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Effect of Polluted Water from the Egyptian Eastern Mediterranean Coast on Reproductive, Toxicological and Hematological Characteristics of *Siganus rivulatus*

S.G. Moharram, O.M. Wahbi and Z.A. El-Greisy

National Institute of Oceanography and Fisheries, Kaiet Bay, Alexandria, Egypt

Abstract: The impact of diluted levels of polluted seawater from the Egyptian Mediterranean coast on reproductive, toxicological and hematological characteristics of *Siganus rivulatus* were determined. Mature fish were exposed to diluted levels of 7.5, 10 and 15 ml L^{-1} polluted seawater. Hematological changes after 4, 15 and 30 days of exposure were measured. Erythrocytes decreased (p<0.05) as well as Packed Cell Volume (PCV) and Hemoglobin (Hb), indicating anemia developed to hypochromic macrocytic anemia at end of experiment. Leukocytes, increased (p<0.05), indicating susceptibility of fish to infection and stress. Granulocytic leukocytes, neutrophil and eosinophil increased. While lymphocytes decreased. Blood parameters of exposed fish revealed compensatory responses. The increase in developing hemocytoplast and myelocytes emphasize the compensatory and defensive reaction of fish to polluted water. Exposure to polluted water levels has a detrimental effect on gonads development, altered endocrine haemostasis, testosterone and progesterone levels decreased in females (p<0.05). While in male, progesterone level increased (p<0.01). Necrosis of spermatogenic cells and atresia of developing oocytes are pronounced at levels of 10 and 15 ml L^{-1} polluted seawater. Also, has necrotic effect on fish organs. Vacuolation and necrosis occurs in liver and kidney. Melanomacrophage aggregates can be seen. Gills showed epithetial lifting and vascular widening. Results showed that, polluted water has serious consequences on *Siganus rivulatus* blood characteristics as well as organs cellular structure. It rendered fish anemiatic, altered reproductive hormones level, leading to necrosis of males spermatogenic cells and atresia of developing oocytes.

Key words: *Siganus rivulatus,* pollution, hematology, reproduction, endocrine disruption

INTRODUCTION

It is well established that many chemicals, both natural and man-made, may adversely affect wildlife, including decrease in fertility, disrupted hormone secretion or reproductive histopathology. Damaged ovarian follicles were observed in female mussels exposed to tetrabromodiphenyl ether (Aarab *et al.*, 2006). Reduced gonad size and delayed sexual maturity have been observed in perch exposed to leachate from Swedish refuse dumps (Noaksson *et al.*, 2005). Inhibition of ovarian development, reduced egg weight and increased atresia, were found in white perch (Richard *et al.*, 2004) in areas affected by domestic and industrial effluents. Inhibition of oogenesis and spermatogenesis were found in medaka exposed to isoflavones (Yiannis *et al.*, 2003). Gonad differentiation and development of salmon were affected by sewage effluents (Luis *et al.*, 2002). Fish living in Bizerta lagoon, downstream of anthropogenic pollution have been found to exhibit an array of altered features in their reproductive development, including sperm abnormality and ovarian atresia (Louiz *et al.*, 2009).

Hormonal disruption in turn, affects reproductive function. Population decline has been demonstrated in wild freshwater fish as a result of exposure to organochlorine chemicals (Jobling and Tyler, 2003). A reduction in reproductive capacity and plasma steroid levels has been established in female zebrafish exposed to contaminated sediment and Atlantic salmon after exposure to sublethal doses of diazinon (Linderoth *et al.*, 2006; Wall, 2000). Reduction in level of testosterone and 17 α-20 β progesterone was found in cod and turbot exposed to crude oil and alkyl phenols (Skilton *et al.*, 2006). Depressions in sex steroids level was detected in cyprinid fish from three sampling sites along the Kor river (Ebrahimi and Taherianfard, 2011). In laboratory experiments, fathead minnows exposed to methyl mercury over their life cycle exhibited a parallel range of effects on circulating steroids (Paul *et al.*, 2006).

Histological investigations may reveal toxic effects of pollutants on fish. The gill is the primary target organ for the toxic action of pollutants. Impairment of the respiratory and the ionoregulatory functions may occur due to the structural changes of gill epithelia. Gill lamellae

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Corresponding Author: O.M. Wahbi, National Institute of Oceanography and Fisheries, Kaiet Bay, Alexandria, Egypt

lesions, including interstitial edema, rupture of pillar cells and necrosis of lamellar epithelium have been reported when fish were exposed to industrial effluent (Wynn and Waring, 2002) and copper (Mazon *et al.*, 2002a). Fish liver not only perform an important role in pollutant metabolism, but also susceptible to damages by their action. The principal lesions observed in the hepatic tissue were focal vacuolation, necrosis, increased pool of macrophages aggregates and changes in hepatocytes structure and distribution (Noaksson *et al.*, 2005; Grinwis *et al.*, 2000). Pathological evidence in fish kidney attributed to pollutants, include breakdown of tubules, vacuolation, haemopoietic tissue reduction and melanomacrophage aggregation (Handy and Grosell, 2002).

Environmental pollution may change the structure of red and white cells (Katalay and Parlak, 2004). Haematological parameters as Red Blood Cells (RBC); Haemoglobin content (Hb); Packed Cell Volume (PCV) and White Blood Cells (WBC) are most commonly used to diagnose fish health (Davidson *et al.*, 2002). Decreased levels of RBC, Hb, PCV and increased WBC count, neutrophilia and eosinophilia were previously recorded in some species of marine and freshwater fish (Barsiene *et al.*, 2006; Jee *et al.*, 2006; Ovuru and Ekweozor, 2004).

Pollution in Egyptian Eastern Coast had a detrimental effect on its fauna and flora. The main source of pollution there is El Tabia pump station in Abu Qir region that receives mixed waste products about 20.55×10^4 m³/day. Untreated effluents, are pumped directly to seawater at the western part of Abu Qir bay that represent the most polluted part of the bay. Beside domestic waste the station receive industrial waste product from fertilizer, electrical, paper manufacture and petroleum factories as well as agriculture drainage (Nessim *et al.*, 1994).

This study aimed to investigate the effect of diluted levels of seawater in front of the outfall of El-Tabia Pump Station effluents on gonadal activity, hematological parameters, as well as histopathological changes in some vital organs of *Siganus rivulatus.*

MATERIALS AND METHODS

Healthy *Siganus rivulatus* fish of average length of 15.4 cm and average weight of 44.2 g were acclimatized for 2 weeks in aerated aquarium (80 L). They were fed on artificial diet (40% protein and 8.5% fat) throughout the holding and experimental period.

Samples of seawater in front of the outfall was collected in plastic containers (during 2008-2009) and stored at 4°C. Analysis was clearly demonstrated in

Table 1: Characteristics of effluent-water mixture

Parameter	Standard limits (Egypt)	Measured values (Mean±SD)
pH	6-9	8.10±0.13
Total solid (mg L⁻¹)	1200	3308.00±158.5
Fixed solid (mg L⁻¹)	30	2140.00±98.70
Volatile solid (mg L⁻¹)	-	987.00±49.50
Phosphate (mg L⁻¹)	1	1.16±0.02
Sulphate (mg L⁻¹)	1	4.59±0.91
Chemical oxygen demand (mg L⁻¹)	40	430.00±26.20
Biological oxygen demand (mg L⁻¹)	-	310.00±14.50
Trace metals		
Copper (mg L⁻¹)		0.12±0.02
Zinc (mg L⁻¹)		0.20±0.04
Lead (mg L⁻¹)		0.15±0.02
Ferrous (mg L⁻¹)		0.90±0.28
Cadmium (mg L⁻¹)		0.12±0.04
Total trace metals (mg L⁻¹)	1	1.49±0.12

Table 1. The medium lethal concentration at which 50% of the fish died was determined (96 hr-LC_{50}) (Franson, 1980).

For the chronic test, seventy two fish were randomly distributed among 8 glass tanks for concentrations of 7.5, 10 and 15 ml L⁻¹ of polluted water and blank (seawater 36‰) for 30 days (2 replicates for each concentration). Blood samples were collected at intervals of 4, 15 and 30 days by caudal excision and stabilized with 50 IU sodium heparin. Erythrocyte count (RBC), Packed Cell Volume (PCV), Haemoglobin content (Hb) and White Blood Cell count (WBc) were determined Lewis *et al.* (2006). Blood sugar level was determined using diagnostic system (COBAS INTEGRA). Blood films and kidney prints were fixed and stained using Giemsa stain.

Unheparinized blood was collected from control and treated fish at end of 30 days of exposure and left at 4°C for 24 h to clot, then sera were collected and analyzed for steroids level using Radio-Immunoassay test (Pantex I^{125} kit).

Biopsy specimens were collected from gills, liver, kidney and gonads, fixed in formal saline, dehydrated, embedded in wax (melting point 56°C), sectioned at 4 μ, stained with Hematoxylin and Eosin and examined according to Culling (1974).

Statistical analysis: Results were analyzed for comparison among different polluted water treatments using one way Analysis of Variance (ANOVA) according to Snedecor and Cochran (1982). Duncan's Multiple Range-Test (Duncan, 1955) was applied to determine statistically significant differences, possibilities less than 0.05 were considered statistically significant ($p<0.05$). Program of MSTAT-C was used to compute the statistical analysis (MStat-C, 1988).

RESULTS

The physico-chemical characteristics of the effluent-water mixture are summarized in Table 1. It is clear that all

Table 2: Mean haematological values recorded at different periods in *Siganus rivulatus* exposed to different effluent-water levels (n = 5)

Haematologial indices	Exposure											
	4 days				15 days				30 days			
	0% control	7.5 ml L^{-1}	10 ml L^{-1}	15 ml L^{-1}	0% control	7.5 ml L^{-1}	10 ml L^{-1}	15 ml L^{-1}	0% control	7.5 ml L^{-1}	10 ml L^{-1}	15 ml L^{-1}
RBCs×(10^6/mm^3)	2.65±0.20[a]	2.57±0.15[a]	2.53±0.12[a]	2.32±0.13[b]	2.58±0.13[a]	2.31±0.10[b]	2.42±0.21[b]	2.01±0.02[c]	2.52±0.12[a]	2.19±0.11[b]	1.97±0.25[bc]	1.81±0.22[c]
WBCs×(10^3/mm^3)	13.21±0.30[a]	14.10±0.10[a]	14.91±0.09[b]	15.40±0.03[b]	13.50±0.21[a]	14.50±0.19[b]	15.70±0.29[bc]	16.23±0.52[c]	13.8±0.10[a]	14.70±0.11[a]	15.50±0.49[b]	16.43±0.71[b]
Haemoglobin content (Hb) (g/100 mL)	6.79±0.03[a]	6.60±0.25[a]	6.04±0.13[b]	5.85±0.14[b]	6.64±0.08[a]	6.31±0.06[a]	5.89±0.27[b]	5.60±0.10[bc]	6.48±0.02[a]	6.20±0.41[a]	5.74±0.13[b]	5.50±0.22[b]
Packed cell volume(%) (PCV)	43.73±0.03[a]	40.95±0.37[b]	39.80±0.69[b]	37.30±1.10[bc]	42.86±0.16[a]	40.27±0.16[b]	38.71±0.32[b]	36.91±0.20[bc]	42.79±0.18[a]	40.02±0.08[b]	37.99±0.01[c]	36.88±0.05[c]
Blood sugar level (m/100 mL)	64.5±0.40[a]	74.80±0.47[b]	78.60±1.89[b]	94.90±2.19[c]	64.75±0.14[a]	63.80±0.09[a]	60.01±1.10[b]	57.50±0.31[c]	63.35±0.14[a]	61.50±0.53[a]	58.90±0.69[b]	51.37±1.25[c]

Values are mean±SE of 3 replicates. Mean values in a row with different superscript letters are significantly different (p<0.05)

Table 3: White blood cells count(mean±SE) in *Siganus rivulatus* exposed to different effluent-water levels for different periods (n = 3)

WBCs (%)	Time of Exposure											
	4 days (acute exposure)				15 days				30 days (chronic exposure)			
	0% control	7.5 ml L^{-1}	10 ml L^{-1}	15 ml L^{-1}	0% control	7.5 ml L^{-1}	10 ml L^{-1}	15 ml L^{-1}	0% control	7.5 ml L^{-1}	10 ml L^{-1}	15 ml L^{-1}
Agran.	76.0±0.1[a]	76.0±0.3[a]	75.5±0.5[a]	73.5±0.2[b]	75.5±0.2[a]	73.5±0.5[b]	72.0±0.3[b]	70.0±0.1[c]	74.5±0.2[a]	71.5±0.5[b]	69.0±0.4[c]	68.5±0.1[c]
L.L.	10.0±0.2[a]	10.5±1.0[a]	10.5±0.5[a]	10.5±1.1[a]	11.0±0.2[a]	11.5±0.1[a]	11.5±0.3[a]	11.5±0.7[a]	10.0±0.9	11.5±0.3[b]	12.5±0.1[b]	12.0±0.5[b]
S.L.	65.0±2.2[a]	65.5±3.0[a]	65.0±2.0[a]	63.0±1.0[b]	64.0±1.1[a]	62.0±1.6[b]	60.1±2.4[c]	58.5±2.1[c]	64.5±2.0[a]	60.0±1.8[b]	56.5±2.3[c]	56.5±3.2[c]
Gran.	24.0±1.0[a]	25.5±1.1[a]	26.0±0.5[b]	26.5±1.0[b]	24.5±0.4[a]	27.5±0.5[a]	29.0±1.0[c]	30.0±2.0[c]	25.5±1.0[a]	29.0±0.5[b]	30.5±1.1[b]	31.5±2.1[c]
Nt.	17.5±0.5[a]	17.5±0.3[a]	19.5±0.5[b]	18.5±0.1[b]	18.0±1.1[a]	19.5±0.5[a]	20.0±1.1[b]	20.0±0.3[b]	18.5±0.2[a]	19.5±1.0[a]	21.0±0.3[b]	20.5±1.2[ab]
Es.	6.0±0.1[a]	6.5±0.3[ab]	7.5±0.16[b]	7.5±0.20[b]	6.0±0.34[a]	8.0±0.11[b]	8.5±0.18[b]	9.5±0.10[ab]	6.5±0.22[a]	9.5±0.15[b]	9.5±0.35[b]	10.0±0.11[b]
Bs.	0.5±0.0	0.5±0.0	-	0.5±0.1	0.5±0.0	-	0.5±0.1	0.5±0.1	0.5±0.0	-	0.5±0.1	0.5±0.1

Values are mean±SE of 3 replicates. Mean values in a row with different superscript letters are significantly different (p<0.05). Agranulocytes: Agran., Large lymphocytes: L.L., Small lymphocytes: S.L., Granulocytes: Gran., Neutrophils: Nt., Eosinophils: Es., Basophils: Bs

the parameters values were higher than the Egyptian Agriculture Ministry standards limits (Law 48/1982). The LC_{50} (96 h) was found to be 40 ml L^{-1} of effluent.

Impact of pollutant on blood constituent: No mortality was observed in the control and treatments throughout the experiment. Table 2 shows the mean values of the haematological indices recorded from exposing *Siganus rivulatus* to effluent- seawater levels (7.5, 10 and 15 ml L^{-1}) for 4, 15 and 30 days. After 4 days of exposure only in concentration 15 ml L^{-1}, the number of Red Blood Cells (RBC) undergone a significant decrease 2.32±0.13 compared to 2.65±0.20 ($10^6/mm^3$) in control. Moreover, fish exposed to the tested concentrations 7.5,10 and 15 ml L^{-1} for 15 and 30 days had significant lower number of RBC compared with their counterparts control. A maximum decrease in RBC number was at concentration of 15ml L^{-1} with values of 2.01±0.02 and 1.81±0.22 ($10^6/mm^3$) after 15 and 30 days of exposure compared to 2.58±0.13 and 2.52±0.12 ($10^6/mm^3$) in control ones. The Packed Cell Volume (PCV) data show a lower significant values for all treated groups, compared to control at all exposure periods. The greatest decrease was at concentration of 15 ml L^{-1} with values of 37.30±1.10, 36.91±0.20 and 36.88±0.05% after 4, 15 and 30 days of exposure compared to 43.73±0.03, 42.89±0.16 and 42.79±0.18% in control .On the other hand, a significant decrease was noted in the Haemoglobin concentration (Hb) after 4 days of exposure, became more pronounced after 15 and 30 days for levels 10 ml L^{-1} (5.89±0.27 and 5.74±0.13 mg/100 mL) and 15 ml L^{-1} with values of (5.60±0.10 and 5.50±0.22 mg/100 ml) compared to 6.64±0.08 and 6.48±0.02 mg/100 mL in control, respectively. The haematocrit and haemoglobin values in the treated fish showed a progressive decrease with time, leading to anaemic condition that progressed to a macrocytic hypochromic type after 30 days of exposure in higher levels (10 and 15 ml L^{-1}).

After 4 days of exposure to effluent- seawater levels of 7.5, 10 and 15 ml L^{-1}, blood sugar level showed a significant increase (74.80±0.47, 78.60±1.89 and 94.90±2.19 mg/100 mL), respectively, compared to (64.50±0.70 mg/100 mL) in control. In contrast, blood sugar level decreased after 15 and 30 days upon exposure to 10 and 15 ml L^{-1}. It became more pronounced in fish exposed to 15 ml L^{-1} of effluent-water mixture (57.50±0.31, 51.37±1.25 mg/100 ml) in comparison to (64.75±0.14, 63.35±0.14 mg/100 mL) the control. A significant increase was observed in the White Blood Cells count (WBC) in all treated groups, compared to control at all exposure periods. A maximum increase in WBC number was at concentration of 15 ml L^{-1} with values of 16.43±0.71 $10^3/mm^3$ after 30 days of exposure compared to 13.80±0.10 $10^3/mm^3$ in control .

Leukocyte profile is given in Table 3. Exposure to effluent-water levels resulted in decrease in Agranulocytes percentage as a result of Small Lymphocytes (SL) decrease and an increase in granulocytes percentage due to increase in Neutrophils (Nt) and Eosinophils (Es) numbers. After 4 days small lymphocytes decreased significantly at 15 ml L^{-1} with value of 63.0±01.00 to 65.00±2.20% in control. In contrary, there was a significant increase in both neutrophilic and eosinophilic numbers at concentration 10 ml L^{-1} with value of 19.50±0.50 and 7.50±0.16% compared to 17.50±0.50 and 6.00±0.15% in control, respectively. While at concentration 15 ml L^{-1} they had values of 18.50±0.10 and 7.50±0.20% compared to 17.50±0.50 and 6.00±0.15% in control. Changes were more pronounced at higher levels (10 and 15 ml L^{-1}) and persisted to the end of experiment where lymphocytes recorded lowest value of 56.50±3.20% compared to 64.50±2.00% in control after 30 days of exposure at concentration 15 ml L^{-1}. While (Nt) and (Es) recorded the highest value 20.50±1.20 and 10.00±0.11% at the same concentration and the same period of exposure compared to values of 18.50±0.20 and 6.50±0.22% in control, respectively.

Blood smears of control fish are shown in Fig. 1. Red blood cells are oval shaped cells with homogenous cytoplasm and round nucleus with compact chromatin materials. Blood smears of fish exposed for 4 days at concentration 15 ml L^{-1} are shown in Fig. 2, revealed an increases in number of neutrophil (Nt) Fig. 2a. Higher percentage of deformed erythrocytes, pear and tear shaped erythrocytes can be seen Fig. 2b, also swelling erythrocytes with fading cytoplasm, indicating a decrease in its haemoglobin content were noticed Fig. 2c. After 15 days of exposure (Fig. 3), blood film of fish at the higher effluent-water levels 10 and 15 ml L^{-1} showed abnormality. An increase in neutrophils number (Fig. 3a), large lymphocytes (L.L) (Fig. 3b) also senile erythrocytes (Fig. 3c) were shown in fish exposed to concentrations of 10 ml L^{-1}. Blood film of fish after 30 days of exposure are seen in Fig. 4. Fish at exposure concentrations of 10 and 15 ml L^{-1} showed neutrophilia. Neutrophils of different developmental stages could be seen in Fig. 4a. Erythrocytes with ragged outlines were seen in blood smears of fish at effluent-water concentrations of 10 ml L^{-1} (Fig. 4b) and 15 ml L^{-1} (Fig. 4c).

Kidney prints of fish exposed to concentrations of 15 ml L^{-1} of effluent-water mixture for 4 days are represented at (Fig. 5). It revealed great numbers of Haemocytoblast (Hcb) which is the mother cell of all blood cells (Fig. 5a) and great numbers of Myelocyte (M), which is the mother cell of white blood cells (Fig. 5b). After 15 days of exposure Fig. 6, Lymphocytes (L) showed high numbers at concentration of 10 ml L^{-1} of
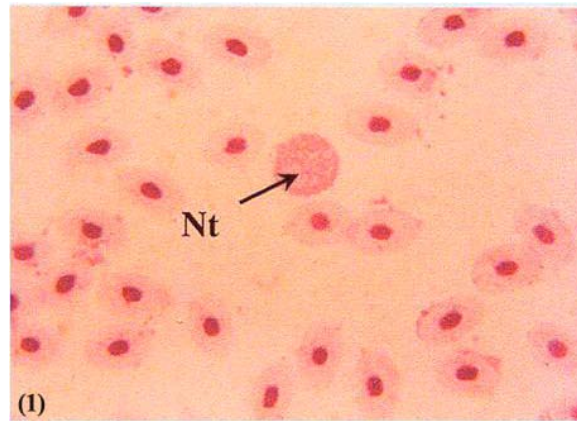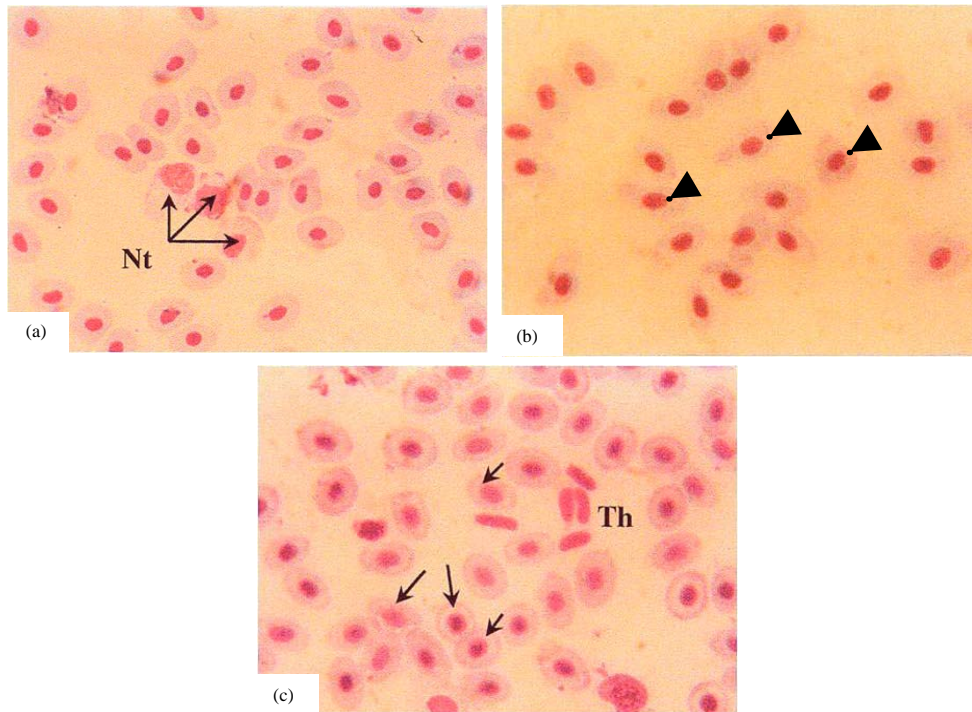
Fig. 1: Blood film of control fish

Fig. 2(a-c): Blood film of fish exposed to 15 ml/l effluent for 4 days. (a) Increased neutrophils (Nt) thrombocytes (Th), (b) pear shaped erythrocytes [head arrows] and (c) Swelling erythrocytes (arrows)

effluent-water mixture (Fig. 6a). Also eosinophilic and neutrophilic myelocytes were higher in fish at 15 ml L^{-1} of effluent water mixture (Fig. 6b). At the end of the experiment, after 30 days of exposure (Fig. 7). Haemocytoblasts of variable sizes could be seen at concentrations of 10 ml L^{-1} of the effluent water mixture (Fig. 7a). The neutrophilic and eosinophilic myelocytes greatly increased at concentrations of 15 ml L^{-1} of the effluent water mixture (Fig. 7b).

Histopathological changes in fish organs
Gonads
Ovary: Histological changes in gonads of exposed fish occurred only at higher concentrations. Histological
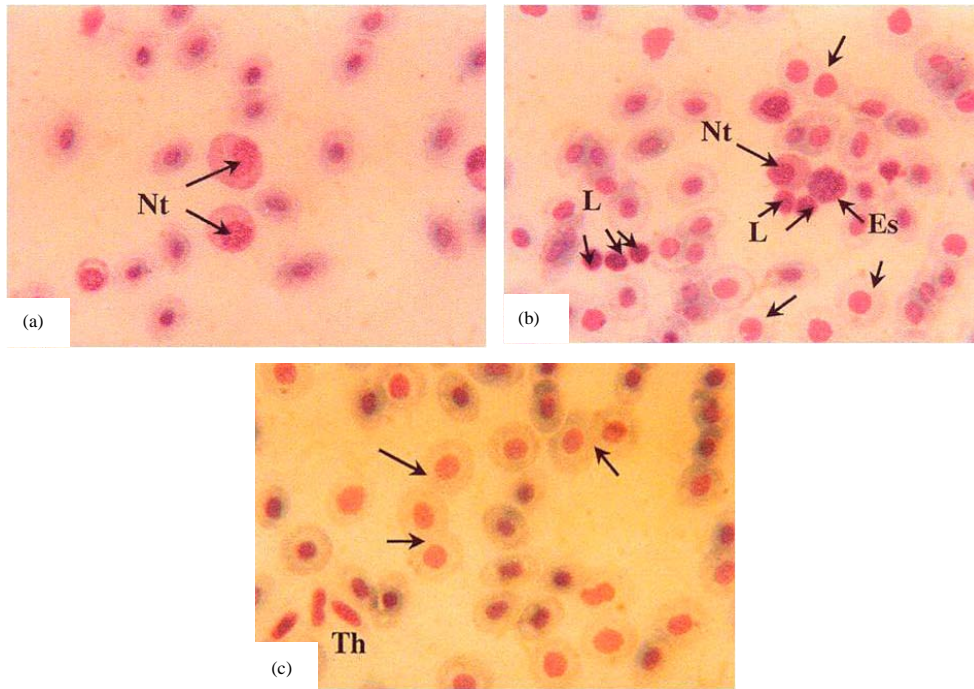
Fig. 3(a-c): Blood film of fish exposed to 10 ml L^{-1} effluents for 15 days, notice (a) Neutrophils (Nt), (b) Eosinophils (Es), large lymphocytes (L) and (c) Senile erythrocytes (arrows)
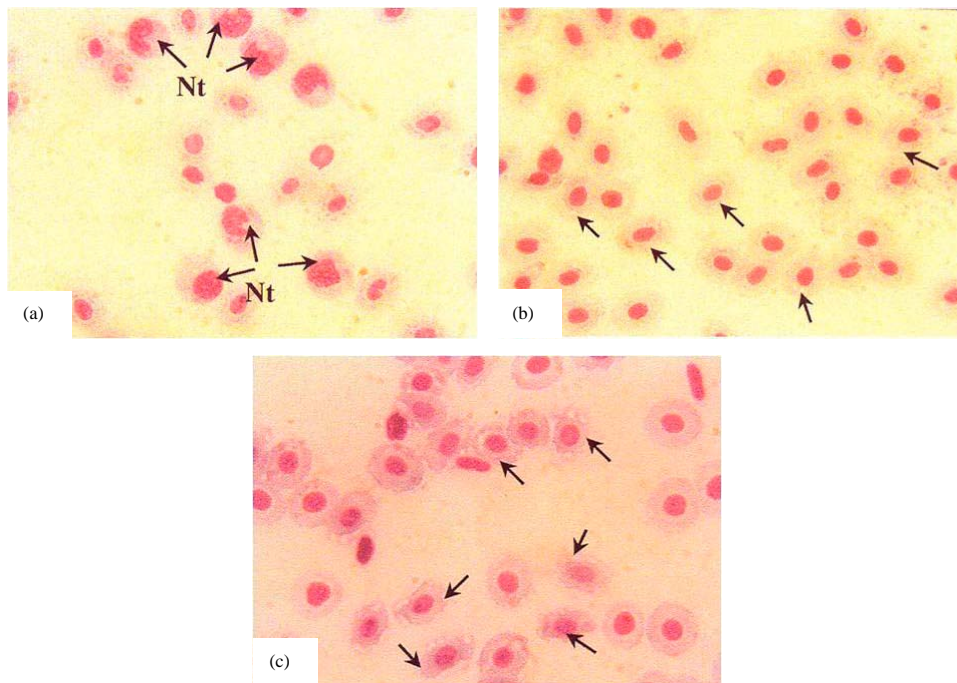

Fig. 4(a-c): Blood film of fish exposed to 15 ml L^{-1} effluents for 30 days, notice. (a) Neutrophilia and (b,c) Ragged vacuolated erythrocytes (arrows)
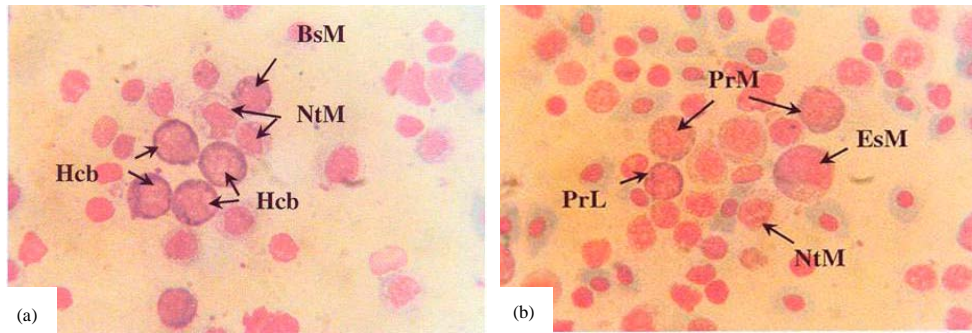
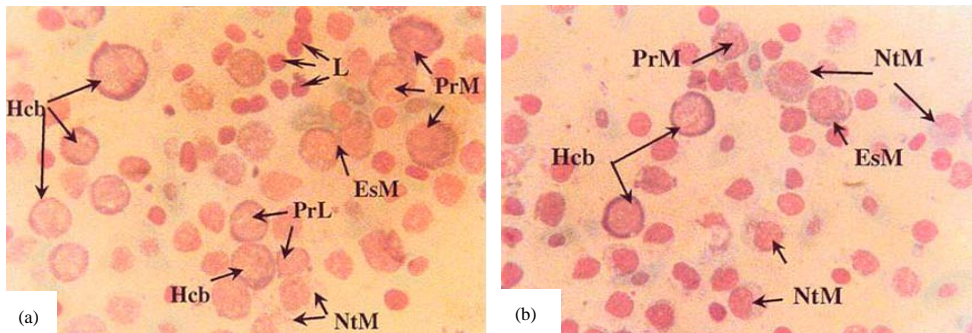Fig. 5(a-b): Kidney print of fish exposed to 15 ml L^{-1} effluent for 4 days Fig. 5a, b

Fig. 6(a-b): Kidney print of fish exposed to 10 and 15 ml L^{-1} effluent for 15 days, 10 ml L^{-1} for 15 days (a) 15 ml L^{-1} for 15 days
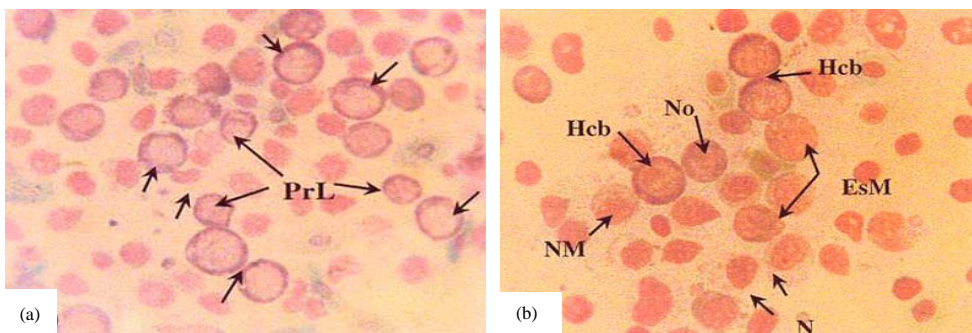

Fig. 7(a-b): Kidney print of fish exposed to 10&15 ml/l effluent for 30 days, 10 ml/l for 30 days (a) 15 ml/l for 30 days, (b) Haemocytoblast (Hcb), Neutrophilic myelocyte (NtM), Basophilic myelocytes (BsM), Promyelocytes (PrM), prolymphocyte (PrL), Eosinophilic myelocyte (EsM), Lymphocyte (L), Neutrophil (N), Normoblast (No)

changes in ovaries are shown in Fig. 8. In control fish, oocytes in the early perinucleolus and late perinucleolus stages are detected. Their cytoplasm is homogeneous and has purple color. The nuclei have distinct nucleoli and normal distribution of chromatin network (Fig. 8a). Ovary of fish exposed to different

effluent-water levels showed abnormalities, including both nuclei and cytoplasm. The cytoplasm of early and late perinucleolus oocytes stained deep purple. The perinucleolus oocytes appeared as solid mass, while the late perinucleolus oocytes became atretic showing liquefied cytoplasm, deformed nucleoli and
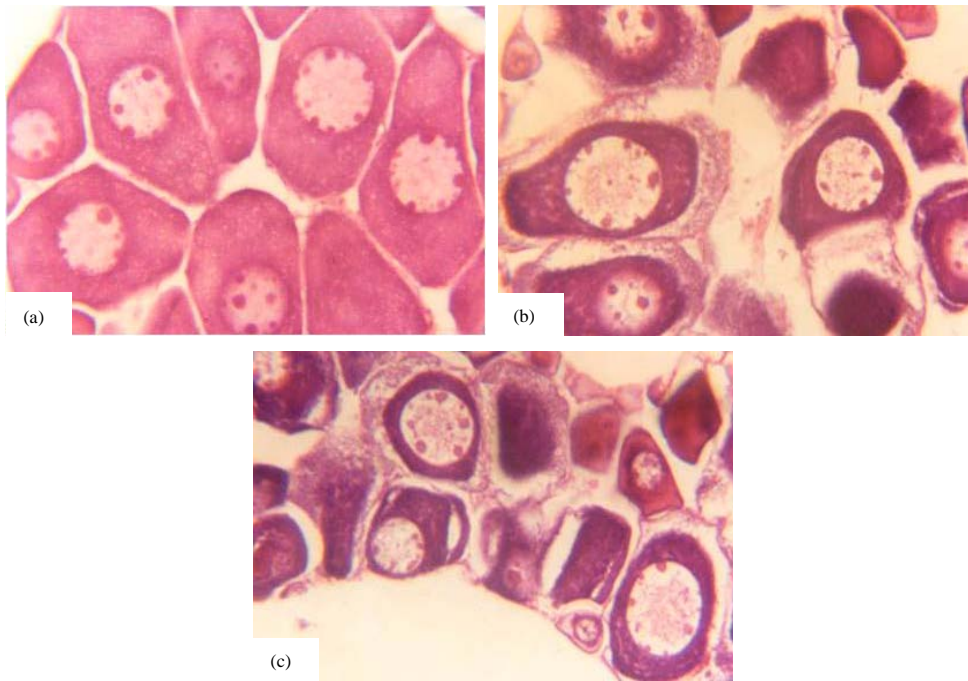
Fig. 8(a-b): Cross section in ovary of fish after 30 days, (a) Control, (b) 10 ml L^{-1} and (c) 15 ml L^{-1}of effluent-water mixture
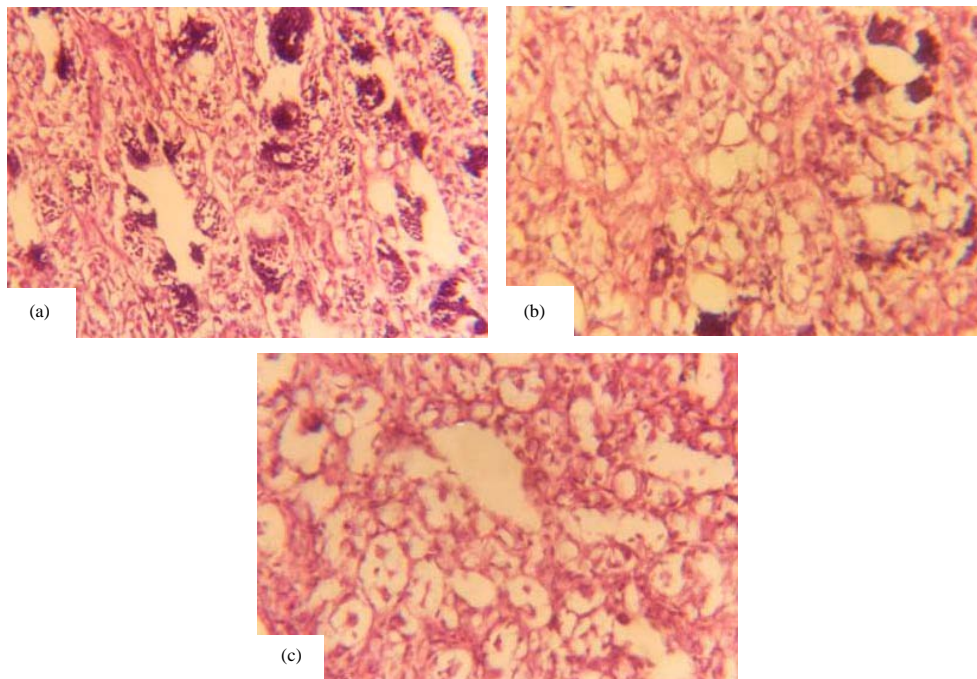

Fig. 9(a-b): Cross section in testis of fish after 30 days, (a) Control, (b) 10 ml L^{-1} and (c) 15 ml L^{-1} of effluent-water mixture

crumpled chromatin network at levels of 10 ml L^{-1} Fig. 8b and 15 ml L^{-1} of effluent-water mixture (Fig. 8c).

Testis: Histological changes in testis are shown in (Fig. 9). In testis of control fish, seminiferous tubules have definite interlobular membrane. The spermatogenic cell layer posses primary and secondary spermatocytes (Fig. 9a). In males exposed to effluent-water levels of 10 ml L^{-1} the lobules have damaged membrane and spermatogenic cell layer (Fig. 9b). In case of 15 ml L^{-1}, great necrosis of spermatogenic cell layer and complete disintegration of interlobular membrane were detected (Fig. 9c).

Sex hormones: No significant changes were found in levels of tested hormones in fish exposed to concentrations of 7.5 and 10 ml L^{-1} after exposure period of 30 days. Radio-immunoassay analysis of steroids level were shown in Table 4. Table 4 revealed that testosterone level in control females was higher than control males. Testosterone level has significantly decreased to 1.65±0.023 ng mL^{-1} in exposed females compared to 2.82±0.071 ng mL^{-1}) in control females, while in exposed males it showed insignificantly decrease.

Progesterone level decreased significantly to 0.31±0.013 ng mL^{-1} in exposed females, it has value of 0.50±0.010 ng mL^{-1} in control females. In contrary, progesterone level in exposed males showed significantly increased value 0.50±0.018 ng mL^{-1} than control males 0.20±0.014 ng mL^{-1}.

Liver: The changes existed in liver of fish due to exposure are seen in all effluent-seawater concentrations (Fig. 10). Liver of control fish showed the normal hepatic acini arrangement in cord of one layer thickening surrounding the bile duct. Hepatocytes are polygonal shaped cells with clear cellular border lines and rounded nuclei (Fig. 10a). In fish subjected to effluent- water concentration of 7. 5 ml L^{-1}, the proper liver structure became obliterated, melanomacrophage aggregated, cytoplasmic vacuoles and pycnotic nuclei were observed (Fig. 10b). Hepatocytes necrosis occurred at concentrations of 10 and 15 ml L^{-1}, cells were hypertrophied with vacuolated cytoplasm and pycnotic nuclei are seen at 15 ml L^{-1} effluent-seawater mixture (Fig. 10c).

Kidney: The changes existed in kidneys of fish due to exposure are seen in all effluent-seawater concentrations (Fig. 11). Kidney of control fish showed uriniferous tubules with distinct epithelial cells and darkly stained nuclei. Haemopoietic tissue existed between the tubules Fig. 11a. At effluent-water concentration of 7.5 ml L^{-1}, cells
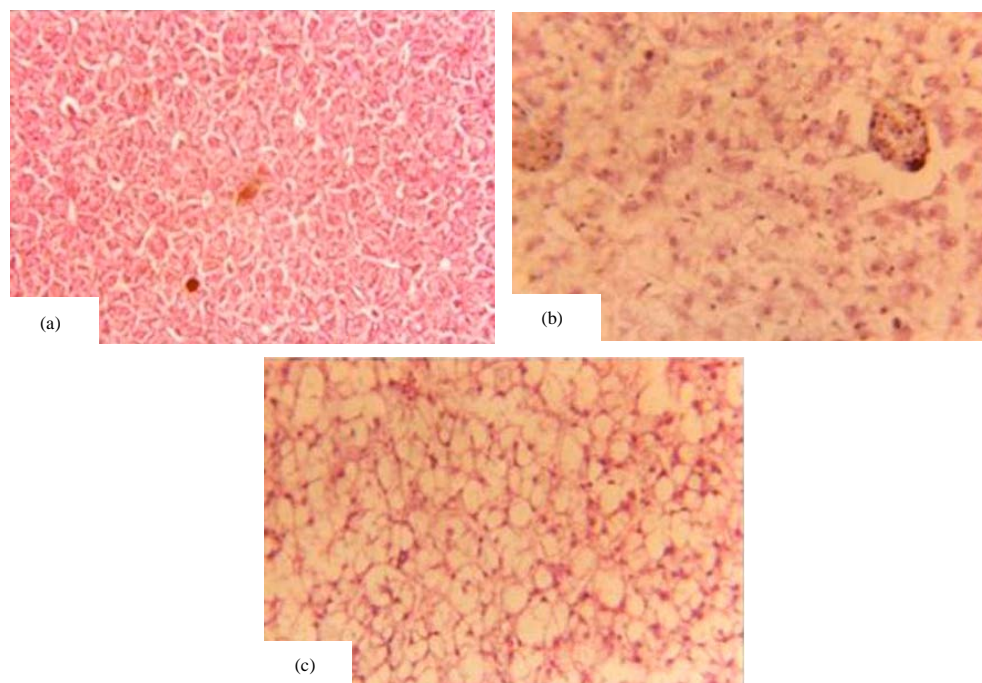

Fig. 10(a-b): Cross section in liver of fish after 30 days, (a) Control, (b): 7.5 ml L^{-1} and (c) 15 ml L^{-1} effluent-water mixture
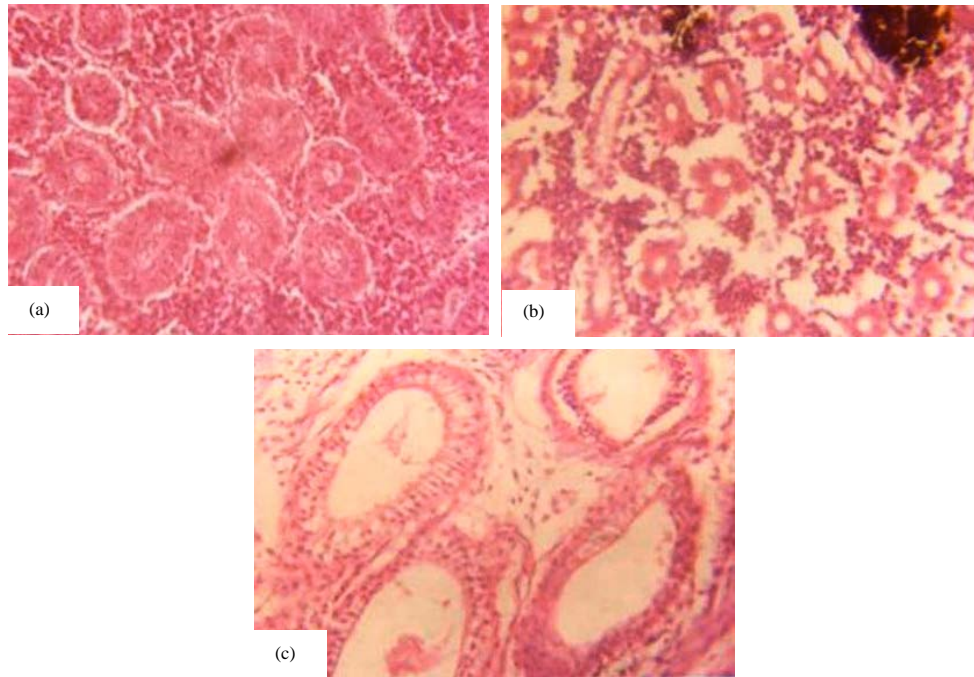
Fig. 11(a-b): Cross section in kidney of fish after 30 days, (a) Control, (b) 7.5 ml L^{-1} and (c) 15 ml L^{-1} effluent-water mixture
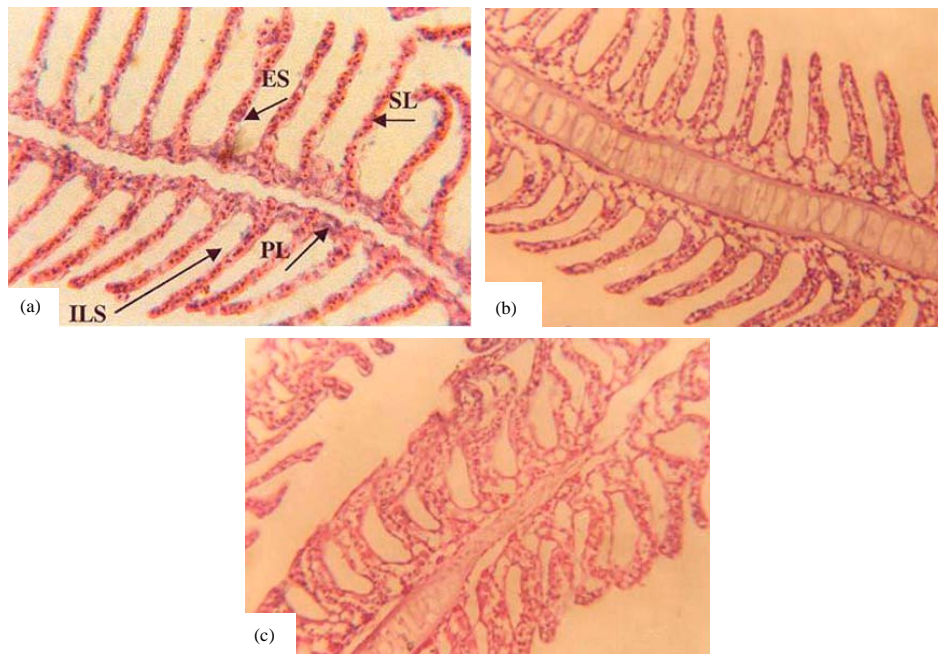

Fig. 12(a-c): Frontal section in gills of fish after 30 days, (a) Control, (b) 10 ml L^{-1} effluent-water mixture and (c) 15 ml L^{-1} effluent-water mixture. Primary lamellae (PL), Secondary lamellae (SL), Inter-lamellar space (ILS), Epithelial cells (ES)

Table 4: Sex hormones levels in control and exposed *Siganus rivulatus* after 30 days of exposure to water-effluent level of 15 ml L^{-1}

	Hormone			
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	Testosterone ng mL^{-1}		Progesterone ng mL^{-1}	
Sex	Control	15 ml L^{-1}	Control	15 ml L^{-1}
Maturing ♀	2.82±0.071[a]	1.65±0.023[b]	0.50±0.010[a]	0.31±0.013[b]
Maturing ♂	2.73±0.065[a]	2.65±0.069[a]	0.20±0.014[a]	0.50±0.018[b]

Values are mean ±SE of 5 replicates. Mean values in a row with different superscript letters are significantly different (p<0.05)

disintegration, vesicular highly staining cytoplasm, nuclear swelling, melanomacrophage aggregation and reduced haemapoietic tissue were detected in the kidney (Fig. 11b). At higher levels of 10 and 15 ml L^{-1}, haemopoietic tissue was greatly reduced Severe destruction of tubular epithelium was observed at 15 ml L^{-1} effluent seawater mixture (Fig. 11c).

Gills: The changes existed in gills of fish due to exposure are seen in all effluent-seawater concentrations Fig. 12. Gills of control fish group exhibit normal architecture, consist of primary and secondary lamellae originated from either sides covered by epithelial cells. Underneath the epithelial covering of secondary lamellae is the supporting layer of pillar cells separated by lacunae where blood flow and gas exchange take place (Fig. 12a). Histological examination of fish gills at concentrations of 10 ml L^{-1} of effluent water mixture showed the most striking features being lamellar sinus widening and a pronounced lifting and desquamation of epithelium of secondary lamellae (Fig. 12b). At concentration of 15 ml L^{-1}, complete denudation of cellular structure of lamellae occurred. Also, pillar supporting layer underneath was greatly reduced (Fig. 12c).

DISCUSSION

The present study revealed an interesting pattern of response of the haematological variables in exposed fish. In addition, different durations of exposure and different concentrations resulted in an anemic condition in fish, as shown from decreased RBC, PCV and Hb concentrations. From the present results, reduction in RBC count, PCV and Hb concentrations of tested fish compared to control may be due partly to the presence of heavy metals and other pollutants. These pollutants may cause RBC lysis . Previous studies had shown a decrease in RBC counts, relative PCV and Hb values when fish were exposed to Cassava effluent (Adekunle *et al.*, 2007), diazinon (Svoboda *et al.*, 2001) and textile dyes (Al-Sabti, 2000). These findings are in agreement with the present results. In contrary an increase in RBC, Hb and Ht value were recorded in African catfish on exposure to Gold crew (Alagoaa and Ekweozorb, 2009), to copper

(Mazon *et al.*, 2002b) and in Nile Tilapia and catfish exposed to lead (Al-Akela and Shamsia, 2000). Deformed RBCs were detected on exposure to cadmium (Witeska *et al.*, 2006) and environmental pollution (Pacheco and Santos, 2002)

The distinct decrease in the level of Hb and PCV, clearly suggests haemodilution. In this instance, the change from normochromic anaemia after 5 days of exposure to hypochromic macrocytic anemia at higher levels of effluent- water mixture (10, 15 ml L^{-1}), after 15 and 30 days of exposure is probably an adaptive response through the influx of immature erythrocytes from the haemopoietic tissue to the peripheral blood to make up to reduced RBC number and decreased haemoglobin concentration. These findings further support the hypothesis that haemodilution is a probable cause for decrease in Hb content in exposed fish. As a conclusion, the diluted effluent-water levels exert a profound influence on health condition of *Siganus rivulatus* after acute and chronic exposures. As a result, normochromic anemia has occurred, eventually leading to hypochromic, macrocytic anemic condition attributable to the swelling of RBC, haemodilution and impaired haemoglobin synthesis. Elahee and Bhagwant (2007) attributed this anemia to hypoxic conditions arising from gills degradation. Also, Kori-Siakpere and Ubogu (2008) reported normochronic anemia in *Heteroclarias* sp. due to zinc exposure.

In the present study, leukocytosis in *Siganus rivulatus* exposed fish may be attributed to increased leukocyte mobilization to protect the body against infection. This result is in agreement with previous studies of *Prochilodus scrofa*, due to copper exposure (Mazon *et al.*, 2002b).

Neutrophils are matured leukocytes that control infections. The observed increase in percentage of neutrophils of the test fish may be due to the presence of pathogens in the polluted water as reflected by high BOD. This suggestion is supported by the increase in the WBC count in tested fish compared to control. WBC fights against infections, therefore the increase observed in the WBC of *Siganus rivulatus* in present work may be due to infection arising from pollution. Lymphopenia and both neutrophil and eosinophil ganulocytosis were reported in *Oreochromis niloticus* exposed to sublethal level of

nickel (Alkahem, 1994). Also, lymphopenia was detected as a consequence of cypermethrin pesticide in *Channa punctatus* (Saxena and Seth, 2002). In contrary lymphocytosis was found on exposing *Heteroclarias* sp. to zinc by Kori-Siakpere and Ubogu (2008).

Exposure of *Siganus rivulatus* to the present effluent-water levels was found to have a detrimental effect on gonadal development. Histological changes in gonads were most pronounced at higher levels. Among the effects observed are inhibition of spermatogenesis, lobule boundary cells disintegration and atresia of early stages of oocytes. These morphological changes occurred in medaka exposed to sewage effluent (Yinnis *et al.*, 2003.) and are qualitatively similar to those documented for *Siganus rivulatus* exposed to copper works effluent (Wahbi, 2004a). The present results predict atresia of developing oocytes. Similar effects have been demonstrated in white perch in areas affected by domestic and industrial effluents (Richard *et al.*, 2004), in salmon chronically exposed to sewage effluent (Luis *et al.,* 2002). Disintegration of lobule boundary cells and alteration of ovarian development were induced by lowering pH values in *Diplodus sargus* (Wahbi, 2004b)

In the present study, levels of testosterone and progesterone in both sexes have altered. This alteration is a direct result whether due to disruptions of the pituitary-gonadal axis or gonadal damage. As a result, it indirectly affects the development of oocytes and yolk formation, so fish can't reach maturity. Reduction in reproductive steroids were reported in cyprinid fish due to heavy metals (Ebrahimi and Taherianfard, 2011), zebra fish exposed to contaminated sediment (Linderoth *et al.*, 2006) and white seabream exposed to lowered pH values (Wahbi, 2004b).

Histopathological alterations in exposed fish in this study were previously described by several authors (Olivaa *et al.*, 2010; Wahbi *et al.*, 2010; Van Dyka *et al.*, 2009; Velmurugana *et al.*, 2009; Fadel and Gaber, 2007), also following metals exposure (Giari *et al.*, 2008; Figueiredo-Fernandes *et al.*, 2007). Increased kidney necrosis was determined in Tilapia (Handy and Grosell, 2002). Macrophages aggregates in kidney of *Prochilodus lineatus*, subjected to a disturbed urban stream was described by Camargo and Martinez (2007). Vacuolation, necrosis and macrophages aggregates in liver of perch and flounder were detected (Noaksson *et al.*, 2005; Grinwis *et al.*, 2000) also liver necrosis were witnessed in *Clarias gariepinus* from polluted aquatic system (Marchand *et al.*, 2009), due to zinc (Loganathan *et al.*, 2006).

Gills alterations, as epithelial layer lifting, necrosis and frequent epithelial rupture were reported by Wynn and Waring (2002). In the present study, the changes recorded in gills of *Siganus rivulatus*, due to exposure, may represent a defense response, as it increase the distance across which pollutant must diffuse to reach the blood stream.

CONCLUSION

The effluent-water levels had negative direct or indirect effects on the haematological parameters, liver, kidney, gills and gonadal development in both males and females *Siganus rivulatus*. The blood parameters of *Siganus rivulatus* not only revealed cellular disturbances but also adaptive responses. Increase in leukocytes implies a mobilization of cell defense, although the reduction of the small lymphocytes percentage suggests a secondary effect of effluent. On the other hand, the decrease in red blood cell parameters (RBC, Hb and PCV) indicates an aneamic condition which gradually progresses upon prolonged exposure eventually cause hypochromic macrocytic anemia attributed to the swelling of the red blood cells, haemodilution and impaired haemoglobin synthesis. The increase in developing haemocytoplast and myelocytes emphasizes the compensatory and defensive reaction of fish to pollution. The pollutant altered reproductive hormones level, leading to necrotic changes in testis and atresia in oocytes. Also it has necrotic effect on fish organs.

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