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EXPERIMENTAL STUDY ON EFFECT OF POLLUTED WATER WITH LEAD ACETATE ON HATCHABILITY, GROWTH AND GONADAL DEVELOPMENT OF NILE TILAPIA, OREOCHROMIS NILOTICUS (TELEOSTEI, CICHLIDAE)

BY

*ABD EL-HAKIM E. EL-GAMAL**

** National Institute of Oceanography and Fisheries, Alexandria, Egypt*

Key Words : Lead acetate – Hatchability – Growth – Histochemistry Gonad – *Oreochromis niloticus*

ABSTRACT

The present data showed that the lowest value of hatchability of Nile tilapia, Oreochromis niloticus was recorded in eggs treated with high dose of 13 ppm of lead acetate/liter of water and its percentage was 58.25 ± 0.229 . However, the highest value of hatching rate of fry was 81.50 ± 0.372 in control group. This value in its comparison with the other values that treated with high dose or with the lower dose (3 ppm of lead acetate / L of water) gave a high significante difference ($P < 0.005$) . A high significant difference was detected in total lengths and body weights of both males and females that treated for a period of 90 days with high dose of pollutant water (13 ppm of lead acetate per/L of water and control group, ($P < 0.005$). The gonadosomatic indices of both of ovaries and testes that treated with high dose level was significantly lower than that control. Histologically, the ovaries in control fish appeared in normal structure and about of 65% of eggs contained yolk granules (Vitellogenic oocyte). However, abnormal structure of oocytes were observed in the treated ovary with high dose. The female of the Nile tilapia, Oreochromis niloticus might be effected after treated with lead acetate at doses ranged from 3-13 ppm/L of water after a period of 90 days. The gametes maturation retarded in the ovaries and and impaired the cell secreting 3β-HSD activity and suggest that the lead acetate are likely to be harmful to juvenile gonadal development and probably effect on reproductive performance .

INTRODUCTION

Heavy metals produce toxic effects on the tissues of various terrestrial and aquatic animals (Sastry and Agrawal , 1979) Among the aquatic fuana fishes are the most

sensitive group . Organic contaminant have been shown to accumulate in fat cells and in tissues such as liver and gonad (Von Westernhagen *et al.*, 1981 and Von Westernhagen *et al.*, 1987). In several flat fish species, this has been related to reduce reproductive success as effect on fertilization in the starry flounder, *P. Stellatus* (Spies *et al.*, 1988) and effect on hatching and the number of viable larvae in the flounder *P. flesus* (Von Westernhagen *et al.*, 1981 and Von Westernhagen *et al.*, 1987). The pollutants may have direct effects on the gonads resulting in a disturbed development of germ cells (Janssen *et al.*, 1997). Indirect effect on reproduction, via interference with regulating hormonal system have also been suggested (Freeman *et al.*, 1980 and Thomas, 1990). Other heavy metal such as methlyl mercury accumulate at lower concentration in gonad of some fishes (Lockhart and Uthe, 1972; Mckim *et al.*, 1976; Hodson *et al.*, 1994 and Pelletier and Audet, 1995) where it inhibits gonadal recrudescence (Dey and Bhattacharya, 1989; Kirubaguaran and Joy, 1988 and 1992) and gametogenesis (Wester and Canton, 1992). It is also reduces growth (Panigrahi and Misra, 1978; Rodgers and Beamish, 1982; Snarski and Olson, 1988; Weis and Khan, 1990 and Niimi and Kisson, 1994) . Lead is more toxic due to its lasting effects on the animals tissues (Sastry and Agrawal, 1979). Very little information is available on the effect of heavy metal especially lead acetate on the gonadal tissues of fish.

The present study is therefore focused on effect of heavy metal " lead acetate on hatchability , growth and gonadal development . Also, its effect on biosynthesis of 3B hydroxysteriod dehydrogenase which is responsible for formation of steroid hormone and its localizing sites from histochemical view that accompany lead intoxication in the ovary of teleost fish , *Oreochromis nilotieus.*

MATERIAL AND METHODS

Natural spawning of *Oreochromis niloticus* was carried out at El-Mattaria Station Researches for fishes during a period from 31 July till the end of October, 2001. After spawning, number of fertilized eggs was removed from mouth of incubated mother fish by using a current of running tap water. After five days of post-hatching, small fry were randomly grouped together in a holding tank and about of 900 fry subjected for experimentation. At the beginning, post hatching fry were divided among three glass aquaria (one control and two aquaria were used for experimentation and replicated). Each glass aquarium occupied (160 x 80cm in area) and about 50cm of depth. For newly hatched fry, each glass aquarium contained 80 liters of dechlorinated water and the density of fry was 150 in each aquarium . About half of dechlorinated water column in each aquarium renewed every two days for a period of three months. The total lengths of newly hatched fry in nearest mm were recorded by using calibrated eye-piece micrometer under Streomicroscope and the body weights of fry in nearest mg were also recorded . The characteristics of test medium were recorded PH (7-7.4), dissloved oxygen (5.3-6.5 mg/L) and water temperature (29 °C – 25°C). Lead used in this study in the form of lead acetate Pb $(C_2H_3O_2)_2$ H_2O. The exposure regimens employed in this study were designed to reflect the effect of lead exposure in the natural environment of

Manzalla lake. The first glass aquarium was used as control without addition any contamination. The second and the third glass aquaria were used for sublethal dose levels which ranged from 3-13 ppm of lead acetate /liter of water. Peliminary bioassays conducted in laboratory under static conditions have shown that lethal dose Lc_{50} equivalent to 30ppm of lead /L of water for about 96 hours. The fish were fed on alternate days with wheat bran and oil cake with about 3-5% of body weigh of fish . The samples were firstly collected after 50 days of posthatching and the second collection was taken after 90 days of posthatching. The average of total lengths and body weights of fish per each treatment was recorded and the gonad was isolated and weighed for the nearest mg.

The condition factor (K) was calculated for each individual fish, and the average of the condition factor was calculated according to the formula recommended by Hile (1936) and LE-Cren (1951) .

$$K = W/L^3 \times 100$$

Where W is weight of fish in gm
L is length of fish in cm

Gonadal development :

Gonadosomatic index (GSI)

The gonadosomatic index was calculated by the following formula according to Clark (1934) .

$$GSI = Gwt / Bwt \times 100$$

Where Gwt is weight of gonad in nearest "gm"
Bwt is body weight of fish in nearest "gm"

For histological study, fish was sacrified and the gonads were isolated and fixed in Bouin's fluid for about 48 hours. After the fixation, the tissues of gonads were washed in tap water to remove any excessive of picric acid, dehydrated in series of ethanol, clearaed in xylen and embedded in paraplast wax (56– 58°C melting point). Sections were cut at 5-6 μ. and stained with harris hematoxylin and counterstained with eosin.

The development stages in the ovary of *Oreochromis niloticus* were described according to Latif and Saady (1973). The cytochemical methods of 3B hydroxysteroid dehydrogenase in the ovary was described according to the methods of (Wattenberg, 1958; Hoyer and Anderson 1977; Swarts and Schuetz , 1980; Chowdhury *et al* . 1985).

Statistical analysis :

In order to calculate the statistical significance between control and experimental studies, a comparison of the parameters were described according to fisher (1950) and Sokal and Rohlf (1969) . Exponential regression analyses were used with the calculation of correlation coefficient (r) between a high dose level of lead in both length and weight of fish after 50 and 90 days after hatching.

RESULTS

Hatchability :

The fertilized eggs in control group, even in 96 hours of posthatching gave a higher value of hatchability and its percentage was 81.50 ± 5.766 . However, the lowest value of hatchability was recorded in eggs of group III that treated with a high dose level of 13 ppm of lead acetale/L and reached to 58.28 ± 0.229. The value of survival rate of fry was 76.75 ± 0.319 in control group (I) . This value in a comparison with the other values that treated with high or low dose levels showed that there was a high significant differences ($P < 0.005$) Table (1) .

Growth :

In early-life stage, it is difficult to detect the gonad of larvae from morphological view. A combined fry having undifferentiated gonads had subjected to low and high contaminant water with lead acetate for a period 50 days after hatching. The average of total lengths and body weights of both males and females were not analyzed separately. A comparison between the average of body weights of fish receiving high dose of lead acetate (13 ppm/L) as pollutant as shown in the group III was highly significant lower than that of control ($P < 0.005$). Table (2). For subjected fish with high doses level , regression analyses revealed a significance inverse correlation between high doses level of lead 13 ppm /L of water and the length and weight of fish . After, the subjected fish to polluted water for a period of 90 days of post-hatching, morphologically, the sex can be easily detected either to males or to females. Consequently, the average of total lengths and total body weights of both males and females of fish treated with high dose of pollutant as in treatment group III was significantly lower than that of control group I Table (3). Inside the same length, the total weight of males in the treatment group III was slightly decreased than females, however, there was significant difference between the weights of two sexes. On the other hand, there was a high significant difference in total weights of both male and female that receiving high dose of pollutant water in treatment group III and control group I ($P < 0.005$) Table (3) . For exposured females to high dose levels of lead ,a significance inverse correlation between high doses level and weight of females

Survival rate :

The experiment showed that the lowest value of survival rate was observed after a period of 90 days of posthatching in treatment group III. There was a high significant difference between the survival rate of this treatment group and that of the control group I ($P < 0.005$) (Table 4) .

Condition factor (K) :

The values of the condition factor are used to assess the impact of environmental alternation on fish performance and health condition of fish. Consequently, fish subjected to high polluted water with lead acetate as in group III showed a lower value of the condition factor than that collected from control (group I) or those subjected to low dose level (Group II) ($P < 0.005$) (Table 4)

Gonadal development :

The gonadosomatic indices of both male and females that treated with high dose level in the treatment group III were lower than control group I . Though, in both males and females, there was a high significant difference in gonadosomatic index was recorded in both treatment group II (low dose level of lead acetate and treatment group, III (high dose levels) ($P < 0.005$). Also, a high significant difference in gonadosomatic index was also recorded between group, III (high dose level) and control group I ($P < 0.005$) (Table (5) .

Histological observations :

The testes collected from fish subjected with high dose level of lead acetate did not contain any predominant lesion in testicular tissue and appeared similar to the collected testes from control fish. The histological study on the ovary yielded more stricking results at the end of three months . In control fish , the ovary appeared in normal architecture and about of 65% of eggs contained yolk granules (Vitellogenic oocytes). Few numbers of these eggs appeard in small size Fig. 1 (a). The ovaries from polluted water with low dose level (3 ppm of lead acetate/L of water) contained less predominant lesions and the most of oocytes appeared in late perinucleolus stage and few of those eggs in were in early perinucleolus stage Fig. 1 (b). Severe abnormalities in ovarian structure were observed in fish collected from polluted water with high doses level of 13 ppm of lead acatate/L of water . The most of oocytes appeared in early perinucleolus stage and appeared in abnormal structure in Fig. 1 (c). The abnormal architecture of those ovaries reflex the lower significant in gonadosomatic index than that in control ovaries as shown in table (5) .

3β- Hydroxysteroid dehydrogenase localization :

The effect of polluted water with various doses level of lead acetate were assayed histochemically for 3 β-hydroxysteroid dehydrogenase (3β-HSD) to show the effect of this material as pollutant on localizing sites of steroid synthesis and its effect on biosynthesis of this active steroid hormones. In the ovary of control fish, a positive reaction of 3 β-HSD was observed as small deep blue colour which are located in the thecal cells of an outer layer of oocytes and interstitial cells. Fig. 2 (a) . Similarly, the ovary from polluted water with low dose level of 3ppm of lead acetate/L of water showed a moderate positive reaction for 3β-HSD and localized in the ovarian wall and interstitial cells Fig. 2 (b). However, the ovaries from reared fish in high concentration of lead acetate (13ppm/L of water) showed a very weak reaction in the wall of young eggs and interstitial cells Fig. 2 (c) .

Table (1) : Mean of hatching and survival rates ± SE of *Oreochromis niloticus* after treated with sublethal doses of polluted water with lead acetate for a period 96 hours after hatcing

Gorups	I	II	III
Concentration of lead acetate "ppm"	Control	3ppm of lead acetat/L	13 ppm of lead acetate/L
Hatching rate	(245) 81.50 ± 0.372	(208) (a) 69.25 ± 0.082	(175) (b) 58.25 ± 0.229
Survival / rate	(185) 76.75 ± 0.319	(126) 60.75 ± 0.354	(72) ‡ 41.250 ± 0.726

Number of fish in each treatment is between the brackets.
(b) represent a high significance difference between control group I and high concentration of lead acetate in group III ($P < 0.005$).
(‡) Mean was highly significant lower than that of control group I ($P < 0.005$).

Table (2) : Mean of total length' and body weights ± SE of *Oreochromis niloticus* after treated with sublethal doses of polluted water containing lead acetate for a period 50 days after hatching

Lead acetate of concentrate (ppm)	**Groups**	**No. of fish**	**Mean of total length (cm) ± SE**	**Mean of total/weight (gm) ± SE**
Control	I	50	6.50 ± 0.064	5.530 ± 0.130
3 ppm of lead /L	II	50	5.03 ± 0.115	(a) 3.870 ± 0.015
13 ppm of lead / L	III	50	4.720 ± 0.056	(b) 3.430 ± 0.140

b) Mean was highly significantly lower than that of control group I ($P < 0.005$) .
A siginificant negative correlation between high dose level of lead (13Pppm /L of water) and both length (r= 0.733) and weight (r= 0.641) .

Table (3) : Mean of total lengths (cm) and body weights (gm) ± SE of both males and females *Oreochromis niloticus* after treated with polluted water with lead acetate for a period 90 days after hatching

Concentration of lead acetate (ppm)	Group	Males			Females		
		No. of fish	Mean of total lengths ± SE	Mean of total weights (gm) ± SE	No. of fish	Mean of total lengths (cm) ± SE	Mean of total weights (gm) ± SE
Control	I	40	8.670 ± 0.117	10.448 ± 0.349	40	9.930 ± 0.154	8.681 ± 0.542
3ppm of lead/L	II	40	6.300 ± 0.08	(a) 5.670 ± 0.025	40	6.500 ± 0.116	(†) 5.919 ± 0.292
13 ppm of lead / L	III	40	5.570 ± 0.185	(b) 4.704 ± 0.465	40	6.625 ± 0.179	(‡) 5.400 ± 0.338

In Males, (b) a high significance difference was detected between control group I and group III ($P < 0.005$).

In females (‡) a comparison between control group I and group III showed a high significance difference ($P < 0.005$) .

A siginificant negative correlation between high doses level of lead (13ppm /L of water) and both length (r= 0.453) and weight (r= 0.622) .

Table (4) : Mean of survival rate and condition factors (K) ± SE of *Oreochromis niloticus* after treated with contaminant water with lead acetate for a period 90 days after hatching.

Groups	I	II	III
Concentration of lead acetate "ppm"	Control	3ppm lead acetate / L	13 ppm of lead acetate/L
Survival / rate	(148) 80.0 ± 0.456	(75) 59.330 ± 0.427 †	(34) (‡) 47.661 ± 0.865
Condition factors (K)	(20) 1.682 ± 0.038	(20) (a) 1.504 ± 0.023	(20) (b) 1.103 ± 0.039

Number of fish is between the brackets.

(‡) A high significant difference $P < 0.005$ was detected between control group I and group III.

(b) A comparison between group I and group III showed a high significant differences ($P < 0.005$) .

Table (5) : Mean of gonadosomatic indices ± SE of both males and females of *Oreochromis niloticus* after treated with lead acetate for a period 90 days after hatching

Sexes	Males			Females		
Groups	I	II	III	I	II	III
Concentration of lead (ppm)	Control	3ppm/L	13 ppm/L	Control	3ppm/L	13 ppm/L
Gonadosomatic index (GSI)	(50) 0.633 ± 0.023	(50) (a) 0.210 ± 0.005	(50) (b) 0.168 ± 0.006	(30) 2.120 ± 0.039	(30) † 0.643 ± 0.015	(30) ‡ 0.243 ± 0.004

Number of fish is between the brackets.

In males (b) a high significant difference was detected between control group I and group III ($P < 0.005$)

(‡) Mean was significant lower than that of control group I ($P < 0.005$)

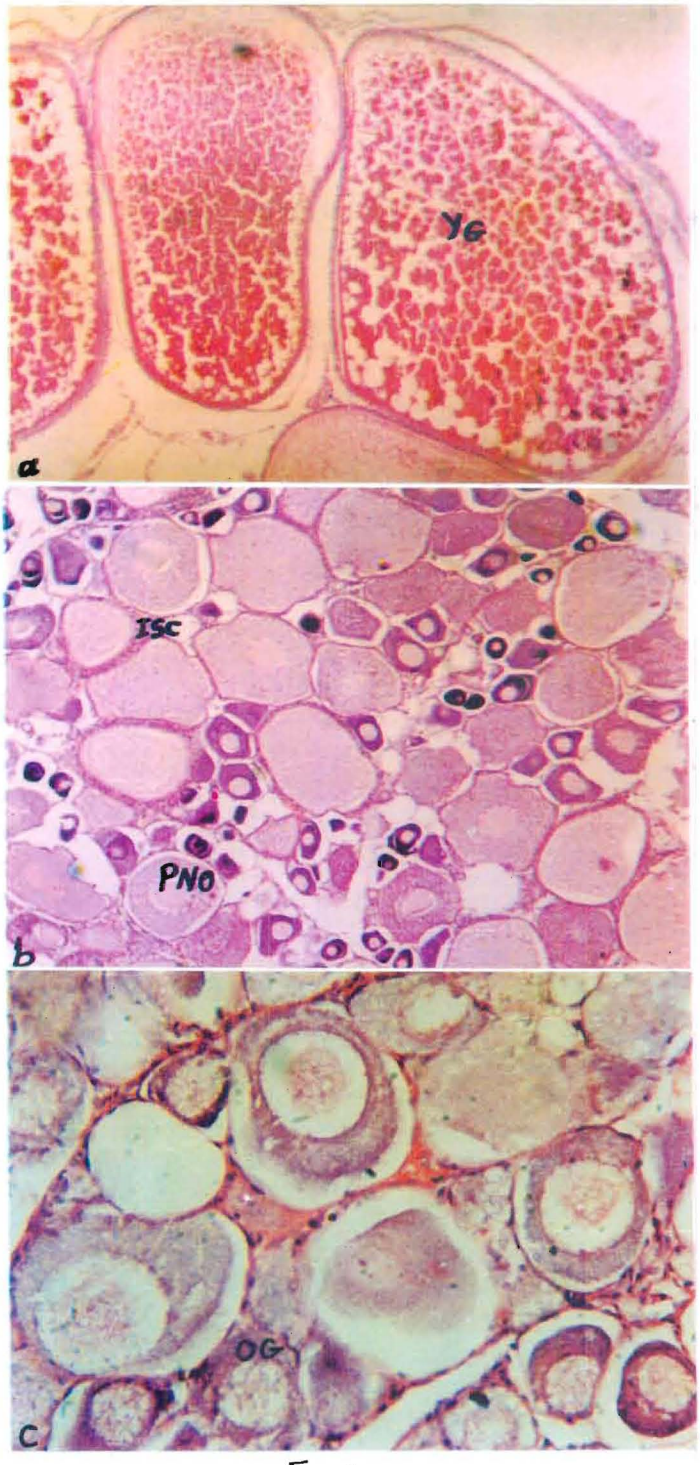

Fig.1

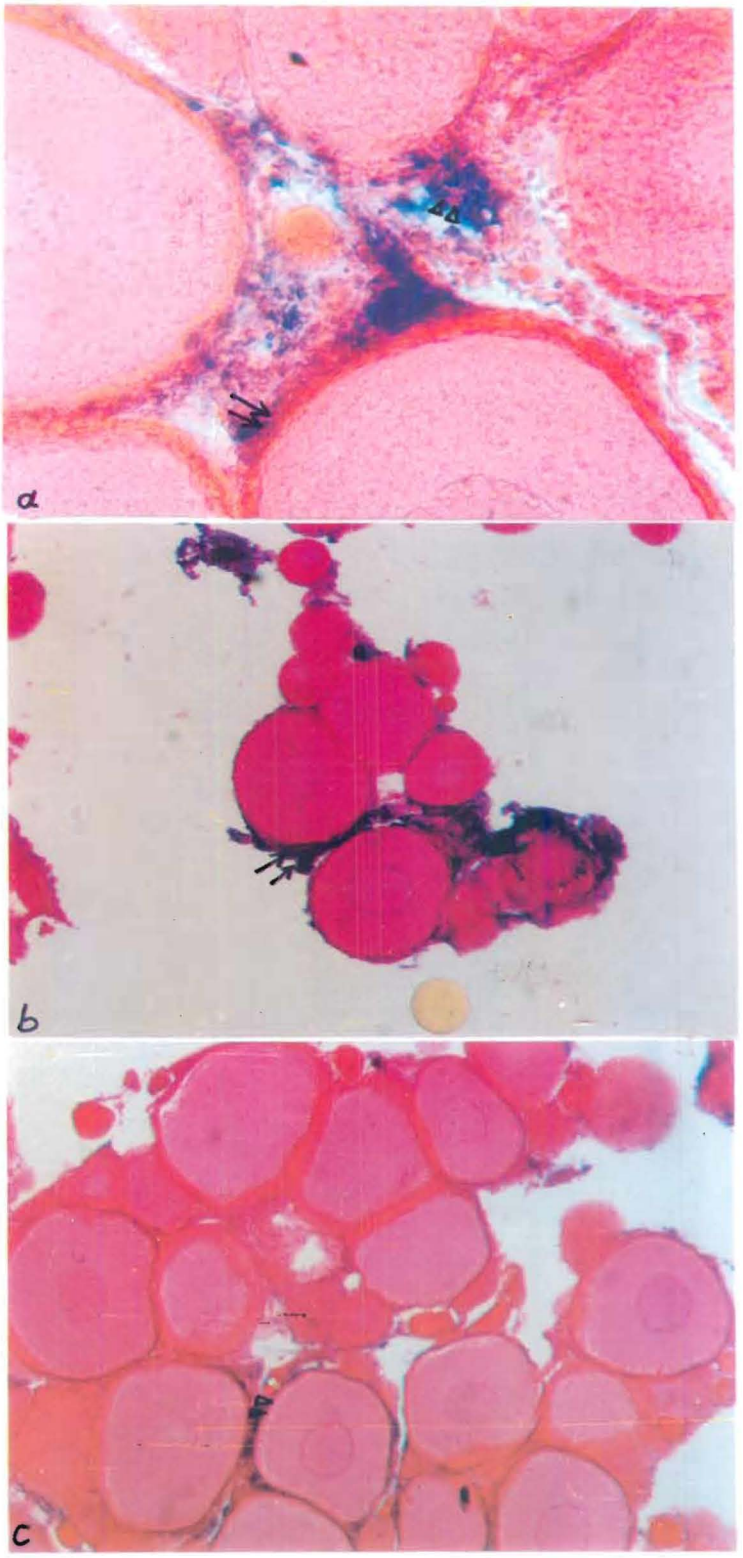

Fig. 2

EXPLANATION OF FIGURES

Fig. (1) : Part of transverse sections in the ovaries of *Oreochromis niloticus* in untreated and treated fishes with lead acetate at various doses level 3-13 ppm/L of water for three months, stained with harris's hematoxylin and eosin showing, (a) . Part of section of untreated ovary (control) in the tertiary oocyte beside Yolk globules (YG) 100 x. (b) Part of section of the treated ovary with 3ppm/L of water showing primary oocyte stages, perinucleolus oocyte (PNO), interstitial cells (ISC), 100 x. (c) Part of section of the treated ovary with high dose of lead acetate (13 ppm/L of water) showing, small oogonia, (OG) appeared in abnomal architecture 500 x.

Fig. (2) : Part of transverse sections in the ovaries of *Oreochromis niloticus* of untreated and treated fish with lead acetate at various doses level 3-13 ppm/L of water for three months after hatching, incubated with substrate of 3B-hydroxy steroid dehydrogenase enzyme and counterstained with eosin . (a) Part of section of control ovary, showing a strong positive of 3B – HSD was observed as small deep blue colour in the thecal cells (Arrows) of the wall of oocyte (Arrows) and interstitial cell (Arrow heads) 500 x .(b) Part of section in the treated ovary with 3 ppm/L of water showing a moderate positive reaction between the young oocytes (interstitial cells (arrows),also in the thecal cells (Arrow head) 100 x . (c) Part of section in the treated ovary with 13ppm/L of water, showing, a very weak reaction appeared in the wall of young oocytes (Arrows) and interstitial cells (Arrow heads) 250 x.

DISCUSSION

The doses level of lead acetate used in our study in glass aquaria were 3-13 ppm /L of water . The doses were designed to reflect the lead exposure in the natural environment of Manzalla lake . In this respect, El-ghobashy *et al* (2001) found that the concentration of lead acetate in the sediment samples of Manzalla lake was 12.77ppm . Ghazaly (1991) in his studies on *Tilapia zilli* used lead as pollutant in the form of lead acetate and its concentration was 8.3 ppm /L of water. The doses level used in our investigation reinforces the general appropriation of exposure regimen for assessing the impact of environmental of lead in the natural environment of Manzalla lake .

The current finding clearly showed that water containing a relatively high concentration of lead acetate (13 ppm/L of water) can affect on the percentage of fertilization and hatching rates of fry, *Oreochromis niloticus*. The precent of both hatching and survival rates reached to 58.25 ± 0.224 and 41.25 ± 0.726 resepectively. Similar observations were reported by Spies *et al.* (1988) in flounder (*P. Stellatus*), Von Westernhagen *et al.* (1981) and Von Westernhagen *et al.* (1987) in Founder (*P.Flesus*) in many of other teleosts. The effect of heavy metal using lead acetate can be affected on growth and gonadal development of *O.niloticus*. The water containing even relatively low concentration of lead acetate can inhibit growth and gonadal development either in male or in female. However, the testes of males were less

effected than the ovaries of females, with exception of the atrophy in testes and decreased in its size resulting from decrease in gonadosomatic index. Similar observations were reported in juvenile walleye (*Stizostedion vitreum*) by using low dose levels of dietary of methyl mercury as pollutant (Friedmann *et al.*, 1996). The inducing of reduction in growth of might be reported from inhibition of both appetite and growth by polluated water with lead acetate. Similar results were obtained by many investigators using methylmercury as pollutants (Panigrahi and Misra, 1978; Rodgers and Beamish, 1982; Snarski and Olson, 1982; Weis and Khan, 1990 and Niimi and Kissoon, 1994). The possibility might be through the ability of heavy metals to reduce circulating levels of cortisol and exhausted cortisol producing endocrine system as suggested by many of workers (Kirubagaran and Joy, 1991; Hontela *et al.*, 1995). Another mechanism might involve an atrophy of pituitary thyrotrophs as in murrel *Channa punctatus* (Joy and Kirubagaran, 1989) and decreased in level of circulating T4 in *Channa punctatus* and the catfish *Clarias butrachus* (Bhattacharya *et al.*, 1989; Kirubagaran and Joy, 1994). The effect of heavy metal on the plasma cortisol and on thyroid function were not involved in our study and will be taken in our consideration and awaits future study .

The current finding indicated that lead acetate is associated with decrease in both of gonadosomati index and healthy state of *O.* niloticus suggesting that this metal can impair gonadal development. The clear picture was observed in ovarian tissues after treated fish with heavy metal of lead acetate for a period of three months after hatching, the early stage of eggs development were predominant and atrophy of ovarian tissue contributed to this decrease of gonadosomatic index. The mechanism by which lead causes this atrophy in the ovaries of *O. niloticus* is unknown. However, many of investigator tried to explain this mechanism in other teleosts and found that mercury as pollutant can alter mitotic activity of gonadal development. (Wobeser, 1975; Dial, 1978; Wester and Canton, 1992) Other mechanism might involve in the ability of mercury to decrease hypothalamic gonadotrophs of both size and number (Joy and Kirubagaran, 1989). In our opinion a clear picture can emerge only when the other key enzymes in the metabolism of oocytes are studied. The mechanism of the biosynthesis of 17β estradiol has been recently proposed by Kagawa *et al.* (1982). According to their proposition , the thecal cells synthesize testosterone that is necessary for granulose to aromatize to 17β estradiol. Similar finding was similar in the ovary of rat (Fortune and Armstrong, 1978) and the same metabolic route is described by Hillier *et al.* (1980) in the human ovaries. The ovary of *Oroechromis. niloticus* under the present study showed that the activity of this key enzyme of 3β –HSD in biosynthesis of testosterone was clearly observed in the thecal cells and interstitials cells of the ovarian wall of control fish. Similar observations were obtained by Mousa (1999) on the ovary of Nile tilapia *Oroechromis niloticus*. The gradual decrease in ovarian 3B – HSD was detected at 90 days of treatment with lead acetate at doses level ranged from 3-13 ppm /L of water resulting in retarded of gametes maturation and impaired the cells secreting 3B hydroxy –steroid dehydrogenase . Similar results were obtained by many workers

(Kirubagaran and Joy, 1988; Kirubagaran and Joy, 1992 and Wester and Canton, 1992) in many of other fishes.

In conclusion, the finding of this study suggest that lead acetate at doses ranged from 3 to 13 ppm / L of water for a period of 90 days after hatching are likely to be harmful on juvenile gonadal development and probably reduce overall survival and adverse effects on reproductive performance of one of the most common fish in Egypt, *Oreochromis niloticus* .

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