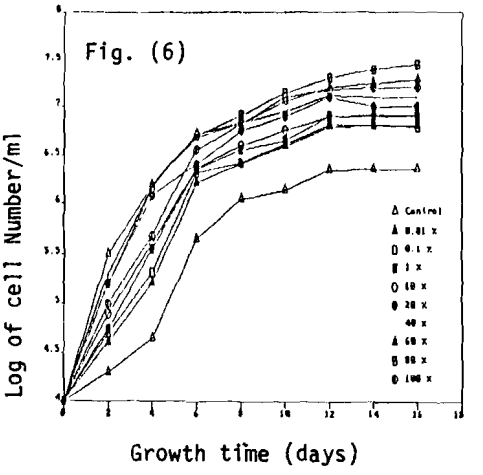
The four test algal species showed to be different in their tolerance against the different pollutants used with their different concentrations.

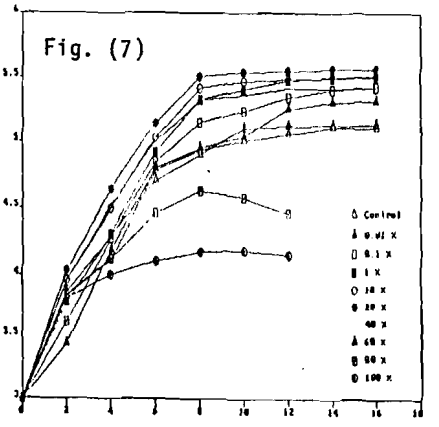
As for natural (unautoclaved) sewage, Cosmarium proved to be the most susceptible one. All its growth curves with different concentrations of sewage were much lower than the control one, with those for cultures with higher concentrations at the bottom (Lowest growth rate). The log

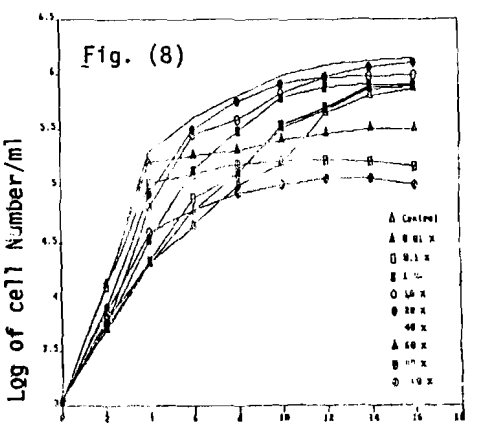


Effect of various concentrations of unautoclaved (natural) sewage on growth of **Chlorella vulgaris** (Fig. 1), **Scenedesmus quadricauda** (Fig. 2), **Pandorina morum** (Fig. 3) and **Cosmarium** sp (Fig. 4).

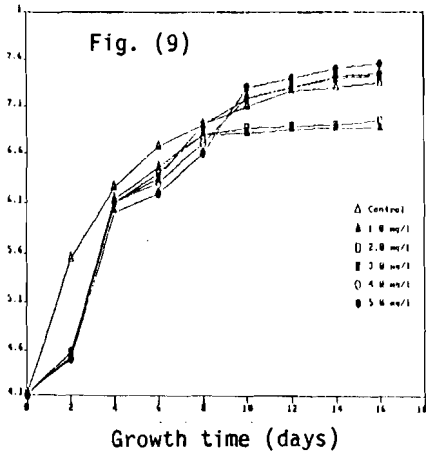


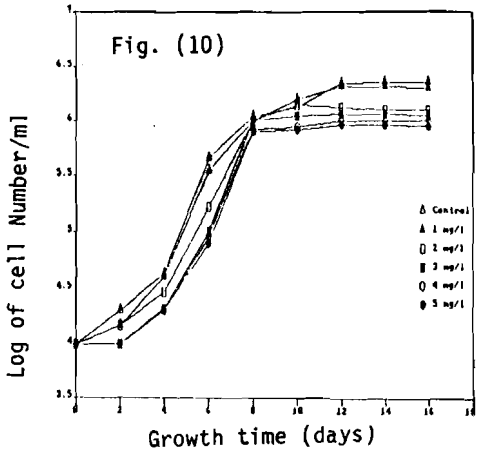


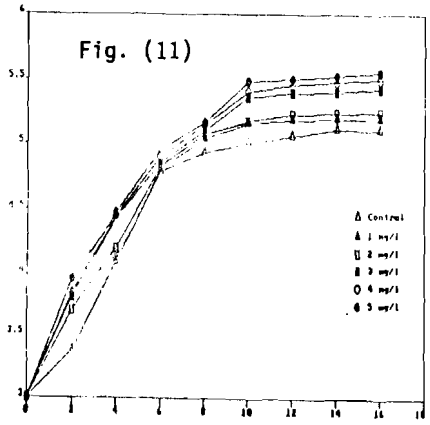


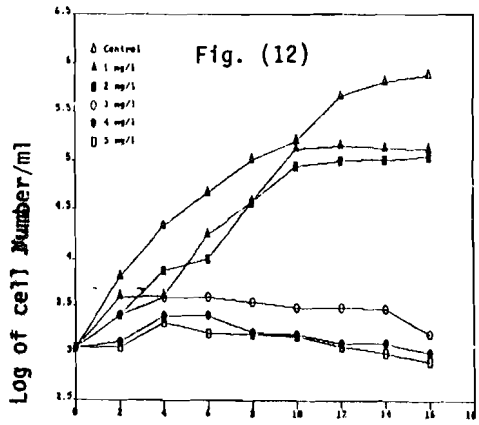


Effect of various concentrations of autoclaved sewage on growth of **Chlorella vulgaris** (Fig. 5), **Scenedesmus quadricauda (Fig. 6), Pandorina morum** (Fig. 7), and **Cosmarium** sp (Fig. 8).

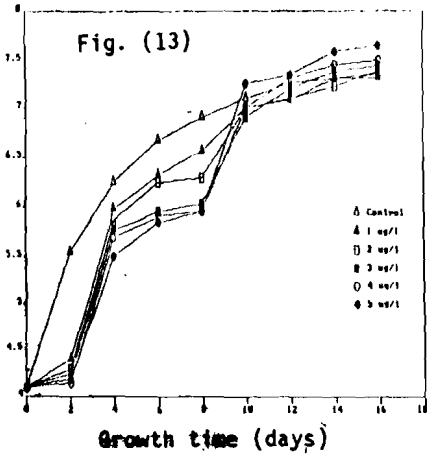


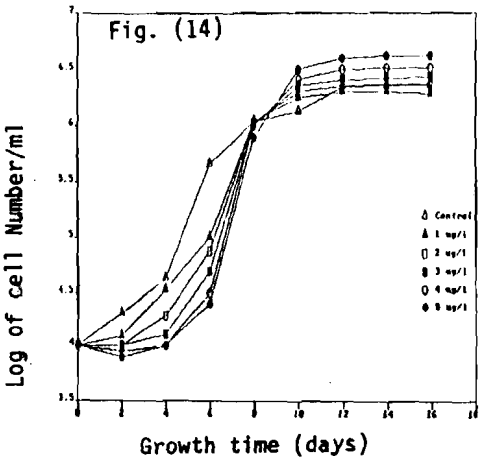


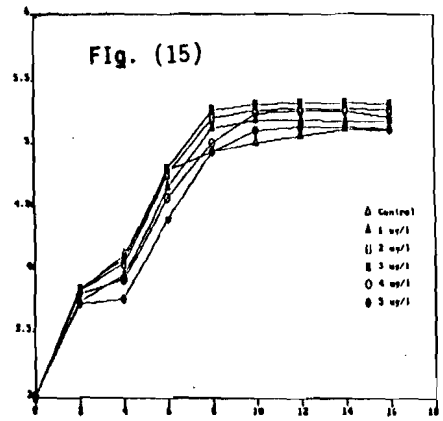


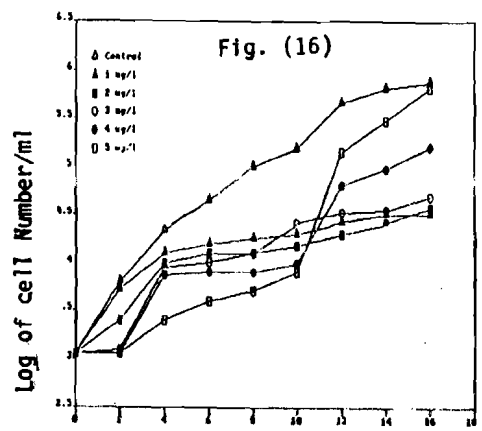


Effect of various concentrations of a mixture of commercial detergents on growth of **Chlorella** (Fig. 9), **Scenedesmus quadricauda** (Fig. 10), **Pandorina morum** (Fig. 11) and **Cosmarium** sp (Fig. 12).

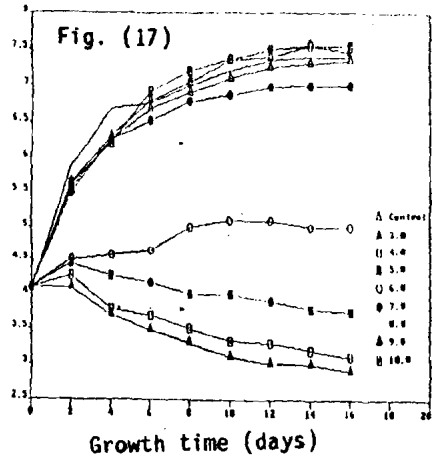


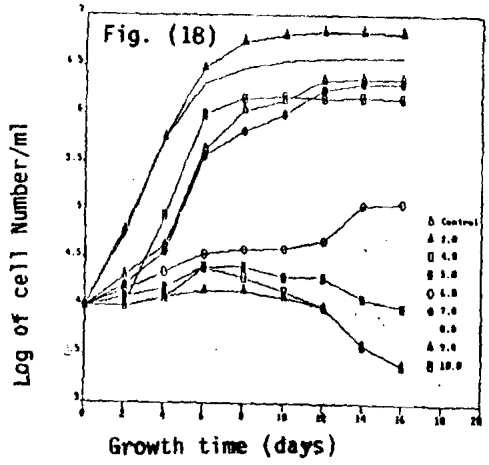


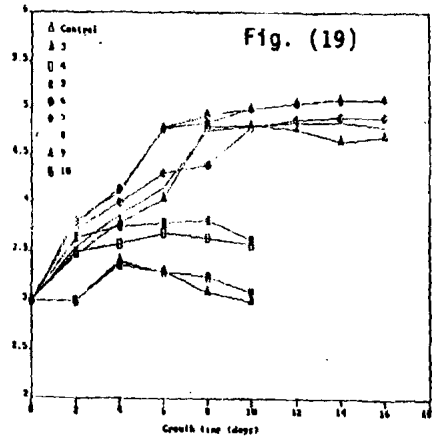


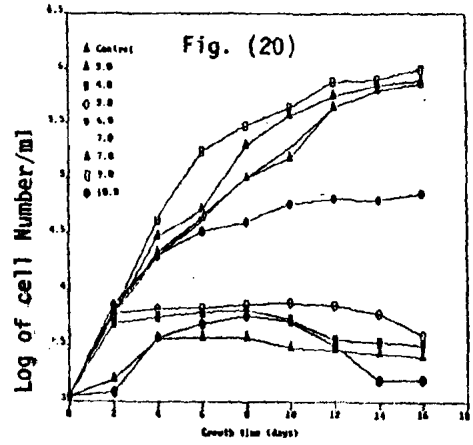


Effect of various concentration of No-$_2$N on growth of **Chlorella vulgaris** (Fig. 13), **Scenedesmus quadricauda** (Fig. 14), **Pandorina morum** (Fig. 15) and **Cosmarium** sp (Fig. 16).









Effect of various pH values on growth of **Chlorella vulgaris** (Fig. 17), **Scenedesmus quadricauda** (Fig. 18), **Pandorina morum** (Fig. 19) and **Cosmarium** sp (Fig. 20).

phase has been reduced to almost half-way where it started a kind of stationary phase whose cell density differed with different concentrations.

Pandorina morum also showed to be very susceptible to natural sewage treatment. All its growth curves for all concentrations were lower than the control one, but the distance between the highest growth curve (with least effect) and that of the control almost half of that in case of Cosmarium. Another clear difference between these two species is that the lag phase extended to 3-4 days in all Pandorina sewage cultures. The lowest growth curves for both Pandorina and Cosmarium are the ones with 60, 80 and 100% sewage which are represented by a longer lag phase, followed by a shorter log one at which they convert to death curves right away at the end of half period of incubation without any stationary phases.

On the other hand, both Chlorella vulgaris and Secendesmus quadricauda showed to be happy with sewage and had all the sewage cultures with higher growth rates compared with control ones, even with the high sewage concentrations (60, 80 and 100%). A clear gap is seen between the control and the nearest growth curve of the treated cultures in case of Scenedesmus. Such a gap is lacking with Chlorella cultures. This shows that Scenedesmus is more tolerant to natural sewage than its cousin Chlorella.

According to Wuhrmann (1975) and Eichenberger (1979), the stimulatory effect of natural sewage on the growth of Scenedesmus and Chlorella could be due to:

1-Direct growth promotion by:

a-Essential macro and micronutrients like carbon, phosphorus, nitrogen, iron, boron, cobalt, copper, manganese, molybdenum and zinc that sewage is rich in.

b-Organic growth substances of vitamin-like character supplied by sewage and known to be essential in culturing of some algae (Shwartz, 1965).

2- Indirect effects like:

a- Solubilization of metals by naturally occurring or man-made complexing agents, thus increasing the supply of essential microelements.

b- Precipitation and co-precipitation of inhibitory metals and organic substances by different compounds, possibly iron hydroxides, phosphates or carbonates (Stumm, 1972).

However, Walsh (1984) concluded that the effects of sewage upon algal growth can not be predicted from a more chemical composition. He suggested that the effect would

rather be the result of additive, synergetic and antagonistic behaviour of the chemicals in relation to physical properties of sewage and physiological and/or genetical response of algal species.

The autoclaved sewage seemed to have a quite different effect on the growth of our test especially with the susceptible ones like Cosmarium and Pandorina. Such a difference is actually expected as the autoclaved sewage lacks bacteria and other microorgansisms and would rather represent a pollutant suspension with high organic and inorganic contents. Our autoclaved sewage proved to have a double phosphate content (450 gm P_{O4} - P/l) comparable to the natural (unautoclaved) one with only 200 gm PO_4 - P/l. The autoclaved sewage exerted a stimulatory effect on the growth rates of all the test algae, even with Pandorina and Cosmarium, when low to moderate concentrations were used. However, higher concentrations (60, 80 and 100%) did inhibit the growth of these two species that showed to be very susceptible when natural sewage was used. This in agreement with the findings of Walsh and Alexander (1980) who reported that autoclaving of some industrial wastes has changed their bioactive properties from highly inhibitory to highly stimulatory. Walsh (1984) referred this change to the bacterial content and other micro-organisms that flourish in natural sewage and compete with algae for nutrients or produce algistatic or algicidal substances. Also autoclaving may lead to getting rid of some harmful gases that normally occur in sewage like ammonia, hydrogen, sulphide, etc. The inhibition with high concentrations of autoclaved sewage could be due to toxic effects of high organic and inorganic contents.

On the other hand, the pleasure of Chlorella and Scenedesmus with autoclaved sewage is quite apparent and the higher the concentration the higher the growth rate would be.

Again with detergent treatments, Cosmarium proved to be a typical susceptible species even with very low concentrations. All its cultures polluted with detergent mixture had growth rates lower than the control. The growth curves for cultures treated with 3 mg of commercial detergent mixture are almost flat, while those for 4 and 5 mg treatments resemble death curves rather than growth ones. Pandorina; a companion of Cosmarium in case of sewage; behaved differently with detergent. It showed higher growth rates with all concentrations used (1-5 mg/l). Yamane (1984) reported that nonionic and anionic washing agents may exhibit an inhibitory effect upon algal growth and the inhibition is mainly species specific. The stimulatory effect of detergents on the growth of Pandorina could be due to its ability to make use of the phosphate content of the detergents. It is well established that a major ingredient of most detergents is phosphate; and according to Ryther (1971) and Kumar (1981); the discharges of detergents into water-ways may support luxuriant growths or blooms of some algal species.

Scendesmus and Chlorella, the two species that were quite happy with sewage proved to be less tolerant with detergent mixture. They are here again more or less similar with growth rates a little bit lower than the control ones. In this sence they are nearer to Cosmarium than Pandorina is.

Concerning the nitrite pollution, Cosmarium is still conservative in being the least tolerant among our test algae. All its cultures with different concentrations had their growth curves completely underneath the control ones all the way from the beginning of the experiment until its end. Attractive features of the treated cultures are extending the lag phase, shortening the log one and break through of the curves with highest concentrations (4 and 5 g/l) after 10 days of the start from being the most susceptible ones with least growth rates to a position much higher than other treated cultures and very near to the control ones. Nitrite had similar effects on the growth of Scenedesmus and Pandorina. It exerted an inhibitory effect with growth curves lower than the control ones uptil 6-8 days, then converted to a stimulation that made the curves of the treated cultures jumb over the control. The inhibition effect of a pollutant or a toxic substance followed by stimulation could be; according to Walsh (1984); due to the development of resistance by algae against the toxicity after being subjected to, for sometime. Another reason that we may suggest is the possibility that nitrite ions be oxidized into nitrate ones by active oxygen resulting from algal photosynthesis.

Concerning pH changes, all our test algae except Pandorina were able to withstand the deviations from neutral towards alkaline. Both Chlorella and Scenedesmus had growth rates higher than the control in alkaline cultures with pH up to 10.0. Chlorella was more tolerant in this sence as the growth curve of Scenedesmus started to go down right after pH 10.0. Cosmarium, the alga that showed to be [illegible] susceptible to all kinds of pollution treatments so far, was able to withstand pH 9.0, but pH 10.0 expectedly had a great inhibitory effect on its growth.

Acid pollution; represented in our treatments by cultures with pH adjusted to 5, 4 and 3; had the most drastic effect on all test algae used. None of our species could show any tolerance against this treatment. All growth curves of all species are either stationary or death curves. Unexpected result here is that both Chlorella and Scenedesmus (the chlorococcalean species) proved to be more susceptible to acid pollution than Pandorina and Cosmarium and had death curves with pH 5, 4 and 3.

It is well established that the availability of CO_2 and bicarbonate for algal photosynthesis is highly pH dependent. The increase in pH decreases the free CO_2 level, thus oligotrophic algae confined to free CO_2 as a carbon for photosynthesis would be unable to grow well under such conditions.

At high pH values (above pH 8), the growth of the most oligotrophic species ceased or was greatly reduced. On the other hand, most of the eutrophic algal species are able to use bicarbonate ions directly and their growth would continue undiminished up to pH values above 9.0 (Fogg, 1965; Raven, 1968 and 1970 and Moss, 1972 and 1973).

According to our findings we may classify our test algae based on the saprobic zones as follows:

a-Cosmarium to be an oligotrophic species.

b-Pandorina morum to be a kind of mesotrophic to oligotrophic one.

c-Chlorella vulgaris to be a mesotrophic with tendency towards the eutrophic zone.

d-Scenedesmus quadricauda to be a real eutrophic species.

The idea that flagellates, in general, are to be considered as species of the eutrophic zone is not a straight rule, and should be dealt with care. Some eutrophic specie supposed to be tolerant against several pollutants, could be more susceptible to a specific pollutant (especially acid pollution) than some oligotropic ones.

In general the classification of algae concerning saprobic zones and their use as eutrophication and\ or water pollution indicators must be specific on the species level; as that one established on the class or generic levels could be dangerously misleading.

REFERENCES

Eichenberger, E., 1979. The study of eutrophication of algal benthos by essential metals in artificial rivers, in: **Biological aspects of freshwater pollution.** Ed. G. Ravera, Oxford, New york, 111-128,P.

Fjerdingstad, E., 1964. Pollution of streams estimated by benthal phytomicro-organisms. 1. Saprobic system based on communities of organisms and ecological factors. **Int. Rev. ges. Hydrobiol.**, 49: 63-131.

Fjerdingstad, E., 1971. Microbial criteria of environment qualities. **Ann. Rev. Microbiol.**, 25: 563-82.

Fogg, G.E., 1965. **Algal cultures and phytoplankton ecology.** Athlone Press, London.

Forbes, S.A., 1928. The biological survey of a river system objects, method and results. **Ill. Dept. Registrat. Edu. Div. Natur. Hist. Surv.**, (17): 277-284.

Forbes, S.A. and R.E. Richardson, 1913. Studies on the biology of the upper Illionois River. **Bull. Ill. Nat. Hist. Surv.**, (9): 481-574.

Kofoid, C.A., 1903. Plankton studies of the Illios River and its basin. **Bull. Ill. 57 Lab. Adat. Hist.**, 6: 95-629.

Kumar, H.D., 1981. The biosphere and its water pollution. In: **Modern concepts of ecology.** ed, H.D. Kumar, Navin Snahdara, Delhi, 163-208.

Moss, B., 1972. The influence of environmental factors on the distribution of freshwater algae, influence of calcium level. J. Ecol. 60: 917-932.

Moss, B., 1973. The influence of environmental factors on the distribution of freshwater algae. The role of pH and carbon dioxide bicarbonate system. J. Ecol. 61: 157-177.

Nichols. H.W., 1973. Growth media-freshwater. In: Handbook of phycological methods, Culture Methods and Growth Measurements Edi: J.R. Stein. Cambridge Univ. Press.

Palmer, C.M., 1969. Tolerance of freshwater algae against pollutions. J. Phycol., 5: 78-82.

Patrick, R., 1949. A proposed biological measure of stream conditions, based on survey of the Conestoga Basin, Lancaster Country, Pennslvonia. Notul. Nat., 1: 277.

Patrick, R., 1971. Diatom communities, In : Carins J., Jr. (Ed.) Am. Microsc. Symposium on the structure and function of freshwater microbiol communities. pp. 151-64. virginia polytechnic. Institute and State University Press, Blacksburg, Virginia.

Phillips, D.J.H., 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments, a review. Environ. Pollut., 13(4): 281-318.

Purdy, W.C., 1930. A study of pollution and natural purification of the Illinois River. II. The plankton and related organisms. U.S.A. Public Health Bull., 198-212.

Raven, J.A., 1968. The mechanism of photosynthetic use of bicarbonate by Hydrodictyon africanum. J. Exp., Bot., 19: 193-206.

Raven, J.A., 1970. Exogenous inorganic carbon sources in plant photosynthesis. Biol. Rev. 45: 167-221.

Round, F.A., 1981. The ecology of algae. Cambridge Univ. Press, Cambridge, 653p.

Ryther, J.H., 1971. Nitrogen, phosphorus and eutrophication in the coastal environment. Science, 171: 1008-1013.

Schwartz, D., 1965. Der einfluss von wirkstoffen auf das wachstum und die vermehrung von algen. Veroffentlichungen der hydrologischen forschungsabteilung der dortmunder stadtwerke AG., 8, 1-206.

Star, R.C., 1964. The culture collection of algae at Indiana Univ. Amer. J. Bot., 51: 1013-44.

Stumm, W., 1972. Die role der Komplexbiolung in naturlichen gewassern und allfallige. Beziehungen zur eutrophierung gewasserschutz-wasser-abwasser, Helft. 57-87. Aachen.

Walsh, G.E., 1984. Algal bioassays of industrial and energy process effluents. In: Algae as ecological indicators. Ed., L. Elliot Schubert. Academic Press. London, 434p.

Walsh, G.E. and S.V Alexander, 1980. Water, Air and Soil pollut. 13: 45-55.

Williams, L.G, 1964. Possible relationships between plankton-diatom species number and water quality estimates. Ecology, 45: 809-823.

Wu, J.T., 1984. Phytoplankton as bioindicator for water quality in Taipei. 25: Bot. Bull. Academia Sinica, 25-214.

Wu, J.T. and W.C. Suen, 1985. Change of algal associations in relation to water pollution. Bot. Bull. Academia Sinica (Taipei), 26(2): 203-212.

Wuhrmann, K., 1975. Experiments on the effect of inorganic enrichment of rivers periphyton primary production. verh. **Internat. Vereim. Limnol.**, 19: 2028-2034.

Yamane, A.M, 1984. The growth inhibition of planktonic algae due to surfactants used in washing agents. **Water Res.**, 18(9): 1101-1106.

Zahran, M.A; Y. A. Azab Mohamad and M. I. Abdel-Hamid, 1988. **Biological assessment of water pollution of Damietta Branch of the River Nile and Proposal for its Control.** Inter. Workshop on Clean Technology and Pollution Treatment., Cairo, 1988, Egyption Environmental Affairs Agency (EEAA), Cairo, Egypt.

Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989: 241 - 258.

STUDIES ON THE ORIGIN, DEVELOPMENT AND FATE OF BLOOD CELLS IN THE TELEOST, CLARIAS LAZERA

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ABSTRACT

Haemopoiesis in Clarias lazera was studied on physiological and cytochemical basis. It was found to be monophyletic since blood cells develop from a common stem cell; the haemocytoblast in the lymphomyeloid tissue of haemopoietic organs. Senile blood cells were also noticed to disintigrate in the circulation and haemopoietic organs.

INTRODUCTION

Many investigators postulated different schemes for the origin and site of stem cells responsible for the formation of blood cells in poikilothermic animals (Jordan and Speidel, 1924; Duthie, 1939; Catton, 1951; Yokoyama, 1960; Watson et al., 1963; Weinreb and Weinreb, 1969; Gardner and Yevich, 1969; Ellis, 1976 & 1977; Mahajan and Dheer, 1979; Cannon et al., 1980; Barber et al., 1981; Hoole and Arme, 1982; El-Feky, 1982; Bergeron and Woodward, 1983; Hightower et al., 1984; Scott et al., 1985; Miller et al., 1986 and Roubal, 1986). However, studies on the haemopoiesis of subtropical fish are very rare and in particular on Egyptian fish species. The aim of this work is to carry out a study on the origin, development and fate of blood cells in the Egyptian catfish, Clarias lazera.

MATERIAL AND METHODS

Fish were collected alive from the unpolluted area of Bab-El-Abid in Lake Mariut, near Alexandria. They were kept for 48 hrs in suitable continuously aerated tanks before examination.

Twelve healthy fishes were examined monthly. Prior to investigation, each fish was measured and weighed. Their lengths ranged between 15-40 cm and weighed between 30-250 gm. Blood smears were made, air dried and fixed in methyl alcohol for 5 minutes and stained by Giemsa, Wright's or panoptic methods. Thereafter, fish were dissected and haemopoietic organs (head kidney, liver and spleen) were removed, cut and applied to clean slides to make tissue imprints. They were fixed in methyl alcohol for 5 minutes, formalin vapour or in a solution of 10 ml formalin and 90 ml methanol, to study periodic Acid Schiff (PAS), Sudan Black B and peroxides reactions respectively (MayHoe et al., 1960 and Pearse, 1972). Sections of the head kidney were made after fixation in 10% neutral formalin and stained using eosin-haematoxylin.

RESULTS

Sections and imprints of the head kidney (Figs. 1,2,5,6,12,13 & 14) showed the presence of stem cells and several developmental stages. The blood smears also showed the presence of some developmental stages in addition to the mature blood cells (Figs. 3,4,7,8,9,10,11,15,16,17,18,19 & 20).

From the study of sections, imprints and smears, it appears that the haemocytoblast in Clarias lazera is the stem cell that arises from a primitive reticular cell which hypertrophies and later separates from the reticular syncytium. The reticular cell (RC) can be seen in areas of blood forming tissue between the uriniferous tubules (Ut, Fig. 1). The haemocytoblasts are formed extravasculary in the stromal areas not within the venous sinusoids. In kidney imrpint preparations (Fig. 2), the outline of the haemocytoblast appears either oval, spherical or irregular. Each cell contains a moderate amount of cytoplasm and a large, centrally located nucleus with one or two nucleoli. The size of the haemocytoblasts varies. It appears that some of the large haemocytoblasts (LHcb) proceed in development towards the granulocytes series, while others form the precursors of erythrocytes series. The medium-sized haemocytoblasts (MHcb) divide to form the small cells (SHcb) which develop into lymphocytes and thrombocytes.

Erythrocytes

The developmental stages of the erythrocyte series are as follows:

a) Pronormoblast

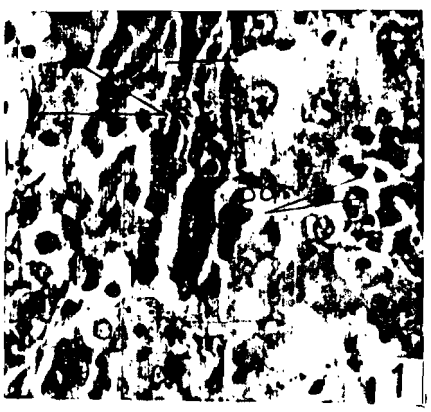
This stage can be recognized in blood smears (Fig. 3, PrN) and kidney imprints (Fig. 5). The large nucleus is still present with thickening of some of the chromatin threads, but no haemoglobin is yet evident.

b) Basophilic Normblast

This stage is often seen in kidney imprints (Fig. 6) and rarely in blood smears (Figs. 3 & 4, BN). It is characterized by homogenous basophilic cytoplasm and a concentric nucleus in which the chromatin forms large clumps. It is smaller in size than that of the pronormoblast, and the nucleus is still large in proportion to the cytostome.

c) Polychromatophil Normblast

As development proceeds haemoglobin appears in the cytoplasm of the erythroblast cell which looses its basophilia. In blood smears and imprints of haemopoietic organs, the cytoplasm may have lighter areas and opaque ones (Fig. 4 PN), and hence the name polychromatophil normoblast. The cell is often rounded and the nucleus is spherical and concentric.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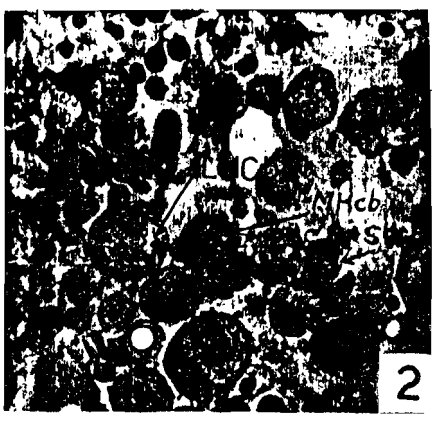
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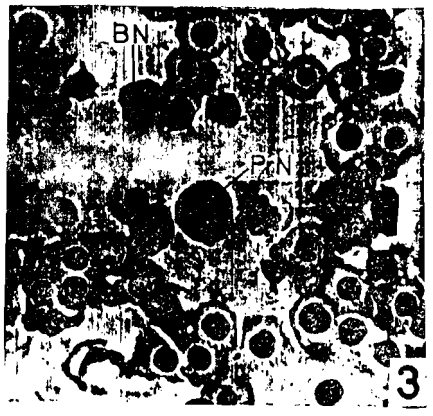
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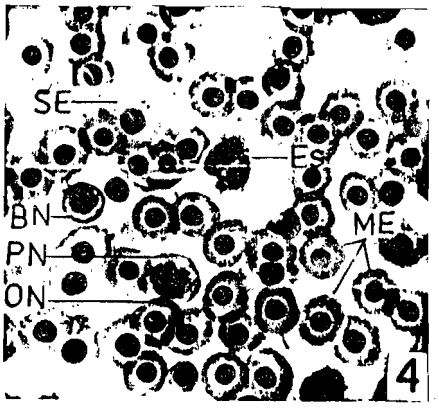
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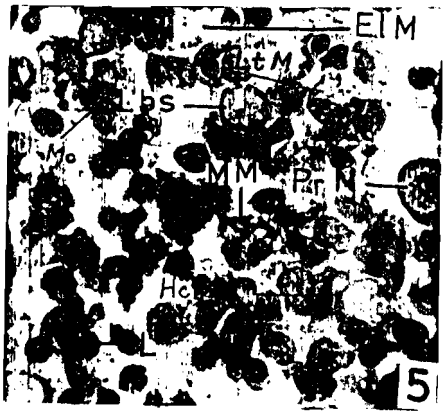
ETM
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5

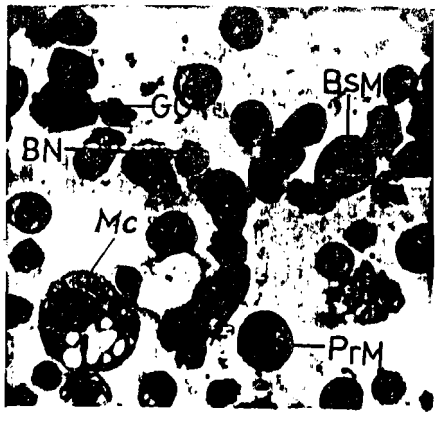

BsM
G
BN
Mc
PrM

FIG. 1.
Formalin -eosin haematoxylin. L.S. in the head kidney showing reticular cells (RC) between renal tubules (UT) and a group of developing cells (DC). X 1250.

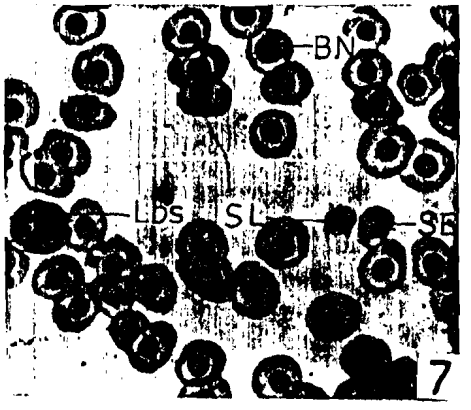
FIG. 2.
Kidney imprints showing large haemocytoblast (LHcb) medium haemocytoblast (MHeb and small haemocytoblast (SHcb). X 1250.

FIG. 3.
Blood smear-Wright's stain showing a pronormoblast (PrN) and a basophilic normoblast (BN), X 1250

FIG. 4.
Blood smear-Panoptic method showing basophilic normoblast (BN), Polychromatophil (PN), orthrochromatophil normoblast (ON), mature erythrocytes (ME) and large senile erythrocyte distended and deformed cytoblasm (SE), eosinophil (Es). X 1250.

FIG. 5.
Kidney imprints-Sudan Black B, showing + ve granules in both early and late myelocytes (ElM) (LtM) and metamylocytes (MM), while -ve in haemocytoblast (Hcb), lymphoblast (Lbs), lymphocyte (L) and pronormoblast (PrN). Notice a macrophage (Mc). with weak + ve reaction. X 1250.

FIG. 6.
Kidney imprints-Wright's stain showing basophilic myelocyte (BsM), macrophage (Mc) with several vacuoles and promylocyte (PrM). Notice Ghost cell (GC) and basophilic normoblast (BN). X 1250.


BN
Lbs
SL
SE
7


S
GC
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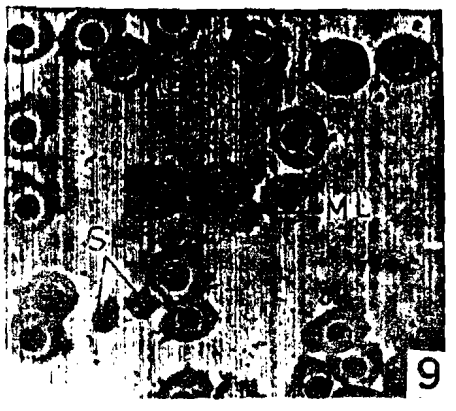
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Th
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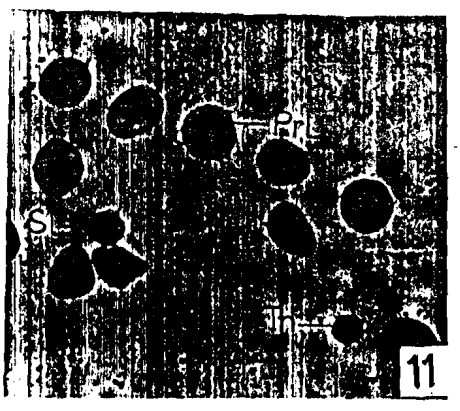

Pr
S
TH
11


Lbs
DL
DT
Pr
DE
12

FIG. 7.
Blood smear-Giemsa stain showing a basophilic normoblast (BN), small senile erythrocyte (SE), small lymphocyte (SL) and lymphoblast (Lbs). X1250.

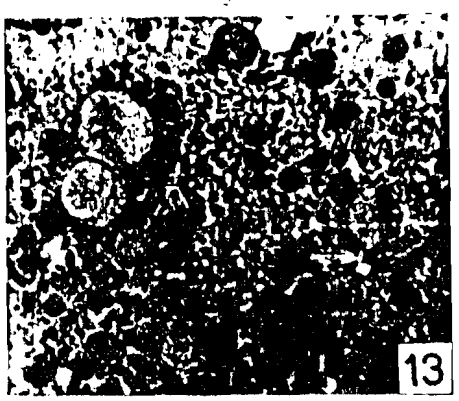
FIG. 8.
Blood smear-Giemsa stain showing poikilocytosis, senile rythrocyte (SE), ghost cells (GC) and early myelocyte (ElM). X 1250.

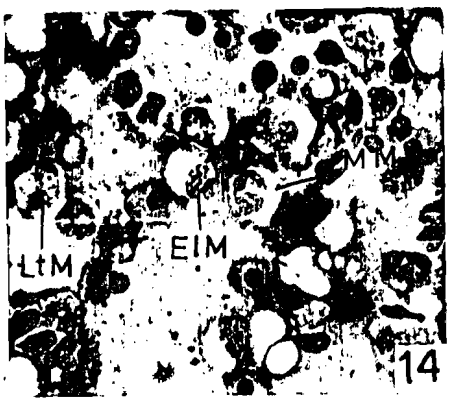
FIG. 9.
Blood smear-panoptic method showing 2 small lymphocytes (SL), a medium sized lymphocyte (ML). Notice that all the cells posses pseudopodia. X 1250.

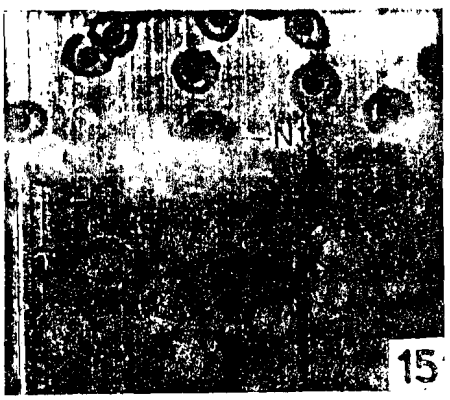
FIG. 10.
Blood smear-Giemsa stain showing a lymphoblast (Lbs) with nuclear details and pseudopodia, small old lymphocyte (SL) and a cluster of spherical thromboctye (Th). X 1250.

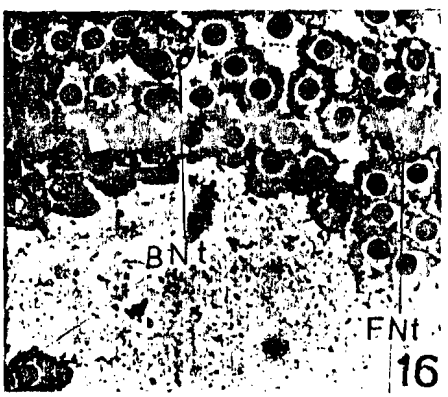
FIG. 11.
Blood smear-Giemsa stain showing prolymphoctye (PrL), senile lymphocyte (SL) and spherical thrombocyte (Th). X 1250.

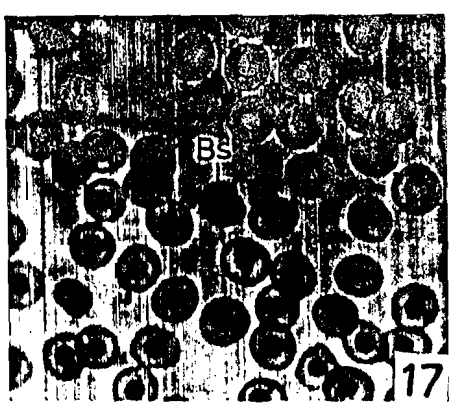
FIG. 12.
Kidney imprints-Wright's stain showing developing lymphocytes (DL), developing thrombocytes (DTh). Notice a prothrombocyte (with kidney- shaped nucleus) (Prth) , lymphoblast (lbs) and developing erythrocytes (DE). X 1250.


13


MM
LtM
EIM
14


15


BNt
FNt
16


Bs
17

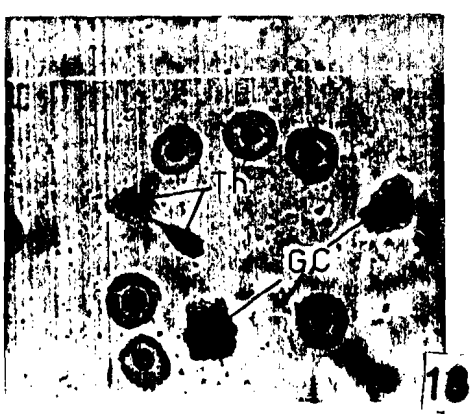

Th
GC
18

FIG. 13.
Kidney imprints-Panoptic method, a neutrophilic myelocyte (NtM), an eosinophilic myelocyte (EsM), promyelocyte (PrM) and haemocytoblast (Hcbl). X 1250.

FIG. 14.
Kidney imprints-Wright's stain showing metamyelocytes (MM) with horse-shoe shaped nucleus (MtM cells), early myelocytes (EsM) and late myelocyte (LM). X 1250.

FIG. 15.
Blood smear-Giemsa stain showing a mature filamented neutrophil (Nf). X 1250.

FIG. 16.
Blood smear-Panoptic method showing three mature neutrophilia, two with bilobed nuclei (BNf), one third with nucleus formed of 4 lobes (FNf). Notice poikilocytosis of erythrocyte. X 1250.

FIG. 17.
Blood smear-Wright's stain showing a basophil (Bs). X 1250.

FIG.18.
Blood smear-Panoptic method showing 2 thrombocytes sending several pseudopodia, one of these pseudopodia is long and links the two thrombocytes (Th). Notice ghost cells (GC). X 1250.

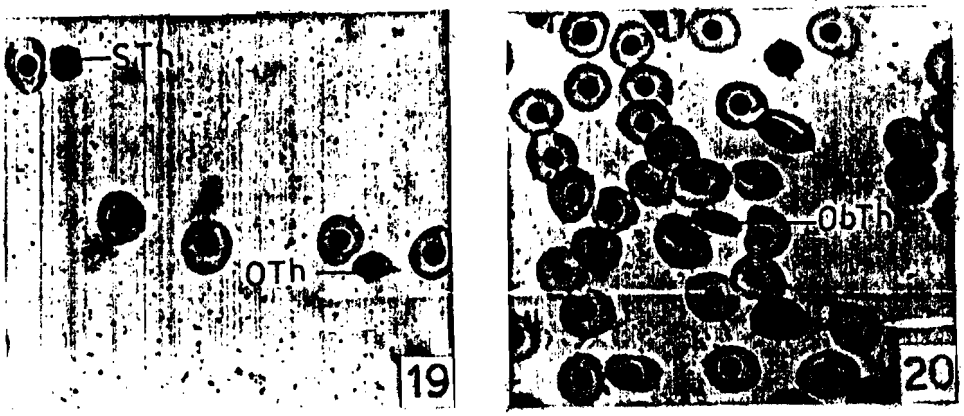


FIG.19.
Blood smear-Panoptic method showing a spherical and an oval thrombocytes (STh) & (OTh). X 1250.

FIG-20.
Blood smear-Panoptic method showing an oblong-shaped thrpmbocyte. (ObTh). X 1250.

d) Orthochromatophil Normoblast

In this stage, the cytoplasm has acquired a further amount of haemoglopin which accounts for its acidophilic or orthochromatic reaction (Fig. 4, ON) but the nuleus is still round with further condensation of chromatin.

e) Mature Erythrocytes

Normal mature erythrocytes contain a centrally located biconvex nucleus and are often round, rarely oval in shape (Fig. 4, ME).

F) Senile Erythrocyte

Senile erythrocytes are characterized by their pycnotic nuclei and cytoplasmic condensation. Thus, a senile cell is much smaller in size than the mature red blood cell and has always a deformed outline (Fig. 7, SE). During their formation, erythrocytes increase in size, stain lightly, loose their normal appearance and show patchy areas which are torn away (Figs. 4 & 8, SE).

It was noticed that all above stages gave negative results with peroxidase and PAS reactions. Only mature erythrocytes showed positive black granules with Sudan Black B, whereas other developmental stages gave negative results.

Leucocytes

Clarias lazera leucocytes are divided into two categories; granulocytes and agranulocytes.

I. Granulocytic leucocytes

The earliest recognizable cell of the granulocyte series is the promyelocyte which gives rise to a sequence of myelocyte, metamyelocyte and polymorph (mature granulocyte).

When a haemocytoblast develops into a promyelocyte stage, several changes take place. The cell size increases due to increased cytoplasm (Fig. 13, PrM). The nucleus assumes an eccentric position, and nucleoli disappear.

In kidney imprints (Fig. 13), the cytoplasm of promyelocyte is pierced in some regions by acidophilic areas which first become evident close to the nucleus and gradually spread irregularly to the periphery of the cell. Thereafter, acidophilic areas enlarge and finally affect the basophilic cytoplasm. Cytochemically the promyelocyte is strongly positive to PAS, Peroxidase and Sudan Black B.

Myelocyte is most abundantly found in the circulating blood. The cytoplasm has lost most of its basophilic nature and possesses a fine spongy appearance. The nucleus contains a coarse network of chromatin, sometimes with heavier clumps. In early myelocyte, the nucleus is oval in shape (Figs. 5 & 14, ElM), while in the late myelocyte, it becomes indented (Figs. 5 & 14, ElM). According to the affinity of the cytoplasmic granules of myelocytes to various days, there are neutrophilic, eosinophilic and basophilic myelocytes. The neutrophilic and eosinophilic myelocytes appear to have similar sizes (Fig.13), where as the basophilic myelocyte is always of a much smaller size (Fig. 6). Myelocytes give positive reactions with PAS and strongly positive reactions with Sudan Black B. With peroxidase reaction, both neutrophilic and eosinophilic myelocytes show positive results, while basophilic myelocyte give a negative one.

In metamyelocyte the nucleus becomes indented and finally attains a horse-shoe shaped (Figs. 5 and 14). The metamyelocytes are of similar size to the mature forms. No nucleoli were observed in their nuclei. All types of metamyelocytes give positive reactions with all cytochemical tests applied, except for the basophilic metamyelocyte which gives negative result with peroxidase reagent.

The nucleus in the neutrophilic metamyelocyte consists of two oval parts joined by a broad band. Later, the two lobes become connected by a thin filament of chromatin (Fig. 15,

Nt). This stage is called the filamented stage. Polymorphonuclear neutrophil with nucleus of four lobes exists in Clarias (Fig. 16). Chromatin clumps of the nucleus are large and easily recognized. The cytoplasm is filled with fine granules. In some cells, small vacuoles may be seen in the cytoplasm.

Neutrophils are positive to PAS reagent. The cell cytoplasm reacts weakly, but the granules are strongly positive. Neutrophils are also positive to peroxidase and Sudan Black B.

The eosinophilis are smaller than the neutrophils and are irregular or oval in shape (Fig. 4 Fs). The mature cells contain a large quantity of cytoplasm. The development of the nucleus resembles that of the neutrophil with the fine chromatin network becoming progressively coarser. Although most cells contain a single eccentric round or oval nucleus (Fig. 4), they sometimes may have a bilobed one. The cytoplasm appears to be acidophilic due to the accumulation of the coarse eosinophilic granules. Mature eosinophilis give positive reactions to all the cytochemical tests applied.

Mature basophils are the smallest and rarest cells in the blood of Clarias. They may be irregular or oval in shape. The basophilic cytoplasm is filled with scattered highly refractile granules (Fig. 17). The basophilic granules are larger than the eosinophilic ones. Certain basophils may contain from 8 to 10 large granules (Fig. 17 Bs), but the majority contains even more than these numbers. The nucleus may be round or oval in shape and stains blue to purple and eccentrically located in the cytoplasm. No polymorphonuclear basophils is observed in the blood of Clarias. Basophils give positive results with both Sudan Blake B and PAS but negative reaction with peroxidase.

When the cells of granulocytic series reach the end of their physiological activity, some disintegrate in the blood circulation as seen in blood smears (Figs. 8 & 18). Others degenerate in the lymphomyeloid tissue of the haemopoietic organs, especially those of the spleen and the kidney (Fig. 6, GC). They appear as dark stained masses of irregular shapes. The term ghost cells is given to these degenerating granulocytes.

II. Agranulocytic leucocytes

The agranulocytic type of Clarias leucocytes includes the lymphocytes which are the prevailing white cells in circulating blood and the macrophage, whose presence is restricted only to the lymphoid tissue of the haemopoietic organs. In tissue smears, macrophages are often seen containing several vacuoles in their cytoplasm (Fig. 6 Mc).

Lymphooytes in Clarias are derived from prolymphocytes (Fig. 11, PrL) whose mother cell is the Lymphoblast, which in turn originates from the stem cell, the haemocytoblast.

The lymphoblasts (Lbs), occur mainly in the haemopoietic tissue, sometimes in the peripheral circulation (Fig. 10). and possess nuclei made up of coarse reticular chromatin network with the one or more rounded nucleoli. Some nuclei are deeply indented (Fig. 12, DL)). Senile lymphocytes (Fig. 10, SL) are characterized by the disappearance of normal nuclear details.

The lymphocytes in the circulating blood of Clarias lazera are predominatly small (Figs. 7 & 9, SL) and occassionlly of medium size (Fig. 9, ML). The nucleus with its clumped chromatin occupies the entire volume of the cell, and is surrounded by thin cytoplasm. The cytostome may be rounded or have an amaeboid shape due to numerous pseudopodia (Figs. 9, 10 & 11). Both lymphoblasts and lymphocytes are weakly positive to the PAS reagent and are negative to both peroxidase and Sudan Black B reactions.

Macrophages are giant cells with a large amount of cytoplasm, a loosely reticular, often of vacuolated (Fig. 6 Mc). It was noticed that the nucleus of the macrophage resembles that of the primitive reticular cell of the lymphoid tissue in the haemopoietic organs. However, it is larger and contains more chromatin granules and two prominent nucleoli.

Cytochemically, macrophages give weakly positive reaction with Sudan Black B (Fig. 5) and negative reactions for both Peroxidase and Periodic Acid Schiff.

Thrombocytes

In the peripheral blood of Clarias lazera, thrombocytes occur in various stages of development. These thrombocytes are represented by prothrombocytes, various intermediate forms and mature thrombocytes. In blood smears the thrombocytes appear either round or oval (Figs. 10 & 11, Th) and contain an oval nucleus and a small amount of cytoplasm.

Thrombocytes are derived from their mother cell, the thrombocyte, which in turn comes from the haemopoietic stem cell; the haemocytoblast, frequently seen in the lymphomyeloid tissue of haemopoietic organs (Fig. 12 PrTh). The size of prothrombocyte is relatively smaller than those of small lymphocytes. The nucleus is kidney-shaped with fine reticular network of chromatin, and has one or more nucleoli. However, the amount of cytoplasm is considerably large than of the thrombocyte, and is moderately basophilic in reaction.

The mature thrombocytes are spheroid or oval in shape (Fig. 19), sometimes oblong (Fig. 20 ObTh). The cytoplasm has a fine reticular flaky appearance. The nucleus is large compared to the amount of cytoplasm. In some cases, thrombocytes contain one polar granule or a single vacuole. Occasionally the vacuole is located in the concavity of the nucleus (Figs. 19 and 20 STh & Oth). Mature thrombocytes are able to radiate cytoplasmic pseudopodia.

Figure 18 shows two thrombocytes projecting several thin pseudopodia, with one pseudopodium elongating to link two thrombocytes. It appears that these thrombocytic networks may serve in blood clotting of Clarias.

DISCUSSION

Blood smears of Clarias lazera showed that erythrocytes, lymphocytes and granulocytes were present. Typical monocytes were absent. Mature and immature cell types at various transitional stages of development were also noticed, a state similar to that reported by Weinreb (1963).

The results show that all different types of blood cells originate from a stem cell, the haemocytoblast, found in the lymphomyeloid tissue of haemopoietic organs, particularly the kidney and spleen. It arises from a primitive reticular cell. This is in agreement with Catton (1951) and Watson et al. (1963). In Clarias lazera, haemocytoblasts vary in size. The large haemocytoblasts develop to erythrocytes and granulocytic leucocytes, while medium or small-sized ones form lymphocytes and thrombocytes. These observations are in accordance with data presented on the perch blood (Yokoyama, 1960) and on Carassius auratus (Watson et al., 1963 and Weinreb and Weinreb, 1969).

During erythropoiesis, the haemocytoblast undergoes marked transformation in nucleus and cytoplasm to give developmental stages (pro-, basophilic, polychromatophil and orthochromatophil normoblasts) until it reaches the mature erythrocyte. Fish haematologists applied various nomenclatures to the erythrocyte developmental stages. However, the terms used in the present work are more or less identical to those given by Yokoyama (1960) in the perch. The polychromatophil of the present work appears to be similar to the proerythrocyte described by Ellis (1976) in the plaice with the exception that the nucleus of the latter is sometimes eccentric. Furthermore, the orthochromatophil normoblast of Clarias lazera was not recorded by the majority of workers, although Ellis (1976) described a similar stage in the plaice which he called young erythrocyte . Catton (1951) and El- Feky (1982) described a similar developmental stage in a number of teleosts, which they called reticulocyte.

All erythrocytic stages gave negative results with peroxidase reaction. This conclusion confirms the observations of many authors such as Yuki (1957); Ellis (1976); and El-Feky (1982), but contradicts the data of Caxton-Martins (1978) and Cannon et al. (1980). Only mature cells showed positive Sudan Black B granules, whereas developmental stages gave negative results. Similar results were reported for the plaice erythroblasts (Ellis, 1976).

Mature erythrocytes of Clarias lazera showed negative reaction with PAS reagent, similar to the findings of Hayhoe et al. (1960); Caxton-Martins (1977, 1978 & 1979) and El-Feky (1982).

During development of lymphocytes in **Clarias lazera**, the haemocytoblast transforms into a lymphoblast which in turn gives rise to the prolymphocyte. From the latter stage, a mature lymphocyte is derived. Similar reports have been given for lymphocyte development in teleost fish (Catton, 1951; Watson et al., 1963; and Weinreb and Weinreb, 1969). However, McKnight (1966) was unable to distinguish lymphoblasts in imprints of haemopoietic organs in the mountain whitefish.

Both lymphoblasts and lymphocytes are weakly positive to PAS reagent, similar to the lymphocytes of the plaice (Ellis, 1976) and Schilbe mystis (El-Feky, 1982). As to peroxidase reaction, lymphocytes are negative. This result agrees with those reported by Ellis (1976) and El-Feky (1982).

The lymphocytes of Clarias lazera were found to give negative results with Sudan Black B reaction similar to the finding of Baillif and Kimbrough (1946), and Blaxhall and Daisley (1973).

Macrophages were noticed to be confined only to haemopoietic organs and were not found in the peripheral circulation. Such observation resembles these reported by Yokoyama (1960); Van Furth et al. (1972); Ellis and De Sousa (1974); Ellis (1976 & 1977) for other fish species. However, Watson et al. (1963) reported the presence of macrophages in the blood circulation as well as in the haemopoietic tissues of the goldfish.

As regards the ontogeny of macrophages in **Clarias lazera**, they appear to be derived directly from primitive reticular cells. This view finds support by Jordan and Speidel (1924). Cytochemically, macrophages give weakly positive reaction with Sudan Black B and negative reactions for both peroxidase and PAS tests.

Cytochemically, promyelocytes, myelocytes and metamyelocytes give positive reaction with PAS in both granules and cytoplasm. This result confirms those of Ellis (1976) and Barber and Westermann (1978). As to the Sudan Black B reaction, all immature stages of leucocytes described in Clarias lazera give moderate positive results. This observation is contrary to that reported by Ellis (1976) in the plaice, where the only Sudan Black positive cells were the mature leucocytes.

The promyelocyte, neutrophilic and eosinophilic myelocytes along with metamyelocytes give positive results with peroxidase reaction, while basophilic myelo- and metamyelocytes reacted negatively. However, Ellis (1976) reported that immature stages of all leucocyte types have a negative peroxidase reaction.

In neutrophils of Clarias lazera, the cytoplasm is faintly stained while the granules show strong PAS reaction, this confirms results of El-Feky (1982), but contradicts the observation of Roubal (1986). Granules also give positive reaction with Sudan Black B, in agreement with Baillif and Kimbrough (1946). They give positive results with peroxidase. This confirms the results of Yuki (1957), Ellis (1976), Cannon et al. (1980) and El-Feky (1982) in various teleosts. On the other hand, the present observations are contradictory to those of Kelenyi and Nemeth (1969) who stated that neutrophilic granules in teleosts were peroxidase negative.

Eosinophils of Clarias lazera give positive reaction to PAS, Sudan Black B and peroxidase. This agrees with results of El-Feky (1982); Baillif and Kimbrough (1946); and Hattori (1958).

Basophils give positive results with PAS, similar to Schilbe mystis (El-Feky, 1982). They reacted positively with Sudan Black B, contrary to the results of Baillif and Kimbrough (1946). As regards the peroxidase reaction, the basophilic granules in Clarias gave negative results, this agrees with the results of Hattori (1958), and El-Feky (1982).

Cytochemically, it was noticed that both prothrombocytes and thrombocytes were negative for PAS reaction. This result confirms these of Duthie (1939) and Caxton-Martins (1979) on some teleosts. Contrary to the present observation is that of Ellis (1976) on the thrombocytes of the plaice. As to Sudan Black B reaction, thrombocytes of Clarias gave negative results to peroxidase reaction. This observation supports the work of Duthie (1939) on teleost fish.

Finally, it is proposed that blood cell formation in Clarias lazera is monophyletic according to the following scheme:

Proposed scheme showing the origin and development of the blood cells of **Clarias lazera.**

Haemocytoblast

Erythrocyte	Granulocyte series			Agranulocyte series		Thrombocyte series
1. Pronormoblast	1. Promyelocyte					1. Prothrombocyte
2. Basophilic Normoblast				Macrophages	Lymphocyte series	2. Mature Thrombocyte
	2. Neutrophilic Myelocyte	2. Eosinophilic Myelocyte	2. Basophilic Myelocyte		1. Lymphoblast	
3. Polychromat Ophil Norm						
4. Orthrochromat Ophil Norm	3. Nt. Meta-Myelocyte	3. Eos. Meta-Myelocyte	3. Bas. Meta-Myelocyte		2. Prolymphocyte	
5. Mature Erythrocyte	4. Mature-non-filamented	4. Mature Eosinophil	4. Mature Basophil		3. Mature lymphocyte	
					Large	Small
	5. Bilobed					
	6. Polymorphnuclear					

REFERENCES

Baillif, R.N and C. Kimbrough, 1946. Studies on leucocyte granules after stainig with Sudan Black B and May-Grunwald Giemsa. I. Lab and Clin., Med., 32 (2) : 155-166.

Barber, D.L and J.E.M. Westerman, 1978. Occurrence of the Periodic Acid-Schiff positive granular leucocyte (PAS-GL) in some fishes and its significance. J. fish Biol ., 12: 35-43.

Barber, D.L.; J.E.M. Westerman and M.G. White, 1981. The blood cells of Antarctic icefish Chaenocephalus aceratus lonnberg : Light and electron Microscopic observations. J. Fish. Biol., 19: 11-28.

Bergeron, T. and B. Woodward, 1983. Ultrastructure of the granule cells in the small intestine of the rainbow trout Salmo gairdneri before and after stratum granulosum formation. Can. J. Zoo., 1, 61: 133-138.

Blaxhall, P.C and K.W. Daisley, 1973. Routine Haematological methods for use with fish blood. Fish Biol., 5: 771-781.

Cannon, M.S.; M.M. Mollenhauer; T.E. Eurell; D.M. Lewis; A.M. Cannon and C. Tompkins, 1980. An ultrastructural study of leucocytes of the channel catfish, Ictalurus punctatus. J. Morph. 164: 1-23.

Catton, W.Y., 1951. Blood cell formation in certain teleost fishes. Blood, 6: 39-60.

Caxton-Martins, E.A., 1977. Cytochemistry of blood cells in peripheral smears of some West African reptiles. J. Anat., 124 (2): 393-400.

Caxton-Martins, E.A., 1978. Cytochemistry of blood cells in two West African amphibians. J. Anat., 125 (2): 231-235.

Caxton-Martins, E.A., 1979. Cytochemical stydies of cell population in peripheral blood smears of two West African teleosts. J. Anat., 128 (2): 269-276.

Duthie, E.S., 1939. The origin, development and function of the blood cells in certain marine teleosts. Part I-Morphology. J. Anat., 73: 396-412.

El-Feky, N.K., 1982. Comparative studies on the blood picture and haematopoietic organs in some poikilothermic animals. M. Sc. Thesis. Faculty of Science, Tanta University, Egypt.

Ellis, A.G., 1976. Leucocytes and related cells in the plaice Pleuronectes platessa. J. Fish Biol., 8: 143-156.

Ellis, A.G., 1977. The leucocytes of fish: A review J. Fish Biol., 11: 453-491.

Ellis, A.E. and M.A.B. De sousa, 1974. Phylogeny of lymphoid system. I. A study of the fate of circulating lymphocytes in plaice Eur. J. Immun., 4: 338-343.

Gardner, G.R. and P.P. Yevich, 1969. Studies on the blood morphology of three estuarine Cyprinodontiforme fishes. J. Fish. Res. Bd. Can., 2: 433-447.

Hattori, K., 1958. An Improved method of peroxidase reaction combined with Giemsa's stain for blood cells. J. Labo. & Clin. Medi., 51, 829.

Hayhoe, J.G.F.; D. Quaglino and J.R. Flemans, 1960. Consecutive use of Romanowsky and Periodic- Acid-Schiff techniques in the study of blood and bone-marrow cells. Brit. J. Haemal., 6: 23-25.

Hightower, J.A.; L.J. McCumber, M.G. Welsh; D.S. Whatly, R.E. Hartivgsen and M.M. Sigel, 1984. Blood cells of fundulus heteroclitus (L.). J. Fish. Biol., 24: 287-298.

Hoole, D. and C. Arme, 1982. Ultrastructural studies on the cellular response of roach, Rutilus rutilus. J. Fish Dis., 5: 131-144.

Jordan, H.E., and C.C. Speidel, 1924. Studies on lymphocytes. II. The origin, function, and fate of the lymphocytes in fishes. J. Morpho., 38 (4): 529-549.

Kelenyi, G. and A. Nemeth, 1969. Comparative histochemistry and electron microscopy of the eosinophil leucocytes of vertebrates. I. A study of the avain, reptile, amphibian and fish. (c.f. El-Feky, N.K., 1982).

Mahajan, C.L. and J.S. Dheer, 1979. Cell types in the peripheral blood of an air-breathing fish Channa punctatus. J. Fish Biol., 14: 481-487.

McKnight, L.M, 1966. A hematological study on the mountain whitefish Prosopium williamsoni. J. fish. Res. Bd. Canada, 23: 45-64.

Miller, N.W.; A. Deuter, and L.W. Clem, 1986. Phylogeny of lymphocyte heterogeneity the cellular requirements for mixed leucocyte reaction with channel catfish Ictalurus punctatus. Immunology, 59 (1): 123-128.

Pearse, A.G.E., 1972. Histochemistry, theoretical and applied. 3rd edn., Vol. II. London : Churchill Livingstone.

Roubal, F.R, 1986. Blood and other possible inflammatory cells in the Sparid Acanthopegrus australis (Gunther). J. Fish Biol., 28: 573-593.

Scott, A.L.; W.A. Rogers and PH Klesius, 1985. Chemiluminescence by peripheral blood phagocytes from channel catfish Ictalurus punctatus function of opsonin and temperature. DEV comp. Imunol, 9 (2): 24-250.

Van Furth, R.; Z.A Cohn; J.G Hirsch; J.H. Humphrey; W.g. Spector and H.L. Langevoort 1972. the mononuclear phagocytic system : A new classification of macrophages, monocytes and their precursor cells. W.H.O., 46: 845-952.

Watson, M.F.; K.W. Guenther, and R.D. Royce, 1963. Haematology of healthy and virus diseased Sockeye salmon, Oncorhynchus nerka. Zoologica. N.Y., 41: 27-37.

Weinreb, E.L., 1963. Studies on the fine structure of teleost blood cells. Anat. Rec., 147: 219-238.

Weinreb. E.L. and S. Weinreb, 1969. A study of experimentally induced Endocytosis in a teleost. I. Light microscopy of peripheral blood cell respnses. Zoologica, 54: 25-37.

Yokoyama, H.O., 1960. Studies on the origin, development, and seasonal variations in the blood cells of the perch, Perca flavescens. Wildl. Disease, 6: 1-103.

Yuki, R., 1957. Peroxidase staining of the leucocytes in rainbow trout. Bull. Fac. Fich. Hokkaido Univ., 8: 36-44.