

IMPACT OF HEAVY METALS POLLUTION ON HISTOPATHOLOGICAL ALTERATIONS IN KIDNEY AND LIVER OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) INHABITING EL-RAHAWY DRAIN

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SUMMARY

This study was performed to evaluate agricultural and industrial wastes water with respect to the potentially some toxic metals on histopathological alterations in kidney and liver of Nile tilapia (*Oreochromis niloticus*) from El-Rahawy drain at different seasons. Samples of Nile tilapia (*Oreochromis niloticus*) were collected seasonally (12 fish / season) from each site. Fish total length and total weight were from 13.5 to 26.1 cm and from 50 to 315 g, respectively. The present results indicated that, the heavy metals due to pollution, increased in tissue of fish caught from the drain. The distribution of heavy metals (Iron, Manganese, zinc, lead and copper) concentrations in water were estimated during four successive seasons were found to be, Fe (0.680, 0.626, 0.380, 0.395 mg/L), Mn (0.214, 0.173, 0.112, 0.214 mg/L), Zn (0.138, 0.120, 0.107, 0.108), Pb (0.041, 0.054, 0.050, 0.049 mg/L) and Cu (0.006, 0.010, 0.009, 0.007 mg/L), respectively. It was found that, the heavy metals were accumulated in the studied fish muscle by various levels. The concentration of the tested metals in fish muscle of (edible parts) followed a sequence of Fe > Zn > Mn > Cu > Pb. Also, the results showed that, elevation in studied heavy metals concentrations caused histological alterations in collected fish. These alterations including: hemorrhage, degeneration, hemosiderin and oedema in hematopoietic tissue, also observed necrosis in renal tubule and glomerulus of the kidney while showed marked histopathological changes in the liver of studied fish revealed hemolysis and hemosiderin in blood vessel. It was concluded that, agricultural, industrial and domestic wastes histopathological alterations were observed in the kidney and liver of Nile tilapia (*Oreochromis niloticus*) collected from El-Rahawy drain.

Keywords: *Pollution, heavy metals, El-Rahawy drain, Nile tilapia, histopathology, kidney, liver.*

INTRODUCTION

Aquatic ecosystems are major recipients of pollutants, which over time, can have serious consequences for the biota that might not become apparent until changes occur at the population or ecosystem level, a point at which it may be too late to take effective countermeasures (Khallaf et al., 2010). In Egypt, the problems of the drainage canals have extremely increased in the past years (Authman et al., 2013). Where disposing of partially treated or untreated domestic and industrial wastewater into agricultural drains deteriorates their water quality [El-Sheikh et al., 2010].

El-Rahawy drains is one of the main drains; it starts as El-Rahawy pump station on Mansouria Rayah lies at 30 km, North to Cairo at El-Kanater El-khayria in area, Egypt. El-Rahwy drain lies between latitudes 30° 0' 10" N to 30° 12' N and longitudes 31° 02' E to 31° 03' E. It is about 12.41 km² and passes through El-Rahawy village and many villages distributed along it receiving agricultural and domestic wastes without purification in addition to sewage of El-Giza governorate and discharge these wastes directly without treatment into Rosetta branch of the River Nile [El Bouraie et al. 2010].

The drain is surrounded by high density of population area and wide agricultural lands. The surface level of the drain is 12.37 m above sea level. This drain receives waste water from El-Moheet drain that passes by a deep under El-Nassery sub-branch of the River Nile to open into a concrete reservoir of about 20m high at El-Rahawy drain. From this reservoir, the drainage wastewater runs to about 4km through El-Rahawy village and opens into Rosetta branch. The drain discharges about 2.800000 m³/day of which about 1.900000 m³/day sewage effluents. The surface level of the drainage wastewater in the reservoir is about 13m higher than the water in Rosetta branch. Gaber et al. (2013) showed that, the heavy metals

concentrations (Cu , Fe , Pb , Cd , Mn and Zn) in water are higher in El-Rahawy drain than in River Nile and this due to sewage and other pollutants discharge , also showed significant changes and histological abnormalities of stomach , intestine and gonads of African catfish *clarias gariepinus* . The need of detect and assess the impact of pollutants , particularly at low , sub lethal concentrations, on environmental quality had led to development of a range of biological responses measured in number of different species [Fen, 2004 and Linde –Arias et al., 2008] . Fish are generally considered to be the most feasible organisms for pollution monitoring in aquatic systems. Fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in aquatic food-webs because of their function as carrier of energy from lower to higher trophic levels [Linde –Arias et al., 2008 and Van der Oost et al, 2003].

Fish live in their environment, are extremely dependent upon it and are affected by changes in it. Thus fish could be used as a "warning system" to indicate the presence of pollutants in natural water [Nusse et al, 1995] . Prior to death or overt sickness, fish may respond to stress by changing molecular, physiological, histological or behavioural responses.

Midhat et al. (2013) observed that , the heavy metals concentrations in water from El-Rahawy drain region were higher than those obtained from River Nile and the Nile catfish *Clarias gariepinus* inhabiting in the drain suffer from many histopathological changes in the kidney and spleen including degeneration, necrosis, haemorrhage, activation of melanomacrophage centers, hyperplasia and hemosiderosis .

The aim of this study to estimate industrial waste water effect on histopathological alterations in kidney and liver of Nile tilapia (*Oreochromis niloticus*) from El-Rahawy drain.

MATERIALS AND METHODS

The present study was extended from winter to autumn 2016 during four successive seasons in El-Rahawy drain at El-Rahawy Village, El-Giza governorate. Samples were collected from El-Rahawy drain, Fig.(1) to represent the drain ecosystem.

Sampling, preservation and experimental procedure of the water samples were carried out according to the standard methods for examination of wastewater [APHA, 1998]. Heavy metals (iron , manganese , zinc , lead and copper) in water samples were determined using atomic absorption spectrometry (Perkin – Elmer 3110 , USA) with graphite atomizer HGA – 600 , after using the digestion technique by nitric acid [APHA, 1998].

Fish Samples Collection and Analysis:

Samples of Nile tilapia (*Oreochromis niloticus*) were collected seasonally (12 fish / season) from each site of about 13.5 to 26.1 cm and from 50 to 315 g, respectively. The fishes were transposed alive back after catching to the laboratory for subsequent analysis. In the laboratory , for each fish , the total length and total weight were recorded.

Heavy metals in water

Samples of water of 1 liter were collected from the selected station in poly ethylene bottles. The bottles were previously rinsed several times with river water before collection. The entire sample was acidified using 5 ml concentrated nitric acid at collection. In the laboratory, 20 ml of nitric acid was added to 500 ml of water sample in a beaker and boiled on a hot plate till the lowest volume, nearly 15 – 20 ml before precipitation occurs, until complete digestion. The remaining volume was completed to 100 ml with deionized distilled water. A portion of this solution was used for the determination of heavy metals. The metals concentration (Fe, Cu, Mn, Zn and Cd) were determined using atomic absorption model (Perkin Elmer 3110 USA) with graphite atomizer HGA-600, according to the method described by APHA (1998). The results were expressed in µg/l.

Heavy metals in fish organ

Specimens of the major organ muscles, spleen, gills, liver and kidney were collected from 5 fishes and were transferred to beaker in a drying oven thermostatically regulated at 105 °C overnight. One gm dry weights of organs were digested according to the method described by Goldberg et al. (1963) in which concentrated HNO₃ and HClO₄ with ratio of 5 ml + 5 ml were used in Teflon beakers on hot plate at 50 °C for about 5 hours till complete decomposition of organic matter. The digested solution were cooled to

renal tubule (E) . There are edema and necrosis in glomerulus, renal tubule , glomerulus and hematopoietic tissue (F) .

Table (1): The water concentrations and muscles of heavy metals in the experimental area at different seasons of the year.

Seasons	Heavy metals concentrations (water)					
	Iron (Fe)	Manganese (Mn)	Zinc (Zn)	Lead (pb)	Copper (Cu)	
Winter	0.680 ±0.124	0.214 ± 0.054	0.138 ±0.020	0.041 ±0.003	0.006 ±0.001	
spring	0.626 ±0.113	0.173 ±0.026	0.120 ±0.018	0.054 ±0.006	0.010 ±0.002	
Summer	0.380	0.112	0.107	0.050 ±0.004	0.009 ±0.002	
autumn	0.395 ±0.120	0.214 ± 0.102	0.108 ± 0.060	0.049 ± 0.004	0.007 ± 0.001	
Egyptian Law No. [16]	Permissible Limits (Mg /L)					
	(Mg/L)	1000	500	1000	50	1000
U.S. EPA [17]		1000	NA	120	2.5	9.0
Seasons	Heavy metals concentrations (Muscle)					
Winter	2.073 ± 0.272	0.086 ± 0.020	0.377 ± 0.108	0.015 ± 0.006	0.044 ± 0.020	
spring	1.094 ± 0.180	0.091 ± 0.016	0.332 ± 0.142	0.005 ± 0.001	0.051 ± 0.012	
Summer	1.765 ± 0.164	0.115 ± 0.060	0.381 ± 0.060	0.020 ± 0.004	0.250 ± 0.073	
autumn	1.557 ± 0.123	0.088 ± 0.001	0.316 ± 0.130	0.146 ± 0.032	0.067 ± 0.018	

Anthropogenic sources such as agriculture run-off industrial and sewage have oriented both localized and regional pollution problems in nearly over country around the world (El Bouraie et al., 2011). In some oases the pollution has been extensive enough to lead to environmental disasters and ecosystem shutdown (Lasheen et al., 2012).

Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff , leaching from landfills, shipping and harbor activities and atmospheric deposits(30).

Present results showed that , most of the heavy metal concentrations in surface water of El-Rahawy drain and River Nile water are found within the permissible limka of both the Egyptian governmental law No. 48 [Abeyede, 1993] and U.S.EPA [Abdel-Baky, 2001]. These results are in agreement with El-Bourale et al.(1993) who studied heavy metals in five drain outfalls and found that the level of metals is within the permissible limits of Egyptian law 48/ 1982. Also, Lasheen et al. [2012] stated that the average concentrations of heavy metals in El-Moheet drain: which discharge in El-Rahawy drain within the permissible range according to the Egyptian law 48/ 1982

The employment of histopathological biomarkers to determine the effects of environmental contamination has been perceived as a highly relevant methodology since they reflect the true health state of the organism Gaber et al. (2013).

Similarly, histopathological investigations have long been recognized to be reliable biomarkers of stress in fish [Midhat et al., 2013 and Adeogun, 2012].

Histopathological analysis has already been tested and proposed as an efficient and sensitive tool to the monitoring of fish health and environmental pollution in natural water bodies [Rajeshkumar. and Munuswamy, 2011].

Fish are notorious for their ability to concentrate heavy metals in their tissues. The metals exist most probably as cationic form in water (Fe, Mn, Zn, Pb, Cu) and tend to form ionic complexes and accumulate in the internal organs of fish (Mears and Eister, 1977). The present results showed that, the concentrations of heavy metals in the studied muscles of the fish ranged between 1.094 – 2.073, 0.086 – 0.115, 0.316 – 0.381, 0.005 – 0.146 and 0.044 – 0.250 for Fe, Mn, Zn, Pb and Cu, respectively. It was found that Fe concentration recorded higher accumulation in the muscle than the water during the winter season. Higher Fe content in muscle of *O. niloticus* can be attributed to the large quantities of Fe in water, this agrees with the findings of Ghazaly et al. (1992), Tariq et al. (1993), Bahnasawy (2001) and Mohamed and Gad (2005). Iron concentrations in the water and muscle of fish is more than the maximum permissible level for Fe (5.0mg/g) cited by Abeyede (1993).

The present study indicated that manganese concentration in the water ranged between 0.112 and 0.214 mg/L at El-Rahawy drain. Also, Mn concentration in the muscle of Nile tilapia ranged between (0.086 – 0.088) in winter and autumn, respectively and (0.091 – 0.115) in spring and summer seasons, respectively. The present results agree with the findings of Gomaa et al (1995) and Khallaf et al (1998). On the other hand, Mn concentrations in the studied organs of *C. gariepinus* were in the following order: muscle < liver < kidney < gills, which agree the results of Abdel-Baky (2001).

Zinc is an essential element and a common pollutant as well. It is taken up by fish directly from water especially by mucus and gills (Skidmore, 1964). The present study showed that Zn concentrations in water and muscle were ranged between (0.107 – 0.138) and (0.316 – 0.381) mg/L during four seasons. The present results indicated that, Zn concentrations higher in the muscle of fish than water of the drain. The present results are in agreement with those obtained by Shenouda et al. (1992), Gomaa et al. (1995), Khallaf et al. (1998) and Mohamed and Gad (2005). Western Australian food and drink Regulations recommended a level of 40mg/kg Zn for human consumption (Marks et al., 1980). Accordingly, the concentrations of Zn in the muscles of the studied fish are still below the permissible level.

Lead is toxic even at low concentrations and has no known function in biochemical processes. The present data explain that Pb concentrations in muscles of the fish ranged between 0.005 and 0.146 mg/g dry wt. While, the concentrations of Pb exhibited in water of the drain during four seasons. The present results agree with the results of Barak and Mason (1990), Ghazaly et al. (1992), Gomaa et al. (1995), Khallaf et al. (1998) and Mohamed and Gad (2005).

Copper is an essential element for all living organisms. It is among the most toxic metals. The present study showed that copper concentrations in muscle of the studied Nile tilapia fish ranged between 0.044 and 0.250 mg/g dry weight for *O. niloticus* while recorded concentration (0.006 - 0.010) mg/L were found in the water of the drain. The present results agree with the results of Khallaf et al. (1998), Abdel-Baky (2001) and Mohamed and Gad (2005). Also Benedetti et al. (1989), Gomaa et al. (1995) and El-Moselhy (1999) found that Cu exhibited its higher levels in the liver and the lowest values in the muscles. The concentrations of Cu in the muscles of the studied fish are still below the permissible level for Cu (30mg/kg) recommended by National Health and Medical Research Council (NHMRC) Marks et al. (1980). Mohamed and Gad (2005) showed that, the levels of Fe, Zn, Mn, Pb, Cu, Cd in the water of Abu Zaabal Lakes ranged between 3022 – 6.01 – 0.21 – 1.50 – 0.67 – 1.64 – 0.62 – 1.67, 0.13 – 0.20 – and 0.03 – 0.06 mg/L, respectively, also who found that, the concentrations of the tested metals in different organs of the studied fish were in the following order Fe > Zn > Pb > Mn > Cu > Cd concentrations in the fish muscles (edible parts) except Cd in the muscles of *T. Zillii* were within the maximum permissible limit.

The liver of *Oreochromis niloticus* collected from El-Rahawy drain showed histopathological features (F. 3).

Degeneration in blood vessel wall, fatty degeneration, pyknotic and necrosis in hepatocytes(A). Hemorrhage, hemosiderin in blood vessels, Congestion in blood sinusoids and necrosis in hepatocytes(B). The present study showed several alterations in the liver included hemolysis, hemosiderin, degeneration in blood vessel wall and necrosis in hepatocytes (C).

Also, appeared hmyolysis, necrosis and degeneration in blood vessel wall and hepatocytes (D) and (E). However, there were microscopic differences that characterize the liver showed congestion in blood sinusoid, pycnotic, necrosis and degeneration in hepatocytes (F) .

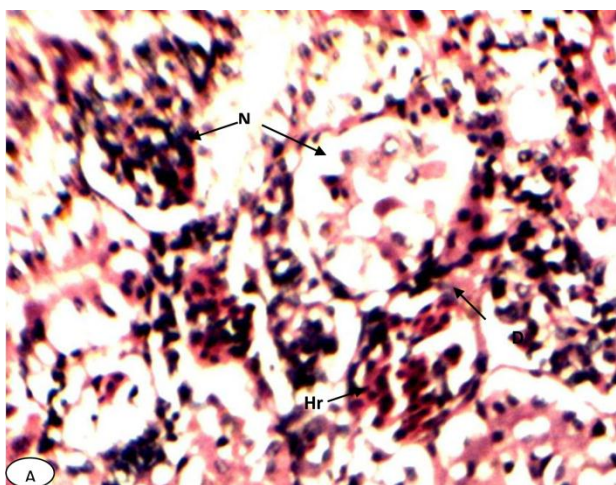


Fig (A): Kidney section of *O. niloticus* collected from El-Rahawy drain showing: hemorrhage(Hr) , and degeneration (D) in hematopoietic tissue, necrosis (N) in renal tubule and glomerulus., HE. X 400

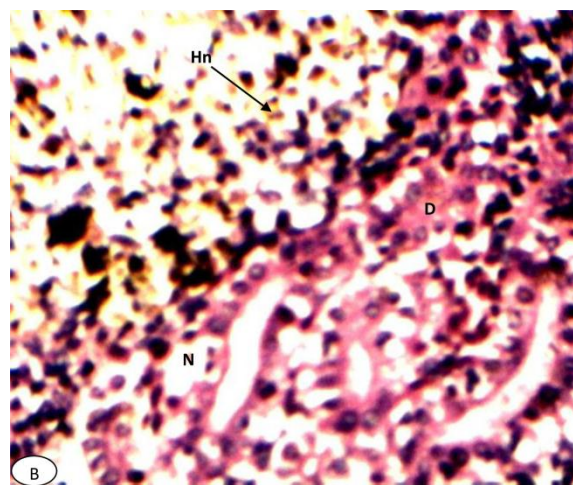


Fig (B): Kidney section of *O. niloticus* collected from El-Rahawy drain showing : degeneration (D) , necrosis (N), hemosidrin (Hn) in hematopoietic tissue., HE. X 400

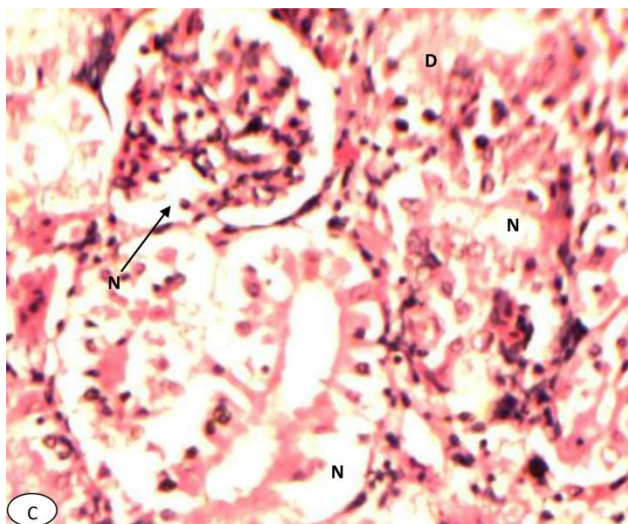


Fig (C) : Kidney section of *O. niloticus* collected from El-Rahawy drain showing : necrosis (N) in renal tubule and glomerulus , degeneration (D) and necrosis (N) in Hematopoietic tissue., HE. X 400



Fig (D): Kidney section of *O. niloticus* collected from El-Rahawy drain showing : hemorrhage (Hr) , hemosidrin (Hn) in hematopoietic tissue , edema (E) in renal tubule , necrosis (N) and degeneration (D) in renal tubule and hematopoietic tissue., HE. X 400

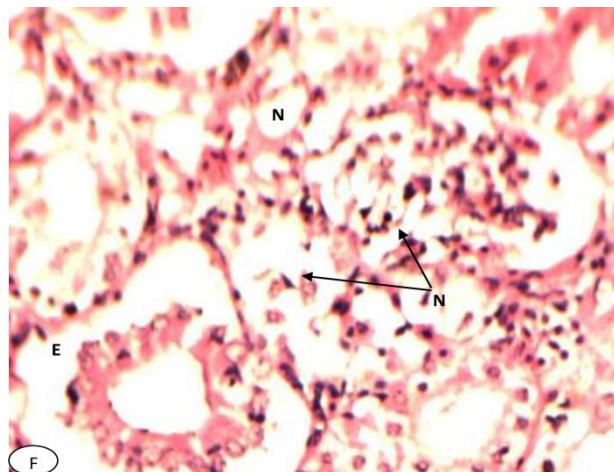
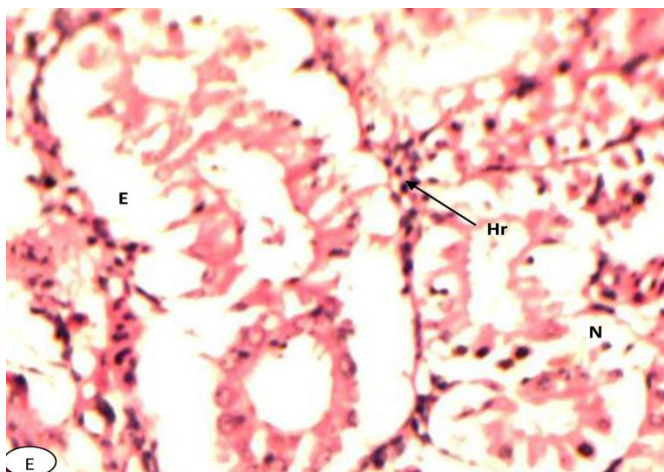


Fig (E) : Kidney section of *O. niloticus* collected from El-Rahawy drain showing : hemorrhage (Hr) in hematopoietic tissue , edema (E) , and necrosis (N) in renal tubule ., HE. X 400

Fig (F) : Kidney section of *O. niloticus* collected from El-Rahawy drain showing : edema (E) in glomerulus, necrosis (N) in renal tubule and glomerulus and hematopoietic tissue., HE. X 400

• Kidney section of *O. niloticus*

A) - haemorrhage (Hr)

[2] - degeneration (D) in hematopoietic tissue

- necrosis (N) in renal tubule and glomerulus

B) - degeneration (D)

- necrosis (N)

- hemosidrin (Hn) in hematopoietic tissue

C) - necrosis (N) in renal tubule and glomerulus

- degeneration (D)

in hematopoietic tissue

- necrosis (N)

D) - haemorrhage (Hr)

[3] - hemosidrin (Hn) in hematopoietic tissue

- edema (E) in renal tubule

- necrosis (N) in renal tubule and hematopoietic

- degeneration (D) tissue

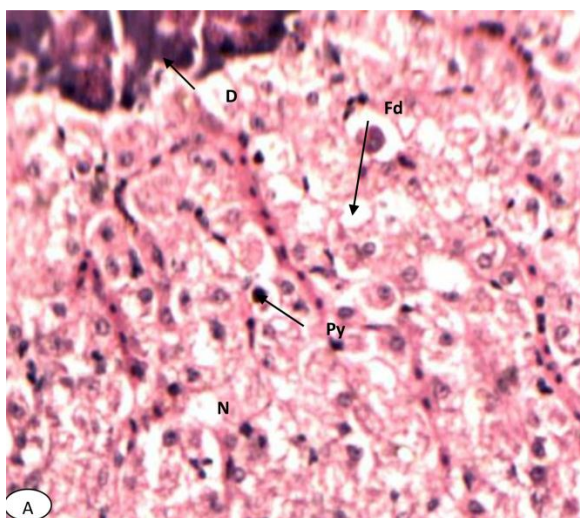
E) [1] - haemorrhage (Hr) in hematopoietic tissue

[4] - edema (E)

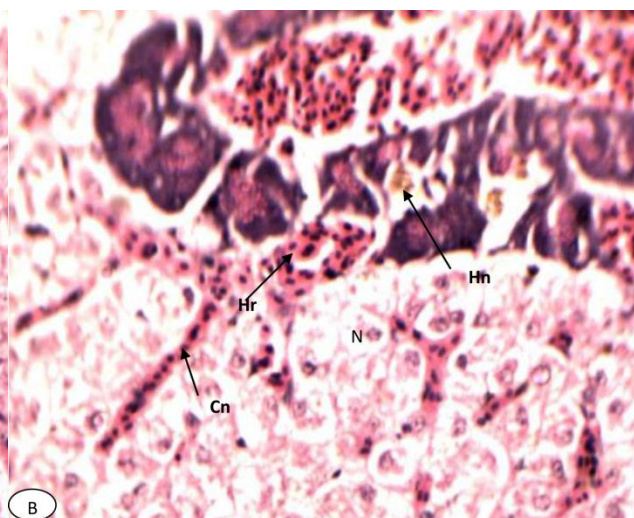
- necrosis (N) in renal tubule

F) - Oedema in glomerulus

- Necrosis in renal tubule
- in glomerulus
- in hematopoietic tissue



Fig(A) : Liver section of *O. niloticus* collected from El- Rahawy drain showing : degeneration (D) in blood vessele wall , fatty degeneration (Fd), pycnotic (Py) and necrosis (N) in hepatocytes., HE. X 400



Fig(B) : Liver section of *O. niloticus* collected from El-Rahawy drain showing : hemorrhage (Hr) , hemosidrin (Hn) in blood vessele , congestion (Cn) in blood sinusoids , and necrosis (N) in hepatocytes. , HE. X 400

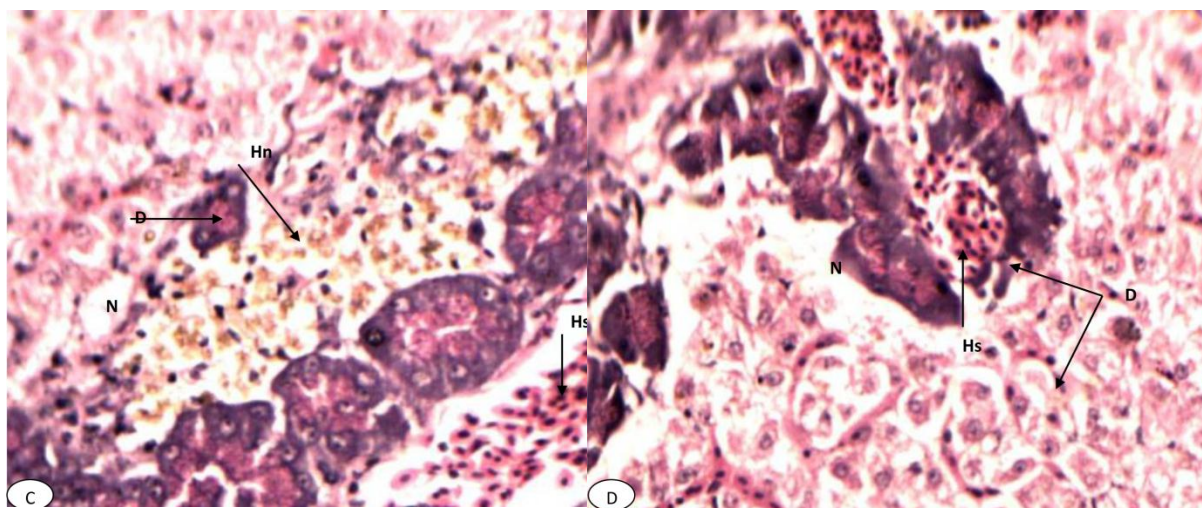


Fig (C) : Liver section of *O. niloticus* collected from El-Rahawy drain showing : hemolysis (Hs) , hemosidrin (Hn) , degeneration in blood vessel wall , and necrosis (N) in hepatocytes., HE . X 400

Fig(D) : Liver section of *O. niloticus* collected from El-Rahawy drain showing : hemolysis (Hs) , necrosis (N) and degeneration (D) in blood vessel wall and hepatocytes., HE . X 400 .

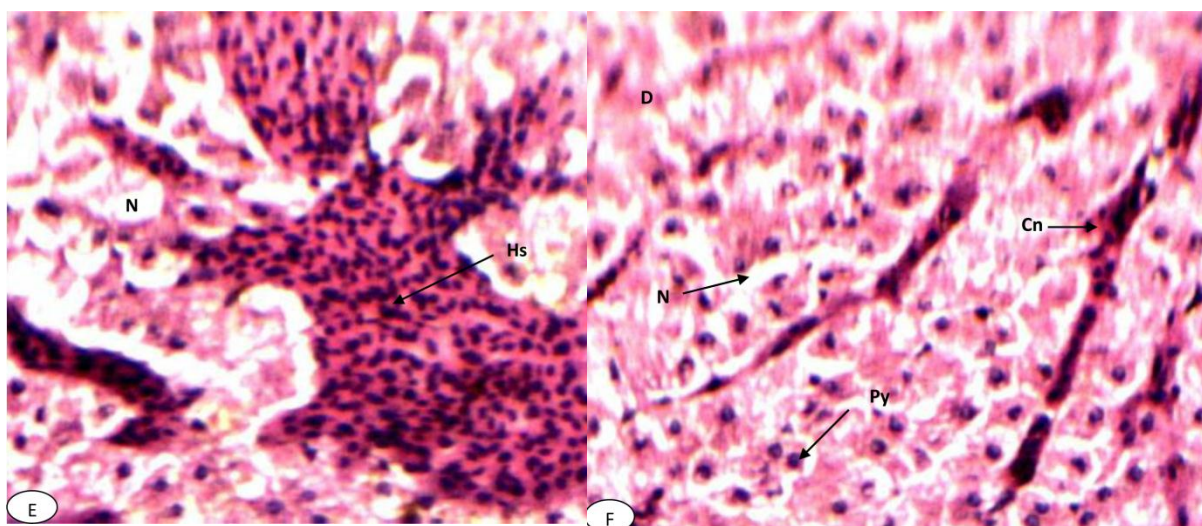


Fig (E) : Liver section of *O. niloticus* collected from El-Rahawy drain showing : hemolysis (Hs), and necrosis (N) in hepatocytes., HE. X 400

Fig (F) : Liver section of *O. niloticus* collected from El-Rahawy drain showing : congestion (Cn) in blood sinusoid , pycnotic (Py), necrosis (N) , degeneration (D) in hepatocytes., HE . X 400

- Liver section of *O. niloticus*

- A. 3➡ - degeneration (D) in blood vessel wall
 - fatty degeneration (FD)
 6➡ - pycnotic (Py)
 and -necrosis (N) in hepatocytes
-

- B. - haemorrhage (Hr)
 2➡ - hemosidrin (Hn) in blood vessel
 4➡ - congestion (cn) in blood sinusoids
 and - necrosis (N) in hepatocytes
-

- C. - hemolysis (Hs)

- hemosidrin (Hn)
 - degeneration in blood vessel wall
 - and - necrosis (N) in hepatocytes
-
- D. - hemolysis (Hs)
- necrosis (N)
- and - degeneration (D) in blood vessel wall and hepatocytes
-
- E. 1 → - hemolysis (Hs)
- and- necrosis (N) in hepatocytes
-
- F. - congestion (cn)
- pycnotic (Py)
- necrosis (N) and - degeneration (D) in hepatocytes

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تأثير تراكم المعادن الثقيلة على كلى وكبد أسماك البلطي النيلي القاطنة بمصرف الرهاوي

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هذه الدراسة تم تنفيذها لتقييم اثر المخلفات الصناعية وخاصة العناصر المعدنية الثقيلة في مياه مصرف الرهاوي (قرية الرهاوي) على تحورات الكبد والكلى لاسماك البلطي النيلي. الاسماك تم جمعها موسميا (١٢ سمكة / موسم) من كل موقع . الطول الكلى لاسماك تراوح ما بين ١٣,٥ الى ٢٦,١ سم والوزن الكلى تراوح ما بين ٥٠ الى ٣١٠ جم على التوالي. اوضحت النتائج أن تركيز التلوث بالعناصر الثقيلة ازداد في انسجة اسماك المصرف. زكما اوضحت النتائج ان تركيز العناصر الثقيلة (الحديد والمنجنيز والزنك والرصاص و النحاس) المقدره موسميا لمياه مصرف الرهاوي كانت مايلي : (0.395 , 0.380 , 0.626 , 0.680) للحديد و (0.173 , 0.214) (0.112 , 0.214 mg /L) للمنجنيز و (0.108 , 0.107 , 0.120 , 0.138) للزنك و (0.041 , 0.054 , 0.050 , 0.049 mg /L) للرصاص و (0.007 mg /L , 0.009 , 0.010 , 0.006) للنحاس على التوالي. فيما كان تركيز العناصر في انسجة البلطي كان متراكما بمختلف المستويات.تركيز العناصر الثقيلة المختبرة في انسجة الاسماك كان كالتالي الحديد<الزنك <المنجنيز<النحاس <الرصاص. ايضا النتائج اظهرت مستويات العناصر الثقيلة احدثت تحورات هستولوجية في الاسماك المجمعة. اظهرت الدراسة ان التركيب الهيستولوجي لهذه الاسماك التي تعيش في منطقه الرهاوي تعاني من الكثير من الامراض في انسجه الكلى والكبد حيث لاحظ العديد من التغيرات النسيجية المرضية في الكلى أدى الى تدهور ونخر في الانابيب الكلويه وانايب ملبيجي وانحلال وركود الدم ونزف وضومور وتليف في الانسجه وظهور صبغه الهيموسيدرين التي تم جمعها من منطقه البحث.وظهرت خلايا الكبد المتحلله وبها بعض المترامات الدهنيه مع نزيف واحتقان في الاوعيه الدمويه وتحلل ايضا لجدار هذه الاوعيه كما لوحظ وجود نخر وفقد للشكل العام للخلايا والاعويه الكبديه وتدمير الخلايا والانوية.

لذا يمكن استخلاص ان المخلفات الزراعية والصناعية احدثت تحورات هستولوجية و مرضية في كبد وكلية اسماك البلطي النيلي المجمعة من مصرف الرهاوي.