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RESEARCH ARTICLE

Effect of Heavy Metal Pollutants on Fish Population in two Egyptian Lakes

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Chromium (Cr), Lead (Pb) and Cadmium (Cd).

Abstract

Heavy metals pollutants are dangerous for the environment. Some of these are essential elements that are required for the normal metabolism of the body such as copper (Cu), iron (Fe) and zinc (Zn), while others are non-essential and play no significant role such asCr),lead (Pb) and cadmium (Cd). Their natural effects can be toxic, carcinogenic, mutagenic or teratogenic. This study investigated and compared the relationship between the concentration of chromium (Cr),lead (Pb) and cadmium (Cd) in Lake Bardawiland Lake Manzalah(Egypt)on fish population. Moreover, the study also investigated the effect of theheavy metals on reproduction and growth ability of thinlipped grey mullet (*Liza ramada*) and the sea bass (*Dicentrarchuslabrax*. Histopathological and histochemical examination of the gonads (testes and ovaries) of the two fish species was conducted and length-weight relationships were studied for both male and female members.The study demonstrated that toxic heavy metals can alter tissue histological structures and cause reproductive defects.

Introduction

Fish is a commodity of potential public health concern because it can be contaminated by a range of environmentally persistent chemicals, including heavy metals (Soliman, 2006). The need for increasing fish production in Egypt is important. Therefore, efforts have been made to increase fish production and to maintain the present level of consumption in view of the rapid population increase (El-Nemaki et al., 2008). The significance of Lake Bardawil fisheries lies not only in the supply of fish for regional domestic consumption (3,300 tons in 2003), but especially in regional employment and in export earnings (El-Bawaab, 1995). Lake Bardawil is a source of good quality fish since it is a protected area. Lake Manzalah is economically the most important delta lake in Egypt. It provides about 30% of domestic fish supply (Abd El-Hakim et al., 1999). Lake Manzalah lies within the borders of four Egyptian governorates. These are Dakahlia, Damietta, Port-Said, and Sharkiya (El-Shebly, 1998). Lake Manzalah is bordered by Suez Canal from the East, Damietta branch of Nile from the West, Mediterranean sea from the North and agricultural lands of Dakahlia and Sharkia Provinces from the South (El-Shebly, 1998). The northern part of Lake Manzalah is affected by marine water invasion through El-GamilBoughaz, while the southern and south-western parts receive sewage effluents, agricultural and industrial drainage water (El-Shebly, 1998). These drains make the lake highly polluted with heavy metals.

Heavy metals pollutants include essential elements such as Cu, Fe and Zn, and other non-essential elements that play no significant role including such as Cr, Pb and Cd. Their effect can be toxic (acute, chronic or sub-chronic), carcinogenic, mutagenic or teratogenic. Heavy metals are one of the five major types of toxic pollutants present in fresh water (Mason, 1991). The important environmental pollutants are those that tend to accumulate in organisms, those which are persistent because of their chemical stability or poor biodegradability, and those which are readily soluble and therefore environmentally mobile (Hellawell, 1986). Heavy metals pollutants have been found to be dangerous to the environment (Phillips, 1991). Some of them are essential elements that are required for the normal metabolism of the organism including Cu, Fe and Zn, while the others are non-essential and play no significant role including Cr, Pb and Cd (Sanders, 1997). Their natural effects can be toxic (acute, chronic or sub-chronic), carcinogenic, mutagenic or teratogenic (Young, 2005). The present study investigated heavy metals levels (Cr, Pb and Cr) in Lake Manzalah and compared them with Lake Bardawil. It revealed the effect of heavy metal pollution on reproductive ability of thin lipped grey mullet fish (*Liza ramada*) which belongs to order Mugiliformes and family Moronidae (McDonough and Wenner, 2003).

Materials and Methods:

Water samples and thin lipped grey mullet fish (*Liza ramada*) were collected from Lake Bardawil (control) and Lake Manzalah (polluted lake) during the summer of 2012.Cr, Pb and Cd concentrations were determined byAtomic Absorption Spectrophotometer using an air-acetylene flame (Model 2380, Perkin-Elmer) according to the method of Zottner and Seligson (1964). Statistical analysis was performed by using the student t-test (Murray, 1982**).** Fish tissue from both lakes was dissected for inspection of the gonads (testes and ovaries). Specimens were fixed in 10% saline formalin, dehydrated in ascending series of alcohol and embedded in paraffin wax. Serial transverse sections at 5µm were stained by Haematoxylin-Eosin (Clayden, 1971).Total polysaccharides and protein contents were identified according to Causan and Pickett (1983) and Launa (1968) respectively**.** Masson's Trichrome Staining method was used for collagen fibers (Masson, 1929) and Methyl Green Pyronin Y method was used for detection of RNA/DNA content (Kurnick, 1955).

Results:

1. **Heavy Metals in Water:** Lake Manzalah water displayed the highest concentration levels (ppm) of tested heavy metals (Cr, Pb and Cd), which was showed by high significant difference (table 1). According to safety limits in the two environmental laws No. 48/1982 and No. 4/1994, concentration means of Cr, Pb and Cd in Lake Manzalah exceeded these recorded safety limits, but Lake Bardawil water not exceeded them.

2. **Histological and Histochemical Analysis:**

I. Testes: On histological examination, Lake Bardawil*Liza ramada* testes showed numerous seminiferous tubules, intensive spermatogenesis, reduced number of spermatogonia and many spermatids and spermatozoa (Figure 1). These seminiferous tubules showed both primary and secondary spermatocytes (Figure 1). Testes of *Liza ramada* from Lake Manzalahshowed decreased number of seminiferous tubules along with some degenerative changes (Figure 2). They appeared empty different from primary and secondary spermatocytes. The interstitial cells and sertoli cells had invaded all seminiferous tubules sperm nests. Accordingly, seminiferous tubules showed lesser number of spermatids which had a radiated shape without any inner septa between nests (Figure 2). This can cause a marked decrease in spermatogonial mitosis and inhibition in the development of early stages of male germ cells resulting in delayed transformation of the spermatids to mature spermatozoa. Histochemically, testicular tissue of Lake Bardawil*Liza ramada* exhibited a strong PAS reaction for polysaccharides (Figure 3). It also showed a large amount of protein after application of mercury-bromophenol blue staining method (Figure 5). However, testicular tissues of Lake Manzalah fish exhibited lesser amount of polysaccharide (Figure 4) and protein after Mercury Bromophenol Blue reaction (Figure 6). Testicular tissues of Lake Bardawil*Liza ramada* showed greater RNA/DNA content (Figure 7) when compared with testicular tissues of Lake Manzalah studied fish after the use of Methyl Green Pyronin Y stain (Figure 8). They also showed a large amount of collagen fiber content by Masson'sTrichrome stain technique (Figure 10). Conversely, very little collagen fiber content was found in testicular tissues of *Liza ramada* from Lake Bardawil by Masson'sTrichrome stain method (Figure 9).

II. Ovaries: Ovaries of *Liza ramada* from Lake Bardawil showed no histological lesions. Minute polygonal undifferentiated oogonia with a little amount of oviplasm were observed in early and late peri-nucleolar stages (Figure 11). Oocytes were increased in size and enveloped by follicular epithelia and vitelline membrane (zona radiate) as a protective boundary for oviplasm (ooplasm). The vitellogenetic stage (lipid-yolk vesicles stage), was noticed with lipid vesicles in oviplasm. There was yolk protein in the central portion of oocytes (yolk spheres). The vitelline membrane (zonaradiata) was visible between oviplasm and follicular layers (granulose cell layer and theca cell layer) as they were called zona granulose (Figure 11). Ovaries of *Liza ramada* from Lake Manzalah showed atresia (degeneration) (Figure 12). Necrotic oocytes, a distinctive yolk and vacuolated cytoplasm; also, broken tunica albuginea were seen at all sites (Figure 12). Histochemically, ovarian tissue of *Liza ramada* from Lake Bardawil exhibited large amount of polysaccharide, proteins and DNA/RNA contents after application of PAS reaction (Figure 13), mercury-bromophenol blue (Figure 15) and methyl green pyronin Y stain (Figure 17) respectively. However, Lake Manzalah*Liza ramada* ovarian tissues showed lower amount of polysaccharides (Figure 14), proteins (Figure 16) and DNA/RNA contents (Figure 18). They also showed less collagen fiber by Masson's stain method (Figure 20). Converesely, ovarian tissueof *Liza ramada* from Lake Bardawil showed a large amount of collagen fiber (Figure 19). These collagen fibers were present in ovarian tunica albuginea.

Table (1): Mean concentrations (ppm) of Cr, Pb and Cd in Lake Bardawil water and Lake Manzalah at summer (2011). Lake Bardawil is a protected area, so it was included as the control. Values are expressed as mean ± standard deviation.(*): Significant: 0.01 $\leq P \leq$ 0.05 and (**): Highly significant: P< 0.01.

	Element					
	Cr		Pb		Cd	
Lake	Bardawil	Manzalah	Bardawil	Manzalah	Bardawil	Manzalah
Mean	0.0053±0.001	0.073±0.01	0.00095±0.0006	0.04±0.015	0.002±0.001	0.036±0.013
Standard Error	0.0005	0.005	0.0003	0.0075	0.005	0.006
t-Test	0.00172		0.00375		0.0054	
Significance	**		**		**	

Figure 1: Cross section of*Liza ramada* testis from Lake Bardawil showing seminiferous tubules **(STU)**, spermatogonia**(SG)**, spermatocytes **(SC)**, spermducts**(SD)**, spermatids **(ST)** and spermatozoa **(SZ)**. (Hx& E., × 250).

Figure 2: Cross section of*Liza ramada* testis from Lake Manzalah showing seminiferous tubules **(STU)** and spermducts**(SD)**. (Hx& E., × 250).

Figure 3: Cross section of*Liza ramada* testis from Lake Bardawil showing strongly stained PAS-positive materials: seminiferous tubules **(STU)**, spermatogonia**(SG)**, spermatocytes **(SC)**, spermducts**(SD)**, spermatids **(ST)** and spermatozoa **(SZ)**. (PAS reaction, × 250).

Figure 4: Cross section of*Liza ramada* testis from Lake Manzalah showing weakly stained PAS-positive materials: seminiferous tubules **(STU)** and spermducts**(SD)**. (PAS reaction, × 250).

Figure 5: Cross section of*Liza ramada* testis from Lake Bardawil showing strongly stained protein materials: seminiferous tubules **(STU)**, spermatogonia**(SG)**, spermatocytes **(SC)**, spermducts**(SD)**, spermatids **(ST)** and spermatozoa **(SZ)**. (Mercury-bromophenol blue stain, × 250).

Figure 6: Cross section of*Liza ramada* testis from Lake Manzalah showing weakly stained protein materials: seminiferous tubules **(STU)** and spermducts**(SD)**. (Mercury-bromophenol blue stain, × 250).

Figure 7: Cross section of*Liza ramada* testis from Lake Bardawil showing strongly stained DNA and RNA contents: seminiferous tubules **(STU)**, spermatogonia**(SG)**, spermatocytes **(SC)**, spermducts**(SD)**, spermatids **(ST)** and spermatozoa **(SZ)**. (Methyl green pyronin Y stain, × 250).

Figure 8: Cross section of*Liza ramada* testis from Lake Manzalah showing weakly stained DNA/RNA contents: seminiferous tubules **(STU)** and spermducts**(SD)**. (Methyl green pyronin Y stain, X 250).

Figure 9: Cross section of*Liza ramada* testis from Lake Bardawil showing weakly stained collagen fibers: seminiferous tubules **(STU)**, spermatogonia**(SG)**, spermatocytes **(SC)**, spermducts**(SD)**, spermatids **(ST)** and spermatozoa **(SZ)**. (Masson trichrome stain, × 250).

Figure 10: Cross section of*Liza ramada* testis from Lake Manzalah showing strongly stained collagen fibres: seminiferous tubules **(STU)** and spermducts**(SD)**. (Masson trichrome stain, × 250).

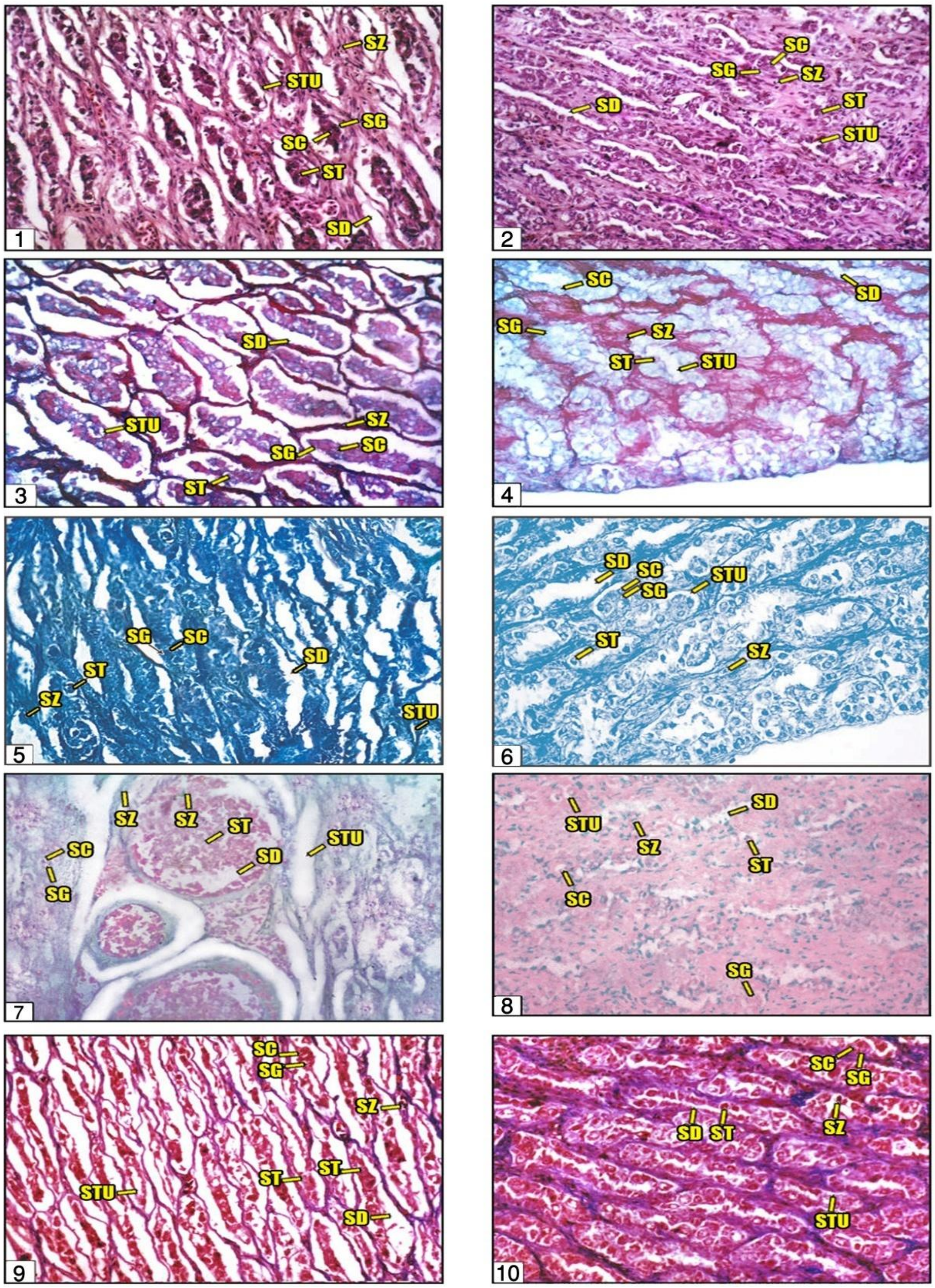


Figure 11: Cross section of*Liza ramada* ovary from Lake Bardawil showing tunica albuginea**(TA)**, oogonia**(O)**, ovarian follicles **(OF)**, yolk spheres **(YS)**, lipid vesicles **(L)**, cortical alveoli **(CA)**, granulose cell layers **(GCL)** and theca cell layers **(TCL)**. (Hx& E., × 250).

Figure 12: Cross section of*Liza ramada* ovary from Lake Manzalah showing broken tunica albuginea**(BTA)**, atresia **(AT)**, vacuoles **(V)**, deformed zona radiate **(DZR)** and necrosis of germ cells **(NGC)**. (Hx& E., × 250).

Figure 13: Cross section of*Liza ramada* ovary from Lake Bardawil showing strongly stained PAS-positive materials: tunica albuginea**(TA)**, oogonia**(O)**, ovarian follicle **(OF)**, yolk spheres **(YS)**, lipid vesicles **(L)**, cortical alveoli **(CA)**, granulose cell layers **(GCL)** and theca cell layers **(TCL**). (PAS reaction, × 250).

Figure 14: Cross section of*Liza ramada* ovary from Lake Manzalah showing weakly stained PAS-positive materials: broken tunica albuginea**(BTA)**, necrosis of germ cells **(NGC)**, atresia **(AT)**, vacuoles **(V)** and deformed zonaradiata**(DZ)**. (PAS reaction, × 250).

Figure 15: Cross section of*Liza ramada* ovary from Lake Bardawil showing strongly stained protein materials: tunica albuginea**(TA)**, oogonia**(O)**, ovarian follicles **(OF)**, yolk spheres **(YS)**, lipid vesicles **(L)**, cortical alveoli **(CA)**, granulose cell layers **(GCL)** and theca cell layers **(TCL)**. (Mercury-bromophenol blue stain, × 250).

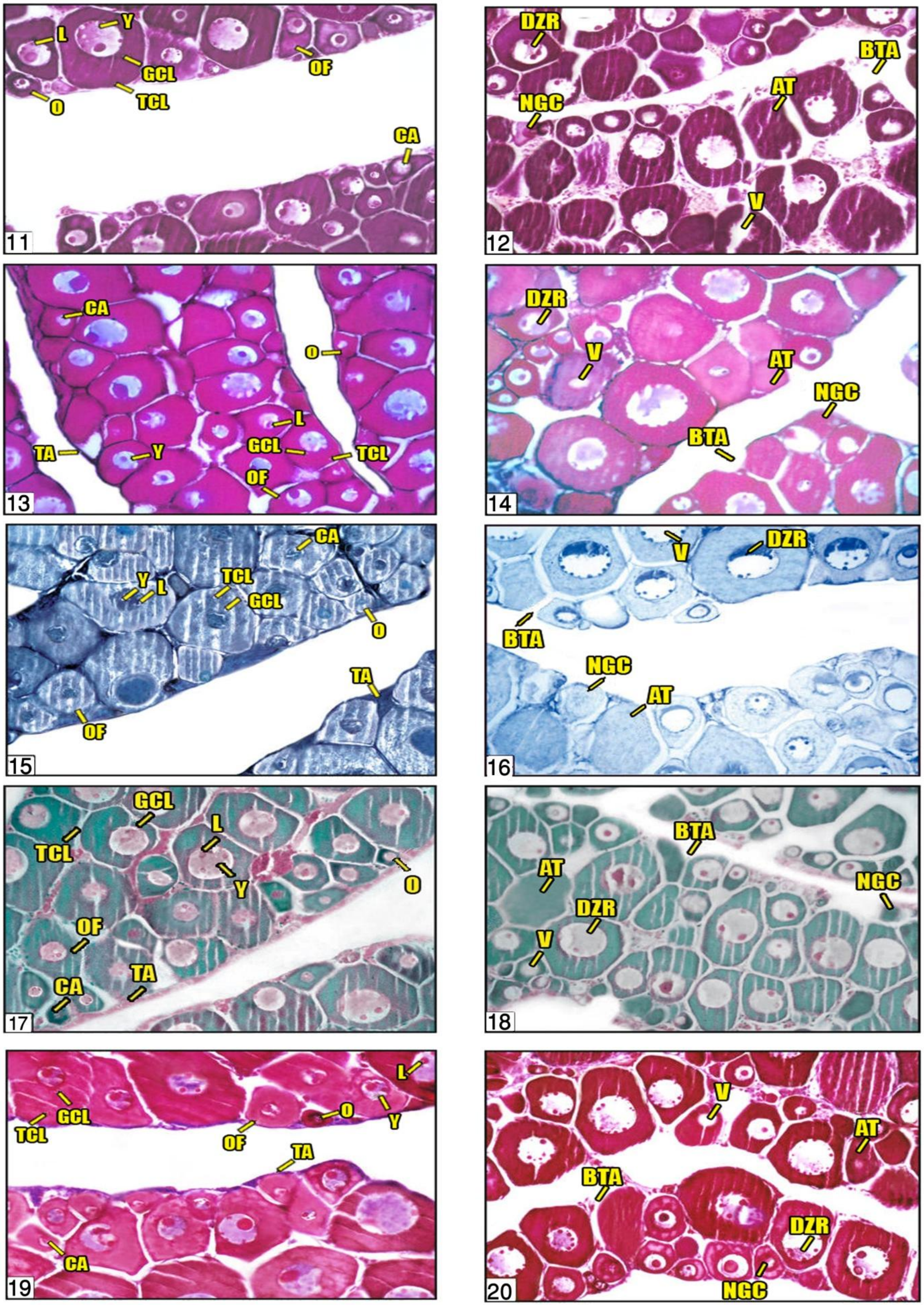
Figure 16: Cross section of*Liza ramada* ovary from Lake Manzalah showing weakly stained protein materials: broken tunica albuginea**(BTA)**, necrosis of germ cells **(NGC)**, atresia **(AT)**, vacuoles **(V)** and deformed zonaradiata**(DZR)**. (Mercury-bromophenol blue stain, × 250).

Figure 17: Cross section of*Liza ramada* ovary from Lake Bardawil showing strongly DNA and RNA contents: tunica albuginea**(TA)**, oogonia**(O)**, ovarian follicles **(OF)**, yolk spheres **(YS)**, lipid vesicles **(L)**, cortical alveoli **(CA)**, granulose cell layers **(GCL)** and theca cell layers **(TCL)**. (Methyl green pyronin Y stain, × 250).

Figure 18: Cross section of*Liza ramada* ovary from Lake Manzalah showing weakly DNA and RNA contents: broken tunica albuginea**(BTA)**, necrosis of germ cells **(NGC)**, atresia **(AT)**, vacuoles **(V)** and deformed zonaradiata**(DZR)**. (Methyl green pyronin Y stain, × 250).

Figure 19: Cross section of*Liza ramada* ovary from Lake Bardawil showing strongly stained collagen fibres: tunica albuginea**(TA)**, oogonia**(O)**, ovarian follicles **(OF)**, yolk spheres **(YS)**, lipid vesicles **(L)**, cortical alveoli **(CA)**, granulose cell layers **(GCL)** and theca cell layers **(TCL)**. (Masson trichrome stain, × 250).

Figure 20: Cross section of*Liza ramada* ovary from Lake Manzalah showing weakly stained collagen fibres: broken tunica albuginea**(BTA)**, necrosis of germ cells **(NGC)**, atresia **(AT)**, vacuoles **(V)** and deformed zonaradiata**(DZR)**. (Masson trichrome stain, × 250).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Discussion:

Pollution of aquatic habitats is a major problem in Egypt. In recent years, more toxic compounds were detected in aquatic ecosystem (Khare and Singh, 2002). Fishing is one of the most important industries and seafood is consumed by a large segment of Egyptian population (Soliman, 2006). However, fish population can be effected by a large range of the environmentally persistent heavy metals (Soliman, 2006).

Heavy metals are present at trace levels in aquatic environment (Al-Weher, 2008). Their levels increase due to industrial, agricultural and mining activities and they contribute to the pollution of aquatic ecosystems (Canli and Atli, 2003). Some heavy metals are essential (Fe, Zn and Cu…etc) and necessary in trace concentrations for normal growth and development (Kalay and Canly, 2000). They become toxic when their levels exceed required levels (Yilmaz, 2005) and be harmful to human health (ATSDR, 2004). However, the other non-essential heavy metals (Pb, Cd and Cr…etc) even at low concentrations are very toxic to aquatic ecosystems and human health (Jayakumar and Paul, 2006).

In the present study, the mean concentrations of Cr, Pb and Cd (non-essential heavy metals) which presented in Lake Manzalah water were 0.073-0.04-0.036 (ppm), respectively as they exceeded the recorded permissible levels in Egyptian Standards of Environmental Laws No. 48/1982 and No. 4/1994 which stated the maximum concentrations of Cr, Pb and Cd in fresh water should not exceed 0.001-0.01-0.05-0.05 (ppm), respectively. These observations showed that there were accumulated heavy metals in Lake Manzalah water. Cd exposure is linked with renal failure, bone fragility as osteoporosis and is considered as a cancer causing agent in the human health (ATSDR, 2004). There is adverse toxic effect of Pb on human health particularly children was recognized (Subramanian, 1988) in addition, neurological defects, renal tubular dysfunctions and anemia (Forstner and Wittman, 1983). Cr complexes, which are bounded to the other lower molecular weight legends, are mostly traversed to the cell membrane (Mertz, 1969) and concentrated in tissue organism and interacts with the cellular macromolecules, including DNA, or may be slowly released from the cell and then causes human carcinogen (Wiegand*et al*., 1985).

Heavy metals accumulate in fish tissues, as they can reach concentration levels up to 2000 fold higher than those found in the surrounding water environment (Popek*et al.,* 2006) and transferred through the upper classes of food chain. Human, obtain the heavy metal contaminations from aquatic ecosystem food, especially fish (Langston, 1990). Toxic heavy metals can alter tissue histological structures, and cause reproduction defects (Weis and Weis, 1989). In addition, Bobek*et al* (1996) showed that pollution affects the normal structure of fish tissues. Heavy metals may have direct effects on fish gonads (testes and ovaries), resulting in a disturbed development of germ cells (Mohamed and Gad, 2008).

The present study demonstrated the histological configurations of *Liza ramada* testes from Lake Bardawil have the normal regular of spermatogenesis process stages (Weltzien*et al*., 2002). The environmental impacts on Lake Manzalah water caused a pronounced decline in gonad activity of the studied fish which reflected by decreasing sperm and spermatids counting in ripe testes, ripe oocytes degeneration (atresia) including spermatogonia, of the seminiferous tubules and necrosis as these histological changes in testes tissue were progressively increased with the increasing degree levels of heavy metal accumulation (Phillips, 1991) and that resulted in permanent testicular damage which reduce the fish ability to reproduce by suppressing sperm production (Hanna *et al.*, 2008). Histochemically, *L. ramada* testicular tissues from Lake Manzalah showed an obvious decreasing of the total polysaccharides and total protein, DNA/ RNA contents and increasing in collagen fibers composition according to the collapsed interstitial cells against the sperm nests due to severe heavy metals effect when compared with those collected from Lake Bardawil. Subsequently, the amount of the PAS-positive materials and mercury-bromophenol blue stained materials in the tissues indicated the degree of the total polysaccharide and protein contents in the testicular tissues. Since, the intensity of staining is greatly dependent on the amount of polysaccharides and proteins in such sites. That applied also on DNA/ RNA contents and collagen fibers (Chandra and Khuda, 2004).

The oogenesis cycle of *Liza ramada* is divided into five stages namely: (i) chromatin nucleolar stage, (ii) perinulceolar stage, (iii) yolk vesicle stage, (iv) vitellogenic stage and (v) ripe (mature) stage (Alne, 1999). These were clearly apparent in Lake Bardawil fish. In the present study, pre-nucleolus stage was characterized by small size oocytes. At the end of this stage, the late per-nucleolus stage distinguished from the early pre-nucleolus stage by the enlargement of oocyte as the primitive wall of the oocyte was composed of one thin layer of flattened follicular cells (Zaki*et al.,* 1993). The second growth phase was the vitellogenic stage which included yolk vesicle stage and was characterized by vacuoles in circular manner and the oocyte wall was consisted of two layers: an externally situated zona granulose and an internally located zonaradiata layer near the cytoplasm (Yammamoto and Yoshioka, 1964). The overall patterns of oocyte development of the studied fish from Lake Manzalah showed marked degenerative changes. There was a distinctive form of yolk, and cytoplasm which was greatly changed and appeared highly vacuolated. Also, broken tunica albuginea was spread at all sites with greatly deformed cytoplasm. The degree of maturation of female fishes from Lake Bardawil was found higher than those obtained from in Lake Manzalah (Hatikakoty, 2002). The reduction in the size and development of oocytes in the fish from Lake Manzalah attributed to the affected vitellogenic and maturational enlargement of oocytes (Mousa and Mousa, 1999). The toxicity of heavy metals was presented as a disruption in gonadal development (Gordon *et al.*, 2000). Histochemically, the ovarian tissues of the studied fish collected from Lake Manzalah gave a clear information about the internal changes in the total polysaccharide and total protein contents as well as DNA and RNA content and collagen fibers composition which resulted from the severe effect of the heavy metal pollution, when compared with those collected from Lake Bardawil as it is a protected area (Mousa and Mousa, 1999).

Conclusion:

Thestudy showed that toxic heavy metals can alter tissue histological structures, and cause reproductive defects. The environmental impact on fish population of Lake Manzalah was delayed spermatogenesis, spermatogenic cell disruption and spermatid reduction. The female fish subjects from Lake Manzalah showed atresia, deformed zonaradiata, necrotic oocytes and broken tunica albuginea.

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