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Impression of Various Water Reserves on Reproductive Performance of the Nile Tilapia "*Oreochromis niloticus*"

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ABSTRACT

This work involved the investigation of the reproductive biology concerning the seasonal variations of the Nile tilapia (*Oreochromis niloticus*) broodstock. A total of 320 apparently healthy fish individuals were collected from four locations (El Manzala and El Abbassa fish hatcheries, Bahr Hadous drain and Ismailia Canal) during the period from 2020- 2021. Water parameters were seasonally evaluated. Besides, all fish samples were subjected to visual and histopathological examinations. The effect of water pollution with various heavy metals on the fish reproductive performance was studied with reference to corresponding bioaccumulation in fish reproductive organs. Water examination recorded a significantly low DO value in El Manzala ($P< 0.05$); whereas, a substantial rise was detected in water salinity, EC, BOD, COD and total ammonia. Water pollution was distinguished with a variety of heavy metals (iron, copper, zinc, cadmium, mercury, lead and arsenic), which exceeded their permissible limits and were significantly high in El Manzala ($P< 0.05$). The results revealed a distinctive surge of gonadosomatic index (GSI) and absolute fecundity of El Abbassa either farmed or wild fish rather than the perceptible retarded performance of both farmed and wild fish of El Manzala ($P< 0.05$), with evident alterations in their reproductive organs. Consistent bioaccumulation of these heavy metals was apparent in the liver and reproductive organs that exceeded their safe limits. Consequential devastating effects on the reproduction function were recorded as the female GSI was mostly affected by elevated water Fe, Pb and Cd and bioaccumulated Fe and Cd in their ovaries. While, their fecundity was influenced by high water Cu, Cd and Hg as well as the bioaccumulated Pb in their ovaries. On the other hand, the male GSI was mostly affected by high water Cu and Pb as well as bio-accumulated Zn and Hg in their testes.

INTRODUCTION

Reproduction is one of the essential biological aspects permitting the ultimate survival and maintenance of species. In aquatic ecosystem, it represents the liberation of unfertilized eggs by female fish. The eggs are fertilized just after release by males. The ripening of eggs and spawning are controlled by hormones, female nutrition and external (environmental) factors **(Akash & Neha, 2017)**.

The Nile tilapia (*O. niloticus*) is the most conventional and prevalent fish in Egypt, where tilapia farming has mostly its roots **(El-Sayed, 2019)**. The Egyptian fish farms are constructed depending on different sources of water with various physical, chemical and biological features affecting the quality of the cultured fish and eventually the human health **(Saeed, 2000; Pulatsu *et al*., 2004**; **Ali, 2007)**. Water pollution occurs when pollutants are directly or indirectly discharged into the water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater) without adequate treatment to remove harmful compounds **(Authman, 2008)**. The presence of pollutants is one of many environmental factors that can result in a dangerously compromised fish reproduction system. Many studies have proven that exposure to pollutants can lead to a decline in gonadosomatic index, shrinking oocyte diameter, dysfunctional reproduction hormones, alteration in reproductive behavior and increased abnormality of fish larvae **(Alquezar *et al*., 2006; Ebrahimi & Taherianfard, 2011; Gerbron *et al*., 2014; Bertram *et al*., 2015; Zhang *et al*., 2016)**. Heavy metals are described to stimulate or reduce the endocrine system, leading to an over-production or under-production of reproductive hormones such as 17β-estradiol in fish. In addition, it might interfere with reproduction via disrupting the endocrine system through the direct cytotoxic effects of metals on gonadal tissues **(Tabb & Blumberg, 2006; Islam *et al*., 2015).** Suffering from stress during the reproductive development could have negative effects on the fecundity, gamete development and reproductive quality in most fish species. For instance, the exposure of many teleost to copper, zinc, and lead during development resulted in the loss of oocytes in the ovaries **(Campbell *et al*., 1992; Mazrouh & Mahmoud, 2009)**.

Therefore, the present study is organized to investigate the reproductive biology concerning the seasonal variations of the Nile tilapia (*O. niloticus*) brood stock from four locations with two different water resources. In addition, it aimed to investigate the impact of heavy metal water pollution on the fish reproductive performance.

MATERIALS AND METHODS

Sampling scene

This study focused on two different water environments (Fig. 1), one depending on drainage water supply in El Manzala: 1) El Manzala hatchery (at 31^{o} 133'793" North and 31^{o} 962'629" East) for the farmed Nile tilapia broodstock. 2) Bahr Hadous drain (at 31^{o} 06'14"North, 31^{o} 58'49" East) for the samples of its wild mature Nile tilapia.

The other is fresh irrigation water environment in El Abbassa: 3) El Abbassa fish hatchery, El Sharqia Governorate (at 30^{o} 515'446"North and 31^{o} 713'235" East) for obtaining the farmed Nile tilapia broodstock. 4) Ismailia canal (at 30^{o} 533'584" North, 31^{o} 710'278" East) for the samples of the wild mature Nile tilapia.

Fig. 1. Sampling locations (by Google Earth) showing the four sites under survey: El Manzala fish hatchery (A_1), Bahr Hadous (A_2), El Abbassa fish hatchery (B_1) and Ismailia Canal (B_2)

Water examination

Water of the four locations under study was tested directly for the following physical parameters: water temperature using dry mercury thermometer, dissolved oxygen using a digital oxygen meter version (AQUALYTIC, OX 24), water transparency (cm) using Sacchi-disc (diameter 25cm), pH using pH-meter (Model Janway 3150), electrical conductivity (EC) using portable conductivity meter (Model S.C.T.33 YSI) and water salinity using portable ORION Salinometer (Model 115). Three estimates were evaluated per time, aligned with the collection of fish samples at the same sites.

A total number of 16 water samples (triplicate one liter capacity dark glass bottles/sample from each location) were collected according to the standard method of **Gregg (1989)**, seasonally together with fish samples collection. All water samples were kept cool in iceboxes and transported immediately to the laboratory for the examination of biological oxygen demand (BOD) using Lovibond BOD System Model BD 600. Chemical oxygen demand (COD) was measured using LAR Process analyzers AG of Quick $COD^{®}_{lab}$ Plus. Total ammonia was measured according to the method of **APHA (2005)**.

The estimation of heavy metals {iron (Fe), copper (Cu), zinc (Zn), Cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As)} in water samples were carried out using Inductivity Coupled Plasma View Spectrophotometer (Thermo Fisher Scientific Model: iCAP 7400). All the analyses of water samples were performed in duplicates to avoid deceptive results.

Fish examination

A total of 320 specimens (apparently healthy mature Nile tilapia *O. niloticus*) were collected through the four seasons (winter, spring, summer and autumn) during the study period 2020- 2021. Farmed broodstock fish were two years old, with mean weight of 500±50g and mean total length of 28±2cm; while randomly collected mature wild Nile tilapia fish individuals that were about one year old, with mean weight of 130±50g and mean total length of 18±2cm. All fish were collected alive and transported immediately to the laboratory belonging to the Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Mansoura University in suitable containers provided with portable aerators for succeeding examinations.

Fish samples were submitted to visual sexing, total length estimation to the nearest millimeter as well as total weight to the nearest 0.1g. Scales from the left side just below the lateral line and behind the pectoral fin were taken, saved in special envelopes with full information for later age reading. Fish were dissected and eviscerated, while° gonad samples were inspected and weighed.

Samples from liver and gonads of ten fishes from each location (spring season only) were taken and sealed separately in labeled plastic sachets and preserved in -20°C for further analysis of heavy metals.

Fish reproductive performance

Gonadosomatic index (GSI)

Gonadosomatic index was calculated for both sexes according to the following equation:

$$\mathbf{GSI} = \frac{Gw}{W} \times \mathbf{100} \qquad \textbf{(Anderson, 1996)}$$

Where, ***Gw*** is gonad weight (g), and **W** is the total body weight (g).

Estimation of absolute fecundity (F)

Absolute fecundity was evaluated as the average number of ripe (opaque) eggs per ovary just before spawning as reported by **Duponchelle *et al*. (2000)**.

The total number of mature eggs in the ovary was calculated following **Khallaf *et al*. (2003)** according to the equation:

$$\mathbf{F} = \frac{\textit{No of mature eggs in the subsample} \times \textit{Weight of the ovary}}{\textit{Weight of the subsample}}$$

Age determination

The age of sampled fish was calculated according to **El-Zarka *et al*. (1970)**. Growth evaluation was not regarded with special concern in this study, data were just used for confirming correlations.

Histopathological studies

Parts of liver and gonad samples (about 80 sample of each organ) were preserved and histopathologically examined according to **Shobikhuliatul *et al*. (2013)**.

A semi-quantitative histopathological evaluation protocol was used, based on the scoring scheme according to **Zimmerli *et al.* (2007)** with some adjustments, following **Zahran *et al*. (2020)** to quantify pathological changes observed in the tested organs.

Heavy metals assessment in fish reproductive organs

The samples digestion was carried out following the method of **Agemian *et al.* (1980)**. Then, the selected heavy metals; namely, Fe, Cu & Zn as essential heavy metals and Cd, Hg, Pb & As as toxic metals in digested samples were estimated using solar atomic absorption spectrophotometer {Thermo (SAAS) Model Techno Sens AA (1.0.2.2.1)} according to the method described by **APHA (2005)**.

Statistical analysis

All the obtained results were statistically analyzed using the one-way analysis of variance (ANOVA) for studying the variance between locations under study. Pearson coefficient was conducted for bivariate correlation analysis. Stepwise multiple linear regression tests for evaluating the correlated influential factor(s) affected the reproductive performance of studied fish.

The effects with a probability of $P < 0.05$ were judged significant, while the probability of $P < 0.01$ was judged highly significant. All the data and graph correlation between various parameters were examined using the SPSS software for windows version 26.0 (SPSS, Chicago, IL, USA).

RESULTS

Assessment of water physicochemical parameters

As shown in Table (1), dissolved oxygen was significantly high in El Abbassa water than in El Manzala ($P < 0.05$), whereas the mean values of DO in the hatchery and Bahr Hadous were below the permissible limit. Water transparency was significantly high in Ismailia Canal, compared to the other locations. Electrical conductivity as well as water salinity was significantly high in the water of El Manzala than El Abbassa water. BOD values exceeded the permissible limit in all locations except for Ismailia Canal. The highest BOD value was recorded in the water of the fish hatchery in El Manzala, which is significantly different from El Abbassa (both the hatchery and Ismailia Canal) ($P < 0.05$). COD as well exceeded its permissible limit. Besides, El Manzala water had significantly higher COD mean values than El Abbassa ($P < 0.05$). Furthermore, the total ammonia in El Manzala water exceeded its permissible limit; besides, they were significantly higher than El Abbassa ($P < 0.05$).

Clinical feature of examined fish

In this study, the Nile tilapia of El Manzala (both farmed and wild fish) showed emaciation, eroded fins, abnormal dark discoloration, eye lesions as blindness with loss of scales (Fig. 2). The female ovaries showed dark discoloration with diffuse dark colored spots of old hemorrhages; elongated thin like shape testes and asymmetry in size with hemorrhagic spots (Fig. 3). While, mature males showed very thin empty like testes and abnormal discoloration (Fig. 4).

Table 1. Estimated water physiochemical parameters (means ±SE) among studied locations

Water Parameter	El Manzala (Drainage water source)		El Abbassa (Irrigation water source)		PL
	Farmed	Wild	Farmed	Wild	
Temperature (^{0} C)	25.25±2.1 [ab]	24.25±2.53[abc]	26.25±1.75 [a]	25.13±2.38[abc]	8 – 28*
DO (mg/l)	4.83±0.47[c]	4.4±0.49[c]	7.83 ± 0.65[a]	7.75±0.34[ab]	5.5*
Transparency (cm)	39.5±2.1[bc]	49.5±2.96[bc]	50.75±2.93[b]	66.75±3.15[a]	> 30**
PH	7.79±0.06 [ab]	7.93±0.11[a]	7.77±0.12[abc]	7.89±0.23[abc]	6.5-9*
EC (ms)	1.18±0.11[a]	1.03±0.11[ab]	0.3±0.02 [c]	0.29±0.02[C]	1400***
Salinity (g/l)	0.75±0.07[a]	0.68 ± 0.07[ab]	0.19±0.01[c]	0.18±0.01[c]	< 5****
BOD (mg/l)	38.3±5.09[a]	33.78±4.44[ab]	18.43±2.63[bc]	7.15±1.67[c]	10**
COD (mg/l)	59.15±3.29[ab]	77.28±8.09[a]	14.6±1.55[c]	13.25±1.3[c]	7*
Ammonia (mg/l)	3.59±0.36[ab]	3.78±0.27[a]	0.74±0.09[c]	0.4±0.04[c]	1.37*

*** CCME (2007)** **** Santhosh & Singh (2007)** ***** WHO (1993)** ****** WHO (2008)**

- Different letters within the same row are significantly different (P< 0.05). **P.L. = Permissible limits**

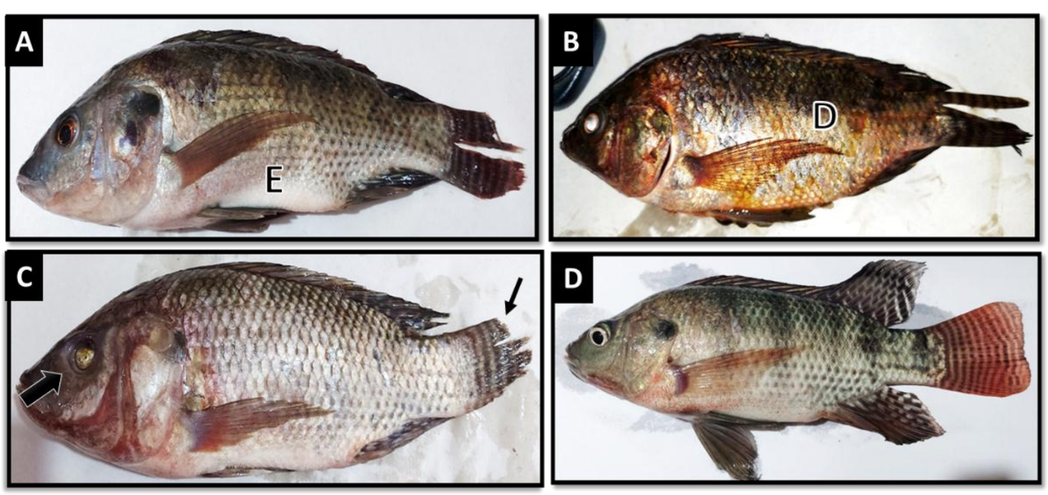

Fig. 2. Mature Nile tilapia (*O. niloticus*) from El Manzala **(A, B & C)** showing emaciation “E”, eroded fins “thin arrow”, abnormal dark discoloration “D”, blindness (thick arrow) with loss of scales, compared to apparently normal shape and color of El Abbassa fish **(D)**

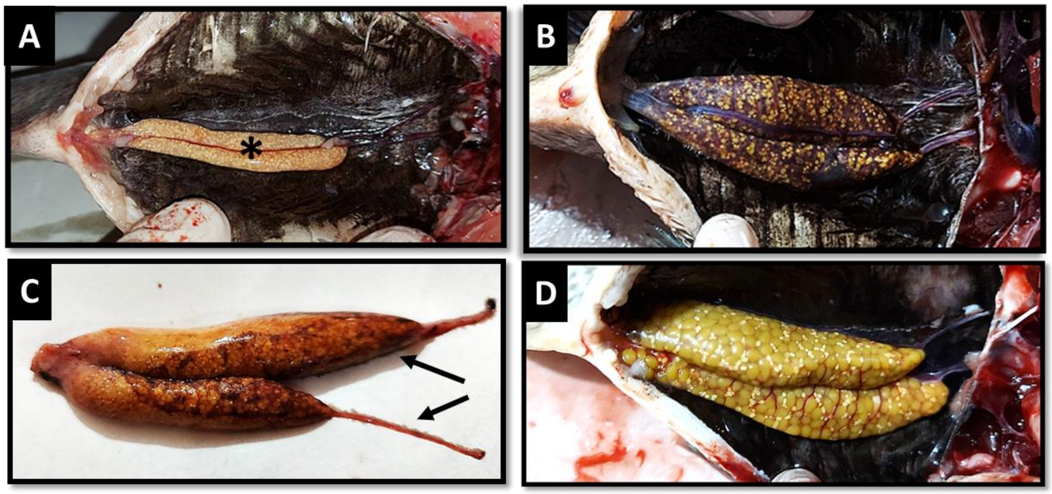

Fig. 3. Ovaries of El Manzala Nile tilapia (*O. niloticus*) mature females showing elongated thin testis like ovary "*" **(A)** dark discoloration **(B)** and asymmetry "arrows" with hemorrhagic spots **(C),** compared to apparently normal shape and color of El Abbassa female ovaries **(D).**

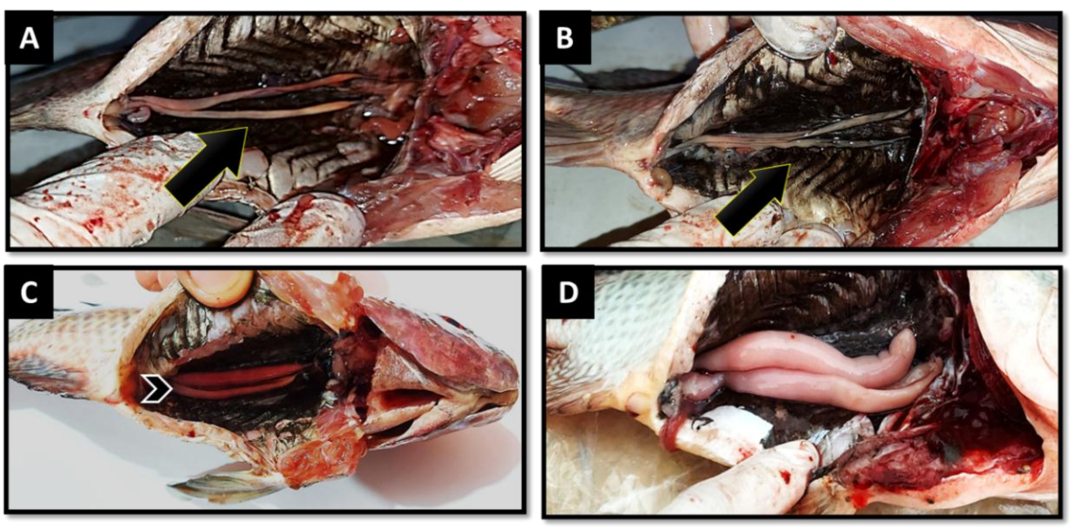

Fig. 4. Testes of El Manzala Nile tilapia (*O. niloticus*) mature males showing very thin empty like shape "black arrow" **(A & B)** and red discolored testes "arrow head" **(C),** compared to apparently normal shape and color of mature male testes of El Abbassa fish **(D)**.

Reproductive performance analysis

Gonado-Somatic Index (GSI)

As shown in Fig (5), the total mean GSI values of both El Abbassa farmed and wild females were significantly higher than both of El Manzala farmed and wild females ($P< 0.05$). While, El Abbassa farmed male breeders were significantly higher than both farmed and wild males of El Manzala ($P< 0.05$) though not significantly variant from El Abbassa wild males. The seasonal investigations of both females and males GSI revealed that, the highest values were in spring followed by summer, while the lowest female GSI values were in winter and those of males were in autumn.

- ***Absolute Fecundity***

It is perceived that, farmed and wild females of El Abbassa were significantly more fecund than both farmed and wild fish of El Manzala (P< 0.05) (Fig. 6), although there was no significant difference between farmed and wild fish of the same region. The seasonal study of total mean fecundity revealed that, there was significant variation only between spring season as it recorded the highest value and autumn season, which recorded the lowest value (P< 0.05).

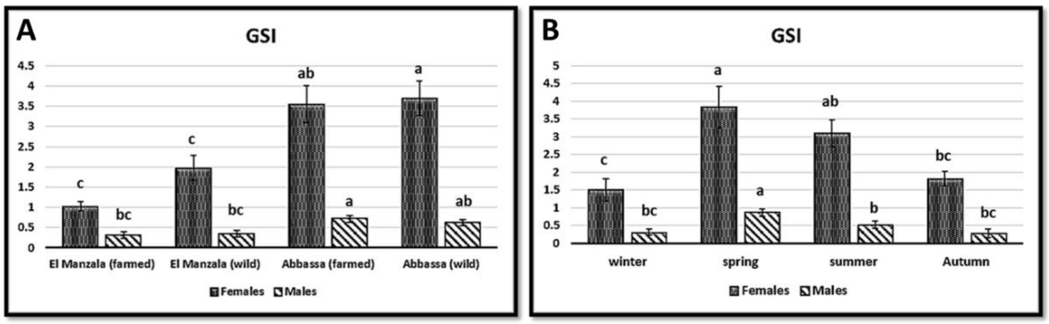

Fig. 5. The total mean gonadosomatic index (GSI) of females and males of the different studied locations **(A)** and the seasonal analysis of total mean gonadosomatic index between females and males **(B)** with the standard error bars.

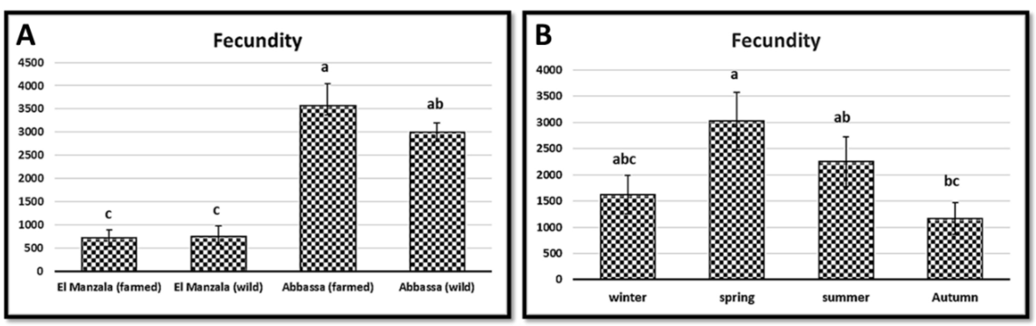

Fig. 6. The total mean absolute fecundity of females of the different studied locations **(A)** and its seasonal analysis **(B)** with the standard error bars.

Heavy metal estimates in water samples

The study of heavy metals polluting water, as recorded in Tables (2, 3) revealed that all the locations under survey are polluted with various heavy metals (Fe, Cu, Zn, Cd, Hg, Pb and As), which exceeded their permissible limits at all sites except Zinc. In addition, cadmium did not exceed its permissible limit in El Abbassa water; while, arsenate in El Abbassa hatchery water was under the permissible limit. It was observed that, El Manzala water is significantly over polluted with the heavy metals under study, compared to that of El Abbassa (P< 0.05), while there was no significant difference between locations at the same region (P> 0.05). It was also noticed that, arsenate showed no significant difference between Bahr Hadous in El Manzala and Ismailia Canal in El Abbassa ($P > 0.05$).

Table 2. Estimated heavy metals in water samples of the four studied locations (Value±SE)

Heavy metal (Mg/l)	El Manzala		El Abbassa		Permissible limit (mg/l)
	Hatchery	Bahr Hadous	Hatchery	Ismailia Canal	
Fe	1.55±0.08^{ab}	1.73±0.07^{a}	0.95±0.02^{c}	0.7±0.02^{d}	0.3[1]
Cu	0.91±0.01^{ab}	0.934±0.01^{a}	0.547±0.007^{c}	0.519±0.06^{c}	0.2[2]
Zn	0.174±0.03^{ab}	0.189±0.07^{a}	0.014±0.002^{c}	0.072±0.01^{c}	0.5[3]

[1] WHO (2008) [2] WHO (1993); FAO (1985) [3] USEPA (2006)

* Different letters within the same row are significantly different ($P < 0.05$).

Table 3. Estimated toxic heavy metals in water samples of the four studied locations (value±SE)

Toxic metal (mg/l)	El Manzala		El Abbassa		Permissible limit (mg/l)
	Hatchery	Bahr Hadous	Hatchery	Ismailia Canal	
Cd	0.025±0.002^{b}	0.059±0.005^{a}	0.005±0.002^{c}	0.002±0.001^{c}	0.01[1]
Hg	0.134±0.02^{a}	0.127±0.02^{ab}	0.035±0.004^{c}	0.041±0.005^{c}	0.002[2]
Pb	0.203±0.009^{a}	0.186±0.02^{ab}	0.081±0.006^{c}	0.082±0.01^{c}	0.015[2]
As	0.086±0.01^{a}	0.056±0.009^{ab}	0.004±0.001^{c}	0.023±0.01^{bc}	0.01[2]

[1] WHO (1993); FAO (1985) [2] Svobodová (1993); Engwa *et al*. (2019)

* Different letters within the same row are significantly different ($P < 0.05$).

Heavy metal assessment in fish organs

The study of bioaccumulation of essential heavy metals (Fe, Cu and Zn), as shown in Table (4), in the Nile tilapia liver, ovaries and testes revealed that these metals exceeded their permissible limits. Iron concentrations in El Manzala fish (both farmed and wild) were significantly higher than both farmed and wild fish of El Abbassa ($P < 0.05$). Copper levels in reproductive organs showed that El Manzala wild fish was significantly higher than other studied locations ($P < 0.05$). Zinc concentrations in El Abbassa fish were significantly lower than both farmed and wild fish of El Manzala ($P < 0.05$).

For toxic metals (Cd, Hg, Pb and As), this study revealed that all bio-accumulated metals in the studied fish organs were exceeding their permissible limits (Table 5). Moreover, El Manzala fish were significantly different from those of El Abbassa, where they had the highest mean values of bio-accumulated toxic metals ($P < 0.05$). While for Hg and Pb, there were significant differences between their levels in the organs of El Manzala farmed and wild fish, where the wild fish had the highest mean values. Arsenate in testes showed significant variations among all studied locations ($P < 0.05$).

Table 4. Estimated essential heavy metals bio-accumulated in fish organs of the four studied locations (mean±SE)

Metal (ppm)	Organ	El Manzala		El Abbassa		P.L. (ppm)
		Farmed	Wild	Farmed	Wild	
Fe	Liver	561.53±31.91^{a}	548.87±32.66^{ab}	273.36±24.39^{c}	161.55±29.71^{c}	****30**
	Ovaries	198.75±11.72^{ab}	233.18±21.59^{a}	122.58±22.21^{c}	78.27±31.97^{c}	
	Testes	341.99±12.06^{a}	318.05±16.61^{ab}	163.54±14.29^{c}	105.92±24.81^{d}	
Cu	Liver	56.39±3.88^{abc}	72.0±3.69^{a}	48.25±1.97^{abc}	61.97±5.13^{ab}	****20**
	Ovaries	20.56±1.76^{bc}	29.90±2.69^{a}	22.52±1.55^{bc}	22.79±2.64^{b}	
	Testes	31.04±2.33^{b}	39.94±2.81^{a}	29.39±1.9^{bc}	28.93±2.8^{bc}	
Zn	Liver	65.67±3.4^{ab}	73.04±7.74^{a}	40.9±4.28^{c}	49.41±6.41^{c}	***40**
	Ovaries	104.11±8.13^{b}	116.32±9.55^{a}	72.05±6.42^{c}	75.37±8.97^{c}	
	Testes	88.79±5.22^{b}	97.89±8.66^{a}	48.33±5.33^{c}	52.04±7.01^{c}	

***Egyptian authority of environmental affairs low48 (1982). **WHO (2008). P.L. = Permissible limits** *******Different letters within the same row are significantly different (*P*< 0.05).

Table 5. The variance of estimated toxic heavy metals bio-accumulated in fish organs among the four studied locations (value±SE)

Metal (ppm)	Organ	El Manzala		El Abbassa		P.L. (ppm)
		Farmed	Wild	Farmed	Wild	
Cd	Liver	2.52±0.32^{b}	2.24±0.29^{a}	0.41±0.14^{c}	0.12±0.1^{c}	***0.5** ****0.1**
	Ovaries	0.59±0.14^{ab}	0.61±0.17^{a}	0.17±0.11^{c}	0.13±0.1^{c}	
	Testes	1.05±0.16^{ab}	1.31±0.12^{a}	0.69±0.18^{c}	0.46±0.12^{c}	
Hg	Liver	16.00±0.99^{b}	18.04±1.32^{a}	1.62±0.45^{c}	1.66±0.54^{c}	***0.5**
	Ovaries	3.52±0.75^{b}	4.56±0.96^{a}	1.3±0.44^{c}	1.34±0.64^{c}	
	Testes	3.27±0.88^{b}	3.9±1.06^{a}	0.48±0.19^{c}	0.71±0.22^{c}	
Pb	Liver	11.51±0.51^{b}	15.43±0.64^{a}	5.13±0.39^{c}	4.91±0.81^{c}	****0.214** ***0.5**
	Ovaries	14.65±0.74^{b}	16.9±0.95^{a}	8.83±0.61^{c}	9.17±0.74^{c}	
	Testes	10.46±0.62^{b}	13.27±0.81^{a}	6.98±0.52^{c}	6.61±0.91^{c}	
As	Liver	2.56±0.16^{a}	2.55±0.17^{ab}	0.92±0.17^{c}	1.33±0.16^{c}	**[1]0.1mg/kg ww** **[2]2µg/g dw**
	Ovaries	1.72±0.15^{a}	1.44±0.17^{ab}	0.46±0.14^{c}	0.62±0.12^{c}	
	Testes	1.44±0.16^{a}	1.08±0.12^{b}	0.21±0.11^{d}	0.4±0.12^{c}	

*** Egyptian authority of environmental affairs, low48 (1982). **FAO/WHO (1999) [1](MHPRC, 2013) [2] (ANZFA 1999).** *******Different letters within the same row are significantly different (*P*< 0.05).

Histopathological studies

Liver. It was noticed that, the liver of both the farmed and wild fish of El Manzala suffered from many histopathological alterations shown in Fig. (7), while both farmed and wild Nile tilapia from El Abbassa showed almost healthy hepatocytes and pancreatic ducts, except for mild degenerative changes noticed as vacuolar degeneration, in addition to edema, leukocytic infiltration and the congestion of hepatic sinusoids.

Ovaies.The histopathological examination of mature ovaries of the Nile tilapia revealed that, El Manzala females suffered from severe retrogressive changes shown in Fig. (8).

Testes.The mature Nile tilapia testes samples from El Manzala showed a reduction in the spermatogenesis, as illustrated in Fig. (9).

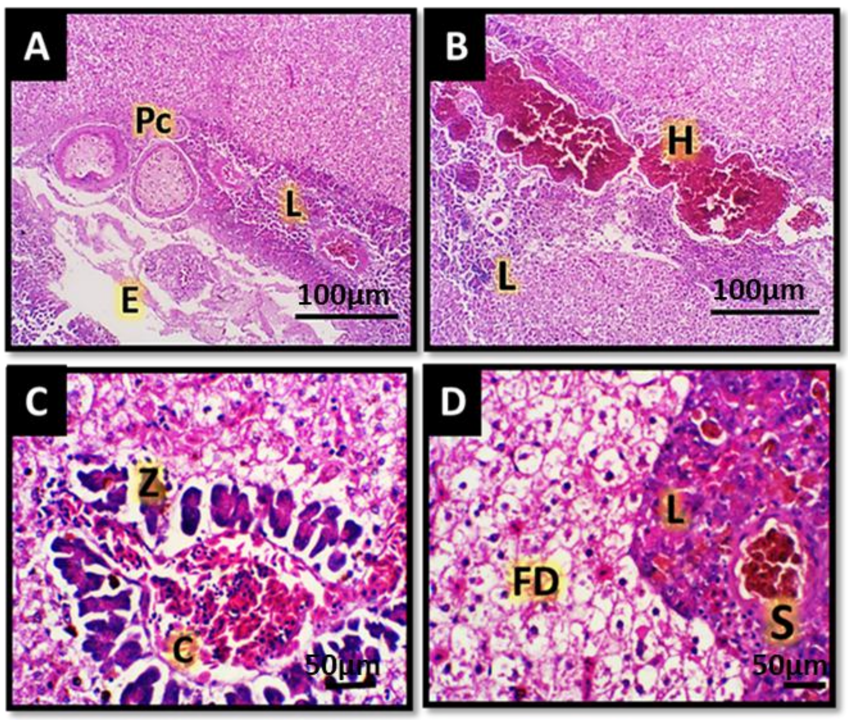

Fig. 7. Microscopic pictures of hepatic sections of farmed and wild Nile tilapia from El Manzala showing parasitic cysts "Pc", with edema "E" **(A)** and leukocytic infiltrations "L" **(A, B&D)**. Other hepatic section showing hemorrhage "H" **(B)**, congestion of pancreatic ducts "C" and aggregation of eosinophilic zymogen granular cells that replacing pancreatic cells "Z" **(C)** beside, fatty degeneration "FD" and hemosidrosis "S" was also common **(D).**

Hematoxylin and eosin (H&E) stain, magnifications X: 100 **(A&B)**, X: 400 **(C&D)**.

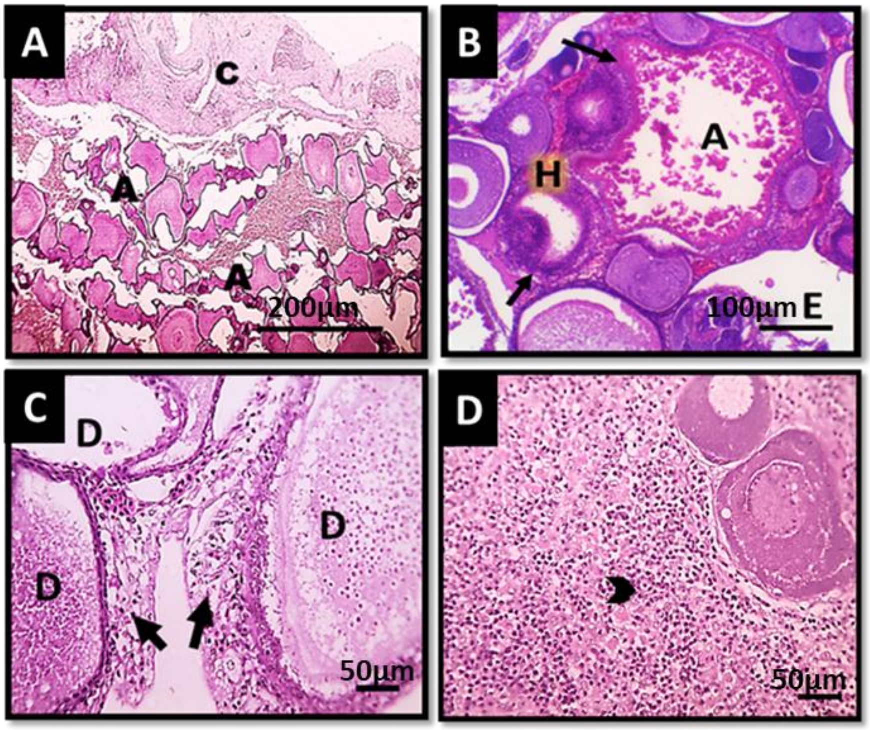

Fig. 8. Microscopic pictures of transverse ovarian section of farmed and wild Nile tilapia from El-Manzala showing thickened capsule “C” enclosed numerous atretic oocytes “A” having irregular wall with absence of mature oocytes **(A)**. Hemorrhage “H”, leukocytic infiltration “L”, edema “E”, with various atretic oocytes in different stages “A” characterized by thickening and separation of basement membrane “arrow” **(B)** and many degenerated “D” vitellogenic oocytes with thickened cell membrane “arrow” **(C)** with the presence of spermatogonia "arrowheads" in stroma **(D)**.

Hematoxylin and eosin (H&E) stain, magnifications X: 40 **(A)**, X: 100 **(B)** and X: 400 **(C&D)**.

Histopathological indices

The analysis of histopathological indices (Fig. 10) revealed that, the examined Nile tilapia from both farmed and wild fish of El Manzala had significantly higher mean values of hepatic, ovarian and testicular indices than both farmed and wild fish of El Abbassa (P< 0.05).

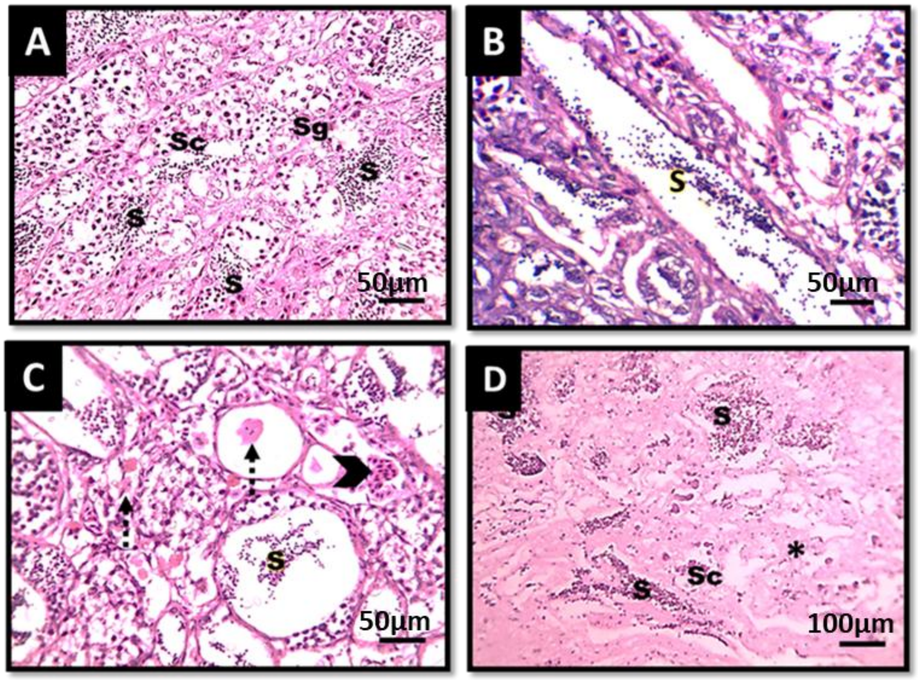

Fig. 9. Microscopic pictures of testicular section of farmed and wild Nile tilapia (El Manzala) showing reduced spermatogenesis; the seminiferous tubules contain many spermatogonia "Sg" **(A)**, spermatocytes "Sc" **(A&D)** and few spermatids "S" **(A, B, C&D)**. Hyalinized spermatids "dashed arrows" and interstitial congestion "arrow heads" **(C)** is seen with thickening of interstitial connective tissue "*" **(D).**

Hematoxylin and eosin (H&E) stain, magnifications X: 100 **(D)** and X: 400 **(A, B&C)**.

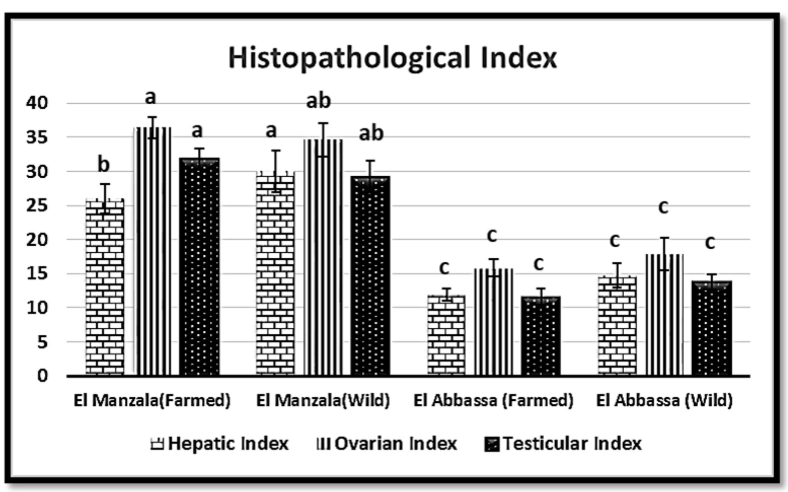

Fig. 10. The mean histopathological index showing the extent of total histological alterations in each organ and its variance between the four studied locations with standard error bars.

Correlations involving heavy metals in water and fish organs

Essential metals

Concerning the study of statistical correlations between heavy metals in polluted water and bio-accumulated metals in the Nile tilapia reproductive organs (Table 6), the results revealed highly strong positive correlations between estimated heavy metals in water and tissues of the Nile tilapia of both sexes ($P< 0.001$). While, copper showed insignificant correlation between water and liver concentrations as well as ovaries ($P> 0.05$) and significant correlation with male testes ($P< 0.05$).

Toxic metals

In this study, the correlations between water pollution with various toxic metals and the accumulation of these metals in the Nile tilapia reproductive organs are highly strong positive ($P< 0.01$), as demonstrated in Table (7).

Table 6. Pearson coefficient analyses showing the correlations between essential heavy metals in water and its bio-accumulation in the Nile tilapia reproductive organs

Essential metal	Liver		Ovary		Testis	
	R	**Sig**	***r***	**Sig**	***r***	**Sig**
Fe	0.956**	0.000	0.925**	0.000	0.938**	0.000
Cu	0.489	0.106	0.386	0.215	0.678*	0.015
Zn	0.916**	0.000	0.916**	0.000	0.928**	0.000

* Correlations are significant ($P\leq 0.05$). ** Correlations are highly significant ($P\leq 0.01$).

Table 7. Pearson coefficient analyses showing the correlations between toxic metals in water and its bio-accumulation in the Nile tilapia reproductive organs

Toxic metal	Liver		Ovary		Testis	
	R	**Sig**	***r***	**Sig**	***r***	**Sig**
Cd	0.952**	0.000	0.858**	0.000	0.929**	0.000
Hg	0.905**	0.000	0.833**	0.000	0.875**	0.000
Pb	0.822**	0.001	0.883**	0.000	0.791**	0.002
As	0.827**	0.001	0.878**	0.000	0.894**	0.000

** Correlations are highly significant ($P\leq 0.01$).

Assumptions of most critical heavy metal(s) on naturally exposed fish reproductive performance

The effects of essential heavy metals on the evaluated fish reproductive parameters, as demonstrated in Tables (8, 9) reveal that, the females' GSI are mostly affected by high water Fe ($F = 41.987$; $P< 0.001$), as well as Fe accumulated in the ovaries ($F = 50.933$; $P< 0.001$). While, the female fecundity are mostly influenced by elevated water Cu ($F =39.181$; $P < 0.001$) and bio-accumulated Zn in ovaries ($F = 84.711$; $P< 0.001$). Additionally, the male GSI are mostly influenced by elevated Cu in water($F =16.282$; $P< 0.01$) and Zn accumulated in testes ($F = 21.023$; $P \leq 0.001$).

For the effects of bio-accumulated toxic metals shown in Tables (10, 11), the female GSI is mostly affected by Pb in water (F = 39.247; P < 0.001) and accumulated Cd in ovaries (F= 37.585; *P*< 0.001). While, its fecundity is affected by Hg in water (F = 38.443; *P*< 0.001) and both effects of Hg and Cd in water (F = 49.068; *P*< 0.001), in addition to the bio-accumulated Pb in ovaries (F = 95.052; *P*< 0.001). Furthermore, the male GSI is mostly influenced by Pb in water (F = 15.090; *P*< 0.01) and Hg bio-accumulated in testes (F = 29.748; *P*< 0.001).

Table 8. Multiple linear regression tests revealing the most influential bio-accumulated heavy metal (s) on the Nile tilapia reproductive performance

Reproductive parameter		Effective variable	*r*	Adjusted r^2	F-ratio	Sig
Female	**GSI**	**Fe**	0.914**	0.819	50.933	0.000
	Fecundity	**Zn**	0.946**	0.884	84.711	0.000
Male	**GSI**	**Zn**	0.823**	0.645	21.023	0.001

** Correlations are highly significant (*P*≤ 0.01).

Table 9. Multiple linear regression tests revealing the most influential heavy metal (s) in water on the Nile tilapia reproductive performance

Reproductive parameter		Effective variable	*r*	Adjusted r^2	F-ratio	Sig
Female	**GSI**	**Water Fe**	0.899**	0.788	41.987	0.000
	Fecundity	**Water Cu**	0.893**	0.776	39.181	0.000
Male	**GSI**	**Water Cu**	0.787**	0.581	16.282	0.002

** Correlations are highly significant (*P*≤ 0.01).

Table 10. Multiple linear regression tests revealing the most influential bio-accumulated toxic metal (s) on the Nile tilapia reproductive performance

Reproductive parameter		Effective variable	*r*	Adjusted r^2	F-ratio	Sig
Female	**GSI**	**Cd**	0.889**	0.769	37.585	0.000
	Fecundity	**Pb**	0.951**	0.895	95.052	0.000
Male	**GSI**	**Hg**	0.865**	0.723	29.748	0.000

** Correlations are highly significant (*P*≤ 0.01).

Table 11. Multiple linear regression tests revealing the most influential toxic metal (s) in water on the Nile tilapia reproductive performance

Reproductive parameter		Effective variable	*r*	Adjusted r^2	F-ratio	Sig
Female	**GSI**	**Water Pb**	0.893**	0.777	39.247	0.000
	Fecundity	**Water Hg**	0.891**	0.773	38.443	0.000
		Water Hg, Cd	0.957**	0.897	49.068	0.000
Male	**GSI**	**Water Pb**	0.776**	0.562	15.090	0.003

** Correlations are highly significant (*P* ≤ 0.01).

DISCUSSION

Reproduction is one of the essential biological aspects permitting the ultimate survival and maintenance of species. The ideal fish production is entirely dependent on the physical, chemical and biological characters of water. All living organisms have acceptable limits of water quality parameters in which they accomplish optimally. A harsh drop or an increase in these limits has antagonistic effects on their body functions **(Davenport & Vahl, 1993**; **Kiran, 2010)**. In our study, the examination of dissolved oxygen in water reveals that, El Manzala mean values in the hatchery and Bahr Hadous are below the permissible limit **(CCME, 2007)**. These findings agree with that of **Aly and El-Sayed (2015)** who assumed that, the depletion in oxygen content of Bahr Hadous and El Salam canal resulted from the decomposing organic matter and oxidation of chemical constituents. Identically, the current results coincide with those of **El-Sayed (2008)** and **Nofal *et al.* (2019)**. Water transparency in this study indicates that in Ismailia Canal this variable is significantly higher than the other studied locations ($P < 0.05$). This suggests the instant flow of fresh irrigation water from the River Nile through the canal, as reported by **El-Sayed (2008)**. Moreover, this explanation agrees with that of **Mahmoud *et al*. (2008)** who mentioned that, water transparency is controlled by depth and turbidity of the water and affected by particulate contents of water from suspended matter and floating substances. Electric conductivity (EC) can be measured to establish a pollution zone around an effluent discharge. Accordingly, the present result concur with that of **Aly and El-Sayed (2015)** who determined the results for the electrical conductivity in Hadous Drain and the mixing point of Hadous Drain and El- Salam Canal as they were higher than the limits of law 48/1982. Additionally, other authors concluded that the high values of EC might be approved for domestic and agricultural wastes containing a high amount of organic and inorganic constituents **(Al-Afify & Abdel-Satar, 2020; Hashem *et al*., 2020)**.

The current findings regarding water salinity correspond with the results of **Reyad *et al*. (2021)**, who estimated the water salinity in El Manzala fish farm and El Abbassa farm and recorded valus of 0.9g/l and 0.2g/l, respectively. Moreover, these results match with those of **Nofal *et al*. (2019)**, who recorded relatively higher salinity value (1.3g/ l) in El Manzala farm ponds, while El Abbassa hatchery recorded a value of 0.3g/ l. Therefore, these findings correspond with those of **Gohera *et al*. (2017)** who assumed that, EC is positively correlated with water salinity.

This study showed that nearly all locations (except Ismailia Canal) have BOD over the permissible limit **(Santhosh & Singh, 2007)**. These estimated high values indicate the presence of high load of organic pollutants consuming the dissolved oxygen via oxidation processes **(Tayel *et al*.. 2014; Ali *et al*., 2015**; **Hashem *et al*., 2020)**. Moreover, the chemical oxygen demand (COD), which represents the total amount of O_2 necessary to oxidize all the organic matter totally to H_2O and CO_2 **(Sincero & Sincero, 2002)**, of which all the water samples from all sites showed elevated values surpassing the permissible limits **(CCME, 2007)**. Besides, El Manzala water had significantly higher COD than El Abbassa ($P < 0.05$) mean values. This agrees with the findings of **Abdel-Satar (2005)** and **Ahmed *et al*. (2011)** with respect to the high values of COD that might be due to the effect of pollution by sewage and agriculture wastes highly loaded with organic matter and the low capacity of its water for self-purification.

By studying the total ammonia, the drainage water type of El Manzala recorded significantly high total ammonia ($P< 0.05$), which exceeded the permissible limits **(CCME, 2007)**. The high values are indicative of the existence of highly active pollutants originated from sewage overflows, industrial discharge and agriculture runoffs and the decomposition of organic matters as reported by **Osman and Kloas (2010)**. Besides, the ammonia level of El Manzala farm was over the range as verified by **Nofal *et al*. (2019)** and **Reyad *et al*. (2021)**.

Concerning the clinical feature of examined fish, the previous clinical observations of El Manzala fish agree with many authors **(Sun *et al*., 2009; Elgaml *et al*., 2019; Nofal *et al*., 2019)**. Besides, the abnormal dark discoloration of the ovaries of El Manzala fish relatively match with the observations of **Azab *et al*. (2019)** who noticed atypical shaped ovaries of *Tilapia zilli* from El-Nasria station at Omar Bey Drain because of the clear greenish fluid under the ovarian epithelium; in addition, oocytes seemed dark in color with white spots on the ovarian wall.

Based on the analysis of gonadosomatic index (GSI), as a guide factor for reproduction condition, our results correspond with those of **Ghannam and Aly (2018)**, who notified that, the GSI values of the brood Nile tilapia were higher in the fresh water farm pond than drainage water farm pond and owed the obtained high values to the precision of the physical and chemical water quality parameters. However, the present data disagree with those of **El-Nemaki *et al*. (2008)**, who obtained higher substantial values of GSI in fish from EL-Abbassa fish farms that received agriculture drainage water. In addition, **Khallaf *et al*. (2003)** and **Khallaf *et al*. (2018)** signified that, *O. niloticus* GSI correlated considerably with heavy metals and pesticides; however, they attributed the delay of sexual maturity to the effect of water pollution. The seasonal investigations of both females and males GSI organized as the highest values were in spring, followed by summer, while the lowest female GSI values were in winter, and in autumn for males. Such results are in accordance with those of many studies **(Njiru *et al*., 2006; Shalaby & Migeed, 2012; Hamed *et al*., 2016; Khallaf *et al*., 2020)**. This study also agrees with **El-Kasheif *et al.* (2013)** and **Khallaf *et al*. (2020)** concerning the fact that the female Nile tilapia individuals have higher value of GSI than males at constant age, length and season; besides, the seasonal variations of both *O. niloticus* sexes following nearly the same trend. While, studying the absolute fecundity of females, our findings approve the environmental effect on the quality of fish reproduction as previously clarified in some studies **(Bell *et al*., 1992; Livingston *et al.*, 1997; Johnson *et al*., 1998)**. However, the seasonal study of the total mean fecundity conflicts entirely with that of **Khallaf *et al*. (2020)** who recorded a gradual decrease in the fecundity from winter to autumn and owed this retreat to the markedly high temperatures of summer months in the canal of Bahr Shebeen.

For the heavy metal pollution in the investigated water samples, this study revealed that, the water of all sites under survey is polluted with various heavy metals (Fe, Cu, Zn, Cd, Hg, Pb and As), which mostly exceeded their permissible limits in all sites. In addition, it was noticed that, El Manzala water is significantly over polluted with studied heavy metals than El Abbassa water ($P< 0.05$), while there was no significant difference between locations at the same region (*P* > 0.05). Such assessments agree with those of **Elgaml *et al*. (2019)** and **Nofal *et al*. (2019)** who postulated that, the water of El Abbassa farm was significantly less polluted with various studied heavy metals than El Manzala fish farm.

With regards to the variance of bio-accumulated heavy metals in fish reproductive organs between studied locations, our findings agree with the results obtained by many authors **(El-Naggar *et al*., 2009; Badr *et al*., 2014; Alawy *et al*., 2015; Ali *et al*., 2015; Aly & El-Sayed, 2015; Abd-Elghany *et al*., 2017; Hamada *et al*., 2018; Mokhamer *et al*., 2019; Nofal *et al*., 2019)**. They proved that, all fish from different polluted waterbodies showed higher levels of bio-accumulated heavy metals in various body organs.

Considering the histopathological examinations, the observed severe degenerative changes of hepatic cells of the affected Nile tilapia from El Manzala as a result of heavy metal accumulation agree with the observations of **Authman and Abbas (2007)** and **Yacoub *et al*. (2021)**. Hemorrhage, hemosidrosis, edema and lymphocytic cell infiltration because of rapid and permanent damage of red blood cells were also common **(Ibrahim & Mahmoud, 2005; Tayel *et al*., 2018; Hashem *et al*., 2020)**. For the fish ovaries, the histopathological findings of El Manzala females agree with the finding of **Mazrouh and Mahmoud (2009)** who observed that, the cytoplasm of early and late peri-nucleolus oocytes appeared as deep purple solid mass. Moreover, the late peri-nucleolus oocytes became atretic in the Nile tilapia females exposed to higher amounts of pollutants. Similar lesions were also explained by other authors **(Ali *et al*., 2015; Mansour *et al*., 2018**; **Azab *et al*., 2019)**. The most conspicuous feature was the incident of testicular developmental stage "spermatogonia" in the stroma of many ovarian samples taken from both the farmed and wild Nile tilapia females of El Manzala. Similar findings was recorded in the study of **Azab *et al*. (2019)**. Reliable to our reflection, instances of intersex status were noticed in testicular tissue of male *O. niloticus*, implying the hormonal disruption that related with the emergence of intersex state **(Ali *et al*., 2017)**. The histopathological alterations of the affected Nile tilapia testes of El Manzala agree with several studies **(Shalaby & Migeed, 2012; Ali *et al*., 2015; Mansour *et al*., 2018; Zahran *et al*., 2020)**. In this context, **Mazrouh and Mahmoud (2009)**, as a result of exposure to pollution, mentioned severe degenerative, necrotic changes in the wall and the cellular components of the seminiferous tubules with focal fibrosis and reduced number of sperms or void demonstrating the lack of active spermatogenesis. The analytical studies of the histopathological index verified the devastative effect of water pollution on various fish tissue and organs as formerly reported **(Elgaml *et al*., 2019; Nofal *et al.*, 2019**; **Reyad *et al*., 2021)**. The effects of heavy metals polluting water on fish reproduction through the histological approach were confirmed. For instance, cadmium is counted as endocrine disrupter since it has been revealed to restrict the formation of steroids, eggs and sperm in rainbow trout where it changes hormone synthesis in testes; while in carp, it reduces steroid formation and ovarian functions **(Mukherjee *et al*., 1994; Vetillard & Bailhache, 2005; Giari *et al*., 2007)**. Whereas, the exposure of post-hatch larvae of *O. niloticus* to (2 and 5ppm) sublethal levels of zinc for 30 days kept undifferentiated gonads with proliferation of oogonia **(Caring, 1992)**, besides the restrictive effect of Pb to steroidogenesis with consequently circulating LH, FSH, and estradiol, causing reduction in the ovarian primordial follicles **(Jackson *et al*.,**

2011). This could demonstrate the incidence of testicular developmental stage "spermatogonia" in the stroma of many ovarian samples taken from El Manzala. Moreover, fish exposure to high levels of the heavy metals as zinc and copper caused atrophy and cytoplasmic leakage in the ova resulting in severe degeneration **(Tang *et al*., 2013)**. The accumulation of mercury in testes caused incompetence of seminiferous tubules, with a reduction of germ cells; besides, the proliferation of interstitial connective tissue in the tropical fish, *Gymnotus carapo* also caused disruptions in blood circulation **(Vergilio *et al*., 2013)**. Furthemore, **Biswas and Ghosh (2016)** described the toxic effect of Pb on *Mastacembelus pancalus,* as it destroyed the oocyst, caused atresia and degeneration of yolk globules, with necrotic oocytes and different necrotic, in addition to degenerative changes in the ovarian tissue. Other researchers perceived that, heavy metals might cause atresia and necrosis in oocytes leading to a decline in egg production. Thus, water pollution with various heavy metals is responsible for distortions and corruptions of fish gonads. These effects might disrupt the development of germ cells and could reduce the ability of the fish to reproduce **(Hanna *et al.,* 2005; Mazrouh & Mahmoud, 2009; El-Morshedi *et al*., 2014)**.

Concerning the study of the correlations between heavy metal water pollution and bio-accumulated metals in the Nile tilapia reproductive organs, the results agree with those recorded in many studies **(Chale, 2002; Boisson *et al*., 2003; Abumourad *et al*., 2014; Riani, 2015)**. They confirmed that the concentrations of examined heavy metals in fish organs was always higher than that in water, which might be due to the diversity of occurrences on the fish body and other aquatic biotas, including the regular dissemination, bio-magnification and bio-concentration. **Sanou *et al*. (2021)** showed that mercury and lead levels in water affected their bio-concentration in the Nile tilapia organs although cadmium concentrations in sediments influenced its bio-accumulation in fish organs as lead was positively correlated with mercury and arsenic in water. While, mercury was positively and significantly correlated with cadmium and lead in the examined farm sediments. Thus, these strong correlations observed between heavy metals in the same medium suggest that they have a common source and mutual dependence **(Suresh *et al*., 2011; Shetaia *et al*., 2020)**.

In this study, the analyses of the effects of essential heavy metals on the evaluated fish reproductive parameters agreed with **Caring (1992)** and **Tang *et al*. (2013).** Arsenic, even though copper and zinc are essential elements, they become toxic when concentrations exceed their permissible limits. For instance, copper influences on fish reproductive system have been noticed at low levels of exposure and included blocking of spawning, reduced egg production rate per female, deformities in newly hatched fry, reduced survival of the offspring, among many other effects **(Sorensen, 1991)**. On the oter hand, zinc at high levels is toxic to fish and might delay or reduce the growth, sexual maturity and reproduction of exposed fish **(Yirgu, 2011; Hama *et al*., 2015)**. Whereas, the effect of Fe on the female GSI might suggest its toxic effects as DNA destruction, lipid peroxidation (LPO) and protein decomposition **(Valko *et al*., 2005)**, as well as its impacts on anti-oxidant enzymes activity as catalase (CAT) in liver and glutathione reductase (GR) **(Bagnyukova *et al.,* 2006)**.

With respects to the effects of water pollution with toxic metals, the detrimental effects of Pb on the female reproductive performance is confirmed by **Jackson *et al*.**

(2011) as lead is capable of restraining steroidogenesis, and consequently circulating LH, FSH, and estradiol, causing reduction in the ovarian primordial follicles. Likewise, its devastating histological alterations as demonstrated by **Biswas and Ghosh (2016)**. Furthermore, the effects of bio-accumulated mercury in testes with corresponding deterioration in its function match with the observations of **Vergilio *et al*. (2013)**. What is more, it was observed that the accumulation of methyl Hg in reproductive organs, even at low concentrations, might influence the survival rate of eggs and fry since it caused degenerative changes in the ovary of zebrafish and reduced egg production. Besides, it caused reduced spermatogenesis and the availability of spermatids as a result of histopathological alteration of the testes **(Raldúa *et al*., 2007; Zhang *et al*., 2016)**. Moreover, the effect of cadmium pollution on reproduction of the exposed Nile tilapia agree with numerous research articles **(Mukherjee *et al.*, 1994; Karels *et al*., 2003; Vetillard & Bailhache, 2005; Giari *et al*., 2007; Luo *et al*., 2015)** as cadmium inhibits egg maturation and therefore reduces the number of spawned ova. Depleted vitellogenin expression has been reported in the fish ovaries exposed to cadmium, counteracting the formation of ova **(Karels *et al*., 2003; Luo *et al*., 2015)**. Therefore, the distinctive increase in the levels of heavy metals mainly (Cd, Hg, Pb and As) as toxic metals and the over elevation of (Fe, Cu and Zn) in water with interrelated bioaccumulation of these metals in liver and reproductive organs of Nile tilapia brood fish, in our study, are associated with the deterioration in their reproductive performance. Since heavy metals pollution, was stated to cause structural deformity of DNA and an increase in liver (ethoxyresorufin ode-ethylase) activity leading to reduction in gonadosomatic index of intoxicated fish **(Martínez-Gómez *et al*., 2012)**

CONCLUSION

Fish health and reproductive performance are ultimately associated with the surrounding water characters and the continual natural exposure to heavy metals resulted in devastative reduction in fish yield. The persistent exposure to heavy metals polluted water environment not only disturbing the reproduction function but also damaging the ultrastructure of reproductive organs as well as its effect as endocrine disruption. Bahr Hadous is carrying extensively polluted water with various heavy metals as (Fe, Cu, Zn, Cd, Hg, Pb and As) beside other organic pollutants delivering its hazardous effects to its natural population so it is misplaced as primary water source supplying El Manzala farm and its hatcheries. So in order to expand the sustainability of the aquaculture division and to produce safer fish for human intake, reliable monitoring of the fish and related environment should be performed by the applicable authorities. An ingrained framework should be established almost immediately to alleviate this critical problem.

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