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Some Haematological Parameters and Blood Picture of *Oreochromis niloticus* in Manzalah Lake, Egypt

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Abstract: *Manzalah Lake is still the largest coastal lake in Egypt; it is situated along the Mediterranean Sea coast between the Damietta branch of River Nile in the west and Suez Canal in the east. It receives daily enormous quantities of industrial and agricultural wastes from different sources of drainage water from Bahr el-Bakar, Hados, Ramses and El Sirew drains. Samples of Oreochromis niloticus were seasonally collected from different areas namely; Ginka, Beshtier and Deshdy. The present work investigates the effect of the environment fluctuations and different pollutants that cause the abnormality in blood picture, hemolysis, deformed and damage of erythrocytes in the peripheral blood and leucocyte cells. The study revealed that Deshdy station was less polluted than the other two studied stations, Beshtier and Ginka.*

***Keywords*:** *Manzalah Lake, Haematology, Haemoglobin, haematocrit, Erythrocyte sedimentation rate, erythrocyte corpuscles, Leucocytes, O. niloticus*

I. Introduction

Lake Manzalah is rectangular in shape with its longer axis (about 65 km) directed from northwest to southeast. Its greatest width is approximately 45 km and has an area of about 700 km2 [1]. It is highly dynamic aquatic ecosystem; there are continuous changes in physical, chemical, hydrological and biological properties as beginning of the 20th Century [2]. Manzalah Lake receives water from different sources, drainage water from Bahr el-Bakar, Hados, Ramses and El Sirew drains. The lake water is generally brackish but range from low salinity in the south 2.03 % to high salinity 16-35% in the outlets, in the north [3].

According to the recent statistics published by the General Authority for Fish Aquatic Resources Development (GAFRD) [4] the maximum catch (63.1%) of tilapia attained in 1997, while the minimum (50.82%) was recorded in 2001.

There are some fluctuations in tilapia catch from year to year. Generally the catch was shown to decrease in the last years. Some studies reveal the effect of different pollutants on fish, in Manzalah Lake, [5-10].

Blaxhall and Daisley [11] examined fish blood by some routine haematological methods. Many reports were published on the toxic effect of some heavy metals on fish growth and haematology, and histological examination by treatment with three toxic metals [12-19].

Four species of tilapia; *Oreochromis niloticus*, *Sarotherodon galilaeus*, *Tilapia zillii* and *Oreochromis aureus*, inhabit Manzalah Lake. The dominant catch is *O.niloticus*.

The present work is destined to determine the potential hazard resulting from the continuous obvious changes in the environment of Manzalah Lake due to the inflow of different pollutants, also due to the poor exchange with seawater. Furthermore, the study also is confined to know the abnormality in blood picture, deformation and damage of erythrocytes in the peripheral blood.

II. Materials And Methods

Three distinguished stations were selected for fish samples collection taking into consideration the effect of Bahr el-Bakar drain on the selected area (Fig.1). The western side, Deshdy station (I) in open area of the lake, Beshtier (II) is in the middle of the eastern side of the lake, where effluent of the drainage and water discharged directly. The third station, Ginka (III) where the lake join the Mediterranean Sea.

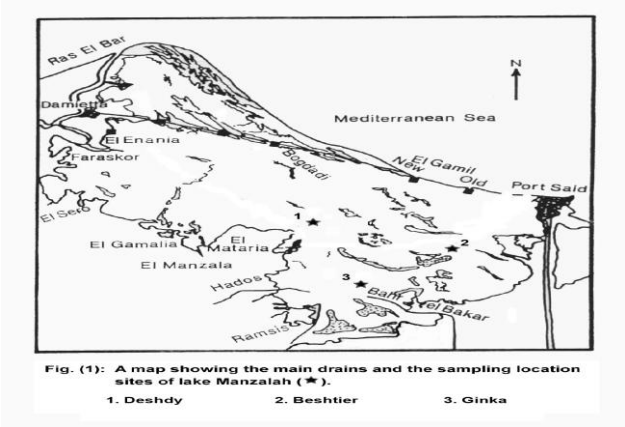

Fig. (1): A map showing the main drains and the sampling location sites of lake Manzalah (★).
1. Deshdy 2. Beshtier 3. Ginka

Samples of *Oreochromis niloticus* were seasonally collected alive during the period from September 1998 to October 1999. About 800, 890 and 678 fish blood samples were seasonally collected for blood estimation. A drop of blood collected by a non-heparinized capillary tube was transferred to make the smear. Heparinzed blood was transferred into acid washed Eppendrof tube (capacity 1.3 ml) for use of routine haematological parameters.

Haemoglobin was determined using the colorimetric method of Wintrobe [20] Haematocrit using Snieszko method [21] Erythrocyte Sedimentation Rate (ESR) was carried out adopting the Wintrobe method [22] and the plasma ions were determined by using atomic absorption apparatus.

Erythrocyte counts were carried out according to Hesser [23] and Leucocytes counts were carried Using Shaw's counting fluid Shaw [24].

The differential leucocytes count is investigated in stained blood films prepared from nonheparinized blood stained with, Giemsa, Leishman, Wright and Periodic acid schift (PAS). The data were analyzed using computer software"Statistica 6", Stat Soft Inc.

III. Results

3.1 Haematological parameters

The response of fish to many external factors is normally followed by certain changes in haematological parameters. These parameters are (haemoglobin concentration (Hb), haematocrit value (Ht), erythrocyte sedimentation rate (ESR), erythrocyte count (RBC_S), leucocyte count (WBCs), mean erythrocyte haemoglobin (MCH) mean erythrocyte volume (MCV) and mean erythrocyte haemoglobin concentration (MCHC %). They were represented and statistically calculated as shown in tables (1 &2) for the four seasons.

There was a significant difference between the estimation of erythrocyte sedimentation rate for Deshdy and Ginka. An obvious significant difference between Deshdy, Beshtier in leucocyte corpuscles count at $P<0.05$.While there was no significant difference between others different parameters in the three stations in winter. In spring, there was a significant difference between Ginka, Beshtier in haematocrit percentage and erythrocyte count at $p<0.05$. There was a significant difference between Deshdy and Ginka in erythrocyte sedimentation rate and leucocyte count at $P<0.05$. While in summer there was a significant difference between Deshdy and Ginka in erythrocyte count at $P<0.05$.In autumn there was a significant difference between Deshdy-Beshtier in haemoglobin concentration and between Ginka and Beshtier in haematocrit % at $p<0.05$. There was a significant difference between Deshdy and Ginka in leucocyte count at $P<0.05$. Deshdy station was less polluted than the other two stations, Beshtier and Ginka.

3.2 The differential count of leucocyte

Agranulocytes behave in a reverse manner to the granulocytes. They decreased in Beshtier when compared with Ginka and Deshdy, lymphopaenia occurs; lymphocytes decrease as concentration of pollutant increase. The maximum percentage of agranulocyte were recorded as 196.4%±3.4, 198.6%±2.3 and 192%±9.4 in Deshdy, Ginka in winter and Beshtier in spring, while the lowest percentage of agranulocytes was 173%±21.9 in Beshtier in summer.

Granulocytes increased as concentration of pollutants increased. The maximum percentage of granulocytes recorded 27.0%±21.9, 26.0%±19.8 in Beshtier during summer and autumn. In Ginka and Deshdy the granulocyte % recorded 20.0%±15.6, 12.6%±9.5 during autumn and spring, respectively. The lower percentage amounted to 3.6%±1.4, 1.4%±0.1 in Deshdy and Ginka during winter and 8.0%±6.4 in Beshtier during spring.

Small lymphocytes were dominant in count during the four seasons, while in Ginka the large lymphocytes percentage was 146.6%±56 in winter. The maximum count of small lymphocyte was

177.2%±12.2, 152.0%±16.9 in Deshdy and Beshtier, respectively during autumn and 127.8%±33.8 in Ginka during spring.

Large lymphocyte recorded percentage was 76.0%±59.9 and 59.2%±30.2 in Beshtier during winter and summer. In Deshdy large lymphocyte amounted to 42.8%±22.5 during spring. Monocyte cell attained 4.0%±1.7 in Beshtier in autumn and 1.4%±0.2, 1.6 %±1.2 in Deshdy in autumn and spring, respectively. Neutrophil percentage was 10% ± 9.3 in Deshdy during spring. While neutrophil percentage was similar in Beshtier during autumn and winter 18.0%±5.2.The minimum percentage was 1.2 % ± 0.8 during winter in Ginka.

The maximum recorded percentage of eosinophil 10.8 % ± 7.4 and 10.0 % ± 8.3 in both Ginka and Beshtier in summer. On the other side, the minimum recorded percentage 0.4 % ±0. 2 during Deshdy in winter. Basophil cells were rare and recorded the maximum percentage in Beshtier. Basophil cells recorded 0.2 %±0.1 in both spring and autumn in Deshdy.

Table (1): The means of length, weight and haematological changes of *O.niloticus* in three stations during winter and spring in Lake Manzalah

Parameters	SUMMER			AUTUMN		
	GINKA	BESHTIER	DESHDY	GINKA	BESHTIER	DESHDY
LENGTH (cm)	18.5± 2.5	19.0 ± 2.7	19.5 ± 02.5	18.8 ± 02.8	16.0 ± 03.9	17.3 ± 03.4
WEIGHT (gm)	142.1 ± 58.2	176.3 ± 67.6	163.3 ± 51.9	148.4 ± 55.4	110.5 ± 41.1	129.9 ± 50.5
HB(g/dl)	5.2 ± 00.7*	4.6 ± 1.9	5.9 ± 0.40*	6.1± 01.9	6.8 ± 02.0	7.9 ± 01.9
HT (%)	52.3± 12.3	39.4 ± 6.8	41.3 ± 8.40	38.2 ± 07.4*	39.9 ± 17.1*	55.8 ± 14.3
ESR (mmh)	0.5 ± 00.1*	1.1 ± 0.1	0.5 ± 0.20*	0.6 ± 00.2*	0.9 ± 00.3	0.6 ± 00.4*
RBC (10^6/MM^3)	1.3 ± 00.3	1.6± 0.3	1.5 ± 0.40	1.5 ± 00.2*	1.6 ± 00.9*	2.1 ± 01.0
WBC (10^3/MM^3)	23.5 ± 7.40	29.8± 3.8*	28.1 ± 7.40*	29.3 ± 05.9*	38.8 ± 12.5	29.8 ± 09.0*
MCH (pg)	39.4 ± 2.30	29.2± 4.8	38.7 ± 1.30	41.1 ± 09.5	42.8 ± 02.2	38.1 ± 03.9
MCV (µ3)	396.4 ± 31.0	250.4 ± 16.0	271.0 ± 25.0	257.4 ± 37.0	251.3 ± 19.0	269.0 ± 19.9
MCHC (%)	9.9 ± 00.1	11.7 ± 00.3	14.3 ± 0.1	15.9 ± 00.3	17.0 ± 00.1	14.2 ± 00.1
N	8	9	8	8	13	9

N = Total mean of samples
* Significant at p<0.05

Table (2): The means of length, weight and haematological changes of *O.niloticus* in three stations during summer and autumn in Lake Manzalah.

Parameters	SUMMER			AUTUMN		
	GINKA	BESHTIER	DESHDY	GINKA	BESHTIER	DESHDY
LENGTH (cm)	17.5 ± 2.5	16.9 ± 2.9	18.5 ± 02.5	21.6 ± 3.6	18.7 ± 03.7	19.5 ± 2.5
WEIGHT (gm)	112.3 ± 44.6	108.2 ± 36.9	120.1 ±39.5	177.4 ± 62.9	182.1 ± 98.1	154.7 ± 59.3
HB(g/dl)	5.7 ± 1.4*	6.4 ± 1.6*	4.6 ± 1.0	5.7 ± 01.2	7.2 ± 1.7*	7.0 ± 1.4*
HT (%)	38.9 ± 10.9	48.1 ± 13.5	30.5 ± 2.7	31.0 ± 11.7*	30.4 ± 8.4*	37.9 ± 5.5
ESR (mmh)	0.8 ± 00.2	0.9 ± 0.3	0.7 ± 0.1	0.7 ± 00.1*	0.7± 00.1*	0.6 ± 00.3
RBC (10^6/MM^3)	1.6 ± 00.5*	2.3± 1.0	1.6 ± 0.3*	1.5 ± 00.3*	1.9 ± 00.7	1.3 ± 0.5*
WBC (10^3/MM^3)	31.4 ± 11.7	32.5±6.9	29.9 ± 2.9	33.6 ± 5.9*	60.1 ± 02.4	35.7 ± 8.1*
MCH (pg)	35.5 ± 2.8	28.4± 1.6	28.1 ± 3.3	39.4 ± 4.0	38.4 ± 02.42	55.1 ± 2.8
MCV (µ3)	242.2 ± 21.8	213.5 ±13.5	185.9 ± 9.0	214.5 ± 39.0	161.9 ± 12.0	298.5 ± 11.0
MCHC (%)	14.7 ± 00.1	13.3 ± 00.1	15.1 ± 0.4	18.4 ± 0.13	23.7 ± 00.2	18.5 ± 00.3
N	8	9	8	9	10	8

N = Total mean of samples
* Significant at p<0.05

3.3 Morphological results of blood cells

3.3.1 Erythrocyte cells

Examination of Giemsa stained uniform blood smears from normal healthy unpolluted fish samples revealed that each erythrocyte is an oval shaped cell with a concentric nucleus with the outer edge of the cell. The cytoplasm stained pale pink and the nucleus deep purplish.

Erythrocyte developmental stages could be seen in blood smears of tilapia. The immature cells were more rounded in shape with greyish-blue cytoplasm and light blue nuclei to violet. These cells are known as normoblast. They have large nuclei more than mature cells, with thick chromatin threads shown in figures (2&3).Observation of erythrocytes in polluted water revealed deviation in both degenerating cells (ghost cells) and senile red cells shown in figure (4). Also Vacuolations in the cytoplasmic zone of the erythrocytes were observed in samples represented in figure (5); such vacuolizations may further aggravate the toxic effect leading finally to death of cells.

The number of circulating immature cells, abnormally shaped cells, and damage of cells exhibited clumped chromatin with increased interchromatic spaces and ragged appearance of cytoplasmic membranes, also increased numbers of poikilocytes took place i.e. Tear- drop shaped erythrocytes and binucleoted cells were represented in figures (5,6&7). Rouleaux formation was observed indicate the abnormal erythrocyte form (Figure 8). These cells were found with large amount in Beshtier and Ginka stations.

Anisocytosis was a predominant feature, whereas a significant variation in shape and size of cells was noticed in fish blood of Ginka and Beshtier samples (Figures 9&10). Parasites were also found in samples collected from Ginka shown in figure (11).

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3.3.2 Leucocyte cells

Leucocytes were of several types differing from one to another in their affinity to dyes .In stained preparations, four main types of cells were observed, one was non granular and three were granular. The agranulocytic type of leucocytes includes lymphocytes which are subdivided into small, large lymphocytes and monocytes.

In figure (13) small and medium sized lymphocyte cells were clear. They are rounded; nucleus occupies most of the cell, leaving a thin rim of clear blue cytoplasm. The chromatin of the nucleus is a compact mass staining dark purple. While the large lymphocyte had a greater amount of cytoplasm and the nucleus tends to be eccentric, occupying about : of the cytoplasm as shown in figures (14&15). Large lymphocyte cytoplasm was more deeply basophilic and abundant than that of small lymphocytes.

Monocyte cells were shown in figure (16) had round or indented eccentric nuclei with an open chromatin pattern. Monocyte cytoplasm was deeply basophilic cytoplasm and often contained clear punctuate cytoplasmic vacuoles.

The granular leucocytes could be divided into three types; neutropil, eosinophil and basophil cells. The first type was the neutrophil, it appeared more or less round, but sometimes may be elongated and have an oval nucleus located close to the edge of the cell as shown in figures (14,15&17). Neutrophil cytoplasm was stippled; gray to lightly basophilic, cytoplasmic borders were irregular.

The second type was eosinophil cells, the nucleus was smaller than that of the neutrophils; it was situated near the edge of the cell membrane they appeared as luminous mass of tiny very bright light bubs (Figure 14,15). Eosinophilic granules ranged from small and few to large and numerous, they occasionally obscured the nucleus.

The third type was distinguished by the basophilic cells they were rounded in shape, in some cases they appeared elongated, and the nucleus was smaller than those of other types, situated close to the edge of the cell. The granules appeared larger in size than those of other granules, stain dark blue with Giemsa and dark purple colour with PAS stain (Figures 14&15).

IV. Discussion

In Manzalah Lake large amounts of drainage water from industrial and urban sewage contribute about 98% of the total annual inflow water to the lake [25] on the other side large quantity of the agricultural drainage water affects the environment of the lake, especially El-Salam Canal project which in turn affected the water budget of the lake. The lake is virtually under high pressure of pollution which affects the physicochemical characteristics of the water and biological components, and furthermore the quality and quantity of fishes.

The lake receives a variety of pollutants from different drains, in particular Beshtier and Ginka areas which represent the extension of Baher El-Bakar drain. This situation took place after the proposed project for the improvement of water quality of this heavily pollutant drain. Deshdy on the other hand is considerably far from the drain's effect.

The hematological and biochemical parameters of fish blood appear as suitable means to indicate the environmental influences, stress effects of anthropogenic origin, condition, health as well as biological manifestations of these aquatic vertebrates. The haematocrit mainly, due to pronounced changes in the structure of the red blood cell morphological and probably physiological damage of erythrocytes showed variations

parallel to haemoglobin data. The fish blood was shown to directly response to different pollutants found in the surrounding habitat.

The difference in location of the three selected areas; Deshdy, Beshtier and Ginka fairly revealed the variation in haemoglobin; erythrocyte corpuscles and leucocyte corpuscles count during the four seasons. Rambhaskar and Rao [26] found that there is an inverse relation between leucocyte numbers and erythrocyte numbers in the ten studied species. Meanwhile, Engel and Davis [27] did not observe any relation in the same species. Such result comes in agreement with the counting of erythrocyte and leucocyte cells of O. niloticus taken from the three stations all over the whole year.

The haematological indices (Hb, Ht, total RBC, MCHC, MCH, and MCV) were used as additional measures to describe the blood oxygen-carrying properties of Rainbow trout acclimated to the dissolved oxygen regimes. The decrease in Ht, Hb and total RBC in hyperoxic fish through acclimation suggests that long-term exposure to hyperoxic conditions resulted in moderate anemia [28]. Such foundations agree with ours in Ginka and Deshdy areas in autumn, summer, respectively.

Hyperoxic and hypoxic fish utilized cellular swelling as denoted by the increase in MCV and normoxic fish responded with cellular as marked by the increase in total red blood cell concentration.

Different haemoglobin levels at different stages of fish development may due to the fish adaptation to different environmental oxygen tensions and acclimatization to different temperatures entails modification in oxygen dissociation characteristics.

The increase of red cell indices like mean erythrocyte haemoglobin MCH and mean erythrocyte haemoglobin concentration MCHC in Deshdy station during autumn reflects the anemic state of fish, a phenomena denoted by Ambrose and Vincent[29].

The noticeable increase in erythrocyte sedimentation rate in Beshtier is shown to be more than in Ginka and Deshdy during the four different seasons which agree with Gautam [30] and Khadre [31] and disagree with Bell *et al*. [32] and James and Sampath [33].

The maximum erythrocyte count was recorded in Beshtier and Deshdy during summer and spring, respectively. Meanwhile the minimum count was recorded in Ginka and Deshdy during winter and autumn.

The increase of immature erythrocyte cells in blood smear of Beshtier fish samples were more than those collected from the other two stations, a result which could be used as indicator of lead pollution in Beshtier. As a verification of these results Sangak [34] stated that, the increase of immature erythrocyte cells in eel blood in the polluted Lake Mariut was more than the immature erythrocyte cells in the less polluted lake Edku and Nozha Hydrodome.

Rouleaux formation as a sign of anemia in Beshtier more than Ginka and Deshdy coincided with Sederak, Faitelson and Jakobsons [35, 36].The extremely obvious increase of total leucocyte count in the collected fish samples is absolutely due to pollutants. The increase in number of small lymphocytes and neutrophil percentage may be the reason of increasing the total leucocyte count percentage. Increasing of large lymphocytes percentages and lower number of small lymphocytes as well as monocytes, when Sarotheroden mossambica exposed to cadmium showed by [37]. The leucocytosis observed in Beshtier station all over the year may due to the increase in total leucocytes count.

Neutropil and eosinophil percentages were highly observed in samples collected from the three stations during four seasons with different amounts. Neutrophilis showed greatest sensitivity to changes in the environment and were the most important of the leucocytes formation.

The deformed erythrocyte corpuscles as anisocytosis or poikilocytosis observed in Beshtier and Ginka more than those appeared in Deshdy, such findings coincide with [38]. On the other side poikitocylosis and damage of cells exhibited clumped chromatin with increased interchromatic spaces and ragged appearance of cytoplasmic membranes.

The observed parasites in fish samples of Beshtier and Deshdy stations may increase the erythrocyte number and decrease of lymphocyte percentage. Such findings were denoted by Martins *et al.* [39].

References

[1]. Zakaria, H.Y., H.A. Mahmoud and F. Roger, Environmental assessment of spatial distribution of zooplankton community in Lake Manzalah. *Acta Adriatica*, 48(2), 2007, 161-172.

[2]. Yacoub, M. A., Study of histopathological changes in the gills of *Oreochromis niloticus* from Lake Manzalah due to water pollution. *Egypt. J. Aquat. Biol. and Fish*. 7 (2), 2003, 71-86.

[3]. Hussien, KH. A., Lake Manzalah Fisheries Development. *Final Scientific Report, ASRT and NIOF*., 1995.

[4]. GAFRD, Statistical reports of the General Authority for Fish Resources Development. A.R.E., 2002.

[5]. Saleh, H.H. and A. Hamza, Study on the health condition of *Tilapia zilli* Gerv. Living in the polluted water of Merghim zone Lake Maruit, Alex. *FAO . Fisheries Rep*. No. 334, 1986.

[6]. Gamal, I.G.M., Biochemistry and mode of action of some insecticides Biochemical interaction between two insecticides and five heavy metals in two types of fish@ *Ph.D. Fac. Agriculture Alexandria, Univ*., 1991.

[7]. Shakweer, L.M.I., Effect of waste disposal on the chemical composition of some aquatic organisms along Alexandria coastal waters. *DH.Faculty of Science. Al-Agar University* (girls), 1993.

[8]. Fishar, M.R.A., Distribution and abundance of benthic assemblages in EL-Gamil basin (Lake Manzalah, Egypt).*Bull. Nat. Inst. of*

Oceanogr. &Fish. (25), 1999, 167-180.
[9]. Anik G., G. C. C. Peter, H. Landis,; Mc. D. Gordon and B.R. Joseph, Influence of lake chemistry and fish age on cadmium, copper and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*). *Can.J.Fish.Aquatic Sci*. /J. *Can.Sci. Halieut.Aquat.*6 (9), 2004, 1702-1716.
[10]. Mohamed, Fatma A.S., Bioaccumulation of Selected Metals and Histopathological Alterations in Tissues of *Oreochromis niloticus* and *Lates niloticus* from Lake Nasser, Egypt. *Global Veterinaria* 2 (4), 2008, 205-218.
[11]. Blaxhall, P.C. and K.W.Daisley, Routine haematological methods for us with fish blood. J. Fish. Biol. 5, 1973, 771-781.
[12]. Srivastava, A.K. and M. Shahikala, Blood dyscrasia in a teleost, (*Colisa fasciatus*) after acute exposure of sublethel concentration of lead. J.Fish Biol., 14, 1979, 199-203.
[13]. Collvin, L., The effect of Copper on growth food consumption and food conversion of perch (*Perch fluviatilis* L.) of feed maximal food rations. *Aquatic Toxicol*. 6, 1985, 105-113.
[14]. Beckman, B. R. and W. S. Zaugg, Copper intoxication in Chinook salmon (*Oncorhynchus tshawytscha*) induced by natural spring glucose. J. Fish. Aquat. Sci., 45, 1988, 1430-1435.
[15]. Cyriac, P.J., A.Antomy and E. Nambisan, Haemoglobin and haematocrit values in the fish *Oreochromis mossambicu* (Peters) after short term exposure to Copper and Mercury. *Bull.Environ.Contam. & Toxicol*. (USA), V43 (2), 1989, 315-320.
[16]. Ezzat, A.A., S.H. Adel Aziz, F. E. EL-Nady and M. Abdel-Barr, Impacts of sublethal concentrations of lead on haematology of *Siganus rivulatus*. *Proceeding of the 8 th Inter.Confer.Environ*. Protection Is a Must.1998.
[17]. Kotsanis, N., J. Lliopoulou-Georgudaki and K. Kapata-Zoumbos, Changes in selected haematological parameters at early stages of the rainbow trout, *Oncorhyncus mykiss*, subjected to metal toxicants: arsenic, cadmium and mercury. *J. Applied Ichthyology*, 16, 2000, 6-276.
[18]. Kruzynski, G.M., A.P. Farrel, K.B. Tierney, S.M. Sanders and I.K. Birtwell, Gill morphometry and haematology of juvenile Chinook salmon chronically exposed to treated (elemental chlorine-free) bleached kraft pulp mill effluent at low temperature to stimulate winter exposure. *Can. J. Zool/ Rev. Can. Zool.* 82(8), 2004, 1187-1193.
[19]. Farrell, A.P., C.J. Kennedy and A. Kolok, Effects of wastewater from an oil-sand-refining operation on survival, haematology, gill histology and swimming of fathead minnows.*Can. J.Zool./Rev.Can. Zool*.82 (9), 2004, 1519-1527.
[20]. Wintrobe, M.M., Clinical haematology.5th ed. Lea and Febiger, *philadelphia*. 1186 p., 1965.
[21]. Snieszko, S.F., Microhaematocrit as a tool in fishery research and Management. *Spec.Sci.Rept.Fish*. 314:15.U.S.*Dept.of Inter. Fish, and wildlife, Serv*., 1960.
[22]. Barnhart, R.A., Effect of certain variables on haematological characteristics of rainbow trout, *Salmo gairdneri* (Richardson).*Trans.Am.Fish. Soc*.98, 1969, 411-418.
[23]. Hesser, E.F., Methods for routine fish haematology. *Prog. Fish Cult*.22, 1960, 16 -171.
[24]. Shaw, A. E., A direct method for counting the leukocytes, thrombocytes and erythrocytes of bird blood. *J. Path. Bract*. 32, 1930, 833-835.
[25]. Khalil, M. and E. Salip, Effect of some water quality parameters on fish composition and productivity in Lake Manzalah, Egypt. *Proc. Zool. Soc. ARE*, 12, 1986, 101-109.
[26]. Rambhaskar, B. and K. Srinivasa Rao, Comparative haematology of ten species of marine fish from Visakapatnam coast. *J. Fish Biol*. 30, 1987, 59-66.
[27]. Engel, D.M. and E.M. Davis, Relation between activity and blood composition in certain marine teleosts.*Copeia*, 1964, 586-587.
[28]. Edsall, D.A. and C.E. Smith, Performance of rainbow trout and Snake River culthroat trout reared in oxygen-saturated water. *Aquaculture*, 90, 1990, 251-259.
[29]. Ambrose, T. and S. Vincent, Chromium induced anaemia in the fresh water carp *Catla catla* (Ham). *J. Nat. Eng. Conserv*, 6 (1), 1994, 67-71.
[30]. Gautam, A.K., Nickel induced haematological anomalies in fresh water teleost, *Channa punctatus Gikoassay*, 5 (2), 1988, 33-34.
[31]. Khadre, S.E.M., Changes in gill structure and blood profile following acute Copper toxicity in two fresh water teleosts. *Proceeding of international Symposium on Biology and Culture of Tilapias, 1990, 27*-31 Alex- Egypt.
[32]. Bell, C.H., J. Davidson and D.A. Smith, The formed elements of blood in "Text Book of Physiology and Biochemistry"p.415 443.*Edinburgh, Churchill Livingstone*, 1972.
[33]. James, R. and K. Sampath, Sublethal effects of mixtures of copper and ammonia on selected biochemical and physiological parameters in the catfish, *Heteropneustes fossiilis* (Bloch). *Bull. Environ. Contam. Toxicol*. 55, 1995, 187-194.
[34]. Sangak, Y.K., Changes in blood picture, digestive and gonadal systems of migrating and non migrating eels when exposed to the effect of industrial pollution in Egyptian lakes. *M. SC.Thesis, High Institute of Public Health, Alex.Univ*. 162p. 1998.
[35]. Sederak, O.M.W., Effect of tanning processing waste water on physiological characteristics of *Solea sp. Ph. Degree in zoology, Fac. Sci. Alex.Univ*., 1998.
[36]. Faitelson, L.A. and E.E. Jakobsons, Aggregation of Erythrocytes into Columnar Structures (Rouleaux) and the Rheology of blood. *J. of Engineering Physics and Thermophysics* 76 (3), 2003, 728-742.
[37]. Ruparelia, SG., Verma, Yogendra, SR. Saiyed and UM. Rawal, Changes in differential white blood cell (WBC) count in fish, *Sarotheroden mossambica* (Peters) under prolonged exposure to cadmium. *Indian.J. Environ Toxico*.1(1), 1991, 59-64.
[38]. Nikinmaa, M., How does environmental pollution affect red cell function in fish? *Aquat.Toxico. (Amsterdam*), 22 (4), 1992, 227-238.
[39]. Martins, ML., Moraes, DM .Miyazaki, CD. Brum, EM. Onaka, J. Fenerick and FR. Bozzo, Alternative treatment for *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) infection in cultivated pacu, *Piaractus mesopotamicus* (Osteichthyes: Characidea) in Barazil and its haematological effects. *Parasite*, 9 (2), 2002, 175-80.

Figures (2 - 7): Blood film of *Oreochromis niloticus* showing erythrocyte and leucocyte cells.

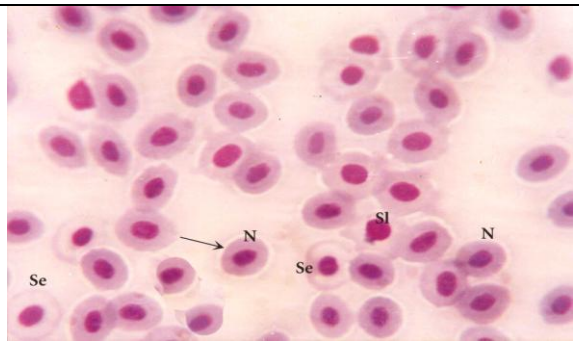

Fig.2: Normoblast (N), small lymphocyte (Sl) senile cell (Se) Giemsa. (100X).

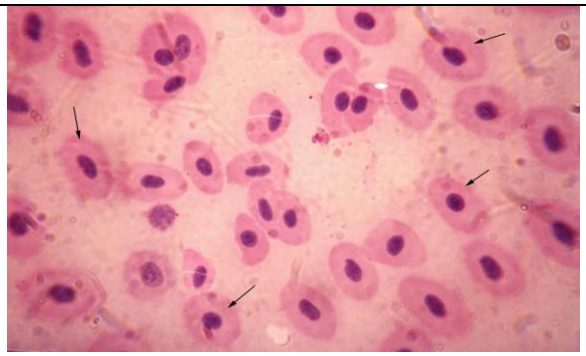

Fig.3: Development of erythrocyte cells, senile cell and anisytosis of cell (arrows) Giemsa. (100X).

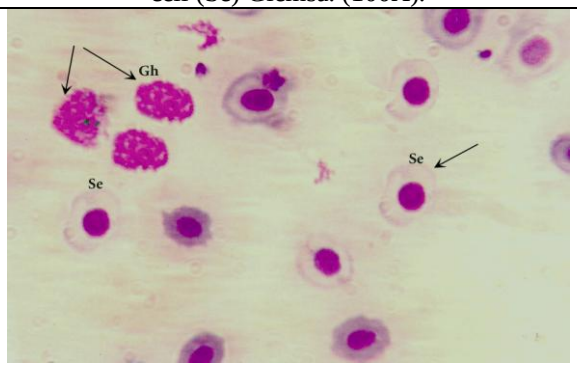

Fig.4: Senile cells (Se) and ghost cells (Gh) (arrows) Giemsa. (100X).

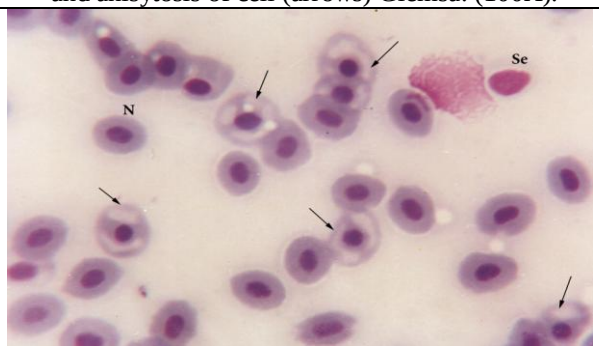

Fig.5: Abnormal erythrocyte with ragged vacuolated cytoplasm appearance and senile erythrocyte (Se) Giemsa. (100X).

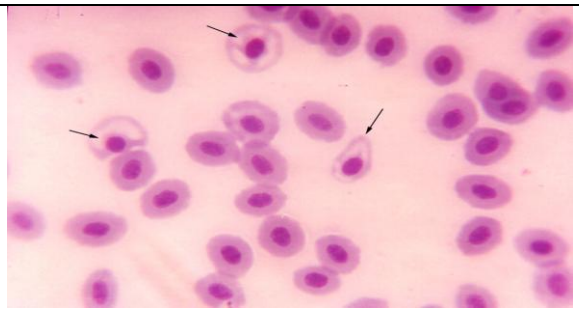

Fig.6: Abnormal erythrocyte cells Tear-shaped, dead cells (arrows) Wright &Giemsa. (100X).

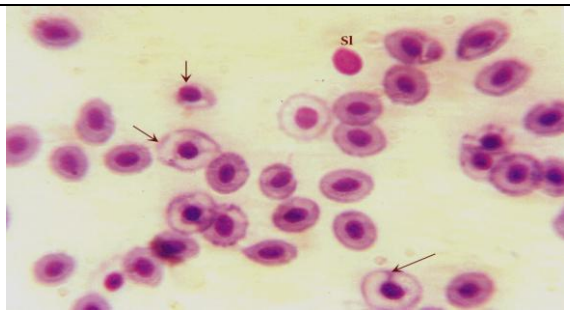

Fig.7: Abnormal erythrocyte cells, senile cells (Se) and small lymphocyte (Sl) (arrows) Giemsa. (100X).

Figures (8 - 13): Blood film of *Oreochromis niloticus* showing erythrocyte and leucocyte cells.

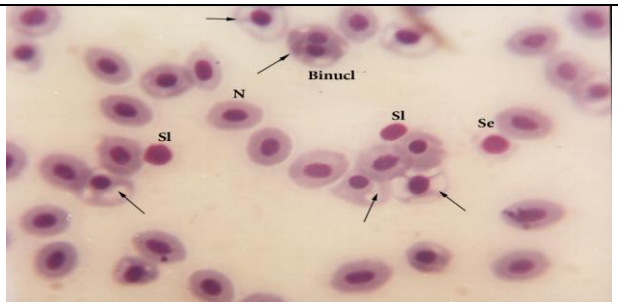

Fig.8: Binucleoted cell, small lymphocyte (Sl) and senile cell (Se) (arrows) Giemsa.(100X).

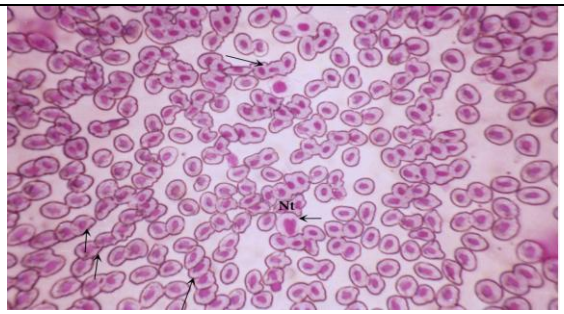

Fig.9: Rouleaux formation of erythrocyte cells, deformed cells and neutrophil cell (arrows) Leishman. (40X).

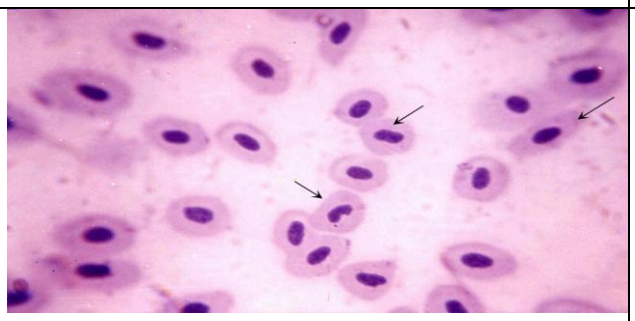

Fig.10: Anisocytosis of erythrocyte cells (arrows) Wright&Giemsa. (100X).

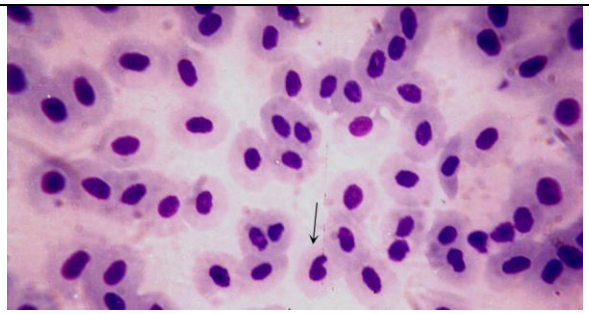

Fig.11: Lobopodial shape of erythrocyte, light stain patches of cytoplasm (arrows) Giemsa. (100X).

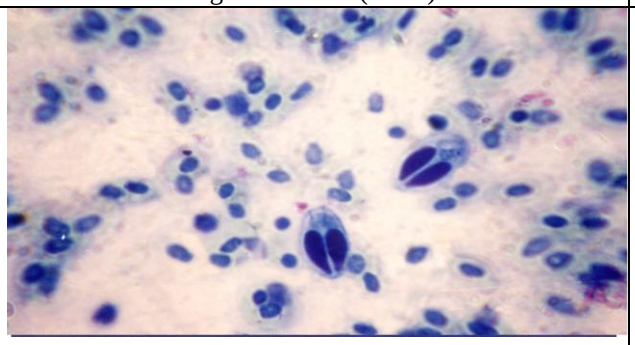

Fig.12: Parasite and deformed erythrocyte cells Wright stain. (100X).

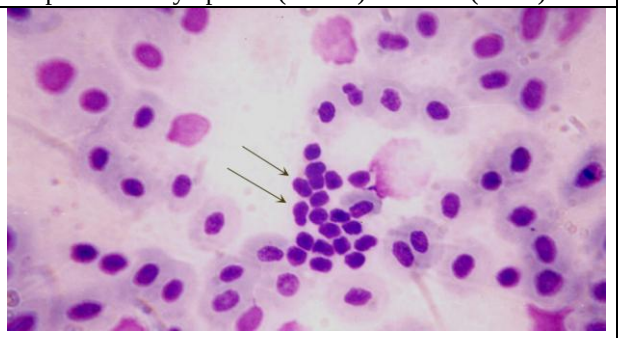

Fig.13: Dead cells, small and medium sized lymphocyte cells (arrows) Wright &Giemsa. (100X).

Figures (14 - 17): Blood film of *Oreochromis niloticus* showing erythrocyte and leucocyte cells.

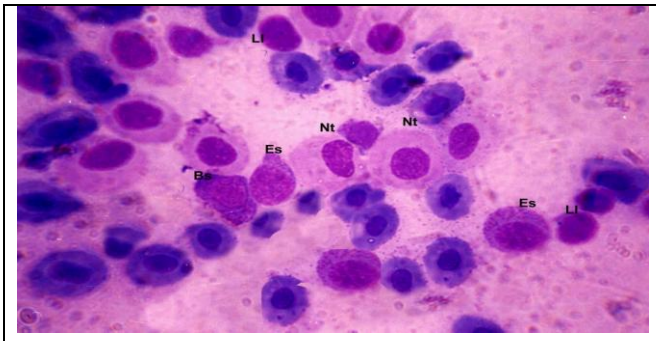

Fig.14: Large lymphocyte cell (Ll), neutrophil (Nt), eosinophil (Es) and basophil cell (Bs) PAS stain. (100X).

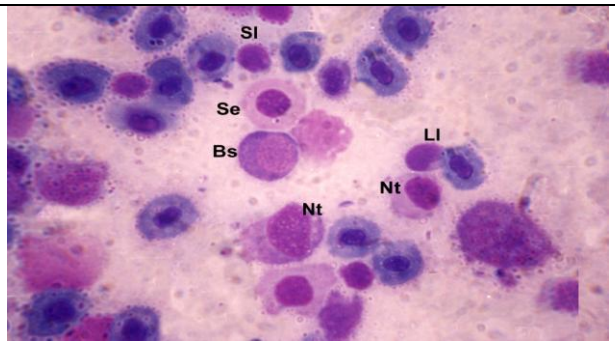

Fig.15: Different stages of neutrophil cells (Nt), large and small lymphocyte, basophil cell (Bs) PAS stain. (100X).

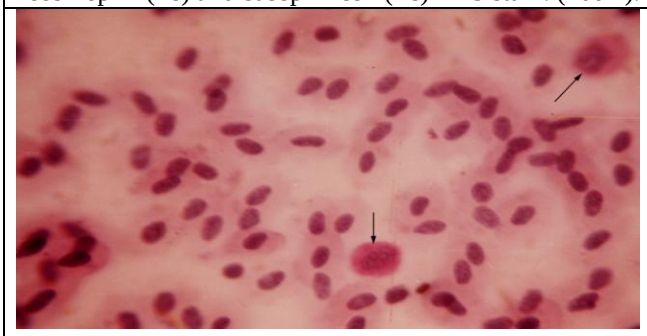

Fig.16: Monocyte cells (arrows) Leishman. (100X).

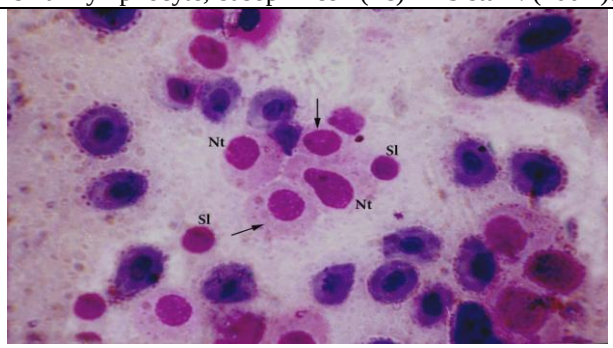

Fig.17: Neutrophil cells (Nt) with variant nucleus shape and small lymphocyte cell (arrows). (100X).