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Effect of heavy metals pollution on some hematological parametersand morphology of red blood cells in *Oreochromis niloticus* (L,) in Lake Maryut

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Abstract

Fishes were collected from three sites, Location (1) East basin (Fish Farm), Location (2) Main basin and location (3) South East basin. Locations 2 and 3 had the highest levels of BOD, COD, turbidity, total hardness and alkalinity. pH values tend to be acidic in both locations 2 and 3 and tend to be alkaline in the reference site (location 1). Highest levels of cadmium, chromium, nickel and lead were found in both lcations 2 and 3. The highest levels of Mn were found in location 1 (fish farm). The number of red blood cells, hemoglobin, hematocrit and MCV decreased significantly in fishes of both locations 2 and 3 compared to fish of control site. Levels of MCH and MCHC concentrations increased significantly in fish of both locations 2 and 3 compared to fish of location 1.

Levels of alanine transaminase (ALT), aspatate transaminase (AST) and creatine phosphokinase (CPK) increased significantly in plasma of *O. niloticus* of both locations 2 and 3 compared to fish of location 1., which may be due to heavy pollution of these two locations.

Keywords: Lake maryut,Oreochromisniloticus, heavy metals, pollution, hematological parameters

Introduction

Lake Mariut (Mariout, Maryut, Mareotis) is a 90-150 cm deep brackish water lake located in the north of Egypt southeast to the Alexandria City, belonging to the Nile river Delta system, and one of the most heavily populated urban areas in Egypt and in the world. In past times, during the Greco-Roman period, Lake Mariut was a pleasure resort and watering spot surrounded by market gardens covering an approximate area of 700 km^2. During antiquity, the lake was fed by the Nile through the canals, but since then the water level has continuously declined, leaving the eastern part of the lake dried up, which is now cultivated land. During the twelfth century, the Canopic mouth of the Nile silted up, blocking the flow of fresh water into the lake, making it unnavigable. As a consequence, Alexandria was cut off from the entire river system of Egypt and was unable to trade as easily as before, and this resulted in the cities decline for many years to come.

Lake Mariut

By the end of the 19th Century, the development of irrigation systems of the adjacent fields made of Lake Mariut an intermediate water body to receive the excess of water from the irrigation channels. Then the water was pumped out to the Alexandria Bay. Nowadays the lake occupies around 250 km^2 due to intense land reclamation for urban and agricultural purposes.

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Life of Lake inhabitants continues to be carried on in much the same way as in ancient times, but now the reed masses covers more than 50% of the lake and fisheries richness has declined both in volume of catches and in fish quality.Lake Mariut is the smallest of these lakes, and the most polluted one.It has suffered over the years from the untreated sewage, agricultural and industrial wastes dumped into it (Amr*et al.*, 2005).Recent studies showed that Egyptian Delta lakes especially lake Mariut are heavily polluted and fishing in these lakes may have some risk (Amr*etal.*, 2005; Adham*et al.*, 2001).Metal contamination of the aquatic environment may lead to deleterious effects from localized inputs which may be acutely or chronically toxic leading to eutrophication. Eutrophication causes increased growth of aquatic plants especially algae that have detrimental effects on aquatic life (Adham, 2002).Lake Mariut is highly polluted with different heavy metals such as iron, copper lead andzinc.It has been changed from a productive lake to a heavily polluted and highly eutrophicated (Hamza, 1999).

Study Area

The Lake is artificially subdivided into four basins (Figure 1); Main Basin, Aquaculture Basin, South West Basin, and South East Basin: Main Basin: 25 km^2 (6,000 feddans), Fisheries Basin: 4.2 km^2 (1,000 feddans), Northwest Basin: 12.5 km^2 (3,000 feddans), Southwest Basin: 31.5 km^2 (7,000 feddans**).**

Lake Mariut is an important fishing lake at the southern area of Alexandria city, Egypt. Main basin (MB), which is lake proper. East basin (EB), which also known as fish farm. It extends 6 km beside the desert road with an average depth of 130 cm. It receives most of its water from New Mariut Hydraulic pumps and Umum Drain. The fish farm connects with Qualaa Drain by a movable gate and at its northern extremity. The main Basin or Lake proper is about 600 acres. Its area has been reduced greatly due to the recent activities of industrial projects on the lake. It is boardered by high ways from three sides and by Nubarriya Canal and Umoum Drain at the west (Samaan and Abdel-Moneim, 1986).

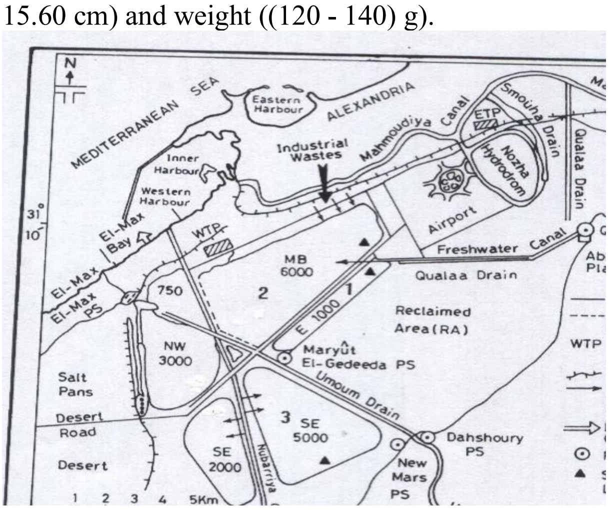
Main Basin receives water flow from three main sources of pollution, the first is Qualla Drain which carries agricultural wastewater, sewage discharge and industrial effluents to the basin. The second is the West treatment Plant (WTP), while the third one Umoum Drain (El-Rayis, 2005).According to El-Rayis and El-Sabrouti (1998), the surplus waterfrom the lake proper is allowed to flow into the lower reach of Umoum Drain before pumping the mixed waters to the MediteraneanSea at El-Max. This pumping process maintains surface water level in the lake at about 2.5m below sea level (Abdel-Moneim, and Abdel-Mohsen, 2010).South west basin is partially contaminated with mineral oils is discharged by the cooling of pipes of El-Nasr Petroleum Company (Abdelmeguid*et al.*, 2002). Fish and water were sampled at locations 1, 2, 3, along the E basin (reference site, MB andSW basins, respectively (Fig. 1).

Material and Methods

Sampling procedures

Water sampling

Water was sampled for heavy metals during months (June, July, August and September, 2010). Each water sample (2...3 L) represented the mean of surface and bottom water. Surface water samples were collected about 20 cm below surface to avoid floating matter. Stoppered, acid-washed, polyethylene bottles were used for sampling. Water samples were filtered in the field using a polypropylene syringe fitted with a 0.45 µm Millipore cellulose acetate filter and acidified for preservation. A total approximate number of 54 individuals of adult *Oreochromisniloticus* (L.) were caught at random from the selected locations Fig. (1). All fish used were of uniform size ((15-15.60 cm) and weight ((120 - 140) g).



Fish sampling

Fish were collected in closed meshed nets before being transferred into large vessels filled up with aerated lake water. In laboratory, fish were allocated into groups (9 individuals per aquarium) and placed in two thirds-filled glass aquaria (80 cm × 40 cm × 40 cm), according to the specific sampling location. Aquaria were supplied by a freshwater system equipped with physical and biological filters and aeration was monitored continuously. These conditions guaranteed quick fish recovery after catch. To avoid handling stress reactions, fish were slightly anesthetized.

Blood collection

Blood was rapidly drawn from the caudal vessel using untreated sterile plastic syringes fitted with 21-gauge needles (Hrubec et al., 1997). For serum preparation, blood was allowed to clot on ice for 1 h. Serum was separated from whole blood by centrifugation at 14 000 g for 5 min. Blood samples from 3 fish were pooled to give one composite specimen. 9 composite specimens for each type of measurement were analyzed for statistical analysis. 1 ml of each boodsample was mixed with anticoagulant for estimation of blood parameters. Total erythrocyte count, Hematocrit value (Ht), white blood cells and platelets (Schalm*et al.*, 1975). Mean Carpuscular Volume (MCV), Mean Carpuscular Hemoglobin (MCH), Mean Carpuscular Hemoglobin Concentation (MCHC) (Hrubec*et al.*, 2000). Blood smear preparation and staining was made according to Houwen (2000) by Leishman stain to be evaluated under light microscope later. One ml of blood was left to coagulate, centrifuged and serum was collected for determination of enzymes alanine transaminase (ALT), aspartate transaminase (AST) and creatinephsophokinase (CPK). Activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of Reitman and Frankel (1975).Creatinephosphkinase was determined according to Oliver (1955).

Analysis of lake water

Chemical determinations in lake water were conducted at three random intervals in each sampling period; one of them was concurrent with fish sampling. Concentrations of heavy metals were measured in filtered lake water by graphite furnace atomic absorption spectroscopy (Perkin-Elmer model 2380) under the recommended conditions and the detection limits (DL) in the manual for each metal. pH, turbidity, DO, COD, BOD, hardness, alkalinity, chlorides, and nutrients (PO4 3–-P, NH3 +-N, NO3– -N, NO2 – -N) were measured by standard methods for the analysis of natural and treated wastewater as described by the American Public Health Association (APHA, 1992).

Statistical analysis

Statistical analysis was carried out by Minitab software statistics. Significance was assessed using two samples T-test analysis. $P<0.05$ is considered significant (Paulson[18], 2008).

Table 1: Physicochemical characteristics of water samples from Lake Maryut (2010)

Locations / Parameter	Fish Farm (control)	Main Basin	South East Basin
PH	$8.3^a \pm 0.08$	$7.4^b \pm 0.82$	$7.35^b \pm 0.03$
Turbidity (NTU)	$18^a \pm 0.17$	$55.5^c \pm 1.8$	$30.5^b \pm 1.06$
DO (mg/L)	$6.1^a \pm 0.07$	$7.75^b \pm 0.18$	$7.55^b \pm 0.2$
BOD (mg/L)	$42.5^a \pm 1.8$	$62^b \pm 1.4$	$59^b \pm 9.2$
COD (mg/L)	$80^a \pm 7.1$	$119^c \pm 0.7$	$108^b \pm 8.5$
Total hardness (mg/L)	$730^a \pm 10.6$	$970^b \pm 21.2$	$890^b \pm 70.7$
Alkalinity (mg/L)	$550^a \pm 35.4$	$900^c \pm 84.8$	$652^b \pm 38.8$
Phosphates (pO_4-P)	0.27 ± 0.01	$1.08^c \pm 0.58$	$1.52^b \pm 0.96$
Nitrates (NO_3-N)	$0.11^a \pm 0.01$	$0.30^c \pm 0.12$	$0.22^b \pm 0.07$

All values are expressed as ppm while turbidity is expressed as NTU (Normal turbidity unit). Data presented

Are means ± SE (standard error). Different superscripts differ ($P<0.05$), while similar superscripts differ

insignificantly. Number of observations in each mean = 3.

Results and Discussion

Physicochemical characteristics

Table 1 shows that locations 2 and 3 had the highest physicochemical characteristics of water eg. turbidity, DO, BOD, COD, total hardness, alkalinity, phosphates and nitrates. pH values tend to be acidic in both locations 2 and 3 and tend to be alkaline in the reference site (location 1). Locations 2 and 3 had the highest levels of BOD, COD, turbidity, total hardness and alkalinity (Table 1).

Heavy metals

Table 2 shows the concentration of heavy metals. Highest levels of cadmium, chromium, nickel and lead were found in both lcations 2 and 3. The highest levels of Mn were found in location 1 (fish farm).

Table 2: Concentration of heavy metals in water of Lake Maryutexpressed as ppm

Element	Fish Farm(F.F.)	Main Basin (M.B.)	South West Basin (S.E.B)	USEPA permissible limit (mg/L)
Cd	0.006^{a} ± 0.003	0.023^{b} ± 0.006	0.022^{b} ± 0.006	0.01
Cr	0.020^{a} ± 0.012	0.14^{b} ± 0.016	0.12^{b} ± 0.04	0.01
Mn	0.044^{a} ± 0.03	0.35^{b} ± 0.11	0.33^{b} ± 0.1	0.05
Ni	0.05^{a} ± 0.005	0.492^{c} ± 0.12	0.143^{b} ± 0.03	0.05
Pb	0.012^{a} ± 0.007	0.11 ± 0.03^{b}	0.12^{b} ± 0.03	0.05

Data presented are means ± SE (standard error). Different superscripts differ significantly ($P<0.05$), while similar superscripts differ insignificantly. Number of observations in each mean = 3.

Hematological parameters

Table 3 shows the number of red blood cells, hemoglobin, hematocrit and MCV,their levels decreased significantly in fishes of both locations 2 and 3 compared to fish of control site. Levels of MCH and MCHC concentrations increased significantly in fish of both locations 2 and 3 compared to fish of location 1.Table 4 shows the number of white blood cells, neutrophils and thrombocytes, their levels decreased significantly in fish of both locations 2and 3 compared to fish of reference site (location 1).

Table 3: Blood parameters of *O. niloticus* in Lake Maryut

Locations	Hb (g/dl) Mean ± SE	Ht (%) Mean ± SE	MCV (fl) Mean ± SE	MCH (pg) Mean ± SE	MCHC (g/dl) Mean ± SE
FishFarm	7.2^{a} ± 0.107	6.3^{a} ± 0.196	50.6^{a} ± 1.71	21.1^{a} ± 0.501	22.9^{a} ± 0.81
Main Basin	5.4^{b} ± 0.193	4.4^{b} ± 0.168	72.2^{b} ± 2.55	29.7^{b} ± 0.815	30.1^{b} ± 1.5
South East Basin	5.2^{b} ± 0.097	4.3^{b} ± 0.21	70.6^{b} ± 2.05	30.5^{b} ± 0.919	32.7^{b} ± 0.83

Hemoglobin (Hb), hematocrit (Ht), Mean capuscular volume (MCV), Mean carpuscular hemoglobin (MCH) and Mean capuscular hemoglobin concentration (MCHC). Values are means ± SE (standard error). Different superscripts differ significantly ($P<0.05$), while similar superscripts differ insignificantly from the control. Number of observations in each mean = 5.

Table 4: Blood parameters of *O. niloticus* in Lake maryut

Locations	RBCs Mean ± SE	WBCs Mean ± SE	Thrombocytes% Mean ± SE	Neutrophils% Mean ± SE
FishFarm	5^a ± 0.15	10.5^a ± 0.19	4.7^a ± 0.19	4.1 ± 0.19^a
Main Basin	3.1^b ± .07	12.9^b ± 0.13	8.2^b ± 0.13	6.3 ± 0.13^b
South EastBasin	3.2^b ± 0.13	13.5^b ± 0.14	9.0^b ± 0.14	6.6 ± 0.14^b

Red blood cells (RBCs× 10^6/µL), white blood cells (WBCs× 10^3/µL), Thrombocytes and Neutrophils. Values are means ± SE (standard error). Different superscripts differ significantly (P<0.05), while similar superscripts differ insignificantly from the control. Number of observations in each mean = 5.

Enzymes

Levels of alanine transaminase (ALT), aspatate transaminase (AST) and creatine phosphokinase (CPK) increased significantly in plasma of *O. niloticus* of both locations 2 and 3 compared to fish of location 1 (the reference site) Table (5).

Table 5: Levels of aspartate transaminase (AST), alanine transaminase (ALT) and creatine phosphokinase (CPK) in plasma of *O. niloticus* in Lake Maryut

Locations	AST (U/L) Mean ± SE	ALT (U/L) Mean ± SE	CPK(U/L Mean ± SE
Fish Farm	91.6^a ± 2.43	22.9^a ± 0.81	1.3^a ± 0.12
Main Basin	140^b ± 2.71	30.1^b ± 1.52	4.7^b ± 0.20
South East Basin	139^b ± 2.83	32.7^b ± 0.83	4.6^b ± 0.22

Values are means ± SE (standard error). Different superscripts differ significantly (P<0.05), while similar superscripts differ insignificantly from the control. Number of observations in each mean = 5.

Hematological parameters

The results of the present study about heavy metals were in agreement with results of Rostom*et al.* (2017). The results revealed that Cr, Mn, Co and Pb metals exceeded permissible limits of USEPA. The results illustrated that the highest concentration of Mn and Fe were distributed in the upper north eastern part of the studied stations, this may be due to human's activities and industrial area. While, the eastern region had high concentrations of Pb, Ni, Cr and Co due to El-Kalaa drain discharges. However, the western part of the study area had high concentration of Zn and Cu due to El-Ommum drain discharges.

In recent years hematological variables have been used more to determine the sublethal concentrations of pollutants (Wedemyer and Yasutake, 1977). The use of hematological parameters to assess alterations in fishes exposed to heavy metals and involving the defense mechanism come from the need to develop a rapidly growing aquaculture industry (Jones, 2001). The results of this study showed that heavy metals caused a drastic reduction in hemoglobin and RBCs count. These results were in agreement with the results obtained by Maheswaran*et al.* (2008), who found that mercuric chloride caused a drastic reduction in hemoglobin and total count of RBCs in fish. Decline in RBCs values and anemia were reported in fishes such as *Salvalinusfontinalis (*Holocombe*et al.*, 1976), Salmogairdneri (Johansson-Sjobcj and Larsson, 1979), *Anguilla anguilla* (Haux and Larsson, 1982), *Barbusconchonius* (Tewari*et al.*, 1987) which were exposed to heavy metals.

The decrease of hemoglobin and hematocrit in *O. niloticus* in this study are indications of anemia and reduced hemoglobin synthesis. The significant erythrocytosis coupled with decreased hemoglobin and hematocrit values and concomitant reduction in MCV, MCH and MCHC in this study are relevant bioindicators of stress and microcytic anemia. Nussey*et al.*, 1995) noted that the observed erythrocytosiscould be triggerered by shortage of oxygen during the exposure period. This according to previous researchers would impose oxygen debt in the fish, there by promoting anaerobic respiration in the fish with the attendat high carbon dioxide level in the blood. Under this prevailing circumstances, the fish would begin to produce immature erythrocytes as a compensatory and adaptive response to cope with the challenge in an attempt to deliver more oxygen to the tissues (Stanley and Omerebele, 2010).

Witeska and Kosciuk (2003) further noted that heavy metals might alter the properties of hemoglobin by decreasing their affinity towards oxygen and reducing their binding capacity thereby rendering the erythrocyte more fragile and permeable which probably results in cell damage. Since the MCH and MCHC are the red blood cell morphological indices reflecting the hemoglobin concentration, the observed decrease in these parameters may indicate that hemoglobin concentration in the control fish was higher than in heavy metals-exposed fish. This may indicate that stress produced by heavy metals may lead to a decreased rate of production of red blood cells. Gill and Epple (1993) have attributed anemia to i) Impaired erythropoisis due to direct effect of metals on hematopoietic centers (kidney and spleen). i) Accelerated erythroclasia due to altered membrane permeability or increased mechanical fragility. iii) Defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions.

The decrease of MCV in *O. niloticus*in locations 2 and 3 was in agreement with the results obtained by Nusseyet *al.*, (1995) in *O. mossambicus* treated with copper and in Labeoumbratusexpoed to pollutants (Van Vuren, 1986). Significant reduction in MCV was reported in *Clariasalbopunctatus* exposed to cadmium (Oluah, 2001). The data on MCV values indicated erythrocyte shrinkage due to exposure to heavy metals and this may possibly be due to impaired water balance as a result of stress (Oluah*et al.*, 2010).

White blood cells play a major role in the defence mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytesfunction as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Ellis *et al.*, 1978; Wedemeyer and Mcleay, 1981). The increase in WBCs observed in the present study could be attributed to be a stimulation of the immune system in response to tissue damage caused by heavy metals. Gill and Pant, (1985) have reported that the stimulation of the immune system causes an increase in the lymphocyte by an injury or tissue damage.Dhanekarand Srivastava (1985) reported increase in large lymphocytes, reduction in small lymphocytes and thrombocyte populations as also elevation monocytes, neutrophil and eosinophil cells in Heteropneustesfossilis, *Channapunctatus and Mastcebaluspuncatus* on long term exposure to least effective concentrations of mercuric chloride.

Enzymes

The results of serum biochemistry revealeda significant increase in serum ALT and AST in serum of fish of both locations 2 and 3. These results were in agreement with the results obtained byGaafar*et al.*(2010) and Dabees*et al.*(1992), who found a significant increase in these enzymes in fish after exposure to toxicants. These finding support the hypothesis that the increased serum tranaminases (AST and ALT) may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation.Creatinephophokinase is significantly increased in both locations 2 and 3 compared to control. The present results were in agreement with the results obtained by almeida*et al.*, (2002) and Al-taee and Al-Hamdani, (2008), who found an increased levels of CPK in fish after exposure to cadmium chloride. Elevation of (CPK) is an indication of the damage to muscles. It is therefore indicative of injuryin fish muscles in both locations 2 and 3, which are heavily polluted.

Effect of heavy metals pollution on morphology of blood cells

It was observed that red blood cells of *O. niloticus* in Lake Maryut exhibited morphological changes. Many of red blood cells appeared in a form of irregular polygons and were elongated. Cell membrane deformations (lysis), vacuole occurrence in the cytoplasm was also observed. Observations also showed round cells. Granulation of protoplasm of some cells was observed. This study showed that heavy metals pollution in fish of Lake Maryut caused anemia (red erythrocyte count, hemoglobin concentration and hematocrit were decreased). These results were in agreement with the results obtained by Arnaudov and Tomova, 2008) after exposure of freshwater fish to heavy metals. There were cases when the impact of heavy metals did not cause any changes in erythrocyte indices, it could even lead to increased values of indices (Witeska and Jezierska, 1994, Witeska, 1998, Mishra and Srivasrava, 1990). Other stress effects, such as transport and handling, which are not related to heavy metal pollution in water basins, also appear to cause changes in hematological indices (Acerete*et al.*, 2004). According to Vosyliene (1999), the influence of heavy metals mixture on hematological indices depends on the concentration of metals and continued exposure to them. When concentration is at low level, freshwater fihes tend to adapt to the presence of metal mixtures in water after a period of time. Witeska, (2004) also provided information about similar pathological changes in erythrocytes of freshwater fishes as a result of their exposure to metal mixture. Figures 2 and 3 show different changes in blood cells in *O. niloticus* in polluted locations of lakeMaryut.

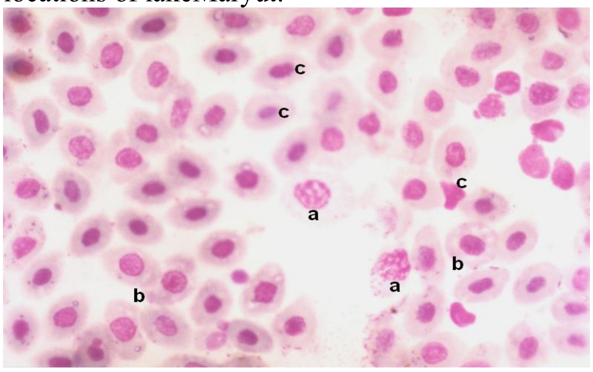


Fig. 2. a: nucleus disintegration b: membrane disintegration c: nucleus shape is changed

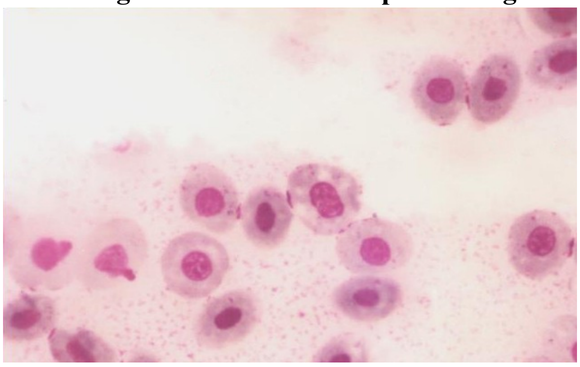


Fig. 3: Hypertrophied cells, cells are enlarged with their nuclei and become circular

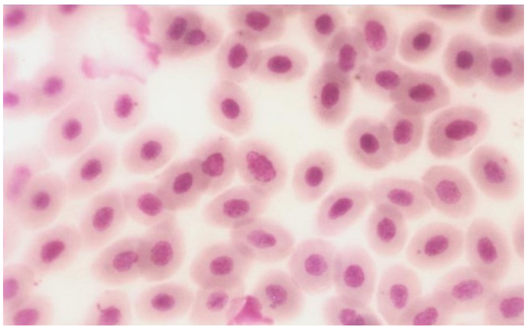


Fig. 4: Blood cells of *O. niloticus* from the control site (Fish Farm)

Conclusion

In conclusion, according to previous researchers the influence of pollution in Lake Maryut could be highly negative to the aquatic biota and affect the biointegrity of the lake and thus needs to be urgently monitored. According to Witeska, morphological changes in erythrocytesand changes in hematological parameters are a serious threatto freshwater fishes and they can be used as indicators of heavy metals related stress in fish after exposure to elevated levels of heavy metals (Witeska, 2004).

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