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

ZEINAB M. EL-SHERIF

National Institute of Oceanography and Fisheries
Alexandria, Egypt.

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.
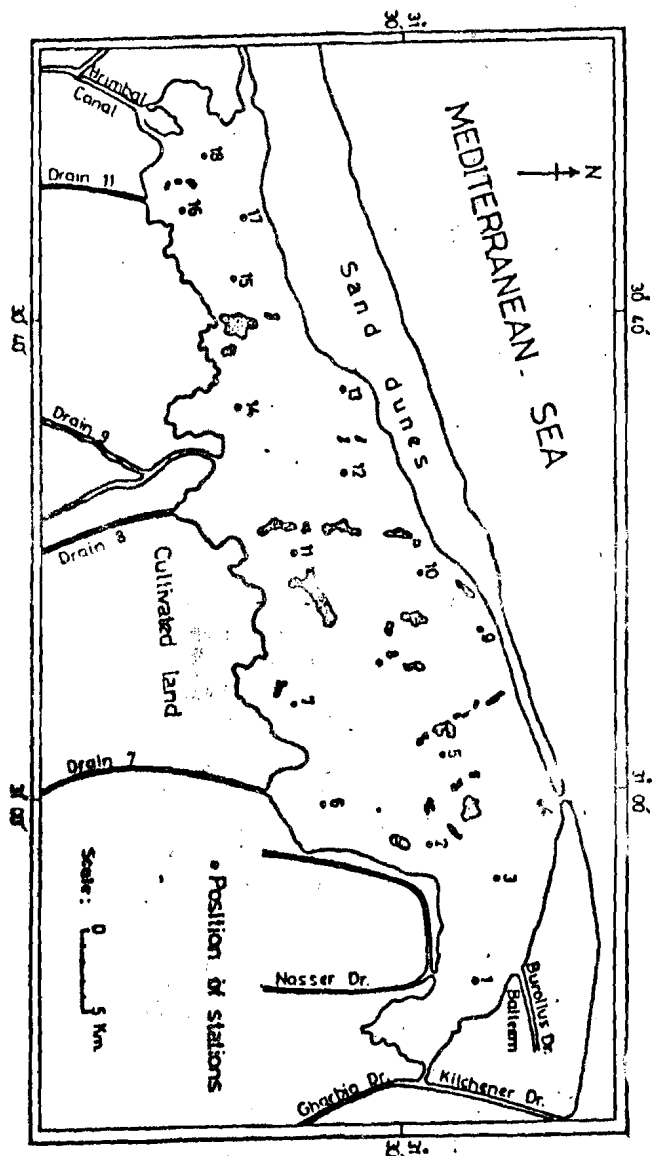
FIG. 1.

Morphometry of Lake Burollus 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 ($=mm^3 \times 10^{-9}$)
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. Variana 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 :	
- Scenodesmus 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: Continue

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.

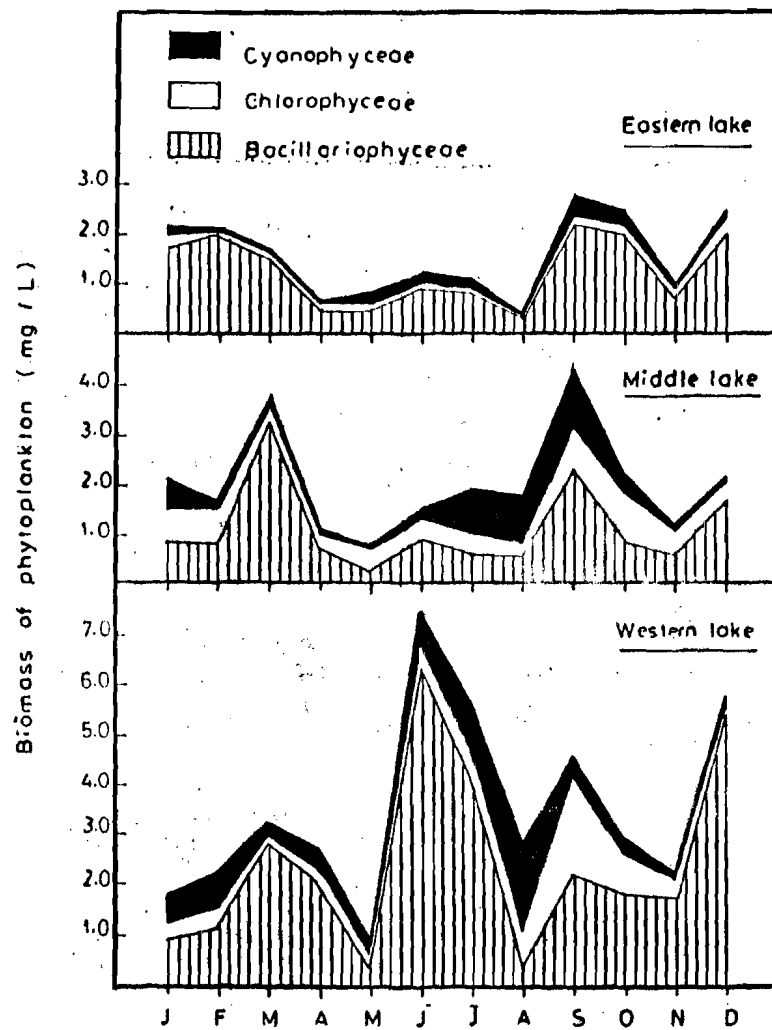| Section Phytoplankton	Eastern Lake	Middle Lake	Western Lake	Average	%
Bacillariophyceae	1.2726	1.1419	2.4398	1.6181	69.0%
Chlorophyceae	0.1251	0.5120	0.5052	0.3808	16.2%
Cyanophyceae	0.1292	0.3869	0.5258	0.3473	14.8%
Total	1.5269	2.0408	3.4708	2.3462	100%

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

184

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.
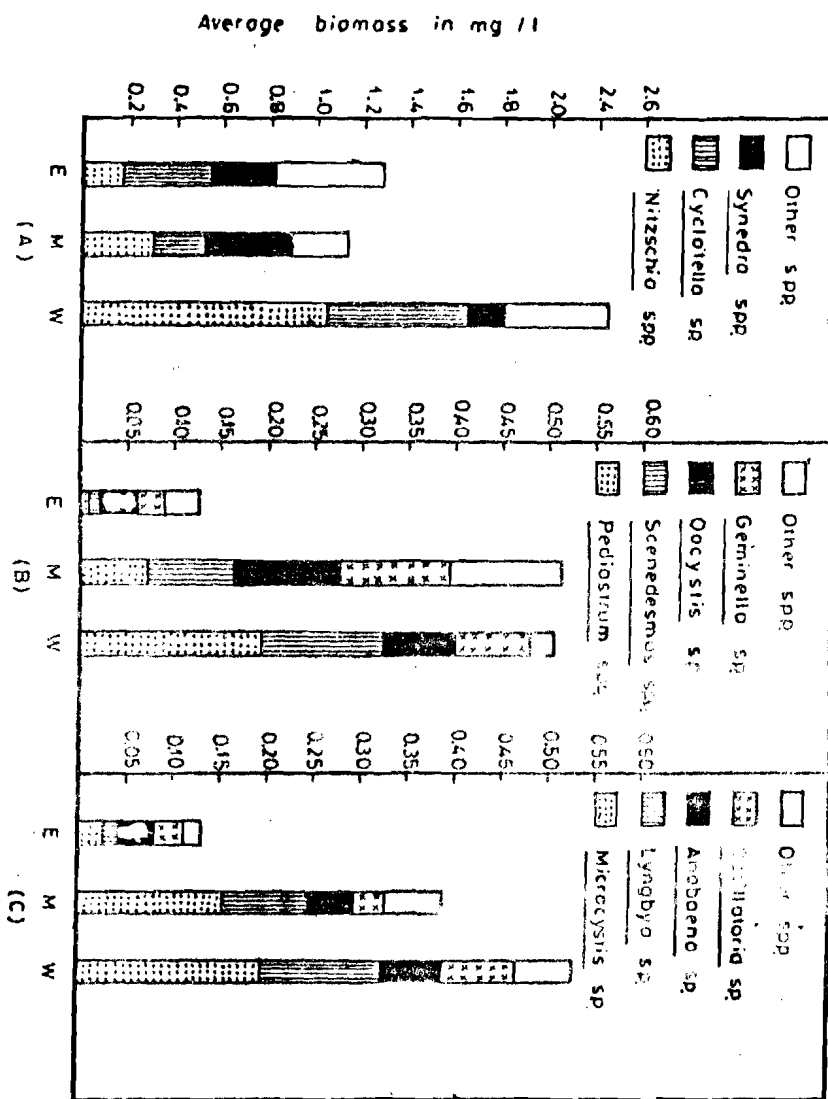
a - Bacillariophyceae :

Diatoms contributed about 69 % by weight to the total phytoplankton (average 1.6181 mg/l). As shown in Table 3 and Fig. 2A 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%

Average biomass in mg / l

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%

187

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.

Blue green algae	Eastern Lake		Middle Lake		Western Lake	
	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.

ROLE OF ANTIFOAMERS IN EXTRACTING OIL
FROM DESALINATED SEA WATERS

HASSAN AWAD AND SAMIR GHAZY*

Department of Oceanography, Faculty of Science, Alexandria
University, Moharam Bey, Alexandria, Egypt.
*MEPA, Jeddah, Saudi Arabia.

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 40^0C).

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 seawat. 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 "-Con antifoamers while their dispersion decrease paralelly 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

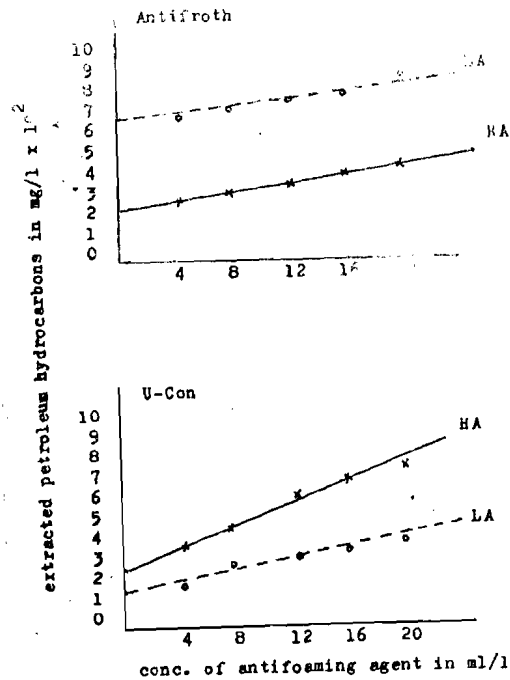$$Y = 6.2 \times 10^2 + 3.49 \times 10^{-3}X$$
(in the case of light Arabian-like crude oil spill)

193

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

194

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

ACKNOWLEDGMENTS

REFERENCES

Anon., 1976. Guide to operational procedure for the IGOSS pilot project on marine pollution (petroleum) monitoring. UNESCO, Manuals and Guides, N^0 7.

Mellhany, 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.

HAEMOPIETIC ORGANS IN THE TELEOST CLARIAS LAZERA

SOHEIR E.M. KHADRE, M.B. SHABANA AND M.M.LOTFY

Department of Zoology, Faculty of Science
Alexandria University, Alexandria, Egypt

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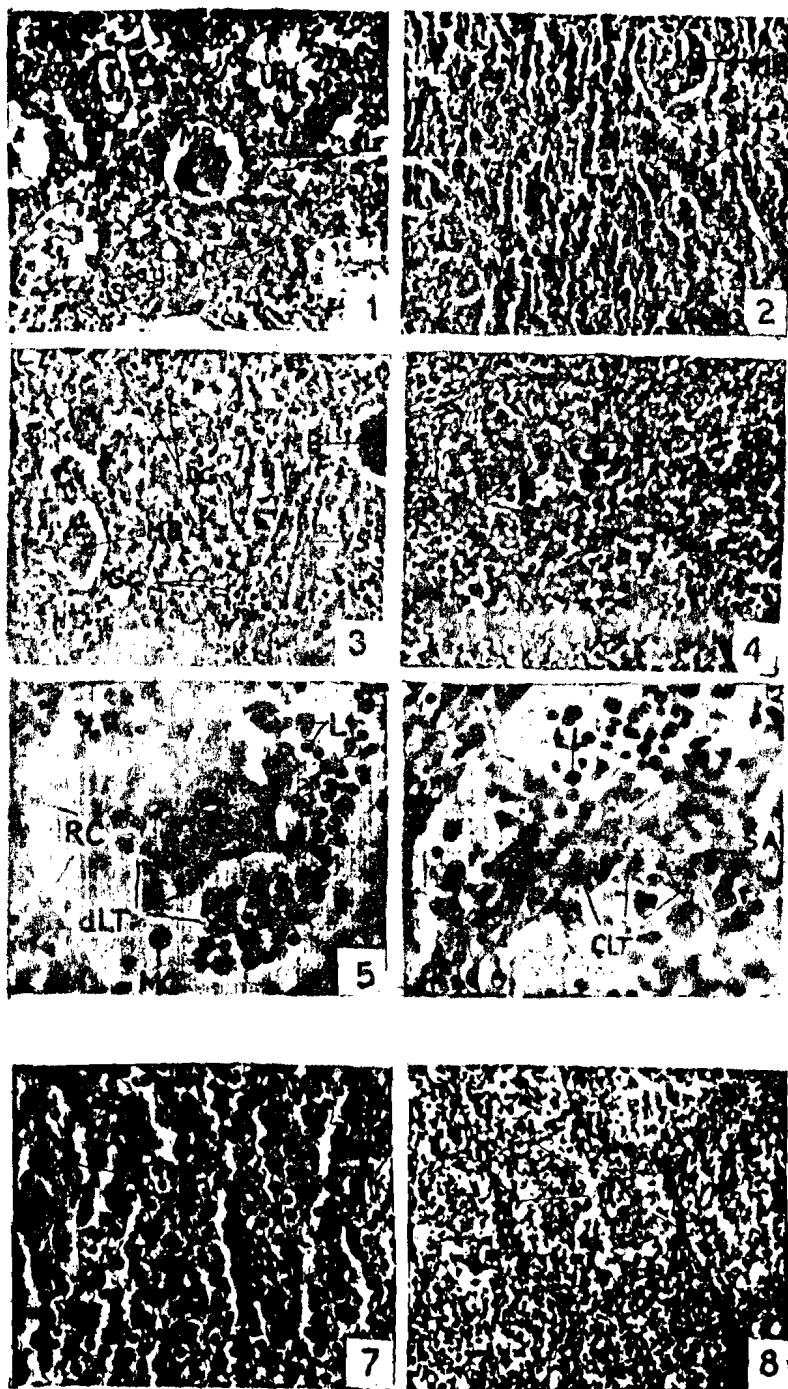
FIG. 1.
Formalin-eosin haematoxylin.
T.S. of head kidney during spring
showing uriniferous tubules (UT),
malpighian body (MB). Haemopoietic tissues (HT),
including lymphocytes (L) and adrenal gland (ag). x 500.

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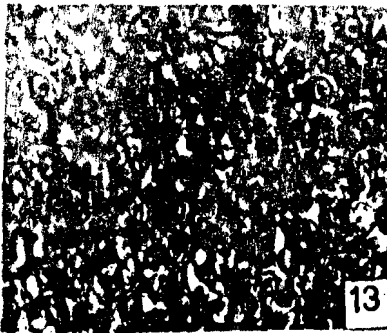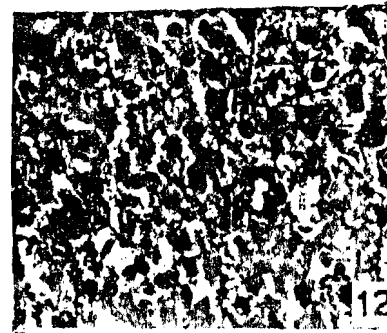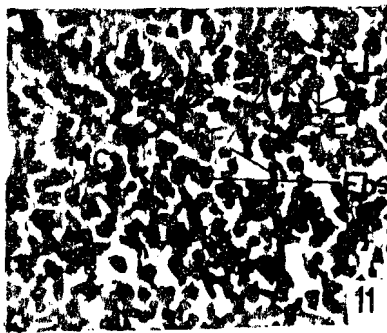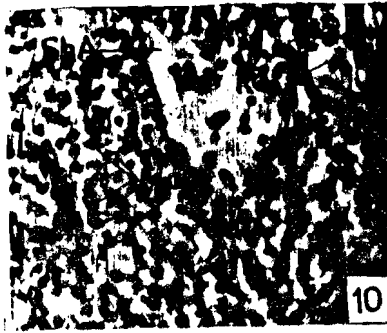
FIG. 9.
Formalin-eosin haematoxylin. T.S. of spleen during spring,
showing the splenic artery (Sa) and splenic vein (SV) traversing
the centeral part of the organ. x 125.

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
(Ebs). 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),
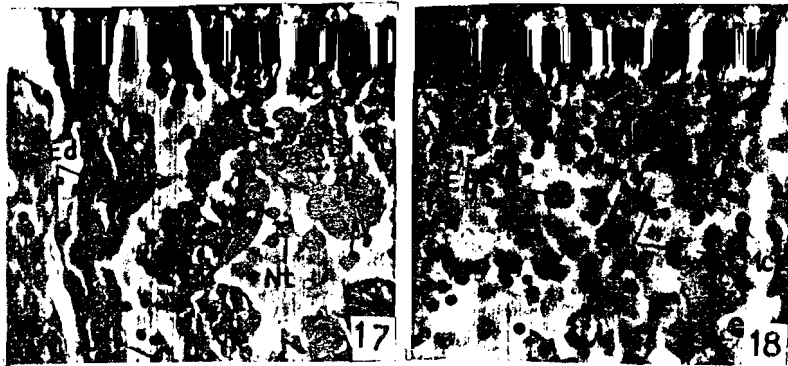lymphocytes (L) and eosinophils (Es), x 1250.

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 idn of
Clarias lazera contains various developmental stages of
red and white blood corpuscles, beside their mature form
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 heamopoiesis 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 Aache 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 haematology, 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., (LEIPZ), 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.M., 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., 48: 107-129.

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

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

Sahai, P.S. and P.V. Rangnekar, 1962. Blood cell formation in the freshwater teleost, Ophiocephalus punctatus (Bloch). J. Animal Morpho. Physiol., 9: 124-130.

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

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

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

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

Welving, F., 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 batrachus (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.

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

EZZAT A. IBRAHIM

National Institute of Oceanography and Fisheries.
Laboratory of Aquatic Plants, El-Zamalek Fish Garden,\
Cairo, Egypt.

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 test 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: 25 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$\pm$1° 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 Mykllestad 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.
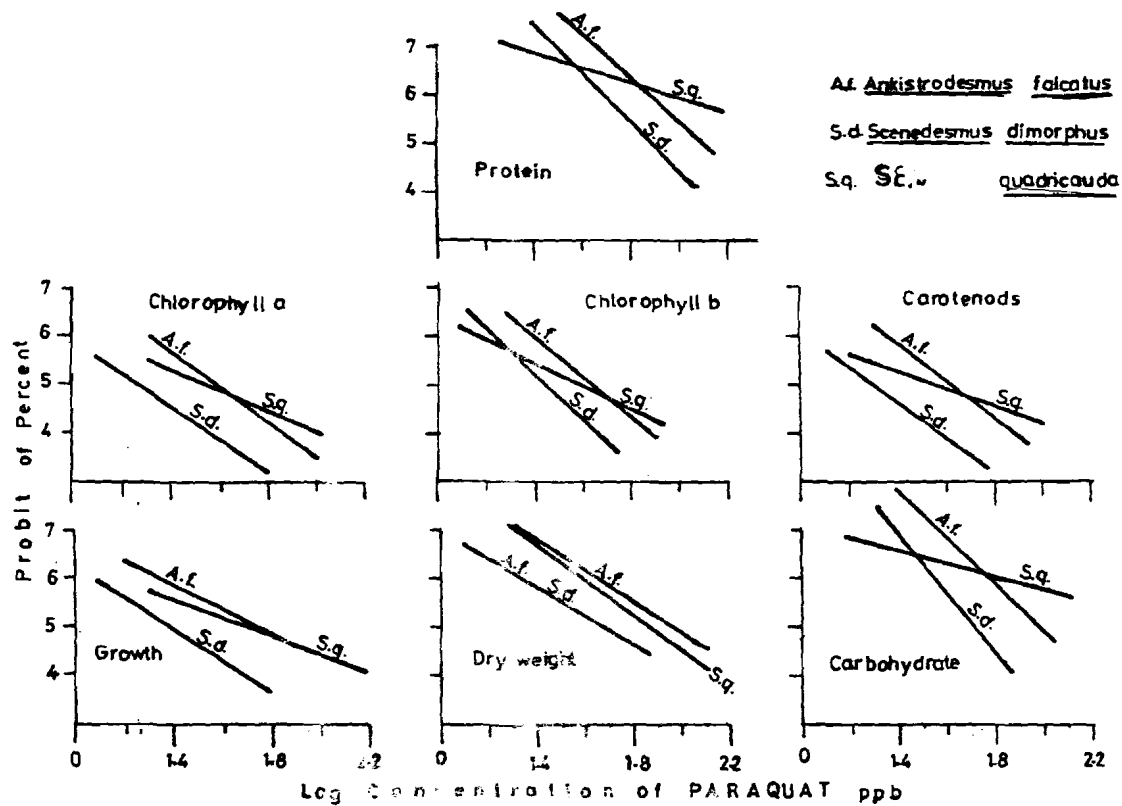
Fig. 1
The regression lines of the responses of the test
parameters to the selected doses of Paraquat.

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	56.8	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, a=12.8,b=-4.78,Log EC50=1.63 a=10.44,b=-3.65,Log EC50=1.49
EC50=29ppb Y=-3.33X+9.68 Y=-4.78X +12.81 EC50=43 ppb Y=-3.65X +10.44, EC50=31 ppb

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	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	64.1	5.4152
	1.8	86±1.2	43.7	4.8414	30 ±0.6	49.2	4.9799	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	15 ±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, a=9.13,b=-2.32,Log EC50=1.78 a=8.02, b=-1.73, Log EC50=1.74
EC50=54.5 Y=-1.99X +8*45 Y=-2.32X +9.13, EC50=60.2ppb Y=-1.73X + 8.02, EC50=54.9 ppb
EC50=54.5 ppb

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	512±5.2	----	----	125±2.6	----	----	278±4.4	----	----
	1.5	443±3.8	86.5	6.1031	114±1.8	91.2	6.3537	219±3.6	88.3	6.2034
	1.8	230±2.4	44.9	4.8718	66±2.2	52.8	5.0702	130±2.8	52.4	5.0602
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 a=12.5,b=-4.1,Log EC50=1.82 a=11.68,b=-3.66,Log EC50=1.83
Y=-3.72X + 11.66. EC50=61.3 ppb Y=-4.1X + 12.5, EC50=66.6ppb Y=-3.66X +11.68, EC50=67 ppb

Table 3

Estimation of the EC50 for Amount 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	45.4±2.2	96.4	6.7991	7.5±0.6	98.2	7.1015	27.2±1.2	97.2	6.9110
	1.4	45.3±2.4	80.3	5.8526	4.6±0.4	60.5	5.2663	18.4±1.4	65.6	5.4016
S. dimorphus	1.9	31 ±2.4	61.8	5.3002	1.4±0.2	18.4	4.0998	7.4±0.6	26.4	4.3489
	1.9	22.5±1.8	44.4	4.8592	0.5±0.04	5.9	3.4368	3.1±0.6	11.1	3.7241
	2.6	16.5±1.8	32.9	4.5570	0.1±0.02	1.4	2.8027	1.4±0.2	5	3.3551

m=18.97, b=-3.99, Log EC50=1.87
Y=3.19X + 18.97, EC50=73.9 ppb

m=15.06, b=-6.11, Log EC50=1.65
Y=6.11X + 15.06, EC50=44.2 ppb

m=13.64, b=-5.17, Log EC50=1.67
Y=-5.17X+13.64, EC50=47 ppb

	Control	42.6±1.4	---	---	8.5±0.4	---	---	26.9±2.2	---	---
	1.3	42.5±1.4	99.7	7.7065	6.2±0.3	73.5	5.6280	18.8±1.4	75.5	5.6403
	1.8	39 ±0.8	91.3	6.3722	5.3±0.4	62.1	3.3081	15.7±1.6	63	5.3310
quadricauda	1.95	32 ±1.2	75.1	3.6776	4.14±0.08	48.7	4.9674	12 ±1.6	48.2	4.9549
	2.08	24 ±0.4	54.3	3.1506	3.7±0.4	44	4.8490	18.5±0.8	42	4.7981
	2.18	17.9±0.4	42.1	4.8007	3.2±0.06	38.3	4.7824	9.5±0.6	38.3	4.7824

m=14.2, b=-4.38, log EC50=2.12
Y=4.52X + 14.2, EC50=132 ppb

m=7.74, b=-1.39, Log EC50=1.97
Y=-1.39X + 7.74, EC50=92.7 ppb

m=4, b=-1.53, Log EC50=1.97
Y=-1.53X + 8, EC50=92.2 ppb

	Control	27.8±1.6	---	---	6.5±0.4	---	---	15.9±1.4	---	---
	1.3	25 ±1.6	91.9	6.3964	6.2±0.6	95	6.7066	14.3±1.2	94.6	6.6072
	1.8	20.2±0.8	76.3	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	2.8±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

m=14.2, b=-3.53, Log EC50=2.06
Y=3.53X + 18.2, EC50=114.2 ppb

m=13.62, b=-4.61, Log EC50=1.87
Y=-4.61X + 13.42, EC50=75 ppb

m=13.5, b=-4.43, Log EC50=1.87
Y=-4.43X + 13.5, EC50=74.5 ppb

213

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 Persoon (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. Landbouw. 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. l-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 alge. Hyacinth Control J., 10: 45-48.

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

AMIN A. SAMAAN, A.F.A. GHOBASHY* AND S.M. ABOUL EZZ.

National Institute of Oceanography and Fisheries
* Faculty of Science, Suez Canal Univ.

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, Mesanthura 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° $30^{/}$ and 31° $10^{/}$ E and latitude 31° $35^{/}$ N. It extends for about 70 Km, with a varying width between 6 and 16 Km and a total area of about 50,000 hectares.

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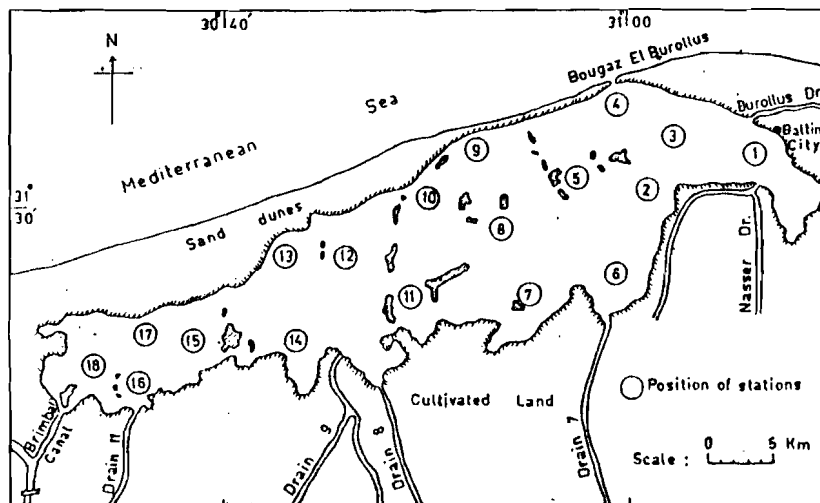
FIG. 1
Morphometry of Lake Burollus and position of stations.

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 littoral 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

218

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.

<div align="center">RESULTS</div>

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 Burollus 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
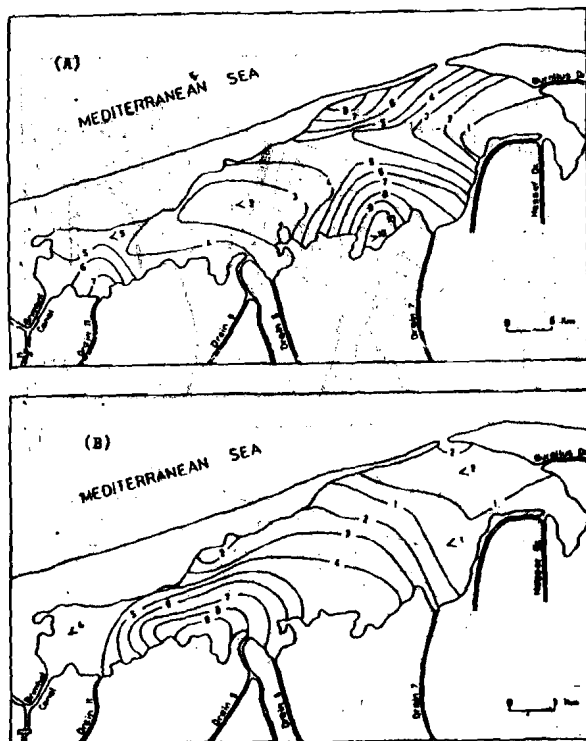
FIG. 2

Horizontal distribution of the total bottom
faunin Lake Burollus (hundred organisms/m²).

(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/m² during 1978 decreased to 6.1 gm fresh wt/m²
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.
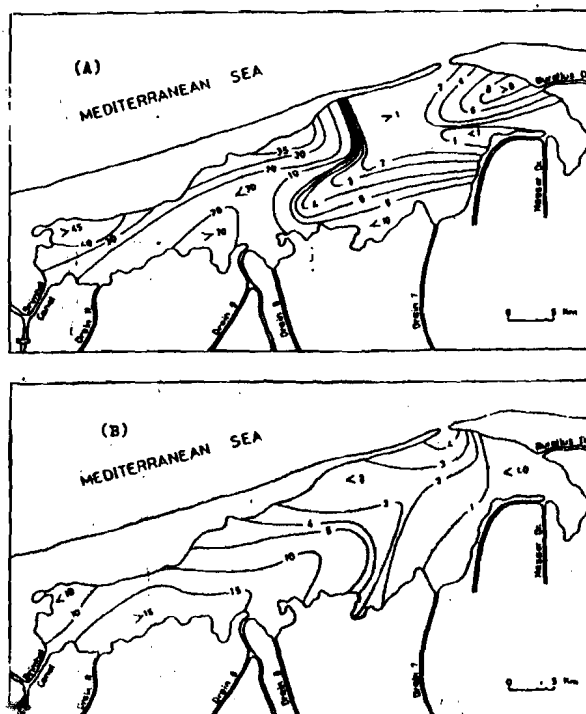
FIG. 3

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.
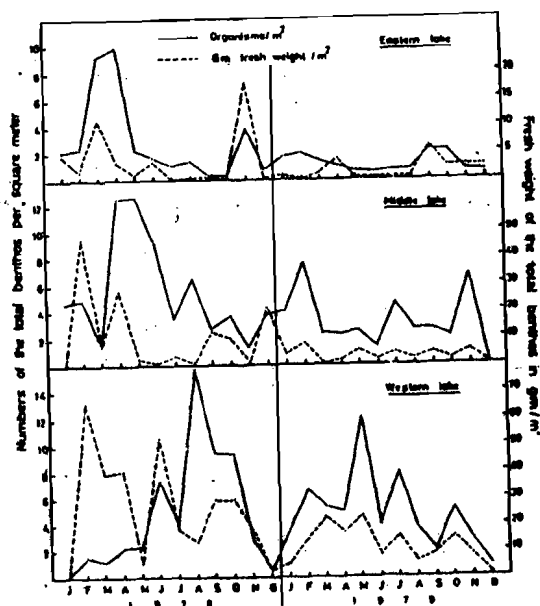
FIG. 4

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.

223

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 111,354 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.

Güerguess, Sh. K., 1979. Ecological study of zooplankton and distribution of macrofauna in Lake Menzalah. 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, K.K. Abdalla, 1988. Distribution of Hydrophytes in Lake Burollus, Egypt. Bull. Inst. Oceanogr. and Fish. ARE, 14(2).

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

Y.A. AZAB MOHAMAD AND M. I. ABDEL-HAMID

Department of Botany, Faculty of Science,
University of Mansoura, Mansoura, Egypt.

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.

227

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 (undautoclaved) 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
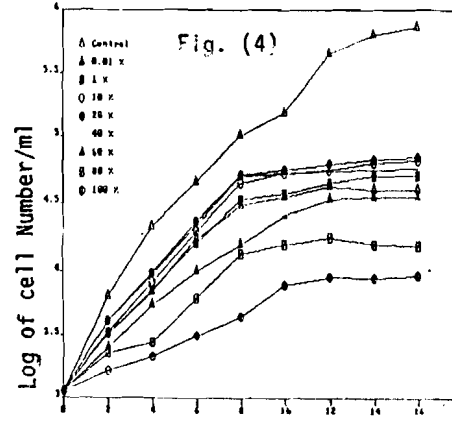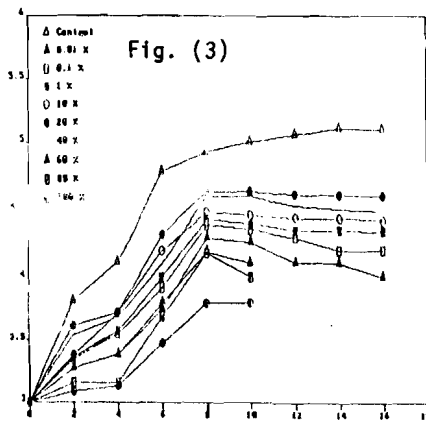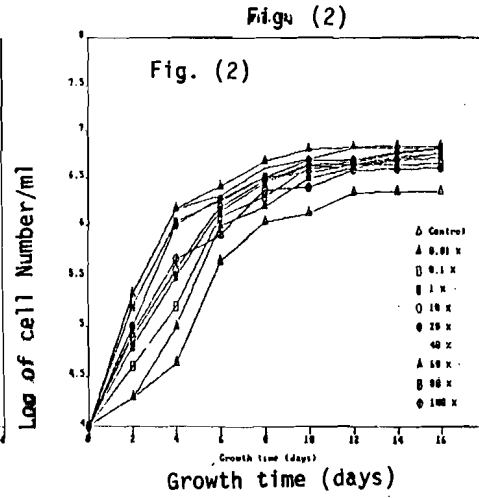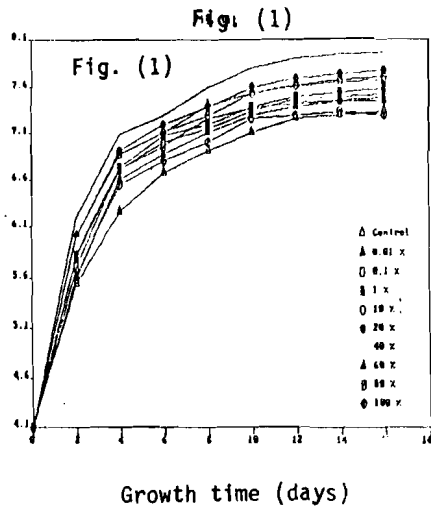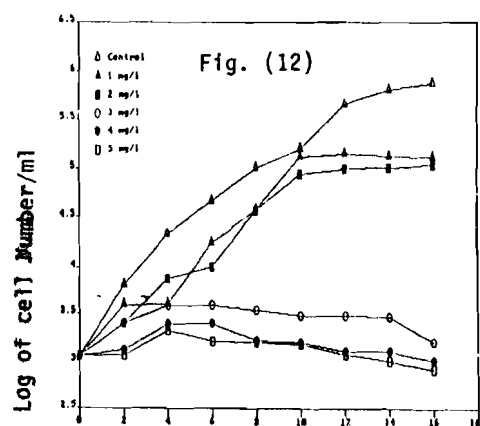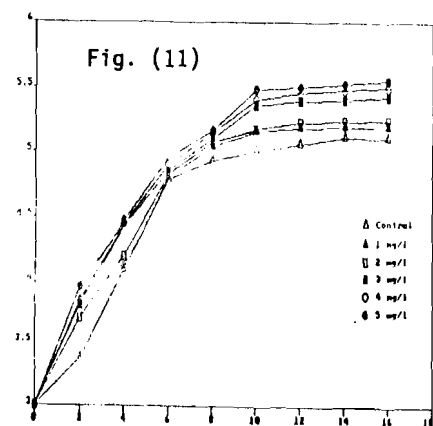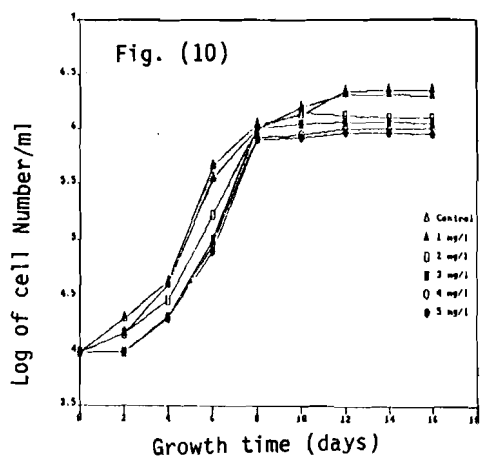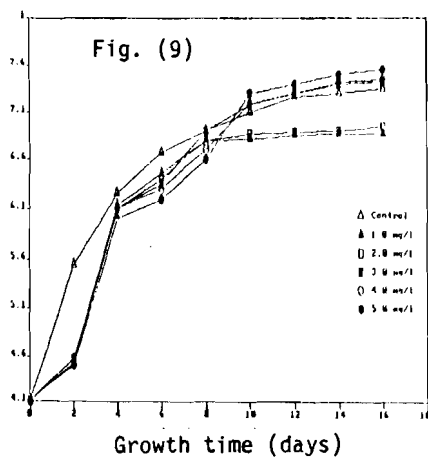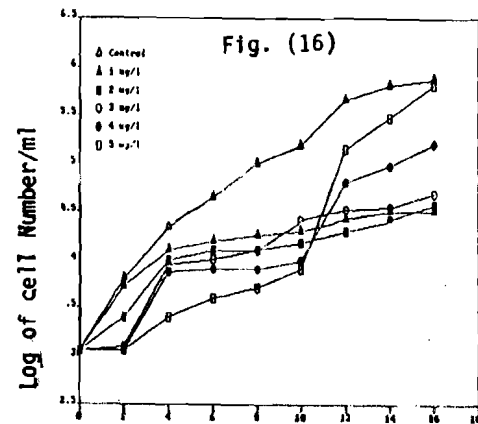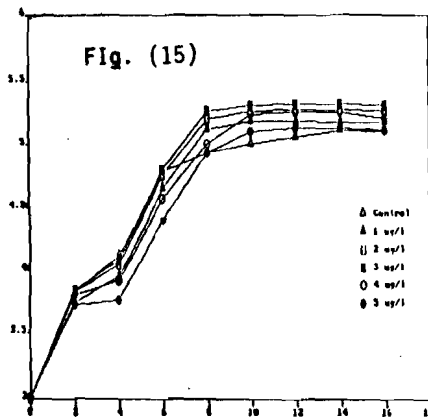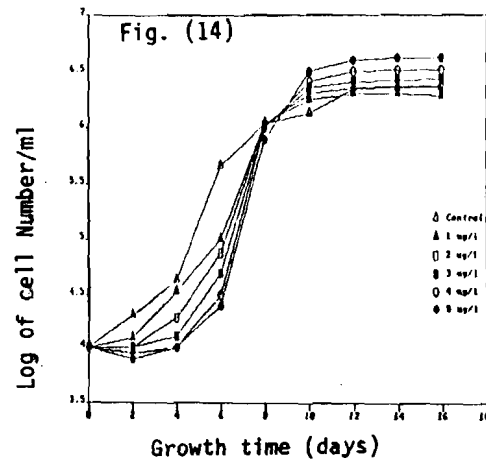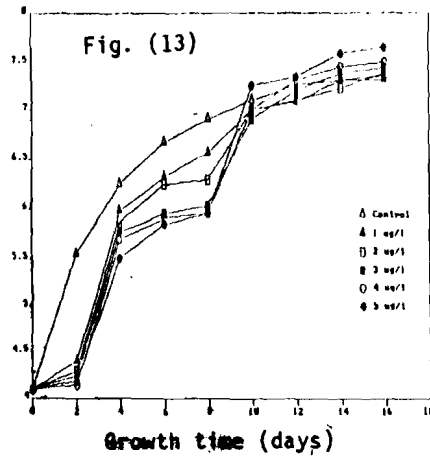
Effect of various concentrations of unautoclaved
(natural) sewage on growth of **Chlorella vulgaris**
(Fig. 1), **Scenedesmus quadricauda** (Fig. 2),
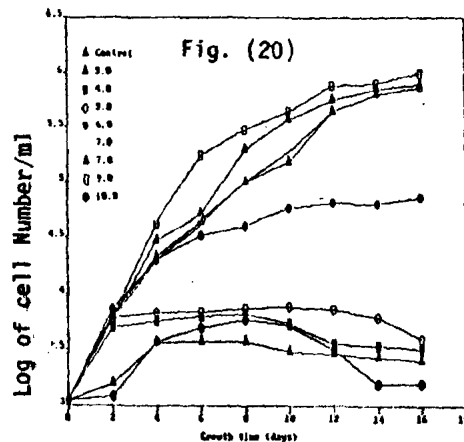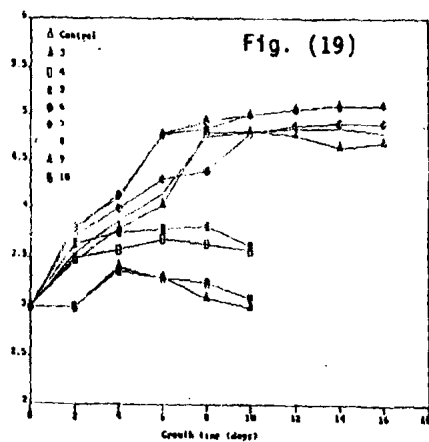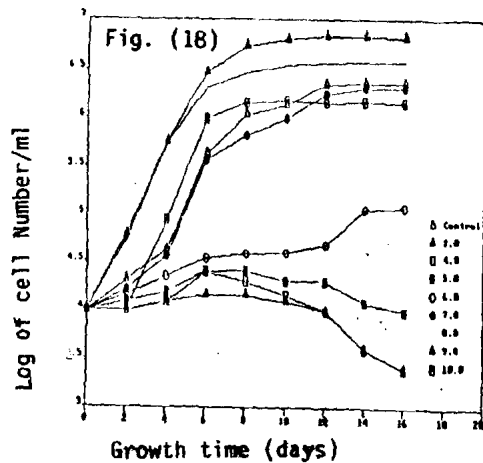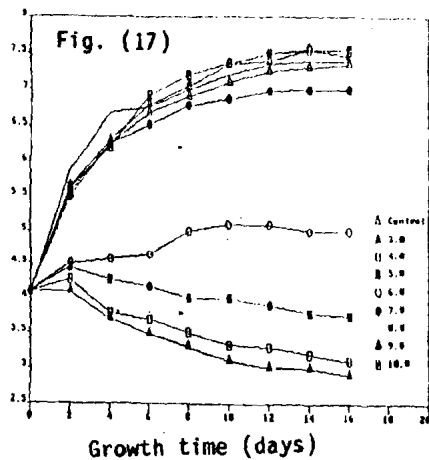**Pandorina morum** (Fig. 3) and **Cosmarium** sp (Fig. 4).
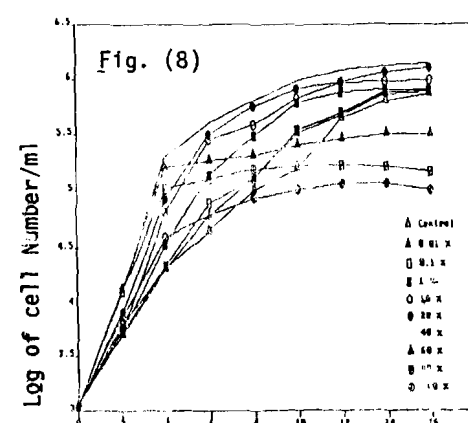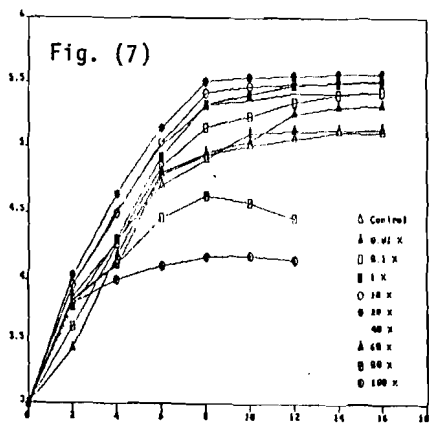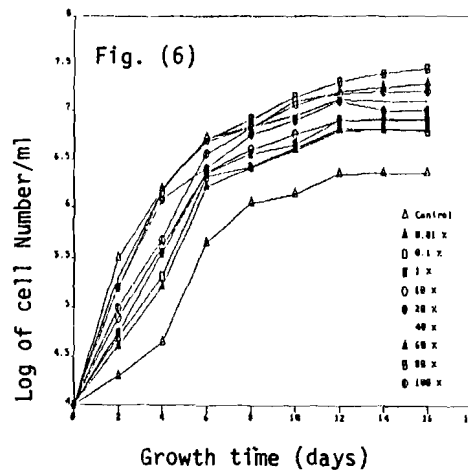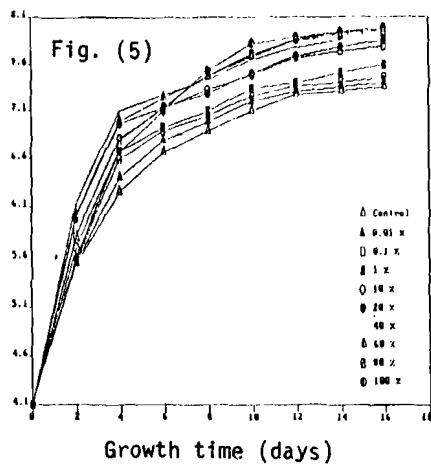
Effect of various concentrations of autoclaved sewage on growth of
Chlorella vulgaris (Fig. 5), Scenedesmus quadricauda (Fig. 6),
Pandorina morum (Fig. 7), and Cosmarium sp (Fig. 8).

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).

231

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).**Pandoring**
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/1) comparable to the natural (unautoclaved) one with only 200 gm PO_4 - P/1. 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/1). 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.

235

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 Scehedesmus 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 very 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 h 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. O. 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: 93-206.

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, 653.

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 for achangsabteilung 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.

Zahren, 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.

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

S.E.M. KHADRE, M.B. SHABANA AND M.M. LOTFY

Department of Zoology, Faculty of Science
Alexandria University, Alexandria, Egypt

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.

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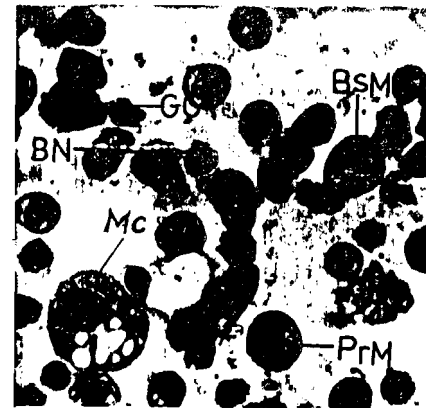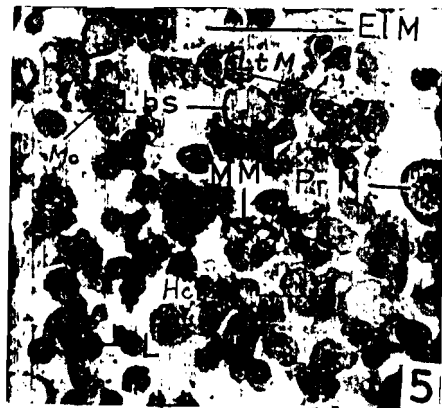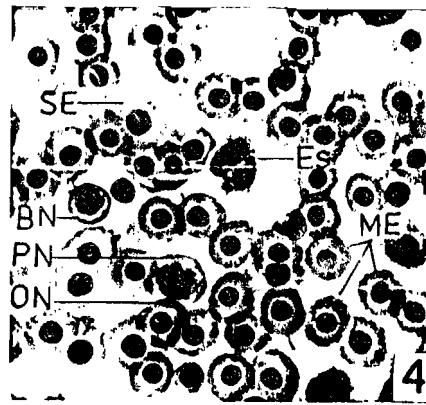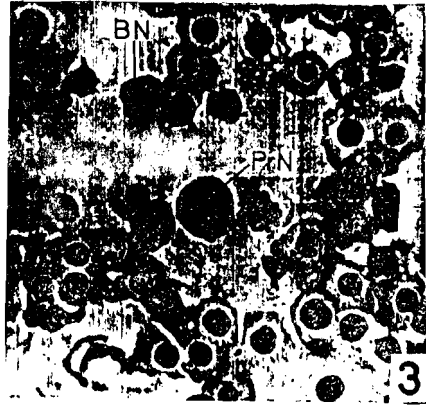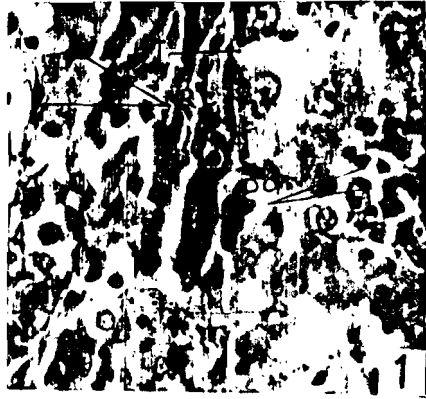
FIG. 1.
Formalin -eosin haematoxylin. L.S. in the head kidney
showing reticular cells (RC) between renal tubules
(UT) and a group of developing cells (DC). X 1250.

FIG. 2.
Kidney imprints showing large haemocytoblast (LHcb) medium
haemocytoblast (MHeb and small haumocytoblast (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 (PH), 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
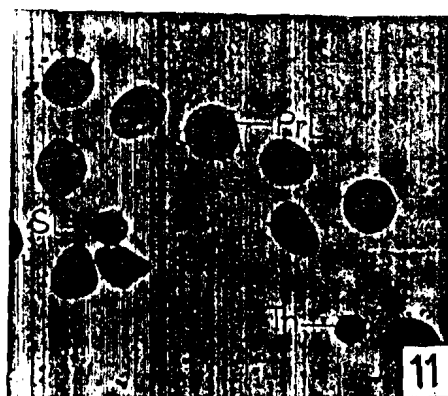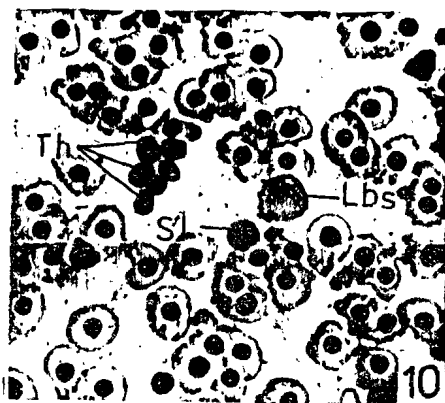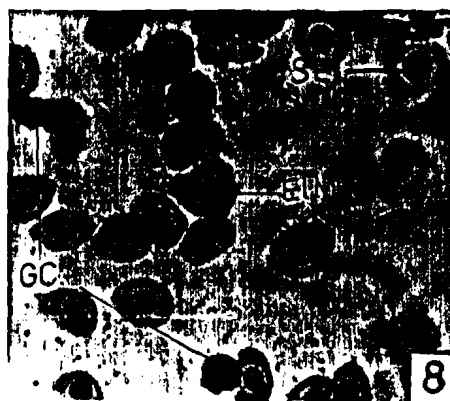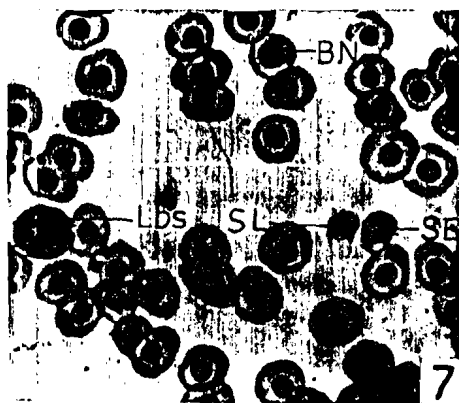(PrM). Notice Ghost cell (GC) and basophilic normoblast (BN).
X 1250.

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
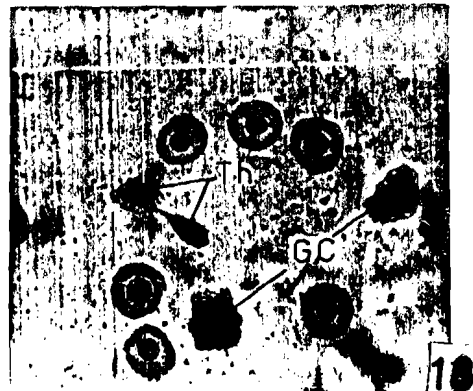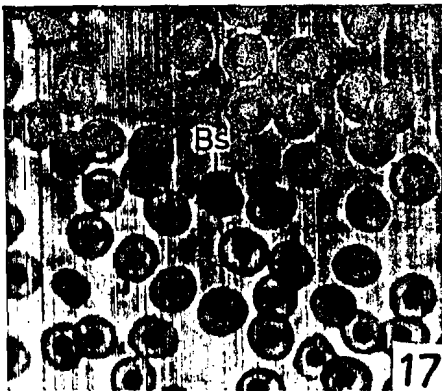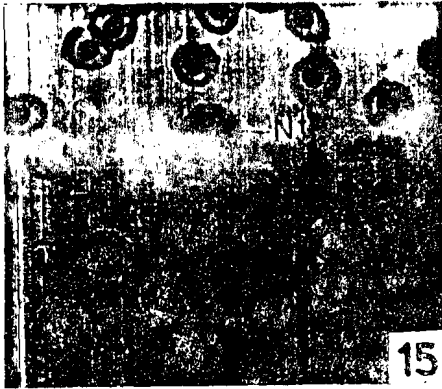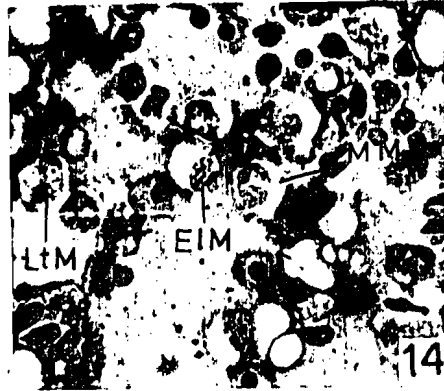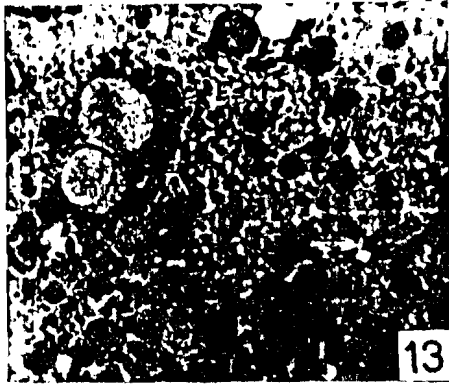erythrocytes (DE). X 1250.

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

FIG. 14.
Kidney imprints-Wright's stain showing metamyelocytes
(Mtr with horse-shoe shaped nucleus (MtM) cells,
dirty myelocyte (ECM) and late myelocyte (LtM).
X 1250.

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

FIG. 16.
Blood smear-Panoptic method showing three mature
neutrophils, two with bilobed nuclei (NtF),
the third with nucleus formed of 4 lobes (FNt).
Notice poikilocytosis of erythrocytes. 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 (Tb).
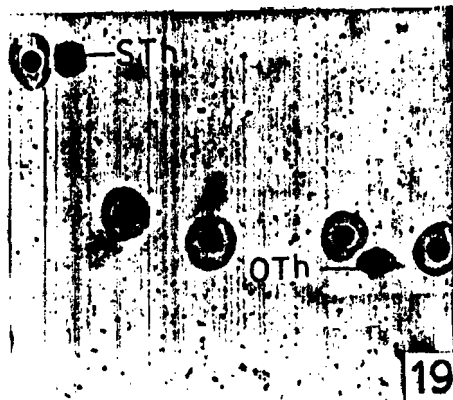Notice ghost cells (GC). X 1250.
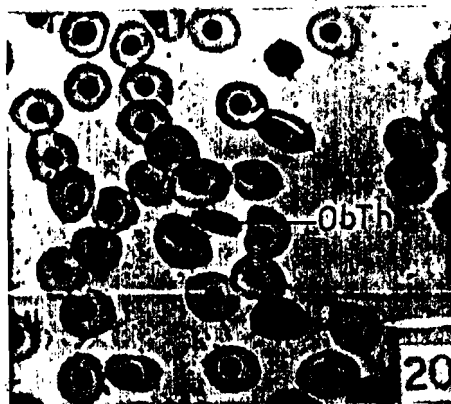
d) Orthochromatophil Normoblast

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 nuolei 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 haemopoi c' 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:

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.; H.H. Mollenhauer; T.E. Eurell; D.H. 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 teleeosts. 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: 587-208.

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 Acanthopagrus 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 taleost. 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.