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Bioaccumulation of Selected Metals and Histopathological Alterations in Tissues of *Oreochromis niloticus* and *Lates niloticus* from Lake Nasser, Egypt

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Abstract: The concentration of some heavy metals (Fe, Zn, Cu, Pb, Cd and Co) in water and liver, gills, intestine, testis, heart and muscle of *O. niloticus* and *L. niloticus* obtained from four khors (El-Ramla, Kalabsha, Korosko and Toushka) of Lake Nasser, Egypt during 2006 was investigated (atomic absorption spectrophotometry) with emphasis on the histological alterations in these organs. Metal concentrations in the water of khors (mg/l) followed an abundance of: Fe>Zn>Pb>Cu>Cd>Co. The highest values of metals were reported in khor Toushka. It was found that the metals were accumulated in different tissues of both fish by various levels, where, the non-edible parts accumulated more metals than the edible muscles. Zn, Cu, Pb and Cd concentrations in the fish muscles were blow the maximum permissible limit, however, Fe in the muscles exceeded the permissible limit. Several histopathological alterations, including vacuolar degeneration with focal areas of necrosis in liver, proliferation in the epithelium of gill filaments and fusion of secondary lamellae, severe degenerative and necrotic changes in the intestinal mucosa and seminiferous tubules, degeneration and atrophy in cardiac muscle fibers and degeneration in muscle bundles were observed in the studied tissues of both fish as a result of the accumulated metals.

Key words: Fish . heavy metals . histopathology . Lake Nasser . Egypt

INTRODUCTION

In aquatic ecosystem, heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Generally, metals enter the High Dam Lake (Nasser and Nubia) from a variety of sources, including: rocks and soils directly exposed to waters, dead and decomposing vegetation and animal matter, wet and dry fallout of atmospheric particulate matter and human activities, including the discharge of various treated and untreated wastes to the water body [1]. Essential metals such as Cu, Zn and Fe have normal physiological regulatory functions [2], but may bioaccumulate and reach toxic levels. Non-essential metals are usually potent toxins and their bioaccumulation in tissues lead to intoxication, decreased fertility, tissue damage and dysfunction of a variety of organs [3, 4].

Heavy metals are non-biodegradable and once discharged into water bodies, they can either be adsorbed on sediment particles or accumulated in aquatic organisms. Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate in various tissues in significant amounts [5] and are eliciting toxicological effects at critical targets. Also, fish may accumulate significant concentrations of metals even in waters in which those metals are below the limit of detection in routine water samples [6], therefore, fish might prove a better material for detecting metals contaminating the freshwater ecosystems. Intensive studies were conducted on the levels of heavy metals in different water bodies [7-12].

The bioaccumulation of heavy metals in the different fish tissues has been studied by several investigators [13-19]. Also [9, 20, 21] studied the concentrations of Fe, Zn, Pb, Cd, Co and Cu in different tissues of *Tilapia nilotica* and *Tilapia galilea* from Lake Nassser, Egypt and stated that the fish tissues accumulated high concentrations of metals. Bioaccumulation of metals may lead to high mortality rate or cause many biochemical and histological alterations in the survived fish.

Histological changes associated with heavy metals in fish have been studied by many authors [22-28]. On the other hand, no histopathological studies have been carried out on the fish of Lake Nasser. Therefore, this study was conducted to determine the levels of some metals (Fe, Zn, Cu, Pb, Cd and Co) in water and tissues

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(liver, gills, intestine, testis, heart and muscle) of *O. niloticus* and *L. niloticus* obtained from four khors of Lake Nasser during 2006. Also, the impact of such metals on the histological structures of tissues of both fish was investigated.

MATERIALS AND METHODS

Study area: Construction of the Aswan High Dam in the Upper Egypt resulted in the formation of a large water storage reservoir, extending for 480km south of the Dam of which 300km as Lake Nasser lies in Egypt and 180km as Lake Nubia in Northern Sudan. Lake Nasser has a long and narrow shape with dendritic side areas called khors, the shallow water of which provides a good habitat for aquatic plant growth [29]. The number of important khors, highly productive in fisheries, is about 85, of which 48 are located on the eastern side and 37 on the western side. The length of shoreline of khors is 969.9 km [30]. The lake lies between 22^{o} 31´ to 23^{o} 45´N and 31^{o} 30´ to 33^{o} 15´E. It has a total surface area of 6200 km^2, with average depth about 25m. The lake as a whole is mostly surrounded by rock terrain, chiefly piedmonts and peneplains of sandstone [31].

Sampling: During 2006, water samples (2L) were collected from khor El-Ramla (I), Kalabsha (II), Korosko (III) and Toushka (IV) of Lake Nasser (Fig. 1), in polyethylene bottles. The samples were acidified by nitric acid and transferred to the laboratory in an ice-box to be analyzed. At the same time, samples of *O. niloticus* and *L. niloticus* were collected from the same khors. The fish measured about 22.7 to 30.2 and 21.0 to 32.1cm in total length and 260.7 to 600.5 and 200.0 to 700.1g in weight for *O. niloticus* and *L. niloticus,* respectively. After dissection of fish samples,

Fig. 1: Map showing sampling stations at Lake Nasser

parts of liver, gills, intestine, testis, heart and muscle were carefully removed and prepared for metals analysis and histological studies.

Determination of heavy metals: Analysis of the heavy metals (Fe, Zn, Cu, Pb, Cd and Co) in the water [32] and in the fish tissues [33] was carried out using Atomic Absorption Spectrophotometery.

Bioaccumulation factor (BAF): The bioaccumulation factor (BAF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water and it was calculated [11] using the following equation:

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{pollutant concentration infish organ (mg/kg)}}{\text{pollutant in water (mg/l)}}$$

Histopathological studies: Specimen of liver, gills, intestine, testis, heart and muscle were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 4-6µm then stained with haematoxylin and eosin and examined microscopically [34].

RESULTS

Concentration of heavy metals in water: Table 1 shows the mean concentrations of the tested metals in the water of the four selected khors of the lake. Khor Toushka had the highest levels of metals, while khor El-Ramla showed the lowest concentrations of Zn, Pb and Cd. The abundance of metals in the khors water followed the order: Fe>Zn>Pb>Cu>Cd>Co.

Concentration of heavy metals in fish tissues:
Iron: Table 2 shows the concentrations of Fe in the studied tissues of *O. niloticus* and *L. niloticus* from the four khors of Lake Nasser. Fe accumulation in the studied tissues of both fish was in the following order: testis>heart>intestine>gills>liver>muscle for khors El-Ramla and Korosko and testis>heart>intestine>liver>gills>muscle for khors Kalabsha and Toushka.

Zinc: Table 3 shows the concentrations of Zn in the studied tissues of both fish. The concentrations of Zn in the studied tissues of both fish were in the following order: testis>intestine>heart>liver>gills> muscle.

Copper: Table 4 shows the concentrations of Cu in the tissues of both fish. The concentrations of Cu in the

Table 1: Heavy metal concentrations (mg/l) in the selected khors of Lake Nasser

	Metals					
Sites	Fe	Zn	Cu	Pb	Cd	Co
I	0.7892	0.0802	0.0122	0.0488	0.0072	0.00028
II	0.4418	0.1520	0.0104	0.0494	0.0074	0.00030
III	0.6038	0.1026	0.0134	0.0502	0.0086	ND
IV	1.2254	0.2126	0.0194	0.1064	0.0100	0.00034
Regional range	0.4418-1.2254	0.0802-0.2126	0.0104-0.0194	0.0488-0.1064	0.0072-0.0100	ND-0.00034
Regional mean	0.7651	0.1369	0.0139	0.0637	0.0083	0.00031

ND = not detectable, I: El-Ramla, II: Kalabsha, III: Korosko, IV: Toushka

Table 2: Concentrations of iron (mg/kg dry weight) and the bioaccumulation factor (BAF) in different tissues of *O. niloticus* and *L. niloticus* from the selected khors of Lake Nasser

	Iron (Fe)											
	Liver		Gills		Intestine		Testis		Heart		Muscle	
Sites	1	2	1	2	1	2	1	2	1	2	1	2
I	181.00	112.15	212.00	204.75	289.11	228.25	581.39	543.06	433.21	400.11	53.22	52.75
	±8.60	±11.76	±13.01	±11.06	±13.14	±11.77	±15.11	±11.96	±13.20	±10.51	±4.01	±4.35
	(229.30)	(142.10)	(268.60)	(259.40)	(366.30)	(289.20)	(736.60)	(688.10)	(8.90)	(506.90)	(67.40)	(66.80)
II	220.01	216.50	98.00	80.00	350.09	279.38	433.10	387.50	383.11	293.91	67.01	63.38
	±10.38	±10.71	±2.33	±10.35	±16.22	±12.65	±16.20	±13.54	±13.39	±9.13	±7.38	±6.18
	(497.90)	(490.00)	(221.80)	(181.00)	(792.40)	(632.30)	(980.30)	(877.00)	(867.10)	(665.20)	(151.60)	(143.40)
III	89.11	62.90	183.11	168.20	280.00	206.43	569.31	511.09	492.01	421.01	55.39	48.22
	±2.00	±3.32	±5.13	±12.25	±15.36	±12.38	±12.11	±13.92	±15.63	±13.03	±3.59	±7.01
	(147.50)	(104.10)	(303.20)	(278.50)	(463.70)	(341.80)	(942.80)	(846.40)	(814.80)	(697.20)	(91.70)	(79.80)
IV	403.50	312.00	217.38	210.00	446.00	362.01	631.25	593.10	621.01	511.09	78.00	68.81
	±10.71	±15.14	±10.53	±13.20	±16.35	±14.55	±10.77	±16.21	±13.55	±15.03	±12.03	±8.11
	(329.20)	(254.60)	(177.40)	(171.30)	(363.90)	(295.40)	(515.10)	(484.00)	(506.70)	(417.00)	(63.60)	(56.10)
Regional mean	223.41	175.89	177.62	165.74	341.30	269.02	553.76	508.69	482.34	406.53	63.41	58.29

Data are represented as mean ± SD. 1= *O. niloticus* 2= *L. niloticus,* Values between brackets are bioaccumulation factors (BAF). I: El-Ramla. II: Kalabsha. III: Korosko. IV: Toushka

Table 3: Concentrations of zinc (mg/kg dry weight) and the bioaccumulation factor (BAF) in different tissues of *O. niloticus* and *L. niloticus* from the selected khors of Lake Nasser

	Zinc (Zn)											
	Liver		Gills		Intestine		Testis		Heart		Muscle	
Sites	1	2	1	2	1	2	1	2	1	2	1	2
I	35.91	31.25	29.93	27.50	113.09	100.75	174.13	166.67	39.11	34.22	13.80	11.63
	±6.22	±5.30	±3.29	±2.71	±9.20	±5.35	±9.91	±7.86	±2.01	±3.90	±1.13	±0.18
	(447.70)	(389.60)	(373.10)	(342.80)	(1410.10)	(1256.20)	(2171.20)	(2078.10)	(487.60)	(426.60)	(172.00)	(145.00)
II	39.20	38.75	26.33	24.88	91.22	81.88	192.90	185.00	55.01	41.13	9.11	8.63
	±2.01	±3.54	±1.23	±3.18	±5.11	±2.65	±11.59	±10.61	±5.31	±6.21	±0.92	±0.53
	(257.90)	(254.90)	(173.20)	(163.60)	(600.10)	(538.60)	(1269.00)	(1217.10)	(361.90)	(270.50)	(59.90)	(56.70)
III	41.72	39.00	35.11	30.10	102.91	121.15	219.01	196.11	46.33	39.91	10.33	8.80
	±5.61	±5.01	±1.33	±3.00	±8.55	±9.10	±5.03	±9.39	±7.01	±2.53	±1.18	±0.39
	(406.60)	(380.10)	(342.20)	(293.30)	(1003.00)	(1180.80)	(2134.60)	(1911.40)	(451.50)	(388.90)	(100.60)	(85.70)
IV	46.50	42.01	39.50	34.05	138.13	129.11	276.25	232.03	52.92	48.01	15.25	12.12
	±3.71	±3.10	±4.35	±5.22	±10.18	±6.93	±10.77	±11.31	±9.00	±3.86	±0.35	±0.81
	(218.70)	(197.60)	(185.80)	(160.10)	(649.70)	(607.20)	(1299.30)	(1091.30)	(248.90)	(225.80)	(71.70)	(57.00)
Regional mean	40.83	37.75	32.72	29.13	111.34	108.22	215.57	194.95	48.34	40.82	12.12	10.30

Data are represented as mean±SD, 1 = *O. niloticus,* 2 = *L. niloticus,* Values between brackets are bioaccumulation factors (BAF). I: El-Ramla. II: Kalabsha. III: Korosko. IV: Toushka

Table 4: Concentrations of copper (mg/kg dry weight) and the bioaccumulation factor (BAF) in different tissues of *O. niloticus* and *L. niloticus* from the selected khors of Lake Nasser

	Copper (Cu)											
	Liver		Gills		Intestine		Testis		Heart		Muscle	
Sites	1	2	1	2	1	2	1	2	1	2	1	2
I	67.10	48.13	14.03	12.25	51.01	48.75	38.71	37.50	5.98	4.11	2.82	2.63
	±5.92	±3.88	±0.93	±0.35	±3.72	±3.35	±2.36	±1.97	±1.20	±0.39	±0.33	±0.18
	(5500.00)	(3945.00)	(1150.00)	(1004.10)	(4181.10)	(3995.90)	(3172.90)	(3073.70)	(490.10)	(336.80)	(231.10)	(215.50)
II	50.35	34.38	9.16	6.13	45.91	26.88	35.01	32.11	3.11	2.37	1.95	1.32
	±6.22	±2.10	±1.03	±0.18	±4.16	±2.88	±1.10	±1.03	±0.23	±0.32	±0.81	±0.10
	(4841.30)	(3305.70)	(880.70)	(589.40)	(4414.40)	(2584.60)	(3366.30)	(3087.50)	(299.00)	(227.80)	(187.50)	(126.90)
III	71.19	53.45	16.32	13.15	59.46	54.35	43.56	39.72	7.22	5.10	3.11	2.90
	±3.00	±6.22	±2.68	±1.09	±2.97	±4.22	±2.95	±3.37	±0.51	±0.81	±0.26	±0.23
	(5312.60)	(3988.80)	(1217.90)	(981.30)	(4437.30)	(4055.90)	(3250.70)	(2964.10)	(538.80)	(380.60)	(232.00)	(216.40)
IV	101.63	85.11	18.63	14.22	63.50	56.22	51.25	44.11	9.29	7.81	3.50	3.00
	±5.53	±3.19	±3.18	±1.34	±5.92	±3.36	±3.77	±2.37	±0.87	±0.39	±0.35	±0.11
	(5238.60)	(4387.10)	(960.30)	(732.90)	(3273.20)	(2897.90)	(2641.70)	(2273.70)	(478.80)	(402.50)	(180.40)	(154.60)
Regional mean	72.57	55.27	14.54	11.44	54.97	46.55	42.13	38.36	6.40	4.85	2.85	2.46

Data are represented as mean±SD, 1 = *O. niloticus,* 2 = *L. niloticus,* Values between brackets are bioaccumulation factors (BAF), I: El-Ramla, II: Kalabsha, III: Korosko. IV: Toushka

Table 5: Concentrations of lead (mg/kg dry weight) and the bioaccumulation factor (BAF) in different tissues of *O. niloticus* and *L. niloticus* from the selected khors of Lake Nasser

	Lead (Pb)											
	Liver		Gills		Intestine		Testis		Heart		Muscle	
Sites	1	2	1	2	1	2	1	2	1	2	1	2
I	7.99	6.14	4.11	2.46	60.10	46.75	143.16	137.30	78.00	66.21	1.38	1.26
	±0.11	±1.14	±0.33	±0.36	±4.50	±2.77	±7.00	±6.57	±5.56	±5.08	±0.08	±0.10
	(163.70)	(125.80)	(84.20)	(50.40)	(1231.50)	(957.90)	(2933.60)	(2813.50)	(1598.30)	(1356.70)	(28.20)	(25.80)
II	8.08	5.94	5.28	1.25	69.98	61.70	156.22	123.25	82.11	74.30	1.46	1.23
	±1.10	±1.22	±0.13	±0.25	±2.10	±4.14	±8.12	±5.35	±2.98	±6.21	±0.12	±0.07
	(163.50)	(120.20)	(106.80)	(25.30)	(1416.60)	(1248.90)	(3162.30)	(2494.90)	(1662.10)	(1504.00)	(29.50)	(24.90)
III	11.33	7.33	5.98	2.98	85.97	75.00	192.08	186.11	113.00	96.98	1.79	1.50
	±2.01	±1.81	±0.17	±0.82	±5.17	±6.23	±10.33	±11.37	±6.39	±10.33	±0.30	±0.25
	(225.70)	(146.00)	(119.10)	(59.30)	(1712.50)	(1494.00)	(3826.30)	(3707.30)	(2250.90)	(1931.80)	(35.60)	(29.80)
IV	13.56	11.10	7.34	5.20	134.50	112.00	256.25	220.39	182.38	152.01	2.38	1.80
	±1.34	±2.01	±0.31	±1.19	±7.23	±5.25	±5.30	±9.23	±11.53	±5.28	±0.02	±0.13
	(127.40)	(104.30)	(68.90)	(48.80)	(1264.10)	(1052.60)	(2408.30)	(2071.30)	(1714.10)	(1428.60)	(22.30)	(16.90)
Regional mean	10.24	7.63	5.68	2.97	87.64	73.86	186.93	166.76	113.87	97.38	1.75	1.45

Data are represented as mean±SD, 1 = *O. niloticus,* 2 = *L. niloticus,* Values between brackets are bioaccumulation factors (BAF), I: El-Ramla, II: Kalabsha, III: Korosko, IV: Toushka

Table 6: Concentrations of cadmium (mg/kg dry weight) and the bioaccumulation factor (BAF) in different tissues of *O. niloticus* and *L. niloticus* from the selected khors of Lake Nasser

	Cadmium (Cd)											
	Liver		Gills		Intestine		Testis		Heart		Muscle	
Sites	1	2	1	2	1	2	1	2	1	2	1	2
I	1.89	1.86	1.70	1.60	1.10	0.99	15.11	13.33	32.09	29.11	0.93	0.81
	±0.07	±0.10	±0.18	±0.09	±0.06	±0.08	±2.21	±2.50	±3.19	±2.50	±0.04	±0.06
	(262.50)	(258.30)	(236.10)	(222.20)	(152.70)	(137.50)	(2098.60)	(1851.30)	(4456.90)	(4043.00)	(129.10)	(112.50)
II	2.12	1.81	1.91	1.72	1.20	0.78	14.93	14.22	37.93	26.91	1.11	0.74
	±0.13	±0.09	±0.07	±0.13	±0.10	±0.06	±1.32	±1.98	±2.71	±3.11	±0.06	±0.08
	(286.40)	(244.60)	(258.10)	(232.40)	(162.10)	(105.40)	(2017.50)	(1921.60)	(5125.60)	(3636.40)	(150.00)	(100.00)
III	2.58	2.45	2.38	2.20	1.34	1.18	18.09	16.39	48.12	39.82	1.35	1.00
	±0.08	±0.19	±0.17	±0.11	±0.09	±0.06	±2.11	±1.93	±2.08	±4.02	±0.11	±0.09
	(300.00)	(284.80)	(276.70)	(255.80)	(155.80)	(137.20)	(2103.40)	(1905.80)	(5595.30)	(4630.20)	(156.90)	(116.20)
IV	2.92	2.89	2.69	2.41	1.49	1.30	21.35	18.91	52.39	46.31	1.61	1.20
	±0.11	±0.08	±0.09	±0.13	±0.16	±0.10	±2.30	±2.01	±1.13	±5.32	±0.09	±0.12
	(292.00)	(289.00)	(269.00)	(241.00)	(149.00)	(130.00)	(2135.00)	(1891.00)	(5239.00)	(4631.00)	(161.00)	(120.00)
Regional mean	2.38	2.25	2.17	1.98	1.28	1.06	17.37	15.71	42.63	35.54	1.25	0.94

Data are represented as mean ± SD. 1= *O. niloticus* 2= *L. niloticus,* Values between brackets are bioaccumulation factors (BAF), I: El-Ramla, II: Kalabsha, III: Korosko, IV: Toushka

Table 7: Concentrations of cobalt (mg/kg dry weight) and the bioaccumulation factor (BAF) in different tissues of *O. niloticus* and *L. niloticus* from the selected khors of Lake Nasser

	Cobalt (Co)											
	Liver		Gills		Intestine		Testis		Heart		Muscle	
Sites	1	2	1	2	1	2	1	2	1	2	1	2
I	0.75	0.51	0.51	0.31	0.46	0.21	0.92	0.87	1.33	0.95	0.13	0.11
	±0.13	±0.09	±0.12	±0.02	±0.06	±0.03	±0.12	±0.21	±0.12	±0.09	±0.04	±0.03
	(2678.50)	(1821.40)	(1821.40)	(1107.10)	(1642.80)	(750.00)	(3285.70)	(3107.10)	(4750.00)	(3392.80)	(464.20)	(392.80)
II	1.13	0.80	0.85	0.52	0.59	0.41	1.49	1.23	1.71	1.42	0.15	0.14
	±0.20	±0.25	±0.08	±0.04	±0.07	±0.02	±0.20	±0.17	±0.16	±0.13	±0.06	±0.02
	(3766.60)	(2666.60)	(2833.30)	(1733.30)	(1966.60)	(1366.60)	(4966.60)	(4100.00)	(5700.00)	(4733.30)	(500.00)	(466.60)
III	0.19	0.15	0.14	0.12	0.16	0.13	0.57	0.36	0.60	0.49	0.07	0.04
	±0.09	±0.02	±0.06	±0.03	±0.01	±0.02	±0.10	±0.09	±0.10	±0.03	±0.003	±0.007
	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
IV	1.38	1.09	1.19	0.7	0.98	0.63	1.92	1.69	2.01	1.80	0.18	0.16
	±0.20	±0.28	±0.10	±0.05	±0.08	±0.11	±0.21	±0.13	±0.09	±0.17	±0.04	±0.08
	(4058.80)	(3205.80)	(3500.00)	(2058.80)	(2882.30)	(1852.90)	(5647.00)	(4970.50)	(5911.70)	(5294.10)	(529.40)	(470.50)
Regional mean	0.86	0.64	0.67	0.41	0.55	0.35	1.23	1.04	1.41	1.17	0.13	0.11

Data are represented as mean±SD, 1 = *O. niloticus* 2 = *L. niloticus,* Values between brackets are bioaccumulation factors (BAF), I: El-Ramla, II: Kalabsha, III: Korosko, IV: Toushka

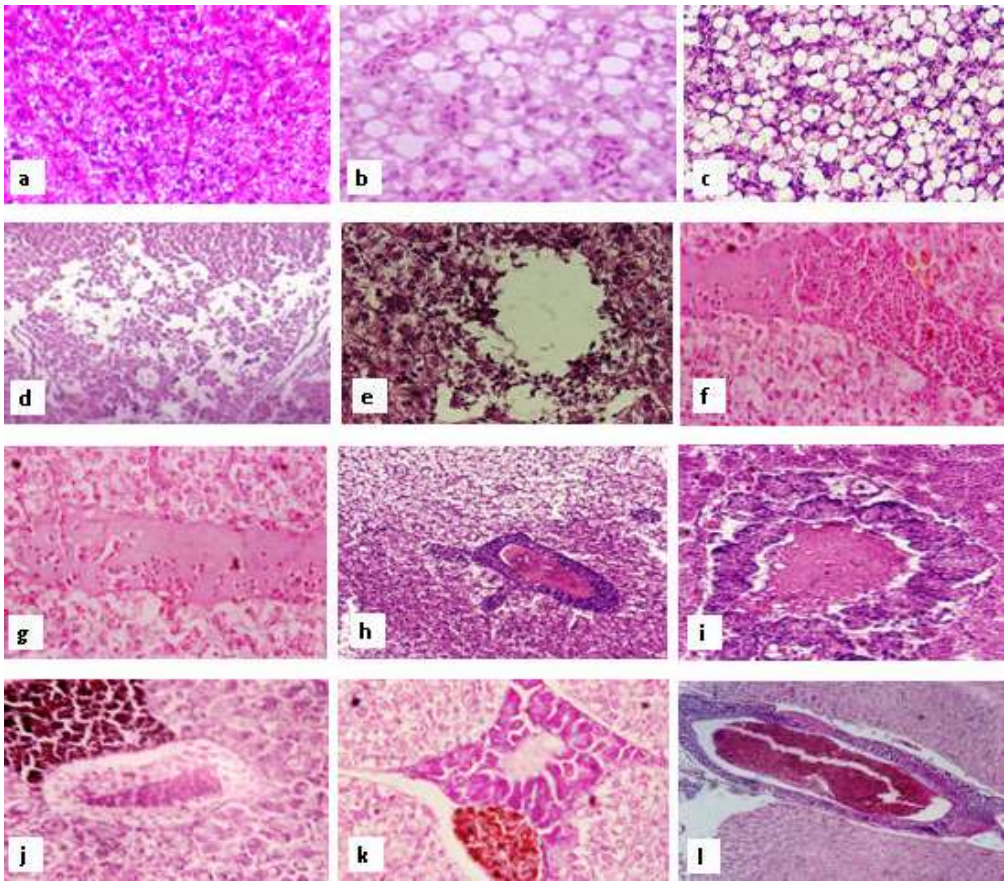

Fig. 2: Liver of fish showing the normal (a) (X400), vacuolar degeneration (b {*L. niloticus*}, c {*O. niloticus*})(X400), focal areas of necrosis (d {X100}, e {X400}{*O. niloticus*}), haemorrhage and haemolysis between the hepatocytes (f,g {*O. niloticus*}{X400}), intravascular haemolysis in blood vessels (h {X100}, i {X400}{*L. niloticus*}), haemosiderin around central vein and hepatoportal blood vessel (j,k {*O. niloticus*}{X400}), thrombosis formation in hepatoportal blood vessel (l {*L. niloticus*}{X100})

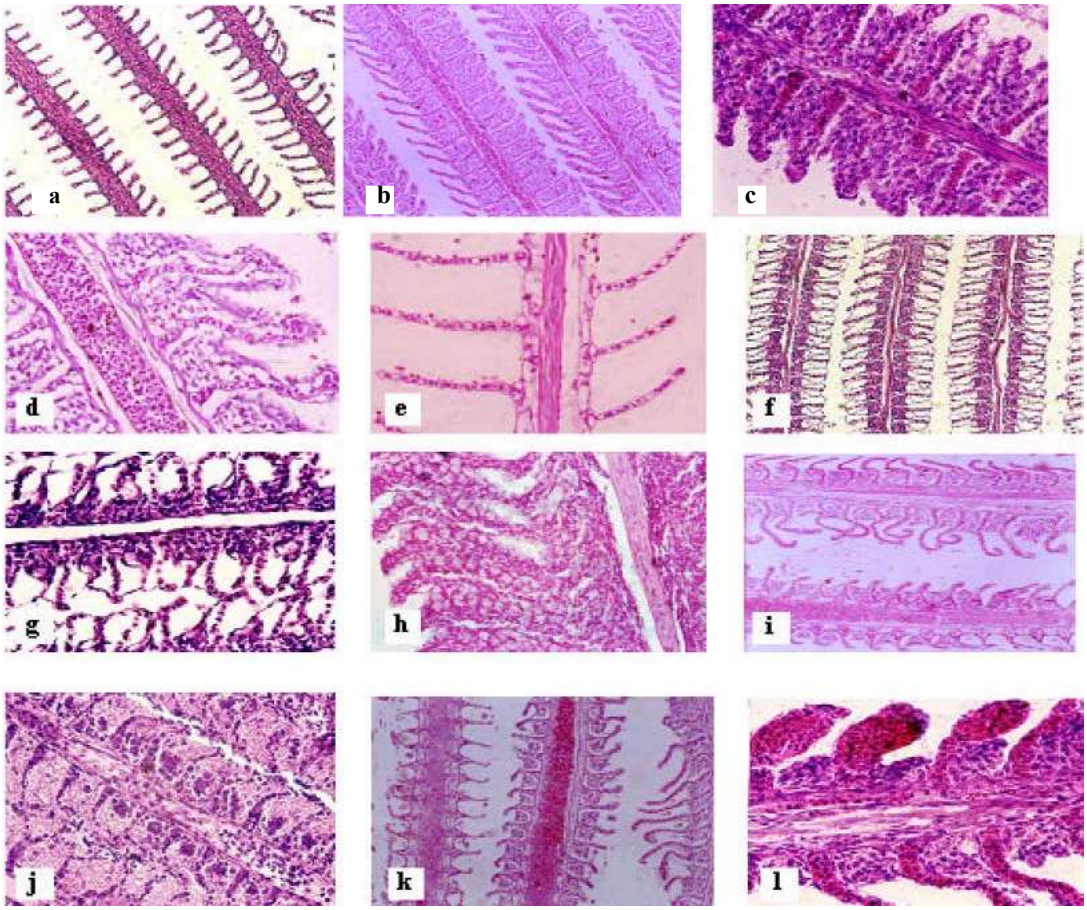

Fig. 3: Gills of fish showing the normal (a) (X100), proliferation in the epithelium of gill filaments and secondary lamellae (b {X100}, c {X400}{*O. niloticus*}), degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae (d,e {X400}{*L. niloticus*}),edema in secondary lamellae (f {X100}, g {X400}{*O. niloticus*}), proliferation of mucous cells (h {X400}{*O. niloticus*}), curling of secondary lamellae (i {X100}{ *L. niloticus*}), haemorrhage between gill filaments (j {X400}{*O. niloticus*}), dilation in blood vessel of gill filament (k {X100}{*L. niloticus*}), telangiectasis in secondary lamellae (l {X400}{*O. niloticus*})

studied tissues of both fish were in the following order: liver > intestine>testis>gills>heart>muscle

Lead: Table 5 shows the concentrations of Pb in the tissues of both fish. Pb accumulations in the studied tissues of both fish were in the following order: testis>heart>intestine>liver>gills>muscle.

Cadmium: Table 6 shows the concentrations of Cd in the studied tissues of both fish. Generally, Cd concentrations in the tissues of both fish were in the following order: heart>testis>liver>gills>intestine> muscle.

Cobalt: Table 7 shows the concentrations of Co in the studied tissues of both fish. In general, Co accumulations in the studied tissues of both fish were in the following order: heart>testis>liver>gills>intestine> muscle for khors El-Ramla, Kalabsha and Toushka and heart>testis>liver> intestine>gills>muscle for khor Korosko.

Generally, the highest values of the studied metals were recorded in the tissues of fish from khor Toushka. The data indicated that the tissues of *O. niloticus* accumulated more heavy metals than *L. niloticus*.

Bioaccumulation factor (BAF): It was found that the concentration of the studied metals (Fe, Zn, Cu, Pb, Cd and Co) in different tissues of both fish were several times higher than their concentrations in water. It is obvious from the data given in Table (2-7) that the highest bioaccumulation factors (BAF) of Fe, Zn and Pb were recorded in the testis of the studied fish, Cu in the liver and Cd & Co in the heart. However, the lowest values of BAF were recorded in the muscles.

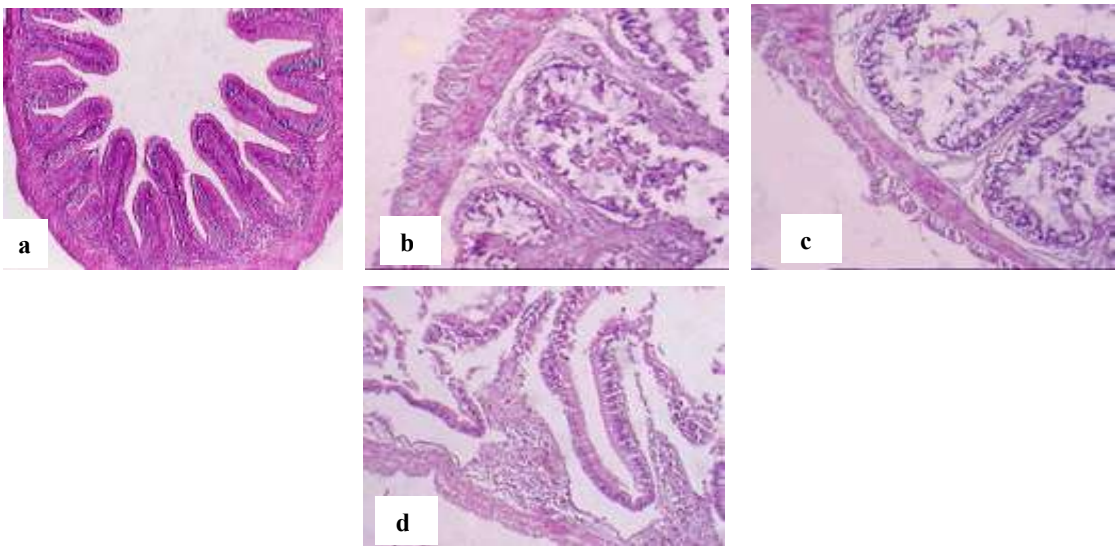

Fig. 4: Intestine of fish showing the normal (a)(X100), severe degenerative and necrotic changes in the intestinal mucosa (b {*O. niloticus*}, c {*L. niloticus*}{X400}), edema between the intestinal submucosa and mucosa (d {*O. niloticus*}{X400})

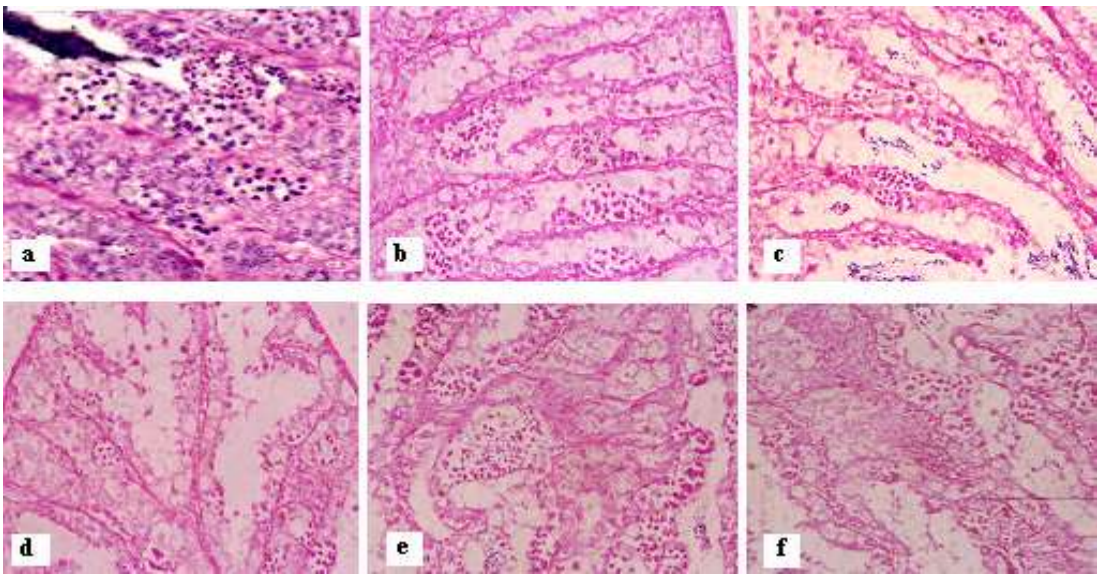

Fig. 5: Testis of fish showing the normal (a)(X400), severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules (b {*O. niloticus*}, c {*L. niloticus*}{X400}), focal areas of necrosis (d {*O. niloticus*}{X400}), focal areas of fibrosis (e {*O. niloticus*}, f {*L. niloticus*}{X400})

Histopathological alterations:

Liver (Fig. 2): Fig. 2a shows the normal histological structures of the liver. The histopathological alterations in the liver of both fish from Lake Nasser were more or less similar. They included vacuolar degeneration in the hepatocytes, focal areas of necrosis, haemorrhage and haemolysis between the hepatocytes and dilation and intravascular haemolysis in hepatoportal blood vessels. Moreover, haemosiderin was seen around central veins and hepatoportal blood vessels. In some cases, thrombosis formation in hepatoportal blood vessels was observed.

Gills: Figure 3a shows the normal histological structures of the gills. The gills of both fish from Lake Nasser showed the same histopathological alterations

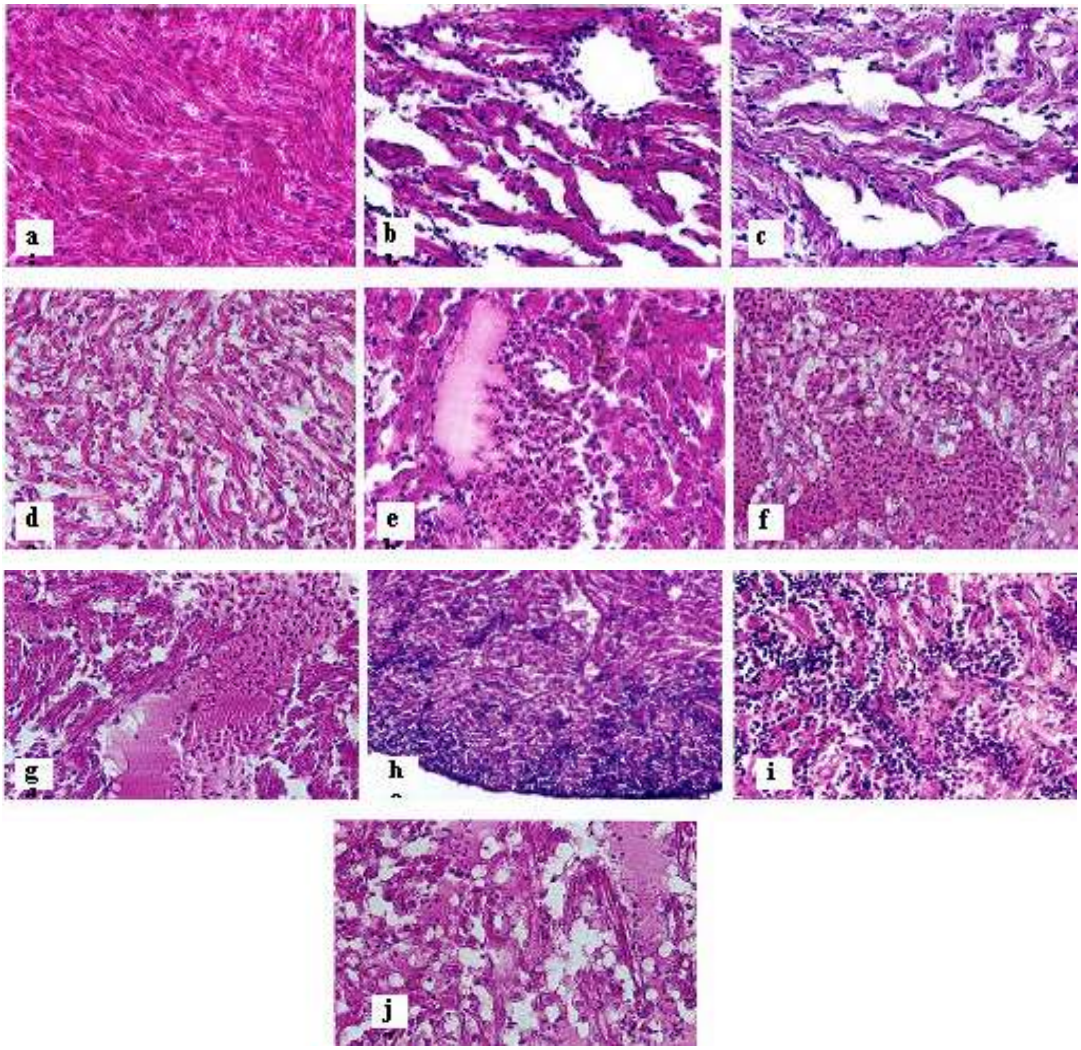

Fig. 6: Heart of fish showing the normal (a)(X400), degeneration (b{*O. niloticus*},c{*L. niloticus*}{X400}) and atrophy (d {*O. niloticus*}{X400}) in myocardial muscle fibers, haemorrhage, haemolysis (e,f {*O. niloticus*},g {*L. niloticus*}{X400}) and aggregations of inflammatory cells (h {X100}, i {X400}{*O. niloticus*}) between myocardial muscle fibers, vacuolar degeneration in myocardial muscle fibers of auricle (j {*O. niloticus*}{X400})

with different degrees of severity. The most common features were proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae and edema in secondary lamellae accompanied with separation of their epithelium from the lamellar supporting cells. Marked proliferation of mucous cells, curling of secondary lamellae, haemorrhage between gill filaments, dilation in blood vessels of gill filaments and telangiectasis in secondary lamellae were observed.

Intestine: Figure 4a shows the normal histological structures of the intestine. The histopathological alterations in the intestine of both studied fish included severe degenerative and necrotic changes in the intestinal mucosa with necrotized cells aggregated in the intestinal lumen and edema between the intestinal submucosa and mucosa.

Testis: Figure 5a shows the normal histological structures of the testis. The histopathological findings in the testis of both studied fish included severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules with focal areas of necrosis and degeneration in the wall of seminiferous tubules. Focal areas of fibrosis were seen. Seminiferous tubules appeared with a lesser number of sperms or lucent indicating lack of active spermatogenesis.

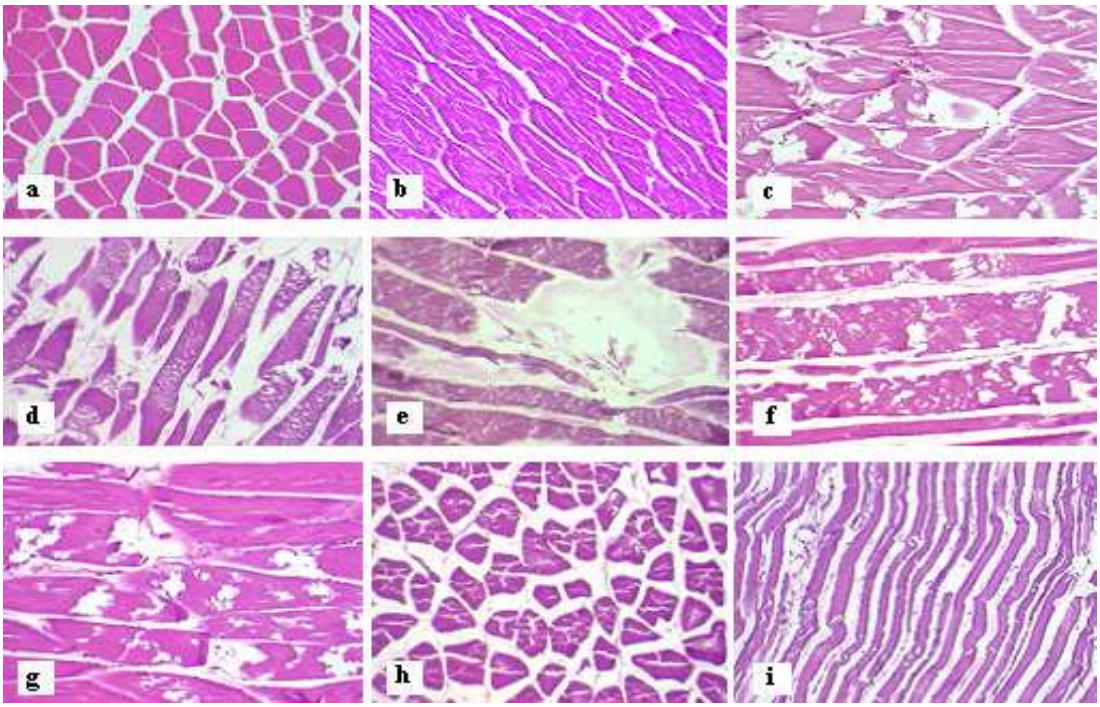

Fig. 7: Muscles of fish showing the normal (a,b)(X400), degeneration in muscle bundles (c,d{*O. niloticus*}{X400}), focal area of necrosis (myolysis) (e {*L. niloticus*}{X400}), vacuolar degeneration in muscle bundles (f {*O. niloticus*}, g {*L. niloticus*}{X400}), splitting of muscle fibers (h {*O. niloticus*}{X400}), atrophy of muscle bundles (i{*O. niloticus*}{X400})

Heart: Figure 6a shows the normal histological structures of the heart. The most common lesions in ventricle and auricle of both studied fish were degeneration and atrophy in myocardial muscle fibers, accompanied with haemorrhage, haemolysis and aggregations of inflammatory cells between myocardial muscle fibers. Moreover, vacuolar degeneration was seen in myocardial muscle fibers of auricle.

Muscle: Figure 7a and 7b show the normal histological structures of the muscle. The histopathological alterations in the muscles of both studied fish included degeneration in muscle bundles accompanied with focal areas of necrosis. Also, vacuolar degeneration in muscle bundles, splitting of muscle fibers and atrophy of muscle bundles were seen.

DISCUSSION

The concentration of Fe in the water of the four khors of Lake Nasser (0.4418-1.2254mg/l) was higher than the permissible level (0.3mg/l) recommended by the Egyptian Organization for Standardization [35], which could be attributed to Fe liberation from sediments as sulphides [1]. Zn (0.0802-0.2126mg/l) and Cu (0.0104-0.0194mg/l) concentrations were lower than the permissible levels (5 and 1mg/l, respectively) permitted by the Egyptian Organization for Standardization [35]. The Egyptian Standards of the Environmental Laws no. 48/1982 and 4/1994 state that the maximum Pb concentration in water is 0.05mg/l. The comparison between the present results (0.0488-0.1064mg/l) and the previous recommended permissible level indicated a higher concentration recorded at khor Toushka. The source of Pb in the lake water and fish may be resulted from gasoline contains Pb from the fishery boats and tour ships travels from Aswan to Sudan [36]. The Cd concentrations in the water of the four khors (0.0072-0.0100mg/l) are still below the permissible level (0.01mg/l) recommended by the Egyptian Organization for Standardization [35]. Co concentrations varied from ND to 0.00034mg/l. By comparing the present Fe, Zn and Pb concentrations in water with the previous levels of the same metals in the Lake waters, the present levels are high (Table 8). This means that metal pollution in the waters has increased with the increase in human activities in the Lake. The concentrations of Cu and Co are lower than the previous levels.

Heavy metals are known to be cumulated in fish tissues, reaching concentrations of up to 20000 fold higher than surrounding water environment [38]. Fe

Table 8: Metal concentrations (mg/l) in Lake Nasser water in years 1980 to the present study

Fe	Zn	Cu	Pb	Cd	Co	References
0.075	-	0.1890	0.0010	0.0120	0.14200	[37]
0.142	-	0.2200	0.0050	0.0100	0.18500	[21]
-	0.0180	0.0142	-	0.0035	0.00650	[10]
0.7651	0.1369	0.0139	0.0637	0.0083	0.00031	Present study

was the most abundant metal in the studied tissues of both fish. The high accumulation of Fe in different fish tissues can be attributed to the large quantities of Fe detected in water, this agrees with the findings of [15]. The data showed that Fe concentration in the studied tissues of both fish was in the following manner: testis>heart>intestine> gills>liver>muscle for khors I&III and testis>heart>intestine>liver> gills>muscle for khors II&IV. Higher Fe content in the testis of both fish agrees with earlier findings of [39]. Whereas, the muscle accumulated the lowest levels of Fe and this agree with those reported by [40]. The present data showed that iron concentrations in the studied tissues were more than US maximum permissible level for Fe (5µg/g) cited by [41].

The concentration of Zn in the studied tissues of both fish was in the following manner: testis>intestine>heart>liver>gills>muscle. The results are in accordance with those obtained by [11, 23] who mentioned that the bioaccumulation of Zn was found to follow the order: liver>gills>muscle. However, [17] found that Zn-gills>Zn-liver>Zn-muscle. The highest levels of Zn were recorded in the testis of both studied fish, this agrees with the findings of [39]. The data indicated that Zn accumulated in the heart of both fish more than the liver, gills and muscle, this agrees with that reported by [42]. Western Australian Food and Drink Regulations recommended a level of 40mg/kg Zn for human consumption [43]. Accordingly, the concentrations of Zn in the muscles of the studied fish are still below the permissible level.

Cu concentration in the studied tissues was in the following manner: liver>intestine>testis>gills>heart> muscle. The present data indicate that liver accumulated higher amounts of Cu and this may be due to its ability to retain Cu. It has been mentioned by [44] that in fish, the liver is the selective organ for storage of Cu. Our results agree with those obtained by [15, 17]. Similarly, [9, 19] found that Cu exhibited its highest levels in the liver and the lowest values in the muscles. The high accumulation of Cu in the liver could be attributed to the specific metabolic processes and enzyme catalyzed reaction involving Cu taking place in the liver. The sulfur legends in the liver also have a great tendency to co-ordinate with Cu *via* oxygen carboxylate amino group nitrogen and/or sulfur of the mercapto group in the metalothionin protein which is in the highest concentration in the liver [45,46]. The concentrations of Cu in the muscles of the studied fish are still below the permissible level for Cu (30mg/kg) recommended by the National Health and Medical Research Council [43].

Pb accumulation in the tissues of both studied fish was in the following order: testis>heart>intestine> liver>gills>muscle. The same trend was observed by [12, 23]. [39] found that Pb exhibited its highest level in the gonads and the lowest value in the muscles. Furthermore, [9] recorded high concentrations of Pb in the intestine of *T. nilotica* from Lake Nasser. The present study revealed that Pb concentrations in the muscles of both fish (except the muscle of *O. niloticus* from khor Toushka) are still below US FDA maximum permissible level for Pb (2.0µg/g) [47].

Cd concentrations in the tissues of the studied fish were in the following order: heart>testis>liver>gills> intestine>muscle. A similar trend was observed in the tissues of *P. pagrus* and *S. aurita* from the Mediterranean Sea [42] and in the tissues of *O. niloticus* and *T. zillii* from Abu Za′baal Lakes [23]. Also, [12] reported that Cd concentrations in the tissues of *C. luteus* from Orontes (Asi) River (southeastern Turkey) were detected in the following manner: gonads>liver>gills>muscle. The concentrations of Cd in the muscles of both studied fish are still below WHO permissible level for Cd (2.0mg/kg) reported by FAO [48]. In the present study, the Co accumulations in the studied tissues were in the following order: heart>testis>liver>gills>intestine>muscle for khors I, II&IV and heart> testis>liver>intestine>gills>muscle for khor III. The same trend was observed by [49]. [50] mentioned that Co concentrations in the tissues of *Tilapia sp.* from Lake Nasser were detected in the following manner: liver>gills>muscle.

It is clear that the tissues of *O. niloticus* accumulated more metals than *L. niloticus.* These observations are mainly due to different fish habitat and the influence of the surrounding ecosystem status [11]. Moreover, the present results indicated that the order of metal concentrations was Fe>Cu>Zn>Pb>Cd>Co in the liver; Fe>Zn>Cu>Pb> Cd>Co in the gills and muscle; Fe>Zn>Pb>Cu>Cd>Co in the intestine and testis; Fe>Pb>Zn>Cd>Cu>Co in the heart. It could be

concluded that the concentrations of metals in the studied fish tissues is dependent upon the target organ and species of fish as well as the type of metal. This is in agreement with that reported by [49, 50]. Bioaccumulation factor gives an indication about the accumulation efficiency for any particular pollutant in any fish organ. Bioaccumulation factors of the studied metals indicate that the elevated concentrations of the measured metals in both fish are derived from water, sediment and aquatic plants as reported by [9, 20]. The results suggested the loss of homeostatic capacity of both fish under chronic metal exposure leading to bioaccumulation.

The liver of both studied fish showed marked histopathological changes. Degeneration and necrosis of the hepatocytes may be due to the cumulative effect of metals and the increase in their concentrations in the liver. These results agreed with [11] who stated that the liver has an important detoxical role of endogenous waste products as well as externally derived toxins as heavy metals. The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood [51]. Many authors have reported similar histopathological alterations in the liver of fish exposed to metals [24, 27, 28].

The present study showed several histopathological alterations in the gills. The observed proliferative changes in the respiratory lamellar epithelium may increase the epithelial thickness which retard or prevent the entry of toxic metals into the blood stream [52]. Such proliferative changes which resulting in fusion of the secondary lamellae may lead to a great disturbance of gas exchange and ionic regulation [53]. According to [54], the observed dilation of the lamellar blood vessels and the presence of edematous fluid in the secondary lamellae may be due to increased permeability induced by the prolonged exposure to the metals. This edematous fluid separated the respiratory epithelium from the underlying tissue and led to its desquamation as well as necrosis. The observed proliferation of mucous cells is in agreement with those reported by [55] who stated that proliferation and stimulation of mucous cells occur in response to metals. The histopathological alterations in the gills of the studied fish are in agreement with those observed in other fish species under the influence of different metals [24-27].

Uptake of metals occurs mainly through gills but may also occur *via* intestinal epithelium. The histopathological alterations observed in the intestine of both fish (severe degenerative and necrotic changes in the intestinal mucosa as well as edema between submucosa and mucosa) may be a result of uptake of toxic metals [56]. The present results are in agreement with those observed by many investigators about the effects of metals on fish intestine [25, 56].

Pollutants may have direct effects on the gonads, resulting in a disturbed development of germ cells. Indirect effects on reproduction, *via* interference with the regulating hormonal system, have also been suggested [57]. According to [58], the testis contains a fixed number of spermatogonia which are responsible, by mitosis, for the production of additional spermatogonia to maintain the processes of spermatogenesis and sperm production. Therefore, the observed degenerative and necrotic changes in the cellular elements, including spermatogonia, of the seminiferous tubules in the testis of both fish result in permanent testicular damage and may reduce the ability of fish to reproduce. High metal accumulation occurring in the testis, affects the process of spermatogenesis and suppressing sperm production. The present results are in agreement with those observed by many authors who have studied the effects of metals on fish testis [56, 59]. After passing through the blood vascular system the metals could have targeted the cardiac tissue, causing extensive damage. The myocardial cell degeneration and necrosis observed in the present study could be attributed to the excessive calcium accumulation and/or increased catecholamine release, as previously reported by [60]. As with gills, muscle tissue come into close contact with pollutants dissolved in water. The muscles of both fish showed degeneration in muscle bundles accompanied with focal areas of necrosis as well as atrophy and vacuolar degeneration in muscle bundles.

In conclusion, the results revealed that the concentrations of the studied metals in Lake Nasser water were in the following order: Fe>Zn>Pb>Cu>Cd>Co, these metals accumulated in different tissues of *O. niloticus* and *L. niloticus*. Generally, the studied metals were more concentrated in the non-edible parts of the fish than the edible muscles. Zn, Cu, Pb and Cd concentrations in the muscles were within the maximum permissible limit; however, Fe in the muscles exceeded the permissible level. It was found that the accumulated metals induced several histopathlogical alterations in the tissues of both fish.

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