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EFFECT OF POLLUTION BY MERCURY, CADMIUM AND LEAD ON REPRODUCTION of TILAPIA NILOTICA FISH

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ABSTRACT

A total of 96 juvenile and 288 mature male and female Tilapia nilotica were exposed to mercury (Hg), cadmium (cd) and lead (Pb) pollution. The effects of such substances on some reproductive parameters were studied. The obtained results in mature treated fish revealed that the gonadosomatic index (GSI)decreased in the females after three weeks in cd and Pb exposed groups while in males a significant decrease in cd treated group and increase in lead treated group was recorded. The sperm count, motility and its duration as well as fecundity showed significant decreases in all treated groups. Concerning Estradiol 17 β and Testosterone, a significant decrease in each of gonads and skeletal muscles of treated males and females were observed. On the other hand, high mortality rate and retarded gonadal development in the juvenile fish were recorded.

INTRODUCTION

The contamination of fresh water with a wide range of pollutant has become a matter of concern over the last decades (Vutukuru, 2005). The natural aquatic system may extensively be contaminated with heavy metal released from domestic, industrial and other man-made activities (Dirilgen, 2001). Heavy metal contamination may have adverse effects on ecological balance of the recipient environmental and a diversity of aquatic organism (Ashraj, 2005; Vosyliene and Jankaite 2006). Among animal species fishes are the inhabitants that can't escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004). Generally these metals are widely distributed in aquatic system and can affect fish population by reducing growth, reproduction or survival (Farombi *et al.*, 2007).The sources of these substances are many and varied.

Mercury is transported to aquatic ecosystem via surface run off and from atmosphere. It is complex and tightly bound to organic and inorganic particles (Amdur *et al.*, 1991). Cadmium has long been recognized as a toxic element. Its concentration in the surface water is high due to mullergic plants, plating operations, cadmium pigments, batteries and plastic manufactures (Rasmussen and Anderson 2000). The use of lead, its mining and its processing dates back several centuries. Industries and agricultural discharges care the primary source of lead poisoning in Egypt (El-Nabawi *et al.*, 1987). Water pipes are the main sources of lead poisoning in aquaculture. Also high ways pose a threat to fish because of lead contamination from automobile exhausts which can be easily leached from soil and contaminate water adjacent to high ways (Diehl *et al.*, 1983).

Depressive effects of lead on steroid biosynthesis and hormone receptor binding were reported by (Weibe *et al.*, 1983). All life stages are sensitive to the toxic effect of metals (Davies *et al.*, 1976).Most published environmental studies involving Hg, cd and Pb exposure of fish focus on uptake and tissue distribution rather than on causal effect. Additional data regarding metal induced effects on reproduction are needed. The present study aimed to focus some light on the relationship between these heavy metal pollution (Hg, cd and Pb) and some reproductive parameters including gonadosomatic index, sperm activity, egg fecundity and steroid hormone concentrations (Estradiol-17β and testosterone) in male and female Tilapia niotica. Beside, its effect on the gonadal development of the juvenile fish.

MATERIALS AND METHODS

A total number of 384 (288 sexually mature and 96 juvenile) Tilapia nilotica with range weight of 55.34 - 96.04 gm for mature and less than 10 gm for immature fish were obtained from Abbassa fish hatchery. They were divided into four equal groups each contained 96 fish (24 male and 72 female). Each group was maintained in glass aquaria 100 cm. x 50cm x 50cm (with 3 replications) and contained dechlorinated and aerated tap water with temperature 28 ± 2°C, pH 7.4 and total hardness of 104 as CaCo3. The fish were kept under natural day light. Half the amount of water in each aquarium was changed every day with the excreta. The fish were fed commercial pelleted ration at a rate of 3% of their body weights. The fish were acclimatized to the laboratory conditions for 15days. At the time of the experiment, the 2^{nd}, 3^{rd} and 4^{th} groups were subjected to 3ug/L, 30ug/L and 50ug/L, of mercuric chloride, cadmium chloride and lead acetate respectively in water according to (El-Bouhy *et al.*, 1994). The clinical signs, mortalities and Post mortem findings were recorded according to Lucky (1977).

Sampling:

Samples from live mature fish in each group were taken after one, two and three weeks where four females and eight males were weighed, then dissected and the gonads were weighed for determination of gonadosomatic index and fecundity according to Munkittnck and Dixon (1988). The ovaries were fixed in neutral buffer formalin for histopathological examination and another part was extracted for hormonal assay. Concerning males, 4 fish were stripped for determination of sperm concentration according to the technique described by Bouk and Jacobson (1976) and sperm activity was determined according to Withier and Lim (1982). The duration of sperm motility was determined by a stop watch. Another 4 males from each group were used for determination of gonadosomatic index, pathological examination and hormonal assay.

Steroid hormone determination:

The ovaries, testes and skeletal muscles were extracted for Estradiol 17β and testosterone determination according to Manlimos and Abraham (1975). Estradiol 17β was estimated using PANTEX PANTEX direct 1125.Estradiol kit according to Powers *et al.* (1985). Testosterone was estimated Using PANTEX PANTEX direct 1125 testosterone kit according Cumming (1985). Analysis of data was performed using student "t" test according to Snedecor and Cochran (1969).

RESULTS

The results of Fig (1) Cleared that, the mortalities reached 80%, 60% and 20% among juvenile fish subjected mercuric (Hg)and cadmium (Cd) in the 1st week respectively for the experimental period. Meanwhile the effect of lead was 20% at the 3rd week from experimental period. In the same time no mortalities were recorded in adult fish.

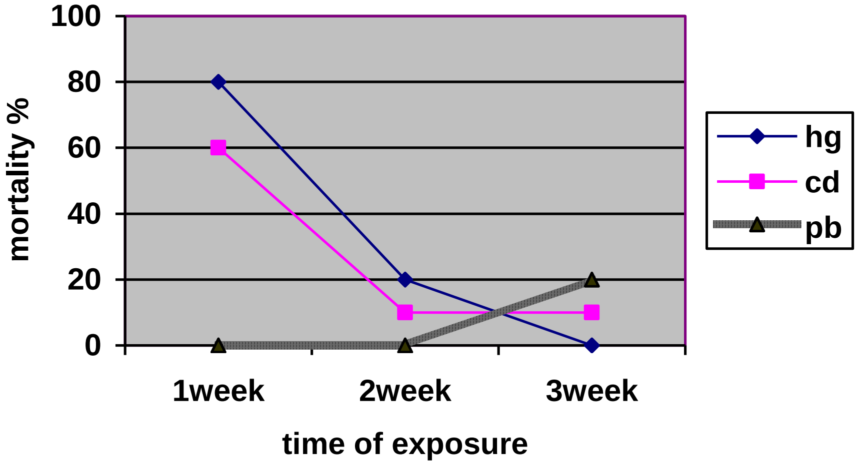

Fig.(1): Mortality among juvenile *Tilapia nilotica* during the period of experiment

The results of clinical examination revealed that after one week of Cd exposure the gonads appeared dark in colour with normal size while after three weeks of exposure, the ovaries appeared very reduced in size in adult fish but could not be detected in juvenile ones. In Hg and cd exposed groups the ovaries appeared dark, homogenous after three weeks.

From the results shown in table (1) it is evident that the female and male fish exposed to cadmium and lead pollution were decreased in body weight after the first week of exposure until the end of the experiment, while exposure to mercury resulted in a decrease only in body weight after two and three weeks of exposure in female and male fish respectively. The gonadal weight in the treated groups did not reveal significant weight changes from control group except the females exposed to lead pollution for three weeks. The gonadosomatic index showed a high significant increase after two weeks of exposure to cadmium and lead in the females followed by a decline after three weeks indicating that the females were more sensitive to these pollutants.

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While in males, a significant decrease resulted after exposure to cadmium chloride pollution for one and three weeks where as significant increase within the second and third weeks in lead acetate.

The sperm count decreased significantly after the first week of exposure to Hg while highly significant decrease in the 2-and 3 weeks of exposure to the three metals was obtained (table 2). The results of table (3) showed that the sperm motility and its duration decreased throughout the experiment in all groups, while no motility was detected after 3 weeks of exposure to Hg and 2 and 3 weeks of exposure to cd. The results of table (4) revealed significant and highly significant decrease in egg fecundity after cd and Pb exposure during the experimental period while in mercury the previous pattern appeared after the 2^{nd} and 3^{rd} weeks. The results recorded in table (5) revealed a significant decrease in Estradiol-17f3 in the ovaries of all groups while its content in skeletal muscles showed a significant decrease in the 2^{nd} and 3^{rd} weeks of cd exposure and in the 3^{rd} week of lead exposure. The male fish showed a significant decrease in Estradiol 17 β content in their testes in response to two and three weeks of Hg, Cd and Pb exposure respectively. The skeletal muscles of males showed a significant decrease in their Estradiol 17 β content after one week of exposure to cd. Comported with female the results in table (6) showed a significant decrease in testosterone content in the ovaries of fish in Pb treated group in the 2 and 3rd weeks while mercury treatment resulted in a significant decrease after two weeks. The skeletal muscles of the female had no change in testosterone content compared to control group.

Table 2: Sperm count x 10^6 in *tilapia nilotica* exposed to Hg ,cd and pb

Time of exposure	Control	Hg	Cd	Pb
One week	4.47±0.30	3.46±0.20*	3.89±0.17	4.01±0.13
two week	4.54±0.27	2.55±0.18**	1.99±0.18	2.84±015**
Three week	4.13±0.23	1.66±0.2 **	1.53±0.11	2.77±0.08**

***P<0.05 ** P<0.01**

Table 3: Sperm motility in tilapia *nilotica* exposed to Hg, cd and, Pb

Time of exposure	Control Motility duration. sec	Hg Motility duration sec.	Cd motility duration sec.	Pb motility duration sec.
One week	++++ 270	++ 124	+ 25	++ 75
two week	++++ 330	+ 90	--	++ 60
Three week	++++ 345	--	--	+ 45

++++ = (>75%activity) +++ = (51%-75% activity) ++ = (50%-26% activity)
+ = (<25% activity) -- = (inactive)

Table 4: Egg fecundity x10^4 in *tilapia nilotica* exposed to Hg, cd and Pb

Time of exposure	Control	Hg	Cd	Pb
One week	67.68±1.41	65.09±1.35	60.19±1.51*	59.35±1.62*
two week	55.99±0.92	50.02±1.20*	51.02±1.11*	47.60±1.28**
Three week	59.10±1.13	48.51±1.15**	49.95±0.97**	41.82±1.54**

***P<0.05 ** P<0.01**

Table 5: Concentration of Estradiol-17β (Pg/g fresh weight) in the gonads and skeletal muscles of female and male *Tilapia niltoca* exposed to Hg, Cd and Pb

Time of exposure	Gonads				Skeletal muscles			
	Control	**Hg**	**Cd**	**Pb**	**Control**	**Hg**	**Cd**	**Pb**
Female								
One week	34.85 ±4.40	17.06± 4.26*	12.38± 3.56*	10.62± 3.09*	1.27± 0.36	1.36± 0.41	1.42± 0.52	1.49± 0.37
Two week	38.61± 5.22	18.42± 2.82*	8.16± 2.12**	17.31± 3.51*	2.19± 0.51	2.16± 0.55	4.51± 0.39*	3.47± 0.26
Three week	28.07± 4.37	11.37+ 2.40*	10.54± 3.06*	13.46± 2.91*	1.48± 0.34	2.04± 0.52	5.23± 0.16**	3.86± 0.43
Male								
One week	4.29± 1.36	1.19± 051	0.91 .23	1.07± 0.45	1.27± 0.29	1.51± 0.31	2.71± 0.40	1.09± 0.33
Two week	5.51± 1.61	1.94± 0.24*	0.99 0.21*	1.06± 0.23	2.10± 0.46	2.06± 0.38	3.05± 0.42	2.22± 0.64
Three week	5.74± 1.41	1.77± 0.06*	1.01 0.47*	1.18± 0.45*	2.62± 0.56	1.94± 0.58	2.90± 0.47	2.08± 0.55*

***P<0.05 **P<0.01 within the same time of exposure for female and male.**

Table 6: Concentration of testosterone (ng/g fresh weight) in the gonads and skeletal muscles of female and male *Tilapia nilotica* exposed to Hg, Cd, and Pb

Time of Exposure	Gonads				Skeletal muscles			
	Control	**Hg**	**Cd**	**Pb**	**Control**	**Hg**	**Cd**	**Pb**
Female								
One week	1.48± 0.64	0.60± 0.21	1.72± 0.44	0.56± 0.22	0.98± 0.22	0.84± 0.34	1.08± 0.41	0.88± 0.14
Two week	1.92± 0.37	054± 0.29*	1.77+ 0.43	0.66± 0.24*	0.81± 0.25	1.15± 0.36	0.91± 0.29	0.69± 0.19
Three week	1.63± 0.41	0.61± 0.28	0.70 ±0.29	0.42± 0.12*	1.10± 0.36	1.61± 0.44	0.82± 0.28	0.77± 0.30
Male								
One week	20.41± 4.27	11.82± 2.53	3.15± 1.04*	16.61± 1.36*	10.15± 3.23	7.60± 1.41	2.32± 0.89	4.08± 0.86
Two week	19.30± 5.01	9.54± 3074	3.42± 0.95*	5.93± 1.36	9.73± 3.51	7.71± 1.72	3.09± 1.14	4.21± 0.97
Three week	18.65± 4.35	7019± 342	4.11± 0.81	5.56± .45*	12.61± 2.62	7.20± 1.55	2.44± 0.90*	3.95± 0.87*

***P<0.05 **P<0.01 within the same time of exposure for female and male.**

The most prominent change in testosterone content in the testes was the cd treated group while lead treatment resulted in significant decreases after one and three weeks of exposure. Testosterone in the skeletal muscles of males showed significant decreases after three weeks of cd and Pb exposure. The histopathological alterations of the ovaries of fish exposed to the three heavy metals were nearly similar and varied from slight degenerative changes in the ova (Fig, 2), few lymphocytic infiltration and slight fibroblastic proliferation which appeared in case of cd. To severe degenerative and necrotic changes with excessive accumulation of fat droplets (Fig, 3).

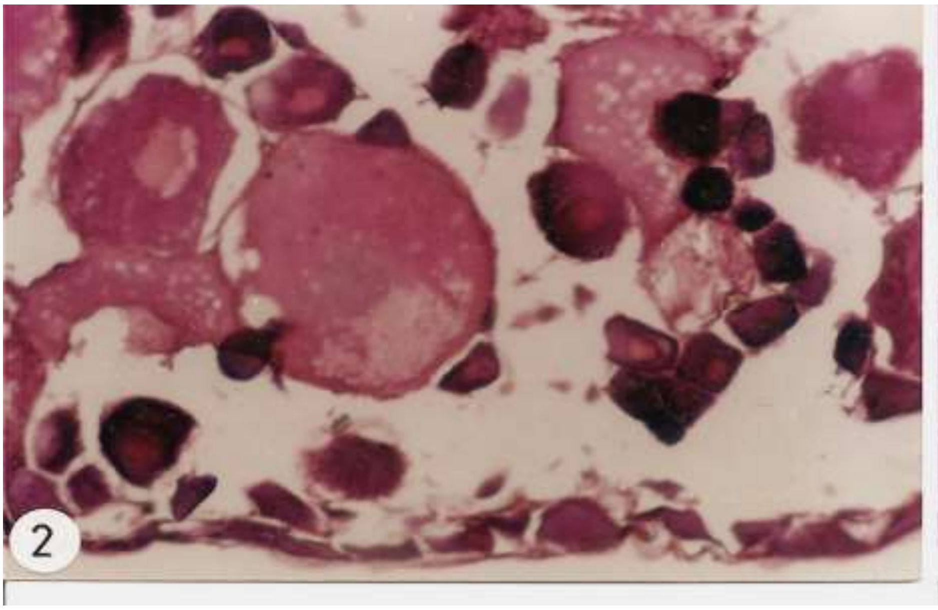

Fig. (2): ovary of *Tilapia nilotica* exposed to cd showing dissociation of mature ova with slight fibrous tissue proliferation of the wall of the ovarian sac.{H&E X300}

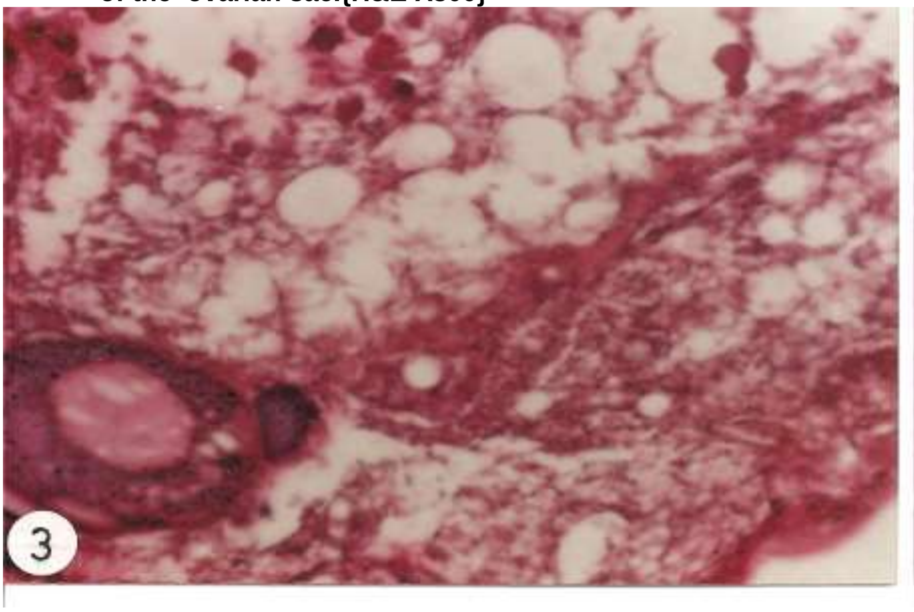

Fig. (3): ovary of *Tilapia nilotica* exposed to Hg showing degeneration and necrotic changes with accumulation of fat droplets. {H&E X300}

Complete lysis of the ova and some necrotic tissues were replaced by fibrous tissue beside excessive leucocytic infiltration in case of Hg and Pb exposure (Fig 4,5).The seminiferous tubules revealed successive developmental stages of the spermatides, the sperm at the center of tubules showed malformation, degenerative and necrotic changes in the nuclear. Elements (Fig.6, 7)

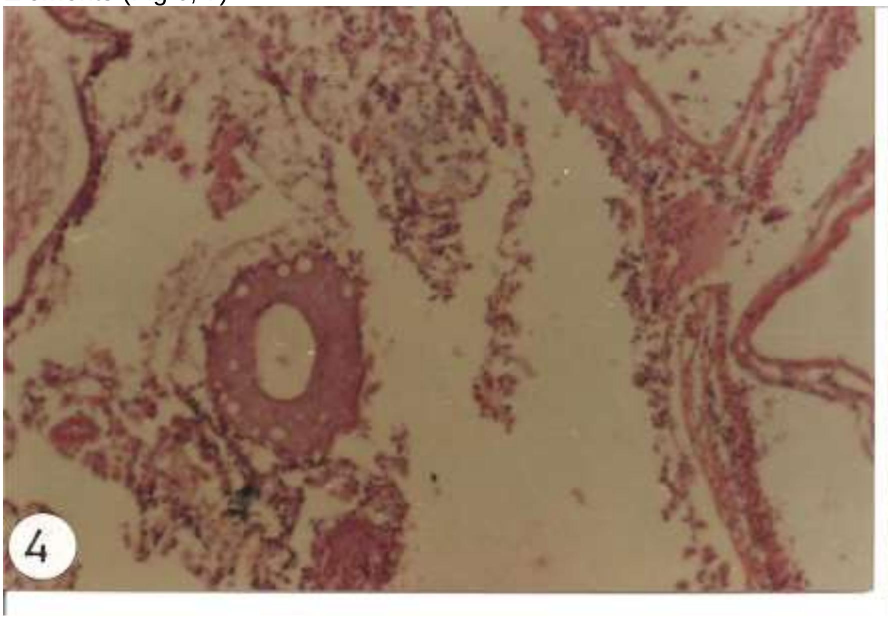

Fig.(4): Ovary of *Tilapia nilotica* exposed to Hg showing lysis of the ova. {H&E X300}

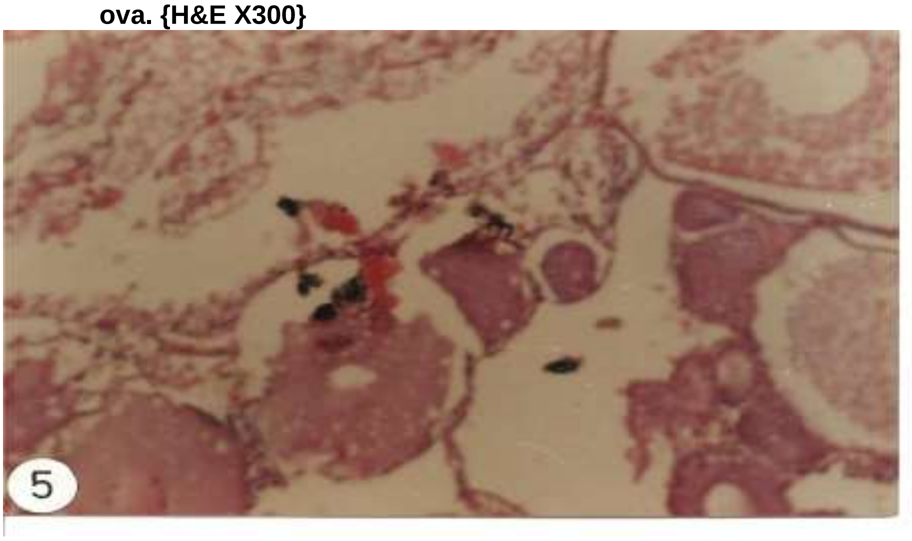

Fig. (5): ovary of *Tilapia nilotica* exposed to Pb showing lysis of the ova and replaced by fibrinoid material with lymphocytic infiltration. {H&E X300}

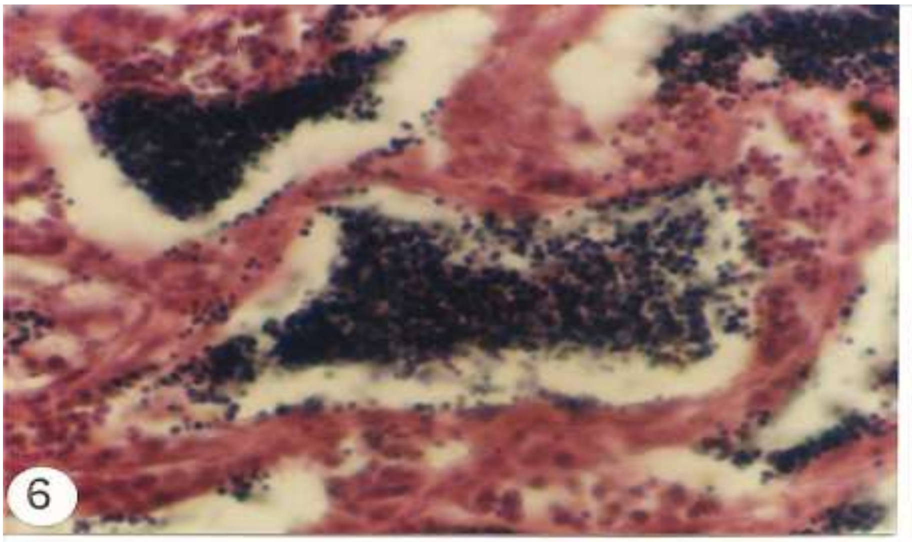

Fig. (6): Testes of *Tilapia nilotica* exposed to Hg showing degeneration of seminiferous tubules. {H&E X300}

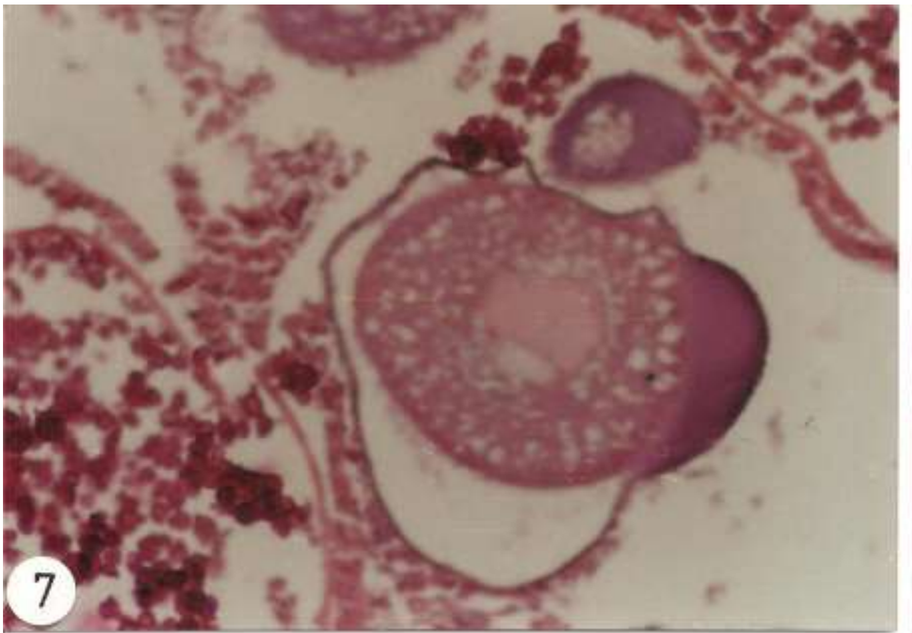

Fig. (7): Testes of *Tilapia nilotica* exposed to cd showing degeneration and necrotic changes in the sperm nuclear element. {H&E X300}

DISCUSSION

Fish are wildly used to evaluate the health of aquatic ecosystems because pollutant builds up in food chain and are responsible for adverse effects and death in aquatic systems (Farkas *et al.*, 2002).

One of the most severe hazards in aquaculture is the poisoning of fish by sublethal concentrations of heavy metals which affect fish population by reducing growth, reproduction or survival and are not under the control of fish farmers (Waqar 2006). Heavy metals contamination definitely affects the aquatic life of the fresh water fish. (Vinodhini 2008)

The results showed that the juvenile fish are more affected to heavy metal toxicity than adult fish which may be due to higher metabolic rate in young fish. These results are similar to that reported by Gill and Pant (1981). The decreased total body weight is response to heavy metal pollution was reported by several investigators. Macleod and Pessah (1973) stated that the active metabolism is inversely related to mercury level in water. Spehar (1976) reported significant reduction in growth rate of the surviving females subjected to cd toxicity while Pb toxicity in fish ranged from mortality to able effect on reproduction, growth and behavior (Katz, 1977). The decreased body weight in all treated groups could be attributed to the stimulating effect of these types of stressors on cortisol secretion (Pickering, 1990). The catabolic action of cortisol is responsible for mobilization of energy reservoirs as gluconeogenesis and lipolysis. However, the net effect of catabolism is a reduction in growth rate, feeding behavior and feeding response resulting in starvation (Rasenthal and Alderice, 1976).

The gonadal weight of the fish was not significantly affected by the pollutants used except in females exposed to lead acetate for three weeks. These results are supported by Katti and Sathyanesan (1985) who reported retarded sexual maturation in Pb polluted fish.

The increased gonad somatic index in the females exposed for two weeks to Cd and Pb are not ralated to increase gonadal weight but to the significant reduction in their total body weight. Otherwise the gonadosomotic index decreased in the females after three weeks. Male fish showed reduction in gonadosomatic index after cd exposure. Similar results were reported by (Sonoko Yamaguchi *et al.*, 2007) which may be due to testicular degeneration. The reduction in sperm number and motility as well as the fecundity in response to heavy metal pollution in the present work could be attributed to the malformation and necrosis of the spermatid and resorption and degenerative changes in the ova, these results are supported by the finding of Tam and Payson (1987) and Sonoko Yamaguchi (2007).

The decreased Strudel 17 β and testosterone in the gonads and muscles due to the used pollutants substantiate what has been found by Katti and Sathyanesan (1985) who reported reduction 3 hydroxysteroid dehydrogenase which is the key enzyme in steroidogenesis due to lead and cadmium pollution: Another explanation of these changes is dependent on cortisol secretion in response to the stress of pollution (Carragher and Sumpter, 1990). Cortisol inhibits estradiol-173 and testosterone secretion from the ovarian follicles also it decreased the concentration of circulating testosterone which was associated with a decrease in the testicular size. Pickering (1990) suggested that the suppressive effects of stress on gonadal steroids are mediated in part at least by hypothalamo - pituitary - interrenal axis whatever the precise mechanism involved and the environmental stress could seem to be capable of inhibiting growth of fish via a reduction in the levels of circulating anabolic gonadal steroids. It could be concluded that contamination of heavy metals such as mercury, lead and cadmium produced adverse effect on fish yield through its direct effect on the reproductive feature in male and female fish and on mortality of young fish. Therefore we aware from the increased limit of pollution in water by metal and other toxic materials which reflect on fish production and humane health.

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تأثير التلوث بمركبات الزئبق والكادميوم والرصاص على التناسل في أسماك البلطي النيلي

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المركز الإقليمي للأغذية والأعلاف - مركز البحوث الزراعية - وزارة الزراعة - مصر

في هذا البحث عرضت 288 سمكة بالغة و96 سمكة صغيرة (غير بالغة) من أسماك البلطي النيلي للتلوث بمركبات كلوريد الزئبق 3 /ميكروجرام /لتر وكلوريد الكادميوم 30 / ميكروجرام /لتر وخلات الرصاص50 / ميكروجرام /لتر لمدة ثلاثة أسابيع لدراسة مدى تأثير هذه المركبات على التناسل ونمو المناسل في البلطي النيلي وقد أوضحت النتائج ما يلي: بالفحص الإكلينيكي وإجراء الصفة التشريحية وصلت نسبة النفوق إلى 80% و60% و20% في الأسماك الصغيرة المعرضة للتلوث بمركبات الزئبق والكادميوم والرصاص على التوالي مع التوقف في نمو مناسل الأسماك الباقية كما ُسجلت التغيرات الهستوباثولوجية على مناسل الأسماك البالغة.

بإجراء قياسات التناسل لوحظ نقصاً معنوياً في دليل المناسل الجسمي في كل من الذكور والإناث في نهاية مدة التجربة خاصة في الأسماك التي تعرضت للتلوث بمركبات الكادميوم والرصاص كما قل عدد الحيوانات المنوية وقد تناقصت حركتها إلى أن انعدمت تماماً في نهاية فترة التجربة خاصة في الأسماك التي تعرضت للتلوث بالزئبق والكادميوم. هذا وقد نقص عدد البويضات في مبايض الإناث التي تعرضت للتلوث بالمركبات الثلاثة.

أظهر كل من هرموني الإستراديول والتستوستيرون نقصاً ملحوظاً في كل من **المناسل** والعضلات الهيكلية للأسماك التي تعرضت للتلوث.

قام بتحكيم البحث

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Table 1: Changes in the body weigh gonads weight and gonadosmatic index exposed to mercury (Hg); cadmium (Cd) and lead (Pb) in *Tilapia nilotic*

Time of exposure	Total body weight				Gonads weight				Gonadosomatic index			
	Control	Hg	Cd	Pd	Control	Hg	Cd	Pd	Control	Hg	Cd	Pd
Female												
One week	59.80± 4.56	52.71± 3.05	21.64± 2.51**	18.50± 1.42**	4.37± 1.11	4.65± 1.20	3.13± 0.92	2.51± 1.11	0.72± 0.001	0.079± 0.003	01.46± 0.004**	0.13± 004**
Two week	63.21± 7.06	42+62± 1.52*	23.69± 1.94**	20.42± 2.61**	5.17± 0.92	3.33± 0.75	2.72± 0.81	1.64± 1.20	0.088± 0.001	0.068± 0.003*	0.136± 0.004**	0.490± 0.003**
Three week	55.34± 2.39	54.60± 2.31	32.31± 1.91**	22.20± 2.13**	5.43± 1.15	2.90± 0.80	1.04± 0.99	1.04± 0.99*	0.092± 0.002	0.080± 0.004	0.071± 0.004**	0.027± 0.003**
Male												
One week	96.04± 6.81	77.23± 4.02	52.41± 2.81**	39.06± 2.1	2.67± 0.80	1.26± 0.41	1.61± 0.62	1.30± 0.45	0.029± 0.003	0.031± 0.002	0.016± 0.002*	0.033± 0.002
Two week	59.50± 5.64	81.50± 5.41	43.91± 3.14**	28.32± 1.94	2.19± 0.58	1.32± 0.51	0.92± 0.43	1.01± 0.37	0.023± 0.003	0.016± 0.003	0.021± 0.001	0.036± 0.001
Three week	88.32± 5.22	64.55± 5.09*	44.32± 3.16**	33.51± 1.83**	1.81± 065	1.13± 0.32	0.61± 0.20	0.61± 017	0.018± 0.001	0.018± 0.001	0.014± 0.001*	0.018± 0.002

***P<0.05 **P<0.01 within the same time of exposure for female and male.**