

## Effect of Different Artificial Diets on Growth Rate Condition and Histological Structure of Nile Tilapia (*Oreochromis niloticus*)

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**Abstract:** The present study on 120 fish (*O. niloticus*) were rearing in three ponds during 5 months (2008) at El-Kanater station. Pond I was untreated groups, ponds II and III were treated by vitamin C and Cobalt chloride respectively. This conducted to demonstrate the effect of vitamin C and  $\text{CoCl}_2$  to illustrate the best treatment that gives the maximum growth rate and alleviation of pollution effects on some tissues (Kidney and Intestine). The results showed noticeable increase in growth rate of fish reared in Vitamin C and  $\text{CoCl}_2$  than in untreated group. In addition to the some histopathological changes in kidney and intestine of fish reared at pond I (untreated group) due to the pollution of the Nile water. However, improvement was observed in the sample collected from the ponds treated with vitamin C and  $\text{CoCl}_2$ .

**Key words:** Treatment, Vitamin C,  $\text{CoCl}_2$ , growth rate, histopathology, *Oreochromis niloticus*.

### INTRODUCTION

Tilapia species is considered one of the important fish in different aquaculture system due to their rapid growth, high tolerance to various environmental conditions, efficient feed conversion, and disease resistance<sup>[1]</sup>.

Many compounds such as hormones, vitamins and mineral salts were added to the fish food to increase metabolic functions and biochemical reactions in fish bodies<sup>[2]</sup>. Vitamins are important nutrients in the supplementary diets of most fish species<sup>[3]</sup>. The absence or relative deficiency of the vitamins in the diets leads to decrease fish appetities, metabolic activities and consequently to the diseases<sup>[4]</sup>.

Vitamin C (L. Ascorbic acid) has a highly efficient role in growth rate and reproduction of different fish species<sup>[5]</sup>. The fish depend upon an exogenous source of vitamin C, as they can not synthesize it and acts as cofactor for many enzymes in animal bodies and is important for formation of bones, cartilage and blood capillaries.

The National Research Council NRC<sup>[6]</sup> indicated that, vitamin C requirement for Tilapia species is 50 mg/kg diet. Whereas,<sup>[4]</sup> reported that, increasing the level of vitamin C to 50 mg/kg diet for *O. niloticus* gives the best growth and feed utilization.

Cobalt salts in the form of cobalt chloride and cobalt nitrate have an important role in fish culture systems for improvement of fish production<sup>[7]</sup> noticed that, the survival rate and growth rate of fish have been increased with cobalt chloride than cobalt nitrate and without cobalt salts. The authors added that, the

addition of cobalt chloride to tilapia diets leads to better growth and optimum feed utilization.

The selection of the Nile tilapia, *O. niloticus* for the present study is due to its important role in the Egyptian fisheries. These fish species were also ideally suitable for different culture systems because they are disease resistant, reproduce easily and can tolerate unfavorable conditions of water<sup>[8]</sup>.

The present study aimed to investigate the effect of vitamin C (ascorbic acid) and cobalt chloride ( $\text{CoCl}_2$ ) on the growth rate and histological structure of Nile tilapia (*O. niloticus*), Also, to illustrate the best treatment that gives the maximum growth rate and normal histological structure.

### MATERIALS AND METHODS

The present study was carried out on hundred and twenty fish of the Nile tilapia (*Oreochromis niloticus*) collected from the River Nile at El-Kanater El-Khayria. Their average lengths were 5.3-6.0 cm and their average weights were 15.5 to 18.5 gm. The fish were divided into three groups (40/ each). Each group was kept in fiber glass rectangular pond filled aerated water. The ponds had an area 370 x 130 x 50 cm<sup>3</sup> and were cleaned daily from faeces and uneaten food by sucking through rubber pipes.

**Basal Diet:** The reared fishes were fed with artificial diet formed from fish meal, soya bean, rice bran, wheat bran, corn yellow and oil, Table (1).

The chemical compounds were added to the basal diet as follows:

**Table 1:** The composition analysis of 10 kg of basal diet (30% protein)

Ingredients	Total protein (%)	Weight (g)	Protein (%)	10 kg of diet
Fish meal	60	20.0	12.0	2.000 kg
Soya bean	48	24.0	11.5	2.400 kg
Rice bran	17	16.0	2.7	1.600 kg
Wheat bran	12	17.0	2.0	1.700 kg
Corn yellow	8.8	20.0	1.8	2.000 kg
Oil	-	3.0	-	0.300 kg
Total	-	100.0	30%	10.000 kg

- Vitamin C: added as 50 mg/kg of basal diet<sup>[4]</sup>.
- Cobalt chloride: added as 0.01 mg to each fish/day<sup>[7]</sup>.

The feeding rate was 3% of body weight for fish of each pond. Pond I: free basal diet (untreated group). Pond II: basal with CoCl<sub>2</sub>.

**Growth in Length and Weight:** A random sample of 10 fish from each pond was taken every month and the mean of total length and weight were recorded for each pond.

Growth rate in length = final in length – initial in length

Growth rate in weight = final in weight – initial in weight

Final length = length at the end of every month.

Initial length = length at zero time.

Final weight = weight at the end of every month

Initial weight = weight at zero time.

**Histopathological Studies:** A randomly 5 specimens from each group were dissected then the kidney and intestine were carefully removed and fixed in 10 % formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into five micrometers thick, and then a half of these sections were stained with hematoxylin and eosin method according to<sup>[9]</sup>. Then sections were examined by light microscopy and photographed by using a microscopic camera (Model No. Ts-242).

## RESULTS AND DISCUSSION

**Kidney:** Normal kidneys of fish (*O. niloticus*) are composed of identical nephrons. Each nephron contains renal corpuscle that leads to a renal tubule. The renal corpuscle contains vascular capillary glomerulus that is enclosed by Bowman's corpuscle. Within the nephron, the kidney contains hematopoietic tissue and blood vessels. The renal tubules have various segmentation and accordingly appear in different shapes.

Some changes in kidney tissue have been observed

in different experimental ponds. At the pond I (untreated group) the kidney revealed degeneration and necrotic change in the renal tubule, malpighian corpuscles and hematopoietic tissue (Figs.1-3). Besides, hemolysis and hemosidrene were seen in the hematopoietic tissue (Figs.4-6).

The kidney of *O. niloticus* rearing in pond II; Vitamin C showed degeneration in some renal tubules and malpighian corpuscles (Fig.8). In addition, slightly degeneration in hematopoietic tissues appears in (Fig.9). However, some renal tubules and malpighian corpuscles were seen in normal shape (Fig.7). In pond III (CoCl<sub>2</sub>), the kidney tissue appeared normal the renal tubule (Figs.10-12).

**Intestine:** The normal intestine of fish (*O. niloticus*) consists of four layers: an outer serosa, muscularis, submucosa and mucosa. The mucosa epithelium consisted of columnar epithelium lined with vascular propria and mucous. Secreting goblet cells thrown into the villi.

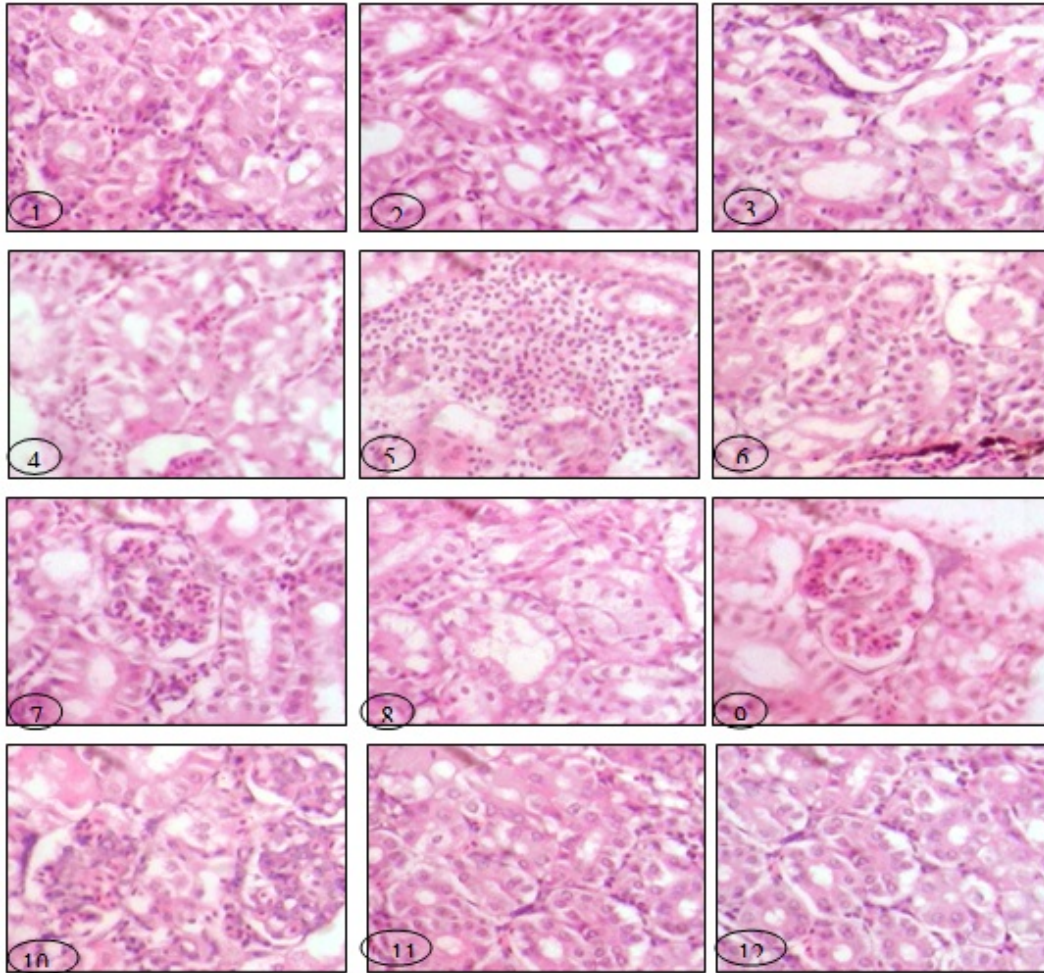
In the present study, histopathological changes in the intestine of *O. niloticus* at pond I (untreated group) included degeneration and necrosis of mucosa and submucosa and destruction of muscularis. Reduction in some villi (Figs.13-17).

In pond II (vitamin C) much degeneration were observed in serosa and submucosa, but muscularis and mucosa appeared healthy (Figs.18-21).

In pond III (CoCl<sub>2</sub>) four layers structures appeared generally in a good healthy (Figs.22-24).

**Growth Rates:** The total length and weight of *O. niloticus* reared in the experimental ponds in shown in Table (2). The study found that, fish reared at ponds II (vitamin C) and III (CoCl<sub>2</sub>) were higher in length and weight than pond I (untreated group). Also, fish reared at pond III (CoCl<sub>2</sub>) showed increase in weight than the other ponds.

**Discussion:** The present study was undertaken to investigate growth rate and histological structures on *O. niloticus* fish under the effect of vitamin C and cobalt

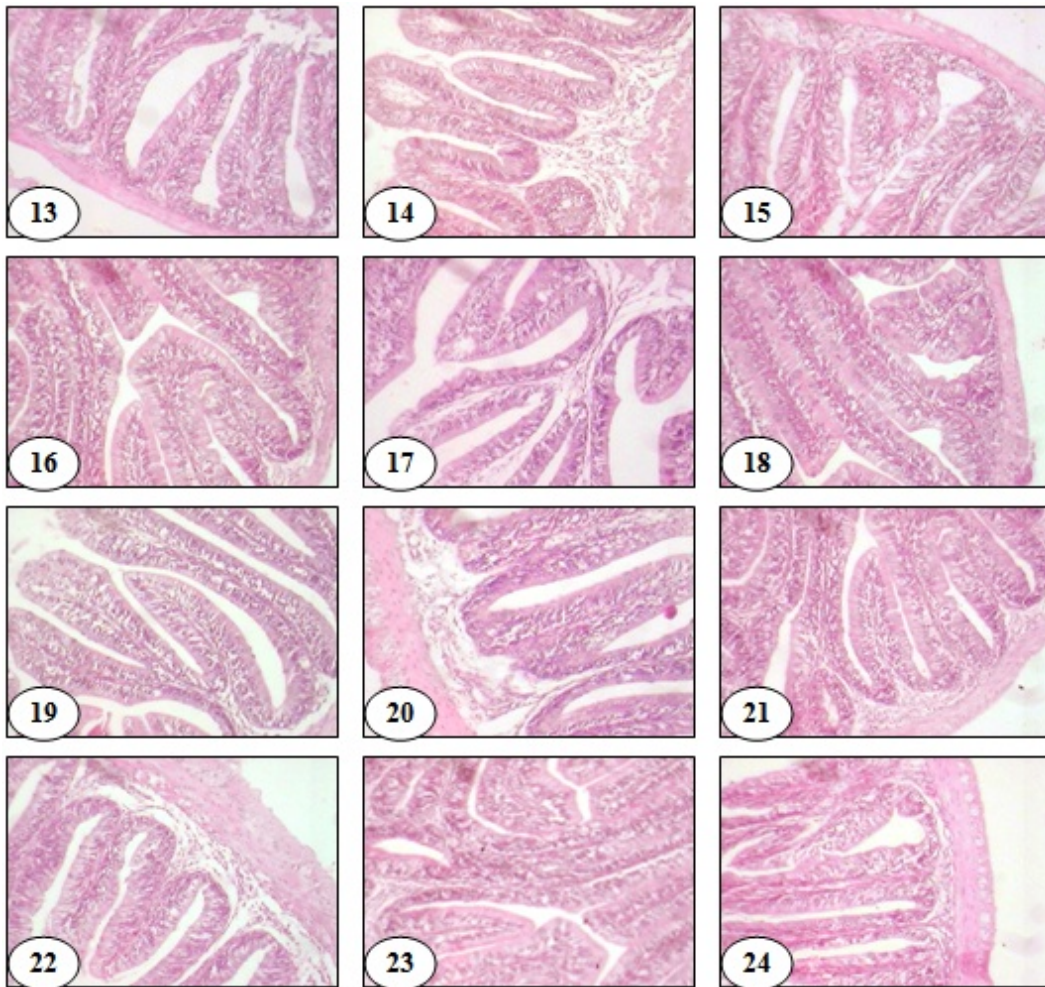


Kidney of *O. niloticus* showing: degeneration and necrotic changes in the renal tubules and glomerulus at pond I (Figs.1-3)[x400] with hemorrhages ,hemolysis and hemosiderin at the same pond (Figs.4-6) [x400]; degeneration in some renal tubules ,malpighian corpuscles and hematopoietic tissues at pond II (Figs.7-9) [x400] ; at pond III, the kidney tissue appeared normal in the renal tubule (Figs.10-12 ) [x400].

**Table 2:** Growth in length and weight of *O. niloticus* fed on diets containing Vit. C and CoCl<sub>2</sub> during 5 months (mean ± SD)

	I		II		III	
	L Mean ± SD	W Mean ± SD	L Mean ± SD	W Mean ± SD	L Mean ± SD	W Mean ± SD
1 Month	0.02±0.06	8.87±3.75	0.68±0.86	12.39±2.31	1.54±0.98	14.56±4.25
2 Month	0.90±0.87	18.23±8.89	2.47±1.02	19.54±5.32	1.30±0.78	27.21±2.87
3 Month	2.50±0.83	23.86±0.71	1.83±0.99	28.37±0.99	3.21±0.72	35.18±8.76
4 Month	2.71±0.73	32.62±7.43	3.07±0.89	36.13±8.68	5.17±0.96	39.68±5.07
5 Month	2.57±1.61	32.66±23.74	4.55±1.97	34.95±9.40	1.92±1.04	37.62±8.30

L : Growth rate in length = final in length – initial in length  
W : Growth rate in weight = final in weight – initial in length  
I : pond I (untreated group).  
II : pond II (vitamin C).  
III : pond III (CoCl<sub>2</sub>).



Intestin of *O. niloticus* showing: degeneration and necrosis of mucosa and submucosa , destruction of muscularis and reduction in some villii at pond I (Figs.13-17 ) [X400]; degeneration in serosa and submucosa at pond II (Figs.18-21 ) [X400]; four layers structures appeared generally in a good healthy at pond III (Figs.13-17 ) [X400];

chloride salt ( $\text{CoCl}_2$ ). At pond II, the addition of vitamin C to the supplementary diets of reared fish Nile (*O. niloticus*) was increase in growth in weight. The same observation recorded by NRC<sup>[6]</sup>, they reported that, the optimal level of vitamin C in diets of reared tilapia species in 50 mg/kg diet. Moreover,<sup>[10-11]</sup> mentioned that, the absence of vitamin C from fish diets leads to the reduction of growth rates.

The growth rate of *O. niloticus* in this study was improved by adding  $\text{CoCl}_2$  to supplementary diets.<sup>[7]</sup> Moreover, the growth rate of *O. niloticus* was increased when the fish were fed on diets containing small doses (0.01 mg/day/fish) of cobalt salt.

<sup>[12]</sup> measured the effect of  $\text{CoCl}_2$  on the cardioventlatory physiology of catfish (*Ictalurus punctatus*), they revealed that, cobalt salts are used in fish culture to stimulate oxygen chemoreceptor and

hypoxic reflexes.

In the present study, many samples of fish kidney and intestine from pond I (untreated group) showed some lesions may be attributed to the nature of the Nile water (that receive different pollutants) from which the samples were transferred to the experimental ponds. During 5 months, of the experimental period the samples from pond II (vitamin C) and pond III ( $\text{CoCl}_2$ ) showed normal structure and fish are healthy.

Similar histopathological lesions were observed in kidney of fish (*Clarias gariepinus*) collected from different areas along River Nile<sup>[13,14]</sup>.

<sup>[15]</sup> studied the effect of River Nile water on kidney of *Tilapia zillii* and *Clarias gariepinus*. They found that, the kidney tissues included degeneration and necrosis of renal tubules and distortion of glomerular capillaries. <sup>[16]</sup> found injuries in kidney tissue of *Liza*

*ramada* fish obtained from water polluted with industrial and agricultural wastes in lake Manzalah. The kidney injuries included degeneration and necrosis of renal tubules and destruction in Malpighian corpuscles and hematopoietic tissue. The lesions in hemopoietic tissue suggest that both the osmotic and ionic regulations are impaired upon exposure to different toxicants<sup>[17]</sup>.

The observed lesions were agreement in the intestine under different exposure conditions, in *Tilapia zillii* after exposure to phenol<sup>[18]</sup>, in *O. niloticus* after exposure to insecticides Reldan and Lunate<sup>[19]</sup>, in *Tilapia* fish after exposure to carbofuran<sup>[20]</sup>.

Majmuder, and Burseson<sup>[21]</sup> found several lesions in the intestine of *O. niloticus* which caught from fish farm at El-Fayoum province. These lesions were degeneration and necrosis of epithelial cells of mucosa, aggregation of inflammatory cells in submucosa and destruction of muscularis. According to Bhatnagar *et al.*<sup>[22]</sup>, the observed irritation and destruction of mucosa membrane of the intestine. The pathological alterations in the intestine of the fish collected from pond I are in agreement with those observed by many investigators about the effects of different toxicants on fish intestine<sup>[23,24]</sup>.<sup>[25]</sup> found several lesions in the villar region after two-week feeding on dietary cadmium. As light vacuolation was observed in the submucosa layer.<sup>[26]</sup>, the study was conducted to investigate the histological structures of the kidney and intestine of *Tilapia zillii* and *Solea vulgaris* obtained from Lake Qarun. He showed some lesions in the kidney vacuolar degeneration in the epithelium of renal tubules, focal areas of necrosis, hemorrhages and hemosidrine between the renal tubules and edema in Bowman's capsules with atrophy in the glomeruli. In the intestine, degenerative and necrotic changes in submucosa and mucosa with edema between them, dilation in blood vessels of serosa and atrophy in the muscularis and submucosa are noticed. Also,<sup>[27]</sup> observed necrosis of tubular epithelium, hypertrophied epithelial cells of renal tubules and construction of the glomerulus in the kidney of *C. mrigala* exposed to Fenvalerate, While<sup>[28]</sup> found cloudy swelling degeneration in the epithelium of renal tubules in the kidney of *P. limeatus* cages in cambe stream, polluted by industrial, domestic and agricultural wastes.

At the end of the experimental period the samples of the pond treated vitamin C and CoCl<sub>2</sub> showed normal kidney and intestine structure. However, the samples of pond I (untreated group) showed some histological alterations.<sup>[29]</sup> reported that, vitamin C acts as a protective agent against DNA damage in normal cells.

I could conclude that, River Nile pollutants induces toxic lesions in the kidney and intestine i.e. alliterating

their fine structure, but used vitamin C or CoCl<sub>2</sub> in fish food lead to treat these lesions. It is recommended that vitamin C or CoCl<sub>2</sub> essential to fish farm to protect the fish organs and human.

## REFERENCES

1. EL-Sayed, A.F.M., 1991. Protein requirements for optimum growth of *Liza ramada* fry (Mugilidae) at different water salinities. Aquatic., Living Resource., 4: 117-123.
2. Dabrowski, K., K. Moreau and D. El-Saidy, 1993. Ontogenetic sensitivity of channel catfish to ascorbic acid deficiency. Journal of Aquatic Animal and Health, 8: 22-27.
3. Dabrowski, K. and J.H. Blom, 1994. Ascorbic acid deposition in rainbow trout (*Oncorhynchus mykiss*) eggs and survival of embryos. Comp. Biochem. Physiol., 108: 129 -135.
4. Abdelghany, A.E. 1998. Feed efficiency, nutrient retention and body composition of Nile tilapia, *Oreochromis niloticus* L., fed diets containing L-ascorbic acid, L-ascorbyl-2-sulphate or L-ascorbyl-2- polyphosphate. Aquaculture. Res., 29: 503-510.
5. Wilson, R.P., 1973. Absence of ascorbic acid synthesis in channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus frucatus*). Comp. Biochem. Physiol., 46: 635-638.
6. National Research Council, NRC, 1991. Nutrition requirements of fishes. National Academy Press, Washington D.C., USA.
7. Hamza, A.K. and S.M. Kenawy, 1997. Effect of growth promoting materials on survival and growth rates of Mullet fish fry *Mugil cephalus* and *Liza ramada*. Bull Faculty of. Sciences., Zagazig University., 19(1): 267-273.
8. Attalla, R.F., 2001. The use of fish meal replaces in fresh water aqua-feeds. Ph. D. Thesis, Faculty of. Sciences., Zagazig University. (Benha Branch).
9. Harris, H.F., 1900. Journal of Applied Microbiology, 3: 777.
10. Fracalossi, M.D., E.M. Allen, K.L. Yuyama and T.O. Oftedal, 2001. Ascorbic biosynthesis in Amazonian fishes.
11. EL-Hammady, A.K.I. and S.M.M. EL-Sayed, 2002. Influence of dietary supplementation of vitamin C to practical diets on growth and some blood parameters of hybrid tilapia (*Oreochromis niloticus* X *Oreochromis aureus*). Journal of Egyptian Academic Society for Environmental Development., 2(1): 29-50.
12. Majmuder, K. and M.I. Burseson, 2006. An evaluation of cobalt chloride as an O<sub>2</sub>-sensitive chemoreceptor stimulant in channel catfish. Compar. Biochem. and Physiol., 142(C): 136-141.

13. Yacoub, A.M., 1999. Effect of pollution in different localities of River Nile on *Clarias lazera* ph.D .Thesis .Faculty of. Sciences Ain Shams University., Egypt.
14. Yacoub, A.M. 2003. Study of histopathological changes in the gills of *Oreochromis niloticus* from lake Manzala due to water pollution .Egyptian Journal of Aquatic Biology and fisheries, 7(2): 71-86.
15. Mahmoud, S.A., S.I. Tayel, A.M. Yacoub, 2008. Histopath-ological changes in kidneys of the fish *Tilapia zillii* and *Clarias gariepinus* under the effect of several pollutants along the River Nile,Journal of the Egyptian German Society of Zoology, 56(C): 219-246.
16. Kadry, Sh. M., A.M. Yacoub, T.A. Siliem, D.B. Mabrouk, 2003. Injuries in kidney tissue of the Mugil fish *Liza ramada* as biomarker for environmental pollution in the lake Manzala. .Egyptian Journal of Aquatic Biology and fisheries, 7(3): 1-24.
17. Jarup, L.B. Persson, C.E. Dling and C.G. Elinder, 1993. Renal function impairments in workers previously exposed to cadmium .Nephron, 64:75-81.
18. Mohamed, F.A.S., 2001.Effects of phenol on the histological structures of the intestine and gonads of the fresh water teleost *Tilapia zillii* (Gervais,1848). Egyptian Journal of Aquatic Biology and fisheries, 5(1): 195-223.
19. Mohamed, F.A.S., 2004. Histological alterations in the intestine and gonads of *Oreochromis niloticus* induced by Reldan (organ phosphorus insecticides) and lannat (carbmate insecticides). Journal of Egyptian Academic Society of Environmental Development, (D- Envi-ronmental Studies), 5(1): 59-88.
20. Soufy, H.M., Soliman, E. El-Manakhy and A. Gaafa, 2007. Some biochemical and pathological investigations on monosex *Tilapia* following chromic exposure to carbofuran pesticides, Global Veterinaria, 1: 45-52.
21. Yacoub, A.M ., S.A. Mahmoud, S.I. Tayel, 2008. Health status of *Oreochromis niloticus* in fish farm irrigated with drainage water in El fayoum province, Egypt Egyptian Journal of Aquatic Research, 34(1): 161-175.
22. Bhatnagar,C.M. Bhatnagar and B. Regar, 2007. Fluoride induced histopathological changes in gill, Kidney and intestine of freshwater teleost, labeorohita .Res.Rep.Fluoride, 40: 561.
23. Hanna, M., I. Shaheed and N. Elias, 2005. Acontribution on chromium and lead toxicity in cultured *Oreochromis niloticus*. Egypt. Egyptian Journal of Aquatic Biology and fisheries, 9: 177-209.
24. Cengiz, E. and E. Unlu, 2006. Sublethal effects of commercial on the structure of gill, liver and gut of mosquito fish, *Gambusia affines*: A microscopic study .Environmental. Toxicology. Phamacology, 21: 246-253.
25. Kruatrachue, M., N. Rangasayatorn, P. Pokethitiyook, E.S. Upatham,and S. Singhakaew, 2003. Histopathological changes in the gastrointestinal tract of fish ,*Puntius gonionotus* fed on dietary cadmium .Bulletin of Environmental Contamination and Toxicology,71: 561-56.
26. Mohamed, F.A.S., 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from lake Qarun Egypt. World Journal of Fish and Marine Sciences, 1(1): 29-39.
27. Velmurugan, B., M. Selvanayagam, E. Cengiz and E. Unlu, 2007. The effects of fenvalerate on different tissues of fresh water fish *Cirrhinus mrigala* .Journal of Environmental Sciences and Health (B), 42: 157-163.
28. Camargo, M.M. and C.B. Martinez, 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop.Ichthyol., 5: 327-336.
29. Blasiak, J., E. Gloc, K. Wozniak and I. Majsterek, 2002. Genotoxicity of indarubicin and its modulation by vitamins C and E and amifostine. Chemical and Biological Interact., 140(1): 1-18.