Reproduction and histomorphology of Nile Tilapia, *Oreochromis niloticus* collected from two different water sources

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Abstract: Nile Tilapia were collected from of two sites. El- Takadom lake (site A) -lies at the east of Suez Canal- which receives fresh irrigation water from Ismailia lake though a siphon underneath the Suez Canal. Baloutha bank (site B) -lies at the south of Sahl El- Tina- which receives agricultural drainage water out of lands irrigated with El-Salam lake fresh water. Heavy metals (Cu, Ni, Fe, Mn and Zn) were significantly higher in the agriculture drainage water and the bioaccumulation of these metals was elevated in the muscles of the studied fishes. The condition factor of site B fishes were significantly (P<0.05) higher in both males (3.85 ± 0.14) and females (3.84 ± 0.10) comparing with fishes of site A. Gonadosomatic index mean values were 0.48 ± 0.04 and 0.26 ± 0.02 for the collected fish males from site A and B respectively, while females GSI from site B was higher (1.57 ± 0.19) than that of site A (1.05 ± 0.17). The mean values of egg diameters (49.68 ± 2.98) and the absolute fecundity (1121.97 ± 73.75) in ovaries of the females from the drainage water site were higher than those of site A. Histopathological changes in liver, kidneys, gills, ovaries and testis of Nile tilapia were described in details. The main alterations found in the liver of Nile tilapia collected from (site B) were cytoplasmic vacuolaion of the hepatocytes, vacuolar degenerated nuclei, blood congestion and dilatation of hepatic vein. While pyknotic nucleus and yellow brown granules of bile stagnation in the cytoplasm were identified in the liver samples of (site A).The male testis showed degenerated spermatogenic cells (site B) and vacuoles within the seminiferous lobules (site A).As for the ovary from fishes of both sites showed necrotic ova and degenerated stroma. The most gill deformed structures were lymphocytic infiltration, epithelial lifting congestion and fusion of gill lamellae (site B) and club shape filaments in (site A). Kidney alterations of tilapia from the two study sites were atrophy of glomerulus and hyperplasia of some renal tubules (site A), besides to expansion of space inside the Bowman's capsule, hematopiotic tissue depletion and degeneration in renal tubules (site B). In conclusion, the present study aims to investigate the effect of irrigation and agricultural drainage water on the reproductive parameter of Nile tilapia besides to the histological alterations of some organs due to the presences the heavy metals.

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1.Introduction

Fishes are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Mansour & Sidky, 2002). Furthermore, fish is one of the most indicative factors in freshwater systems, for the estimation of trace metals pollution and risk potential of human consumption (Barak & Mason, 1990; Papagiannis *et al.,* 2004). Heavy metals are taken up through different organs of the fishes. (Rao & Padmaja, 2000).

Heavy metal pollution in rivers has become a matter of great concern over the last few decades, not only because the threat to public water supplies, but also the hazards to human consumption of fishery resources (Terra *et al.,* 2007). Although many heavy metals are considered as essential macro and micro elements especially at non adverse effect levels (Borovick,1990 & Hossain and Khan, 2001), they can exert toxic effects at concentrations encountered in polluted environment.

Therefore, heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to the next trophic level of the food chain. Trace metals are introduced into the environment by a wide spectrum of natural and anthropogenic sources. Metals are non-biodegradable and once they enter the environment, bio-concentration may occur in fish tissues by means of metabolic and bio-absorption processes (Hogstrand & Haux, 1991 and Adeboyejo *et al.,* 2013).

The degree of contamination depend on pollutant type, fish species, sampling location, trophic level and their mode of feeding (Asuquo *et al.,*2004). For fish, the gills, skin and digestive tract are potential sites of absorption of water borne chemicals (Jovanovic *et al.*, 2011; Yacoub and Gad, 2012. The chemicals once absorbed are transported by the blood to either a storage point such as bone or to the liver. In the liver, it may be stored, excreted in the bile or passed back into the blood for possible excretion by the kidney or gills or stored in extra hepatic tissues such as fat (Dimari *et al.,*2008).

Similarly, histopathological investigations have long been recognized to be reliable biomarkers of stress in fish (Elahee and Bhagwant, 2007). Histopathological analysis has already been tested and proposed as an efficient and sensitive tool to the monitoring of fish health in natural water bodies (Costa *et al.,* 2009; Gaber and El-Kasheif, 2013).

The present study aims to investigate the effect of irrigation and agricultural drainage water on the reproductive parameter of Nile tilapia besides to the histological alterations of some organs due to the presences heavy metals.

2. Materials and Methods

About 80 wild Nile Tilapia were collected from each of two sites. The first site is El- Takadom lake (site A) -lies at the east of Suez Canal- which receives fresh irrigation water from Ismailia lake though a siphon underneath the Suez Canal. The second site Baloutha bank (site B) -lies at the south of Sahl El- Tina- which receives agricultural drainage water out of lands irrigated with El- Salam lake fresh water.

1–Heavy metals concentrations

Cu, Fe, Mn , Ni and Zn were measured in the water and fish muscles according to the method reported by American Public Health Association APHA (1989) using flame atomic absorption spectrophotometer (Perkin Elmer, 2280).

2- Growth and reproductive parameters

The total weights of the fish samples ranged from 36 to 55g and the lengths from 10.5 to 11.19 cm.

a. Condition coefficient (K)

According to Fulton's (1902), the calculation of condition factor (Cube Law) is expressed by the following equation:

$$K = W/L^3 \times 100$$

Where: W = the total fish weight (gm.), L = the observed fish body length (cm.)

b. Gonado - somatic index (GSI)

GSI was determined by the following formula:

GSI = GW / TW× 100 (Berhaut, 1973), where: GW = wet weight of the gonad to the nearest (0.001gm) ,TW = wet total weight of the fish to the nearest (0.1gm).

c. Egg diameter

Oocytes were separated from ovarian tissues and put in a saline solution (0.9% NaCl). About 50 oocytes were taken randomly, diameter was measured to the nearest 0.01µm by using an eye-piece micrometer on the binuclear microscope at a power magnification of 4X.

d. Fecundity

Absolute fecundity was estimated in the laboratory by counting all ripening eggs found in sub-samples (0.01 gm.) which was taken separately from anterior, posterior and middle region of each ovary lobe.

3- Histological studies

Gills, liver, kidneys and gonads were fixed in 10% formalin , then were processed and sectioned at 5 µm (ovaries were sectioned at 7 µm). Sections were stained with haematoxylin- eosin. The slides were then microscopically examined and photographed with a Leitz Diaplan microscope.

4- Statistical analysis

The obtained data were statistically analyzed by using one-way analysis of variance (ANOVA) procedure. Analysis system were done using SPSS program version SPSS Statistics ver. 18.0 (SPSS, Richmond, USA) as described by Dytham (1999). Means were compared using Duncan's test (1955). All data were expressed as means Standard Error. The significance the significance level was set at the probability level of $P<0.05$.

3.Results and Discussion

Table1, showed that higher significance ($P<0.05$) differences in the concentrations of recorded heavy metals (Cu, Ni, Fe, Mn and Zn) were in the agriculture drainage water (site B) rather than in the irrigation water (site A). The bioaccumulation of these metals was elevated in the studied muscles of the site B fishes. Fe recorded the highest values 1.18 ± 0.04 and 0.66 ± 0.01 in both water and muscles respectively. These results are in agreement with Zaghloul (2000) who attributed the increase of the heavy metals in drainage water to the decomposition of the organic matter and the use of fertilizers and chemical in agriculture.

Furthermore, Saeed (2000) stated that the agriculture drainage water usually contains higher Fe level than the irrigation water the same as El-Nemaki *et al.* (2008) findings of the impacts of different water resources on the quality and production of *Oreochromis niloticus* fish reared in two different farms at EL–Abbassa, Sharkia governorate.

Although zinc is an essential element, at high concentrations, it can be toxic to fish, cause mortality, growth retardation and reproductive impairment (Sorenson, 1991). Fish can accumulate zinc from both the surrounding water and from their diet (Eisler, 1993).

Table (1): Heavy metals concentrations (ppm) in both water and muscles of tilapia collected from two water sites

Heavy metals (ppm)	Site A	Site B
Water		
Cu	0.09[c]±0.03	0.20[a]± 0.01
Ni	0	0.36±0.02
Fe	0.93[c]±0.04	1.18[a]±0.04
Mn	0	0.09±0.02
Zn	0.06[c]±0.03	0.12[b]±0.04
Muscles		
Cu	0.15[c]±0.01	0.24[a]±0.03
Ni	0	0.48±0.02
Fe	0.43[b]±0.02	0.66[a]±0.01
Mn	0	0.09±0.02
Zn	0.14[c]±0.03	0.20[b]±0.03

Means followed by the same superscript in the same row are not significantly different according to Duncan's multiple range test ($P<0.05$).

The condition factor (k) is an index reflecting interaction between biotic and abiotic factors in the physiological condition of the fishes (was calculated to assess fish health productivity of fish populations) (Effendi, 1997; Shobikhuliatul *et al.,* 2013).

In the present study the average value of the allometric condition factor (k) of studied Nile tilapia were significantly ($P<0.05$) higher in the fishes of site B (3.85 ± 0.14 and 3.84 ± 0.10 for males and females respectively) comparing with fishes of site A which receives water from Ismailia Lake (Table2). Similar results were obtained by Ali (2007) who reported that the condition factor (2.49) of Nile tilapia grown in agriculture drainage water was higher than those grown in irrigation water. The same was El- Nemaki *et al.,*2008 who observed higher significant values of Condition factor (2.01± 0.11) for fishes from ponds water that received agriculture drainage water supply**.**

Studying fish reproduction based on some parameters such as the gonado-somatic index (GSI) which reflects the physiological activity of the gonads, where the increase is an indication of the beginning of the breeding season of the fish **(**Laban,, 2007**).**

Another parameter is fecundity which shows a wide variation among different species, and also within the individuals with the same length and age**.** It also fluctuated according to different environmental conditions (Amein, 1996). Also, detection of the oocyte diameter frequency helps to understand the nature of reproduction**.** It gives the information on either the fish contributes once or several times in the breading season (Amein, 1996).

In this work variations in the gonadosomatic index mean values were 0.48 ± 0.04 and 0.26 ± 0.02 for the collected fish males from site A and B respectively, while females GSI collected from site B was higher (1.57 ± 0.19) than that of site A (1.05 ± 0.17). The mean values of egg diameters (49.68 ± 2.98) in the ovaries of the females and the absolute fecundity (1121.97 ± 73.75) from the drainage water site were higher than those of site A Table 2. Similarly, higher significant values of Gonadosomatic index were observed by ponds water that received agriculture drainage water supply (El- Nemaki *et al.,*2008) which confirm the present results.

Table (3) : Growth and reproductive parameters of Nile tilapia collected from two water sites

Water sites	Sex	SL	Wt	K	GSI	Egg diameter	Fecundity
(Site A)	Male	10.52[b]±0.10	36.20[b]±0.59	3.13[b]±0.14	0.48[a]±0.04	---------	-------------
	Female	11.14±0.10	52.44[b]±2.63	3.72±0.14	1.05[b]±0.17	48.65[a]±4.36	440.75[b]±43.59
(Site B)	Male	11.19[a]±0.14	54.02[a]±1.23	3.85[a]±0.14	0.26[b]±0.02	-----------	--------------
	Female	11.27±0.11	54.48[a]±0.98	3.84±0.10	1.57[a]±0.19	49.68[b]±2.98	1121.97[a]±73.75

Means followed by the same superscript in the same row are not significantly different according to Duncan's multiple range test ($P<0.05$).

Pollutants such as industrial and agriculture wastes, pesticides (environmental estrogenic) and also different types of bacteria have histopathological effects on the reproductive tissue of fish gonads. These may disturb the development of germ cells and may reduce the ability of fish to reproduce, while metal accumulation occurring in the testis, affects the process of spermatogenesis and suppressing sperm production (Shobikhuliatul *et al.,* 2013). Therefore, in the present study, normal structures and histomorphological changes in liver, kidneys, gills, ovaries and testis of Nile tilapia were illustrated in figures 1-16.

In fact, liver is the most organs associated with the detoxification and biotransformation process due to its function, position and blood supply (Van der Oost *et al.,* 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues & Fanta, 1998 and Camargo and Martinez, 2007). The main alterations found in the liver of Nile tilapia collected from agriculture drainage water (site B) were cytoplasmic vacuolation of the hepatocytes, vacuolar degenerated nuclei, blood congestion and dilatation of hepatic vein (Figs.2 and 3). Anomalies such as pyknotic nucleus and yellow brown granules of bile stagnation in the cytoplasm (Fig.4) were identified in the liver samples of agriculture irrigation water (site A).

The current work being confermed by Pacheco & Santos (2002) who the described increased vacuolisation of the hepatocytes as a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water. In addition, Camargo and Martinez (2007) reported that the histopathological changes in the liver cause metabolic problems such the bile stagnation in liver. This lesion, characterized by the remains of the bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes. (Pacheco & Santos, 2002), indicates that the bile is not being released from the liver.

The results presented here revealed that the histopathological findings in the male testis were degenerated spermatogenic cells (Fig.6 , site B) and vacuoles within the seminiferous lobules were clearly visible (Fig.7 , site A). These results are in accordance with Gaber and El-Kasheif (2013),whose study was conducted to assess the effect of the water quality of El-Rahawy drain at El-Rahawy village on the histology of digestive tract, testis and ovaries.

The histological examination of the ovary of collected fishes from both sites showed necrotic ova and degenerated stroma (Figs.9 and 10).

Many authors observed the effect of pesticide on ovary. Also, Kumar and Pant (1984) found that 2-4 month exposure of an Indian teleost to lead caused disappearance of oocyes in the ovaries. In addition, Magar and Bias (2013) investigated histopathological effects of sublethal concentration of insecticide (malathion) which widely used in agriculture in the ovary of fresh water teleost, Channa punctatus. They observed complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles, and fragmented ova with abnormal shapes.

Gills participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Mazon *et al.,* 2002; Fernandes & Mazon, 2003). In this study, gill deformed structures such as lymphocytic infiltration, epithelial lifting congestion and fusion of gill lamellae were illustrated in (Fig.12, site B) and club shape filaments in (Fig.13, site A).

Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Fernandes & Mazon, 2003; Camargo and Martinez ,2007).

Kidney histological changes of tilapia from the two study sites were atrophy of glomerulus and hyperplasia of some renal tubules (Fig.15, site A), besides to expansion of space inside the Bowman's capsule, hematopiotic tissue depletion and degeneration in renal tubules (Fig.16, site B). Similar alterations of the kidney tubules and glomerulus,were found in the perch (*Lates calcarifer)* exposed to cadmium (Thophon *et al.,* 2003). The teleostean kidney is one of the first organs to be affected by contaminants in the water. Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space (Takashima & Hibiya, 1995).

In conclusion, the present study aims to investigate the effect of irrigation and agricultural drainage water on the reproductive parameter of Nile tilapia and to find out the possible histological effects of the detected heavy metals on the gills, liver, kidney and gonads.

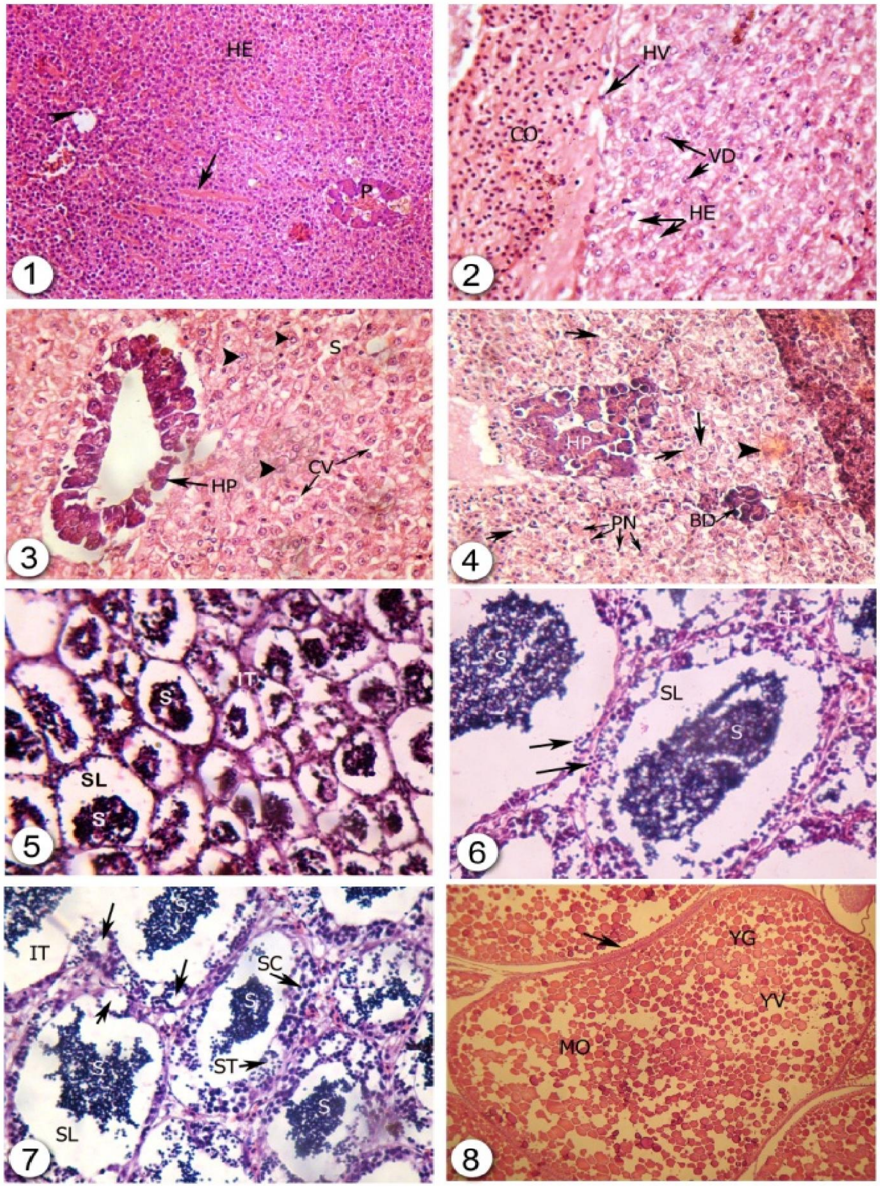

Fig.1: T.S in the liver showing normal structure of the hepatocytes (HC), the exocrine pancreas (P), central vein (➤) and the sinusoids (→) . x 200.
Fig.2: Cytoplasmic vacuolaion (HC), blood Congestion (CO), vacuolar degenerated nuclei (VD) and dilatation of hepatic vein (HV) in fish liver from site (B). x 400
Fig.3: Cytoplasmic vaculation (CV), sinusoid (S), hepatopancrease (HP) and vacuolar degenerated nuclei (➤) in fish liver from site (B). x 400.
Fig.4: Hepatopancrease (HP), cytoplasmic vaculation (→), pyknotic nucleus (PN) , bile duct (BD) and yellow brown granules of bile stagnation (➤) in fish liver from site (A). x 400.
Fig.5: T.S in the testis showing normal structure of seminiferous lobules (SL), spermatozoa (S) and interlobular tissue (IT). x 400.
Figs.6 and 7: Seminiferous lobules (SL) , interlobular tissue (IT), spermatid (ST), spermatocytes (SC), spermatozoa (S), notice the degenerated spermatogenic cells (→) and seminiferous lobules vacuolations (→) in fish testis from site (B) and (A) respectively . x 400.
Fig.8: T.S in the ovary showing normal structure of of a mature oocyte (MO), which contains yolk granules (YG) yolk vesicles (YV) the chorion (→). x 400.

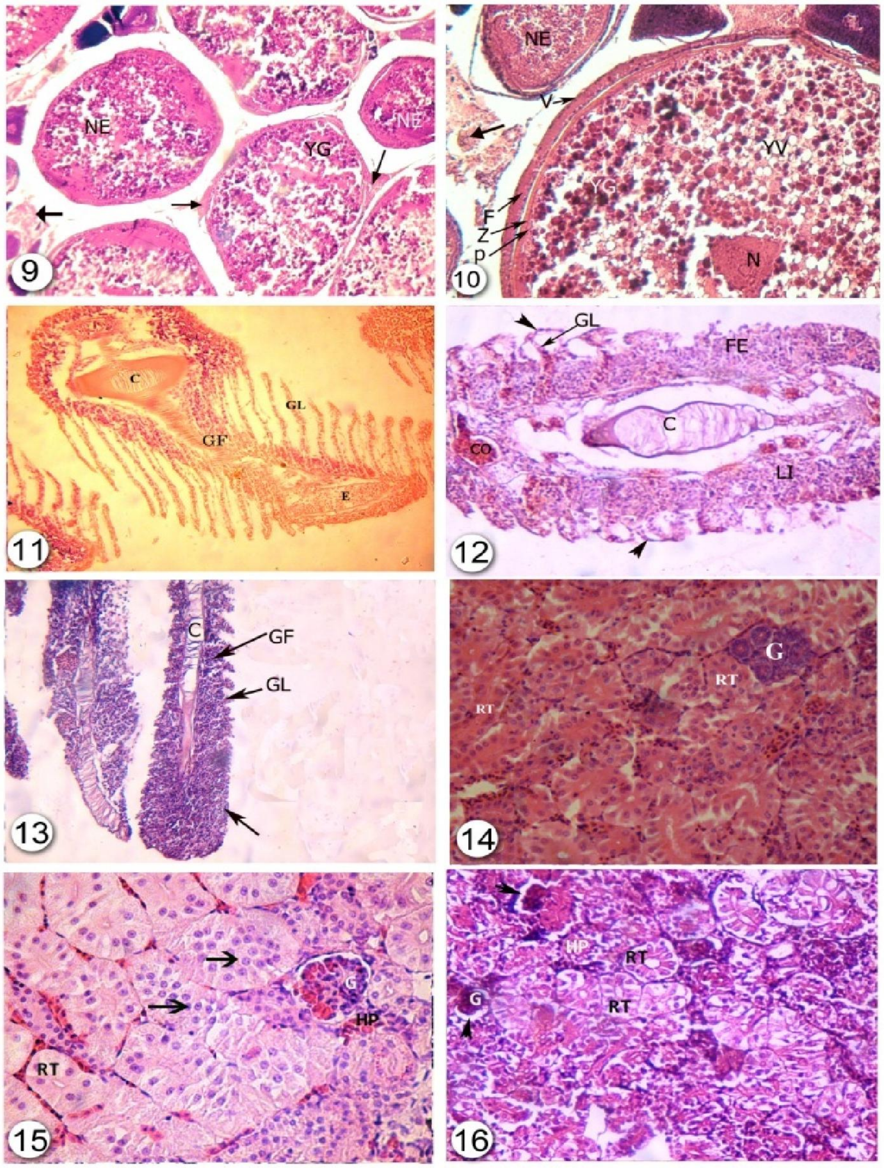

Fig.9: Yolk granules (YG), necrotic ova (NE) and degenerated stroma (→) in fish ovary from site (B). x 400.
Fig.10: The mature oocyte (MO) surrounded by vascular connective tissue (VT), follicular layer (F), zona radiata (ZR) and plasma membrane (PM),Yolk granules (YG), yolk vesicles (YV), nucleus (N), necrotic ova (NE) and degenerated stroma (→) in fish ovary from site (A). x 400.
Fig.11: T.S in the gills showing normal structure of gill filaments (GF), gill lamellae (GL) and cartilage (C) efferent arteriole (E). x 400.
Fig.12: Gill lamellae (GL), cartilage (C), Lymphocytic infiltration (LF), epithelial lifting (➤) congestion (CO) and fusion of gill lamellae (FE) in fish gill from site (B). x 400.
Fig.13: Gill lamellae (GL), gill filaments (GF), cartilage (C) and club shape filaments (→) in fish gill from site (A). x 400.
Fig.14 : T.S in the kidney showing normal structure of glomerulus (G) and renal tubules (RT) . x 400.
Fig.15: Atrophied glomerulus (G), hematopiotic tissue (HP), hyperplasia (→) of some renal tubules (RT) in fish kidney from site (A). x 400.
Fig.16 : Atrophied glomerulus (G) and expansion of space inside the Bowman's capsule(➤), depletion of hematopiotic tissue (HP) and degeneration in renal tubules (RT) in fish kidney from site (B). x 400.

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