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The Toxic Effect of Pollutants in the Aquatic Environment on the Kidney and Blood Picture of Rabbit Fish *Siganus rivulatus* (Forskal) from the Red Sea, Jeddah, Saudi Arabia

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ABSTRACT. *Siganus rivulatus* is an important food fish and possesses certain desired aquacultural characteristics.

The present study was carried out to document possible histopathological changes in the kidney and blood of the fish *Siganus rivulatus*. The tissue change is thought after reported changeable in water quality due to industrial effluents discharges and accumulation of some heavy metals in the Red Sea coast water at Jeddah, Saudi Arabia.

The majority of fish examined showed some forms of lesions in kidney and blood. In kidney such lesions include hyaline droplets degeneration, separation of the tubular epithelium from their basement membrane and tubular vacuolization, reduction of haemopoietic tissue, granuloma inflammation lymphocytic infilteration and hyalinization of the glomerulus's and Bowman's capsule. These degenerative changes indicate impairment of normal kidney functions including blood picture and formation. Also, decrease in hemoglobin content, total erythrocyte count and increase in granular leukocytes were clearly detected in blood samples of the majority of *Siganus rivulatus* examined. Pathological alterations of erythrocytes in examined blood smears included deformation sticking, lysis and abnormal presence of ghost, senile and blast cells were clearly observed.

KEYWORDS: Fish / histopathology, hematology, kidney, hemopoietic tissue.

Introduction

Aquatic animal health has been shown to be a useful indicator of water quality. Physicochemical alterations of the aquatic medium induce pathological lesions in fish organs and general blood picture. Gonzalez *et al.*, 1993; Abdel- Aziz, 1994; Husqy *et al.*, 1996; Zaki *et al.*, 1999 a,b; Bin Dohaish 2003).

The kidney in teleosts is important organ comprising excretory and endocrine elements as well as, haemopoietic tissue responsible for blood formation (Roberts, 1978; Takashima and Hibiya, 1995).

Blood alteration in fish or damage of haemopoeitic tissue organs may be associated with changes in environmental condition. Many investigation were carried out to study the effects of different pollutants on the kidney (Gill *et al.*, 1991; Hilmy, 1996; Husqy *et al.*, 1996; Abdel-Rahman, 1997) and blood of fishes (Khadre, 1990; Gill *et al.*, 1991; Kumar and Banerjee, 1991; Abdel-Rahman, 1997; Zaki, *et al.*, 1999b; Bin Dohaish, 2003).

Thus, the present study was carried out to assess the histopathological changes in the kidney and blood of the fish *Siganus rivulatus* captured from the Red Sea coastal water of Jeddah, Saudi Arabia and identify possible causal agents of these changes.

Materials and Methods

The study area (21º29'-21º30'N and 39º10'-39º11'E) is located in the middle part of the Jeddah city coast and extends between the Islamic Harbour in the south and the desalination plant north of the city (Fig. 1).

The coastal water of the study area (Jeddah fish market) receives different pollutaning discharges from four main sources: untreated domestic sewage waste, oil pollution from a petroleum refinery, fish wastes from a big coastal fish market and desalination plants effluents.

The study area was divided into five random sites as represented in Fig. 1. Mean values of temperature and relative humidity were observed through the seasons of sampling (autumn and winter). The recorded data in Table 1 found that there are slight differences in these values between both seasons.

Some ecological studies were carried out on water and sediments of the area includes estimation of some physicochemical parameters in water according to APHA (1995) methods (Table 2) as well as determination of concentration of some heavy metals in water and sediments of the chosen sites (Tables 3 & 4). The heavy metals are represented here by eight elements; Fe, Ni, Mn, Cr, Zn, Cd, Pb and Cu. They were estimated by using atomic absorption spectrophotometer.

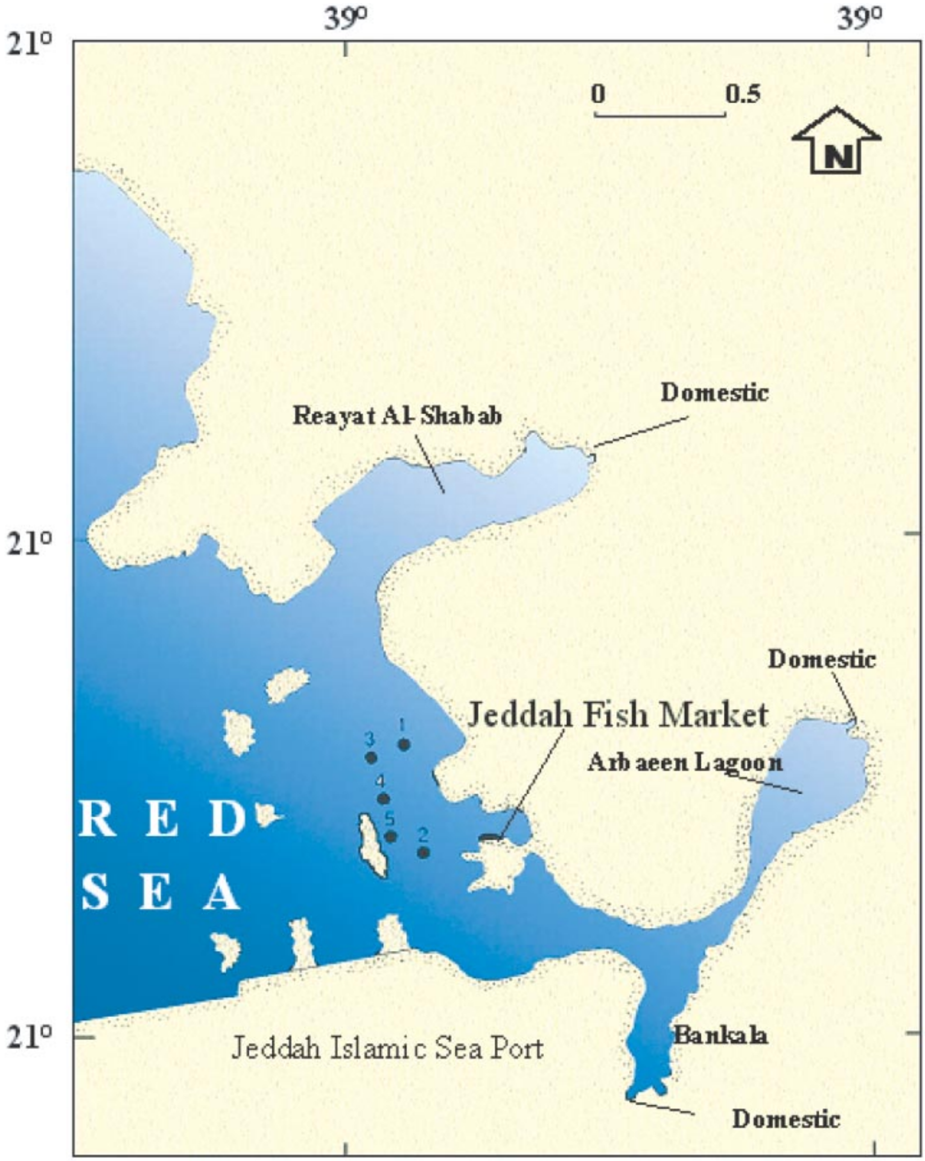

FIG. 1. Map showing the five sampling stations of the study area of Jeddah fish market.

TABLE 1. Meteorological records in the study area through autumn and winter seasons.

Season	Months	I	II	III	IV
Autumn	September	32.4	32.1	79.9	0.0
	October	30.0	29.0	80.0	0.0
	November	27.6	27.2	77.0	0.0
	Mean	**30.0**	**29.4**	**78.7**	**0.0**
	SD	**2.4**	**2.5**	**1.5**	**0.0**
Winter	December	27.4	24.7	78.0	10.0
	January	25.9	21.0	76.0	11.0
	February	25.6	22.0	70.0	11.0
	Mean	**26.3**	**22.6**	**74.7**	**10.7**
	SD	**1.0**	**1.9**	**4.2**	**0.6**

I Air temperature (ºC)
II Water temperature (ºC)
III Relative humidity (%)
IV Precipitation (mm/month)

TABLE 2. Mean (and range) values of the physico-chemical parameters measured in surface waters of the five sites of the study area.

Parameters		PH	Hardness (mg/l)	DO (mg O_2/l)	BOD (mg O_2/l)
Sites	1	7.80	6,850	4.95	3.81
	2	8.14	9,450	3.93	5.02
	3	8.16	7,750	5.35	2.68
	4	8.14	10,750	6.29	6.13
	5	8.17	15,250	7.17	7.93
Range		7.80-8.17	6,850-15,250	3.93-7.17	2.68-7.93
X ± SD		8.08 ± 0.16	10,010 ± 3,294	5.54 ± 1.25	5.11 ± 2.04
Parameters		COD (mg O_2/l)	NO_2-N (µg at /l)	NH_4-N (µg at /l)	PO_4-P (µg at /l)
Sites	1	20.00	0.01	0.13	0.08
	2	20.00	0.15	0.11	0.78
	3	20.00	0.39	0.18	0.11
	4	40.00	0.17	0.13	0.12
	5	40.00	0.17	0.13	0.07
Range		20.00-40.00	0.01-0.39	0.11-0.18	0.07-0.78
X ± SD		28.00 ± 10.95	0.18 ± 0.13	0.14 ± 0.03	0.23 ± 0.31

About 150 fish were caught by trammel and impounding (trap) nets which were fixed at random distances along the study area. Fish length to the nearest (mm) and weight to the nearest (g) were recorded. Excised pieces of kidney were removed from fish immediately after necropsy and fixed in 10% buffered formalin. Tissues were dehydrated in an ethanol series, infiltrated and embedded in paraffin wax and sectioned on a rotary microtome at 3 µm. Tissues were stained by Meyer's haematoxylin and eosin (H&E).

Blood samples were collected immediately from caudel artery by heparinized capillaries and the kidneys were dissected out.

Total erythrocyte counts were made on a spence's brightline hematocytometer in diluted blood. The hemoglobin content was determined according to Shale-Helliege method (Hesser, 1960). Blood smears were air dried, stained with 0.2% Giemsa's stain and examined.

Results and Discussion

The recorded data in Tables 3 & 4 shows that the concentration of heavy metals in sediments is higher than the concentration in water. Fe occupy the first order of abundance in water and the sediment and followed by P_b. In comparison with the safety limits for heavy metals concentration we can conclude that water and sediment of the studied area are polluted with heavy metals.

TABLE 3. Concentration of the heavy metals (µg/l) wet weight in the surface water of the studied sites.

Sample	Fe	Ni	Mn	Cr	Zn	Cd	Pb	Cu
1	19.64	5.36	1.00	0.52	1.44	0.84	3.20	0.52
2	53.68	4.36	1.88	0.72	1.40	1.40	10.00	2.24
3	19.44	1.60	1.84	1.00	1.44	1.92	5.20	2.40
4	48.96	3.28	0.88	0.92	2.00	0.80	12.80	1.32
5	39.36	9.00	0.52	1.24	1.68	1.04	4.80	1.44
Range	19.44-53.68	1.60-9.00	0.52-1.88	0.52-1.24	1.40-2.00	0.80-1.92	3.20-12.80	0.52-2.40
Mean ± SD	36.22 ± 16.07	4.72 ± 2.77	1.22 ± 0.61	0.88 ± 0.27	1.59 ± 0.25	1.20 ± 0.47	7.20 ± 4.03	1.58 ± 0.76

TABLE 4. Concentration of the heavy metals (μg/g) in the surface sediments of the studied sites.

Sample	Fe	Ni	Mn	Cr	Zn	Cd	Pb	Cu
1	1,050.00	19.11	17.61	13.85	8.44	4.75	50.88	7.59
2	1,040.00	22.76	17.41	13.28	5.25	5.16	53.50	6.84
3	705.00	20.64	15.43	12.75	4.39	5.45	46.63	6.75
4	779.35	25.00	18.09	16.47	9.40	5.81	64.05	8.96
5	1,243.75	21.70	19.11	12.56	4.55	5.23	48.63	39.71
Range	705.00-1,243.75	19.11-25.00	15.43-19.11	12.56-16.47	4.39-9.40	4.75-5.81	46.63-64.05	6.75-39.71
Mean ± SD	963.62 ± 219.44	21.84 ± 2.22	17.53 ± 1.35	13.78 ± 1.58	6.40 ± 2.34	5.28 ± 0.38	52.74 ± 6.83	13.97 ± 14.42

Kidney

Normal histological structure was observed in the kidney sections of 17% of fish examined (Fig. 2 & 3). This is comparable to the percentage in other marine fish (Roberts, 1978 ; Takashima and Hibiya, 1995).

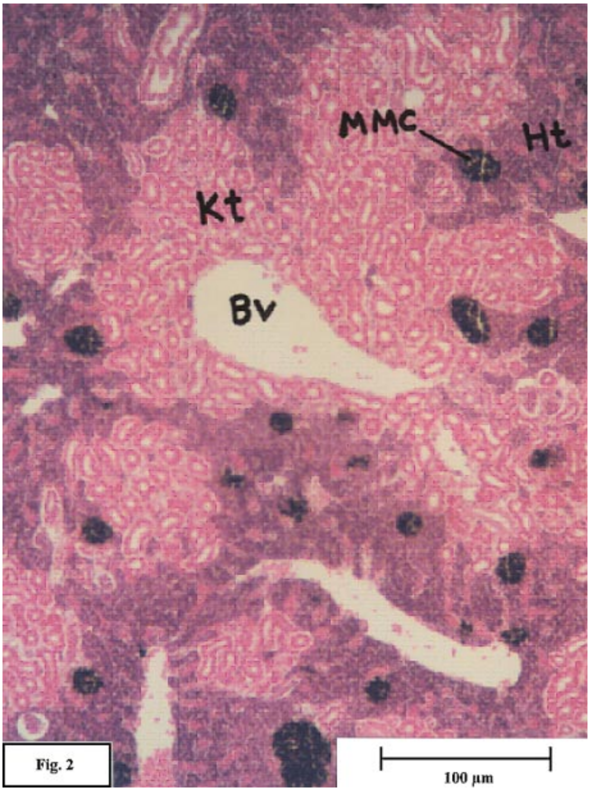

FIG. 2. Kidney section of *siganus rivulates* showing normal architecture. Notice marginal haemopoietic tissue (Ht); renal tubules (Kt); melanomacrophage center (MMC) and blood vessels (Bv) (H. & E.; bar = 100 μm).

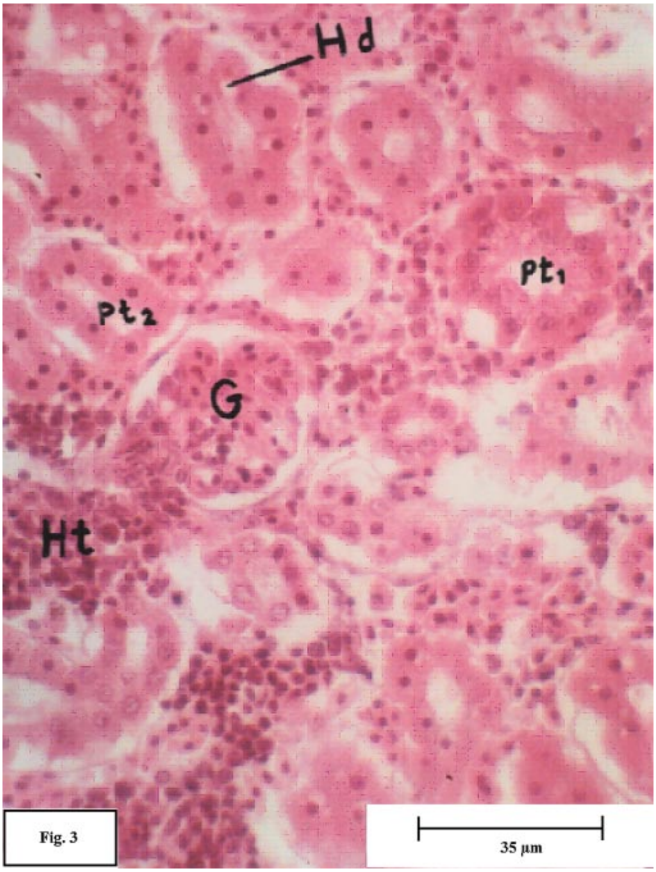

FIG. 3. High power view from Fig. 2 showing glomerulus (G), 1st segment of proximal tubules (Pt_1) with brush boarder; 2nd segment of proximal tubules ((Pt_2); hyaline droplets (Hd) & haemopoietic tissue (Ht) (H. & E.: bar = 35 µm).

Tissue changes were evident in 83% of kidney of captured fish. Granuloma formation was evident in many fish and was characterized with caseous matter within layers of epithelium cells and fibrous tissue. Granuloma formation is a flammatory response. This may be due to parasitic infection (Husqy *et al.*, 1996) and the necrotic debris within these granuloma represent successful host reaction to parasitic infection. Roberts, (1978) mentioned that haemopoeitic tissue in fishes is considered a common site of parasitic infection which appear in the form of encapsulated or necrotic granuloma chronic infection (Burkitt *et al.*, 1996).

Hyalinization of Bowman's capsule and glomerulus was clearly detected (Fig. 4). This may be attributed to chronic infection (Burkitt *et al.*, 1996).

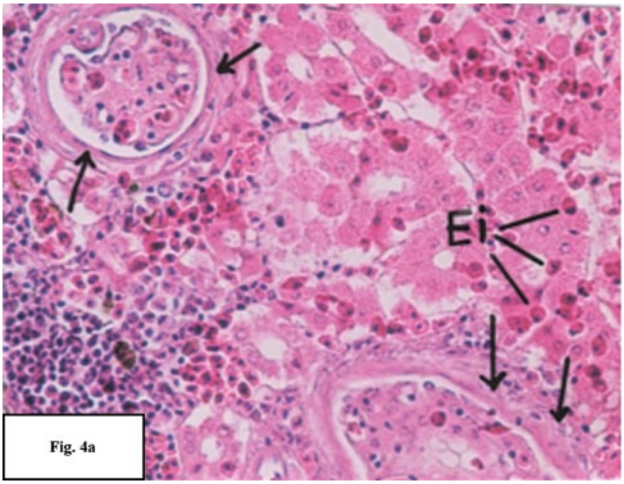

FIG. 4a. Notice: Hyalinization of Bowman's capsule (arrows) (bar = 20 µm).

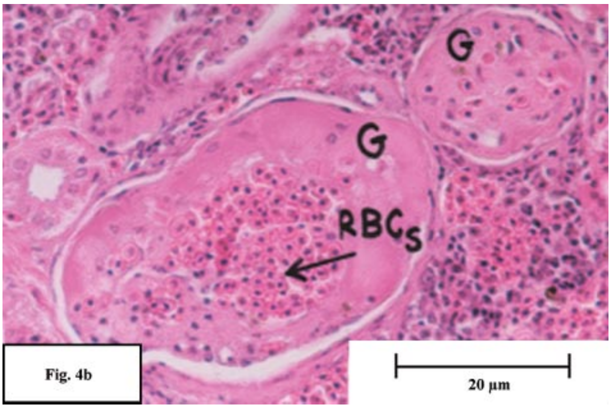

FIG. 4b. A high power from (4). Notice hyalinization of glomerulus and Bowman's capsule (bar = 20 µm.)

Separation and necrosis of tubular epithelia were evident accompanied in kidney tubules (Fig. 5 & 6).

Vacuolization of tubular epithelial cells was cleary discernible in kidneys of some examined fish with reduction of haemopoeitic tissue (Fig. 7). These degenerative changes indicate impairment of normal kidney function and would eventually lead to renal failure. Similar histopathological lesions were recorded previously in kidney tissues of fish treated with different pollutants. They included necrosis and disintegration of hemopiotic tissue and vacuolation of renal tubular cells in winter flounder poisoned by copper (Baker, 1969). Cloudy swelling of tubular epithelium, focal necrosis of glomerular tufts and dilation of urinary space were described in guppy exposed to methyl mercury (Wester *et al.*, 1985). Also, Hilmy (1996) mentioned vacuolar degeneration and lysis of renal tubules; accumulation of hyaline casts within the tubular lumen, atrophy of glomeruli and reduction of hemopoeitic tissue in *Clarias lazera* treated with pesticides.

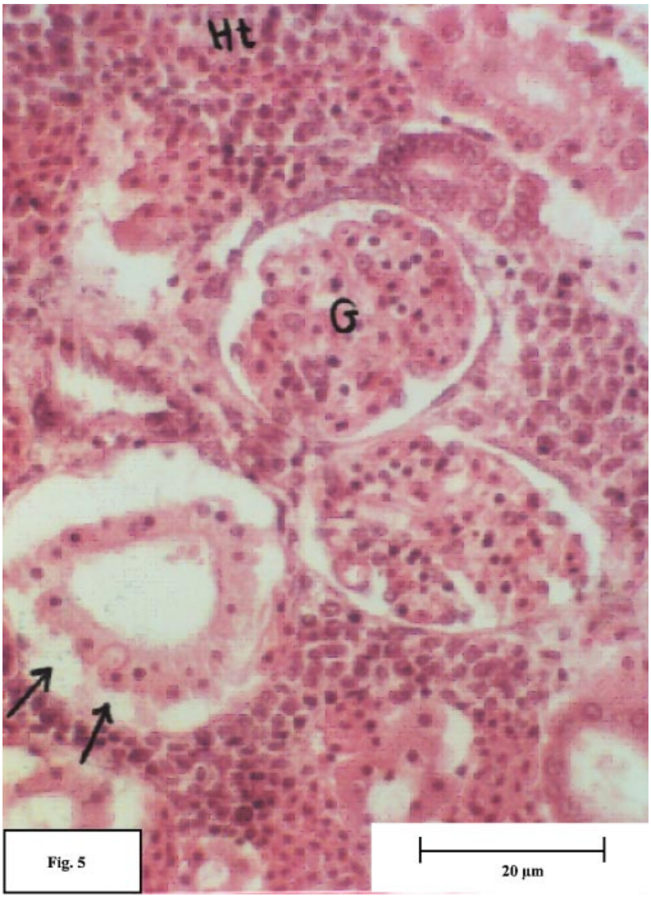

FIG. 5. Kidney section showing lobulated glomeruli (G); separation of tubular epithelia (H. & E., bar = 35 µm).

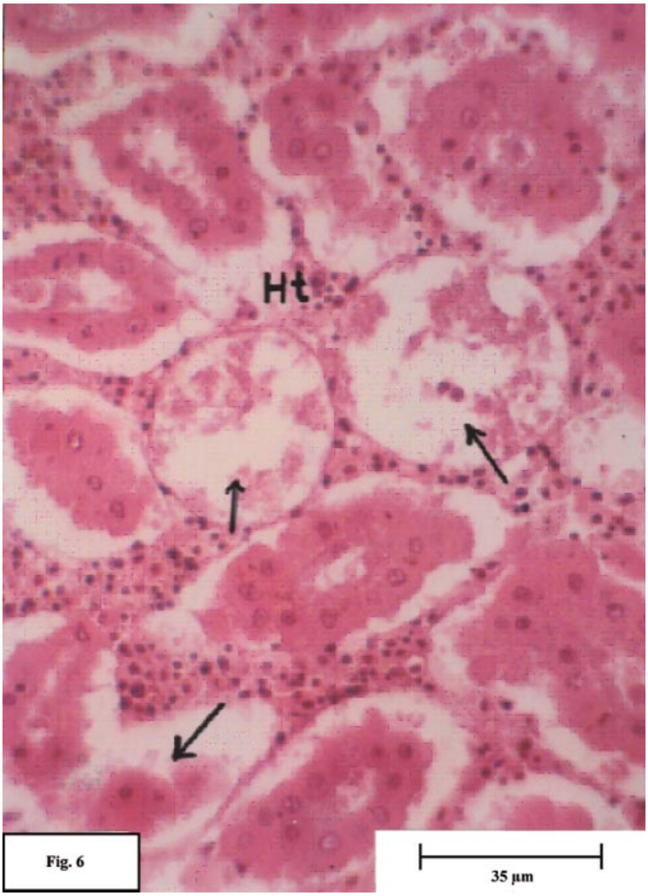

FIG. 6. Kidney section showing necrosis of tubular epithelia (H. & E.: bar = 35 µm).

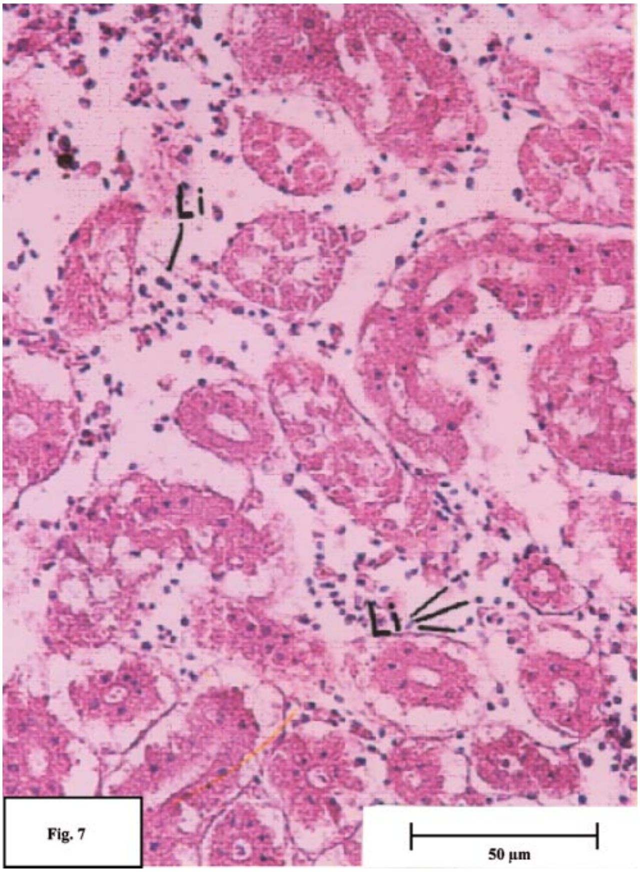

FIG. 7. Kidney section showing Hematopoietic tissue shows a decrease of hematopoietic cells and infiltration of lymphocytes Tubular epithelial cells show vacuolization (bar = 50 μm).

Blood

Determination of hemoglobin content (HC) and total count of red blood cells (RBCs) in peripheral blood of examined fish revealed great individual variability. HC range from 3.941 to 8.124 g/100 ml blood with mean value of 5.497 ± 1.124 g/100 ml blood and the total count of RBCs range from 1.426×10^6 to 3.027×10^6/ml with a mean value of $2.167 \times 10^6 \pm 0.517 \times 10^6$/ml of blood.

Examination of peripheral blood smears of examined fish revealed presence of erythrocytes, thrombocytes and leukocytes. Nearly normal blood picture was observed in blood of some fish examined (Fig. 8 & 9), while pathological changes in RBCs were detected in the majority of blood smears of examined fish (Fig. 9 & 12).

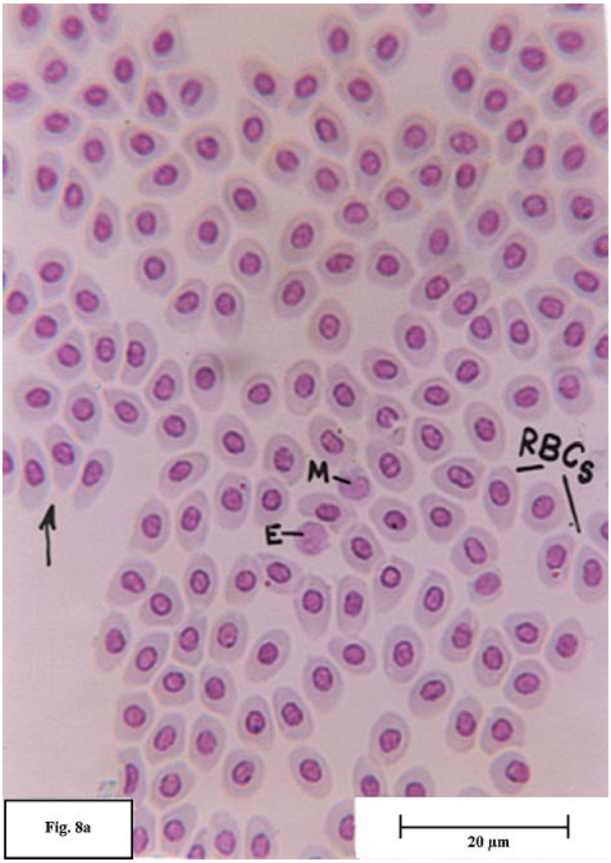

FIG. 8a. Blood smear of *Siganus rivulatus* showing ovoid nucleated red blood cells (RBCs); boat shaped erythrocytes (arrow), monocyte with kidney shaped nucleus (M) & eosinophil (E) (bar = 20 µm).

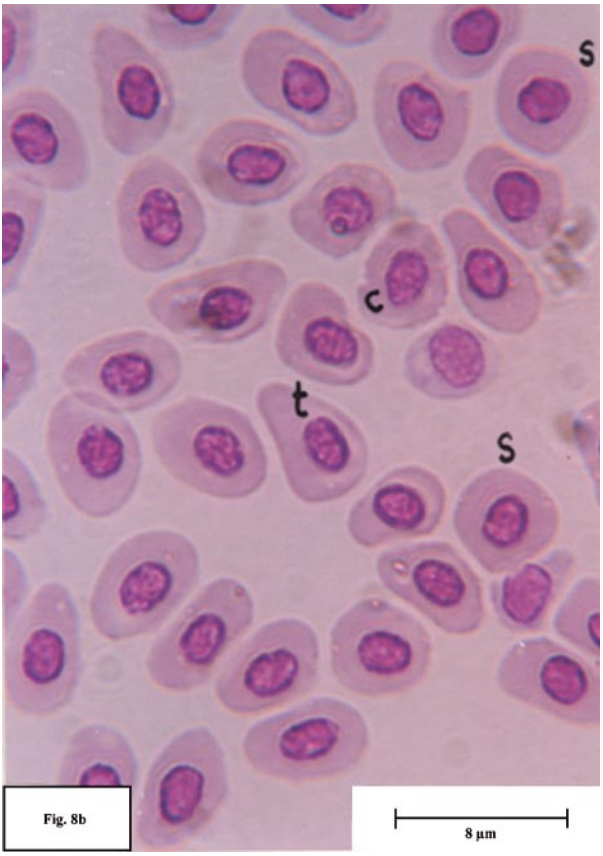

FIG. 8b. High magnification for *Siganus rivulatus* showing ovoid spheroid (S), crenated (C), tear-like drop (t), boat shaped RBCs. Note also monocyte (M) (bar = 8 µm).

Normal erythrocytes of *Siganus rivulatus* are ellipsoidal in shape and contain large central nuclei. However, spheroid, crenated, tear drop like and sickle shape cells were detected (Fig. 8a,b & 9). Also, ghost cells, senile and normoblasts characterized by lightly stained spheroid shape with marginal blue ring were frequent in examined smears (Fig. 9). Sticking of RBCs were detected and the stacked cells were deformed (Fig. 10). Lysis of RBCs was also frequent in blood smears of examined fish and the lyzed RBCs appear as scattered deformed nuclei without distinct outlines (Fig. 10). Presence of parasitic infection was likely in blood smears of some fish (Fig. 11a,b & 12).

Previous studies showed that starvation, stresses, infection and toxins change general blood picture of fish (Gill *et al*., 1991; Abdel-Rahman, 1997; Zaki *et al*., 1999b). Poisoning of *Clarias lazera* with lead resulted in clumping and ly-

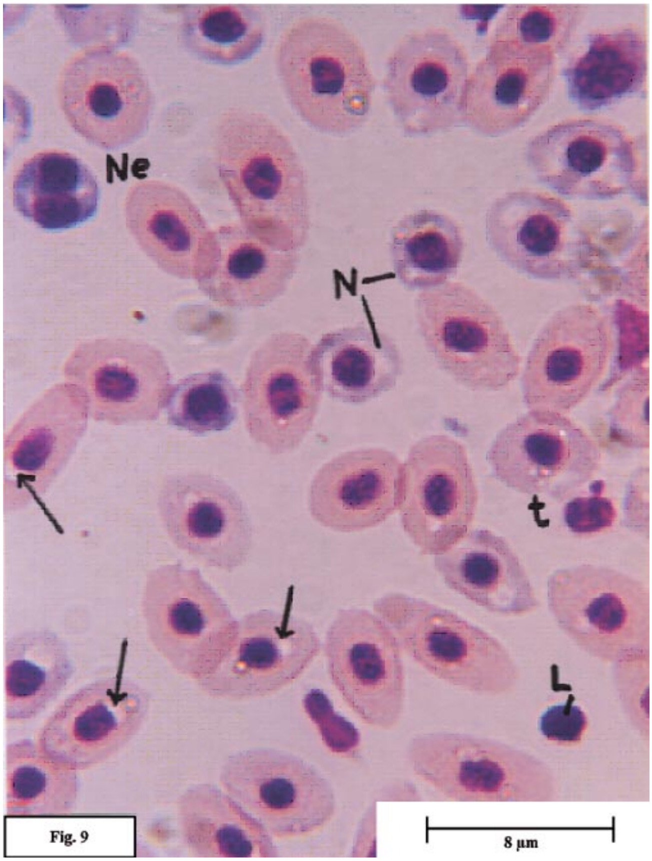

FIG. 9. Showing numerous normoblasts (N); neutrophils (Ne); Lymphocytes (L); thrombocyte (t). Note also pale areas inside erythrocytes (arrow) (bar = 8 µm).

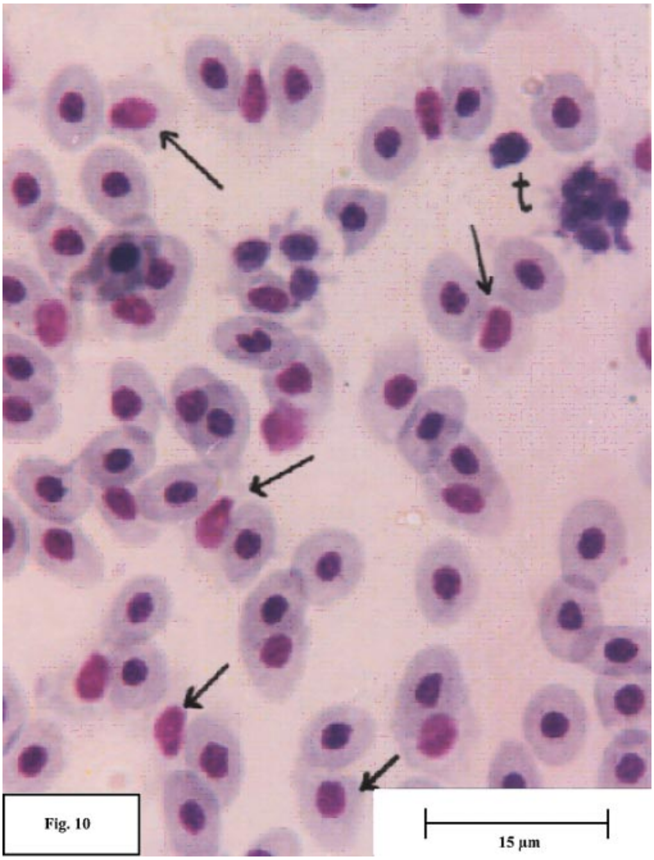

FIG. 10. Notice sticking and lysis of RBCs; scattered acidophilic nuclei of the lyzed cells (arrows) and thrombocyte group (t) (bar = 15 µm).

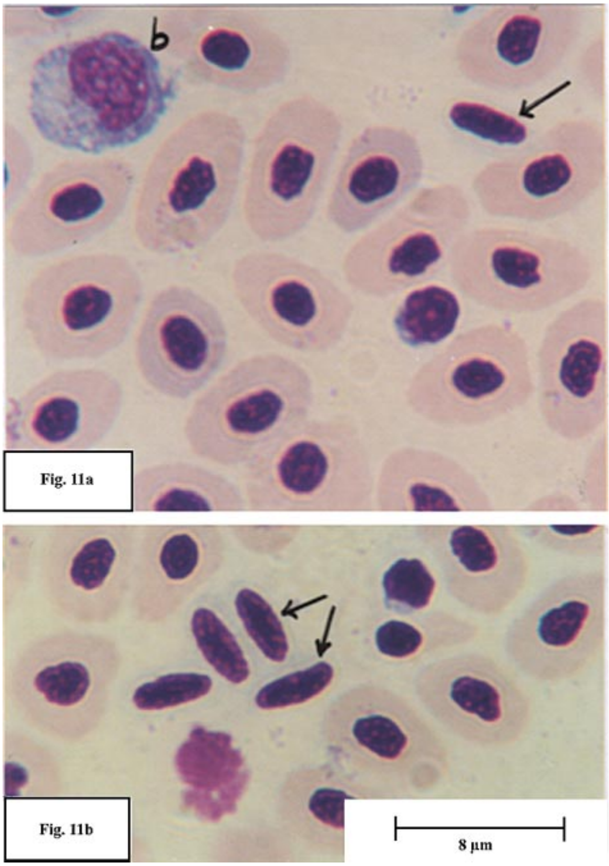

FIG. 11a&b. A possible parasitic infection in blood film (arrows). Notice also; hypertrophy and hypochromatic RBCs and blast cell (b) (bar = 8 µm).

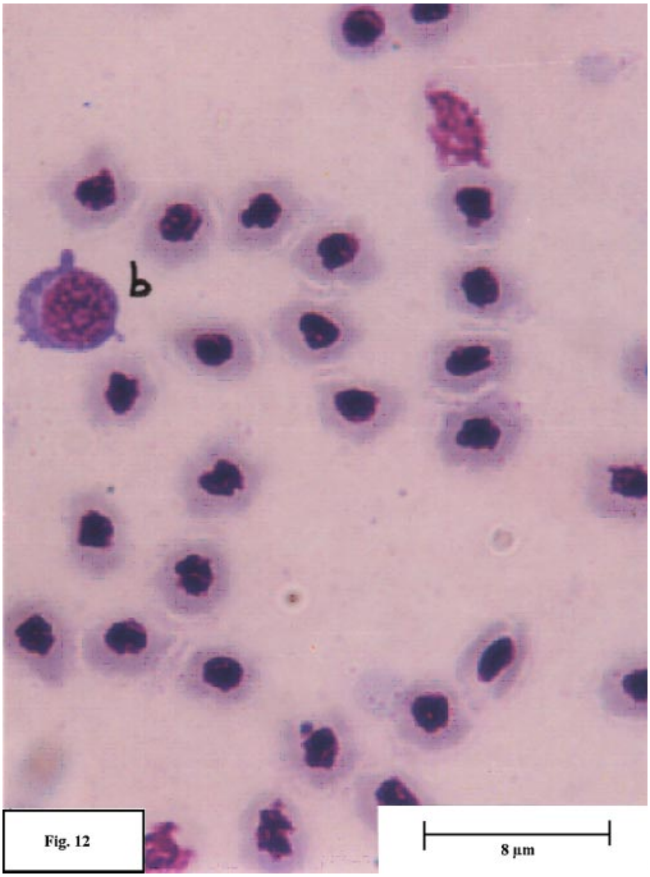

FIG. 12. Showing deformed erythrocytes and blast cell (bar = 15 µm).

sis of erythrocytes. (Shabana, 1983) Shrinkage, crenation, anisocytosis and overlap of erythrocytes were seen in the Indian carp *Catla calta* treated with sublethal concentration of copper (Cu^{++}) (Ahmed and Munshi, 1992). Exposure of fish to pesticides lead to hypochromasia, vacuolar degeneration, crenation anisocytosis and atrophy of erythrocytes (Kumar and Banerjee, 1991; Gill *et al*., 1991) and similarly exposure of fish to tanning processes of waste water (Zaki *et al*., 1999b).

Differential Count of White Blood Cells

In the examined fish the total granular leukocytes represent 70.060 ± 6.460% of the total leukocyte count. Thrombocytes represent 43.598 ± 2.624% of the total leukocytes count and take ovoid or fusiform shape with cytoplasmic extention (Fig. 10). Lymphocytes and monocytes (Fig. 8 & 10) represented 20.941 ± 4.562% and 5.521 ± 1,164 of the total leukocyte count respectively (Fig. 10). Granylocytes were abundant in examined fish and amounted to 29.940 ± 2.689% of the total leukocyte count and include neutrophils, eosinophils and basophils with mean values equal 9.213 ± 1.246%, 7.614 ± 1.226% and 13.113 ± 2.411% of the leukocyte count respectively (Fig. 9, 10 & 12). Lower level of hemoglobin content and total count of RBCs and higher granular leukocytes were found in the peripheral blood of *Siganus rivulatus* inhabiting Red Sea coastal water of Jeddah compared with those obtained for the other fish species and for the same species inhabiting the Mediterranean sea water of Egypt (Abdel-Rahman, 1997). These differences as well as pathological lesions that are detected in blood smears in the present study may be a direct result of pollution in the coastal water of Jeddah.

The pathological lesions in blood picture of *Siganus rivulatus* in the present study may be due to damage of hemopoeitic tissue in kidney; damage of intestinal mucosa and hemorrhage; damage of gills and permeability of Pb into blood which united with hemoglobin and decrease oxygen carrying capacity of blood (Johnson *et al*., 1993).

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التأثير السمي للملوثات في البيئة المائية على التركيب النسيجي لكل من الكلى والدم في أسماك السيجان من البحر الأحمر – جدة ؛ المملكة العربية السعودية

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المستخلص. يهدف البحث إلى تتبع المتغيرات التي تحدث بنسيجي الكلى والدم بأسماك السيجان من ساحل البحر الأحمر بجدة المملكة العربية السعودية ، خاصة في وجود عدة مؤشرات لاحتمال تلوث هذه المياه بالعديد من العناصر الثقيلة . أوضح الفحص النسيجي لكلى الأسماك تغيرات مرضية حادة في نسيج الكلى تمثل في التحلل الفجوي ، وانفصال الطلائية الأنبوبية عن الغشاء القاعدي ، ونكرزه النسيج الطلائي للأنيبيات وتقشرها ، وحدوث التهاب حبيبي ورشح الخلايا الأيوسينية واللمفاوية . وقد لوحظ أيضًا ضمور وتحلل زجاجي وموت للعديد من الكبيبات ، وانفصال وتحلل في مراكز الخلايا الأكولة . وهذه التغيرات الانحلالية تدل على الإعاقة الوظيفية الطبيعية للكلى ، والتي تؤدي إلى الفشل الوظيفي وتثبيط في عملية تكوين الدم . ومن التغيرات المرضية التي لوحظت عند فحص مسحات الدم التشوه والتحلل ، وظهور خلايا كرات الدم الحمراء غير الناضجة والخلايا الهرمة بها . كما أمكن تمييز نقص في المحتوى الهيموجلوبيني والعدد الكلي لكرات الدم الحمراء وزيادة في كرات الدم البيضاء للعديد من أسماك السيجان .