Impact of saturn herbicide on histological structures and residual aspects of Nile tilapia (*Oreochromis niloticus*)

By

*World Fish Center, Egypt.
**Central Laboratory for Aquaculture Research, Agricultural Research Center, Egypt.

SUMMARY

This investigation was performed to demonstrate the effect of Saturn herbicide on Nile tilapia (*Oreochromis niloticus*). Experimental studies were done to determine of LC$_{50}$ of Saturn and evaluate its acute and chronic toxicity (1/2 LC$_{50}$ for 7 days and 1/10 LC$_{50}$ for 60 days). The pathological changes were recorded. The residual contents of the fish tissues (the gills, brain, skin, muscles and ovaries) were estimated. The clearance test was studied by transferring some of chronic Saturn-exposed fish in clear water for 21 days.

From the obtained results LC$_{50}$ after 96 hrs. for Saturn was 8.2 mg/L. Circulatory and degenerative as well as necrotic changes were seen together with tissues residues in the gills, brain, skin, muscles and ovaries. These residues were decreased and some of the histological alterations observed were completely restored. The brain revealed perivascular melanomacrophages proliferation after the recovery period (21 days).

We can conclude that the investigated Saturn herbicide induced a negative impact on the quality of Nile tilapia as manifested by the marked changes in fish morphology, tissue structures and residues.

INTRODUCTION

Fish and fish products are considered as the cheapest important feedstuffs of high protein quality, minerals and vitamins contents. The method of culturing fish in rice fields provides an additional income as well as food for farmers and reduces the risk of rice crop failure (*Huat and Tan, 1980*). Nile tilapia (*Oreochromis niloticus*) is a common popular fish cultured sometimes in rice fields in which herbicides were used for controlling the undesirable weeds and grasses so fish may be exposed to several chemical toxins. Herbicides, including Saturn (S-4-Chlorobenzyl)-N,N-diethyl carbamothioate, are widely used in agricultural fields. They reach the neighbouring water bodies with runoff water and pollute the aquatic environment (*Pluta, 1989*). They cause toxic effects to fish. The suspected accumulation of herbicides residues in the fish flesh
has a public health hazards for the human consumers (Mason, 1991).

Fish exposed to herbicides showed skin darkening with focal sloughing surrounded by inflammatory area and excessive mucus secretion all over the body. Flabby and watery muscles were also observed (Nouh, 2003 and Marzouk et al., 2006). Acute and chronic exposure to Saturn produces microscopical changes in the gills, brain, skin, muscles and ovaries with residues in the tissues of exposed fish (Abd El-Azzim, 2001 and Gafar, 2006).

Altinok and Capkin (2007) demonstrate the pathological changes induced by exposure of fish to sublethal concentrations of methiocarb (carbamate herbicide). Histological recovery was noticed in some organs following clearance period.

Very little data on Nile tilapia toxicity from exposure to sutern are available. So, this study was undertaken to investigate the pathological changes as well as the retained residues of Saturn herbicide in some organs of Nile tilapia (Oreochromis niloticus) following acute, chronic and clearance periods of exposure.

MATERIALS AND METHODS

Fish: A total number of 270 apparently healthy sexually mature Nile tilapia (Oreochromis niloticus) of both sexes were used in this study, with an average body weight of 90 ± 10 gm. Fish were transferred alive from the ponds of Central Laboratory for Aquaculture Research to laboratory. Fish were held in full glass aquaria containing dechlorinated tap water and acclimatized for two weeks before beginning of the experiment.

The fish were kept in 9 glass aquaria 180 liters with continuous aeration. The physical parameters of water were 7.9 ± 0.6 mg/L dissolved oxygen, 7.8 ± 0.3 pH and 23 ± 2°C water temperature. The fish were fed on a commercial fish food at a level of 3% of their body weights according to Eurell et al. (1978).

Saturn herbicide: A carbamates herbicides available in the market under the commercial name Saturn was used in this study. The chemical name is (S-4-chlorobenzyl)-N,N-diethyl carbamothioate. This herbicide is a compound of Kumiai Chemical Industry Co., LTD. Japan and supplied by Egyptian Kafrel-Zayat pesticide and chemicals.

Experimental design:

1. Determination of half lethal concentration dose: It was determined according to Behrens and Karbar (1953) using 90 Nile tilapia divided into 9 equal groups.
2. **Acute toxicity** was performed to study the effect of exposing the tested fish to high concentration of Saturn for 7 days. Nile tilapia stocked in two aquaria were exposed to the 1/2 LC\textsubscript{50} concentration. A third aquarium was used as non-exposed control group (each aquarium contains 30 fish).

3. **Chronic toxicity** was performed to study the effect of exposing Nile tilapia to low concentration of Saturn for 60 days. Two aquaria each of 30 fish were exposed to 1/10 LC\textsubscript{50} concentration of Saturn for 60 days. A third aquarium containing 30 fish was used as a control group. Siphoning of 95% of water from the aquarium and replacing by an equal volume of water containing the same concentration of the toxicant was performed according to Sprague (1973).

4. **Clearance test** was studied by maintaining the chronic Saturn-exposed fish in clear, Saturn-free water for an additional 21 days to check for recovery response and clearance of Saturn residues.

5. **Fish sampling**: After 7, 30 and 60 days from exposure and following clearance test 7 fish samples were collected from each group for investigation of histological changes and determination of tissues residues. Where specimens from the gills, brain, skin, muscles and ovaries were kept frozen at -20°C for estimating the herbicide residual content.

6. **Histological examination**: Tissue specimens from the gills, brain, skin, muscles and ovaries of experimented fish were fixed in 10% phosphate buffer formalin. Five micron thick paraffin sections were prepared, stained with hemotoxylin and eosin, (H&E) (Bancroft et al., 1996) and examined under light microscope.

7. **Residual analysis**: The levels of Saturn residues in the examined samples from the gills, brain, skin, muscles and ovaries were determined according to AOAC (2002) using Thin Layer Chromatography (TLC) plates coated with Silica gel.

8. **Statistical analysis**: Three replications of each trial were performed for Saturn residues in the gills, brain, skin, muscles and ovaries. Data were analyzed using analysis of variance (ANOVA) and means were separated by Duncan at a probability level of < 0.05 (SAS, 2000).

**RESULTS AND DISCUSSION**

1- **The half lethal concentration dose (LC\textsubscript{50})** for 96 hrs for Nile tilapia are illustrated in table (1).
Table (1) Determination of 96 hrs. half lethal concentration dose (LC$_{50}$/ 96 hrs.) of Saturn in Nile tilapia.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of fish/gp.</th>
<th>Conc. mg/L</th>
<th>No. of mortality</th>
<th>a</th>
<th>b</th>
<th>Σ (axb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>4</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>1.5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>13</td>
<td>5</td>
<td>4.5</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>17</td>
<td>6</td>
<td>5.5</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>21</td>
<td>9</td>
<td>7.5</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>9.5</td>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>29</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

a = Mean of dead fish between 2 successive concentration.
b = Dose difference between 2 successive concentration.
Σ (a x b)= Summation of (a x b).
N = No. of fish/ group.

LC$_{50}$ = Lowest concentration caused 100% mortality dose - $\frac{\sum(axb)}{N}$

LC$_{50}$ = 25 - $\frac{168}{10}$ = 25 – 16.8 = 8.2 mg/L

$\frac{1}{2}$ LC$_{50}$ = 4.1 mg/L

$\frac{1}{10}$ LC$_{50}$ = 0.82 mg/L
From the collected results, it is evident that the half lethal concentration dose (LC\textsubscript{50}) after 96 hrs. for Saturn on Nile tilapia was 8.2 mg/L. This result was compared with that mentioned by Abd El-Azzim (2001) who reported that LC\textsubscript{50} of Saturn to common carp was 9.4 mg/L for 48 hours. Gafar (2006) demonstrated that LC\textsubscript{50} of Saturn to the Nile catfish for 96 hrs was 4.25 mg/L. These results may be attributed to the difference in the dose and period of exposure of herbicide used, fish species, sex differences, body weight and age of fish as well as environmental condition as represented by Zbinden and Flury-Roversi (1981).

2- Acute toxicity:

**Morphological changes:** Fish in the control group (non treated) revealed normal morphology (Fig.1). While the acute exposed fish showed, excessive mucus covering the skin and gills. Loss of scales as well as focal petechial hemorrhage with erosion of fins and tail were observed after 7 days post exposure (PE) (Fig.2). Congestion of gills and brain as well as hemorrhages in the ovaries were observed. These findings were similar to those mentioned by Abd El-Azzim (2001).

**Microscopically:**

The gills showed congestion, mononuclear cells infiltration in the gill arch and lamellae with hyperplasia in the secondary lamellae along the 7 days PE. Edema in primary lamellae and desquamation and/or fusion in secondary lamellae were also observed (Fig., 3). These results might be attributed to the direct contact with Saturn in water and response to hypoxic conditions. These results were in agreement with Abd El-Azzim (2001).

**The brain** revealed severe congestion in the meningeal blood vessels with perivascular edema along 7 days PE. Encephalomalacia, focal gliosis, neuronophagia and neuronal degeneration were observed after 7 days PE (Fig., 4). These findings were attributed to Saturn bounded with acetylcholinesterase resulted in acetylcholine accumulation as well as hypoxemia. These results were reported by Mahmoud (1997) and Marzouk et al., (2006).

In the skin and the underlying musculature along 7 days PE, the epidermis showed hyperplasia with desquamation of epithelium, proliferation of melanomacrophages and mucous cells as well as dermal edema (Fig., 5). The muscles suffered hyaline degeneration and Zenker’s necrosis. Severe edema, mononuclear leukocytic cells and few melanomacrophages infiltration were observed among the muscle bundles (Fig., 6). The lesions may be attributed to the direct contact to Saturn in water, an osmoregulatory
dysfunction and hypoxemia with increased muscle lactate levels induced the above mentioned lesions Crestani et al. (2006). The present findings are in agreements Gafar (2006) for muscles of the Nile catfish exposed to 0.425 mg/L Saturn for 96 hours.

The ovaries at 7 days PE revealed congestion of blood vessels, few leukocytic cells infiltration, edema and hemorrhages in the stroma. Some ovarian follicles were atretic as well as losing the typical round configuration and degeneration of follicles were observed (Fig., 7). These results might be attributed to influences of stress, sex hormone disturbance and alterations in protein and carbohydrate metabolism Begum (2004). These findings were in partial agreement with Hazarika and Das (1998) and Dutta and Maxwell, (2003).

3- Chronic toxicity:

Morphological changes: The mucus covered the gills, darkening of skin with erosion of fins and tail compared to control from 30 days PE till the end of experiments were observed (Fig., 8). Similar morphological changes were mentioned by Nouh (2003).

Microscopically:

The gills showed mild congestion and numerous mononuclear cells infiltration in the gill lamellae along the 7 days PE. After 30 days PE till the end of the experiment hyperplasia in the secondary lamellae was seen (Fig., 9). Some cases revealed desquamation of epithelial cells in the secondary lamellae after 60 days. These results were in agreement with Jiraungkoorskul et al. (2003).

The brain on 7th days PE revealed mild congestion. After 30 days PE perivascular edema in cerebral and meningeal blood vessels, lymphocytic infiltrations and focal gliosis were seen (Fig., 10). Focal malacia and neuronal degeneration were also seen at the end of 60 days. Similar lesions were mentioned by Abd El-Azzim (2001) in common carp exposed to 0.9 ppm for 6 weeks Saturn and those by Altinok and Capkin (2007) in rainbow trout (Oncorhynchus mykiss) after exposure to (2.5 and 3.75 mg/L) methiocarb for 21 days.

The skin and the underlying musculature at 7th days PE revealed mild sub-epidermal edema. From 30 days PE till the end of experiment the skin showed moderate activation of the mucous and alarm cells with vacuolar degeneration of epithelial cells (epidermal spongiosis) (Fig., 11). Melanomacrophages and mononuclear leukocytic cells infiltration mainly lymphocytes were seen in the dermis. The unde-
rlying muscles exhibited focal hyaline degeneration and Zenker’s necrosis with severe edema among the muscle bundles. The connective tissue appeared wider (Fig., 12). These findings may be attributed to Saturn affect on mitochondria lead to disturbance in ionic concentrate-on in the cells followed by accumulation of water inside the cells which induced vacuolar degeneration in epithelial cells. Moreover the long-term exposure affected on enzymes activity and oxidative stress in fish tissues Moraes et al. (2007) and Crestani et al. (2007). These formentioned lesions were recorded and in agreement with Gafar (2006) in Nile catfish exposed to 0.2 mg/L. for 90 days sumithion.

The ovaries after 7 days PE showed congestion, leukocytic infiltration mainly lymphocytes. From 30 days PE till the end of experiment edema in the ovarian stroma, atretic oocytes and degeneration and/or necrosis of some follicles were observed (Fig., 13). Mild hemorrhage and decrease in different stages of oocytes in the stroma were seen. These findings may be due to impairment of steroid sex hormone secretion and interference with mitotic activities of follicles and direct inhibition of follicular growth. The present results are in agreement with Tripathy and Verma (1993) in Tilapia mossambica fish exposed to 1 ppm Sumicidin for 60 days.

5- Residual analysis of experimented Nile tilapia (the gills, brain, skin, muscles and ovaries):

Our data of residual analysis Table (2) showed that Saturn has the greatest cumulative character in examined organs following acute exposure. The limit of residues depending on the concentration of herbicide in water, the fat content of each organ, dose, period of exposure and the degree of damage in tissues caused by the direct toxic effect. In chronic exposure this cumulative effect showed gradual increase of the residues from 7 to 60 days PE. These results may be attributed to the ability of fish to eliminate Saturn residues from their tissues by an enzyme-related detoxification in liver. The tissue structural damages caused by long period of exposure lead to accumulation of Saturn in the gills, brain, skin, muscles and ovaries (Abd El-Azzim, 2001 and Marzouk et al., 2006).
Table (2): Residual analysis of Saturn herbicide in the gills, brain, skin, muscles and ovaries of Nile tilapia.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>Acute</th>
<th>Chronic</th>
<th>Clearance test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 days</td>
<td>7 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Gills</td>
<td>ND</td>
<td>0.331 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.075 ± 0.003&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.121 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain</td>
<td>ND</td>
<td>0.083 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.019 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.030 ± 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skin</td>
<td>ND</td>
<td>0.189 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.043 ± 0.003&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.069 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscles</td>
<td>ND</td>
<td>0.117 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.027 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.043 ± 0.005&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ovaries</td>
<td>ND</td>
<td>0.053 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.012 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.019 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SE.

<sup>a-c</sup> Means within a row with the different superscript are significantly different (P < 0.05).

ND = Not Detectable.

6-Recovery response:

Saturn residues showed significant decrease in fish maintained in Saturn free water in comparison with both acute and chronic treated groups. Table (2). The present results are in agreements with Marzouk et al. (2006).

Meanwhile, some histological lesions in the examined organs after clearance exhibited significant improvement in comparison with those of acute and chronic treatment (Table, 3 and Figs., 14, 15, 16 & 17). Except the brain revealed, the perivascular melanomacrophages proliferation which could play a role in the clearance of tissues from this compound (Fig., 18).

These findings may be attributed to that most histological alterations observed during chronic exposure were reversible changes. A rapid elimination process by detoxification as well as acetylcholinesterase activity restored gradually when fish transferred to clean water Chandrasekara and Pathiratne (2007). These results were in agreement with (Begum, 2004; Altinok and Capkin, 2007 and Crestani et al., 2007).
**Table (3):** Saturn herbicide induced histopathological changes in Nile tilapia.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Lesions</th>
<th>Acute 7 days</th>
<th>Chronic 7 days</th>
<th>Chronic 30 days</th>
<th>Chronic 60 days</th>
<th>Clearance test 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>proliferative changes</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>desquamation</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mononuclear cells</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>infiltration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Malacia</td>
<td>++ +</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Neuronal degeneration</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Gliosis</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Skin</td>
<td>Mucous cells</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Melanomacrophages cells</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hyperplasia</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Epidermal edema</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Muscles</td>
<td>Zenker’s necrosis</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hyaline degeneration</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Ovaries</td>
<td>Atretic oocytes</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ovarian hemorrhage</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Oocytes degeneration</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Absent  
+ = Mild  
+++ = Moderate  
++++ = Sever

**Conclusion:** The investigated herbicide Saturn induced a negative impact on the quality and the reproductive activity of Nile tilapia even in low concentrations especially upon long term exposure. We recommend transferring rice field breeding fish to clean Saturn free water for at least 21 days before marketing to avoid the suspected hazards on public health. The consumers has to be advised must reject the head of fish reared in rice field before cooking them as it is the main target site for accumulation of the toxic herbicide.
<table>
<thead>
<tr>
<th>Fig. (1): Nile tilapia (control) exposed to Saturn free water showing normal morphology.</th>
<th>Fig. (2): Nile tilapia, exposed to 1/2 LC$_{50}$ Saturn for 7 days, showing scales fall, petechial hemorrhage with erosion of fins with tail.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. (3): Gill of Nile tilapia exposed to 1/2 LC$_{50}$ Saturn for 7 days, showing congestion, hyperplasia with fusion in secondary lamellae (H.&amp;E., X 300).</td>
<td>Fig. (4): Brain of Nile tilapia exposed to 1/2 LC$_{50}$ Saturn for 7 days, showing satellitosis, encephalomalacia and gliosis (H.&amp;E., X 250).</td>
</tr>
</tbody>
</table>
Fig. (5): Skin of Nile tilapia exposed to 1/2 LC50 Saturn for 7 days, showing hyperplasia with desquamation of epidermis, subepidermal melanomacrophages and dermal edema (H.&E., X 250).

Fig. (6): Muscles of Nile tilapia exposed to 1/2 LC50 Saturn for 7 days, showing Zenker’s necrosis, hyaline degeneration, severe edema, and leukocytic cells infiltrations (H. & E., X 250).

Fig. (7): Ovary of Nile tilapia exposed to 1/2 LC50 Saturn for 7 days, showing edema with losing the typical round configuration and degeneration of ovarian follicles. (H. & E., X 250).

Fig. (8): Nile tilapia, exposed to 1/10 LC50 Saturn after 60 days, showing erosion of tail with darkening skin discoloration.
| Fig. (9): Gills of Nile tilapia exposed to 1/10 LC₅₀ Saturn after 10 days, showing congestion and hyperplasia of some secondary lamellae (H. & E., X 100). |
| Fig. (10): Brain of Nile tilapia exposed to 1/10 LC₅₀ Saturn after 30 days, showing mild congestion perivascular edema and gliosis (H. & E., X 250). |
| Fig. (11): Skin of Nile tilapia exposed to 1/10 LC₅₀ Saturn after 30 days showing epidermal spongiosis and dermal edema (H. & E., X 300). |
| Fig. (12): Muscles of Nile tilapia exposed to 1/10 LC₅₀ Saturn after 60 days, showing moderate edema, hyaline degeneration and focal Zenker’s necrosis (H.&E., X 250). |
Fig. (13): Ovary of Nile tilapia exposed to 1/10 LC₅₀ Saturn after 60 day, showing edema leukocytic cells and necrosis in some follicles (H. & E., X 250).

Fig. (14): Gills of Nile tilapia transferred to clear, Saturn - free water for 21 days showing leukocytic infiltration, mild hyperplasia and some secondary lamellae desquamated (H. & E., X 100).

Fig. (15): Skin of Nile tilapia transferred to clear, Saturn - free water for 21 days showing, mild mucous cells activation and hyperplasia of epidermis (H. & E., X 250).

Fig. (16): Muscles of Nile tilapia transferred to clear, Saturn - free water for 21 days showing, focal Zenker’s necrosis (H. & E., X 100).
Fig. (17): Ovary of Nile tilapia transferred to clear, Saturn - free water for 21 days showing, normal developmental stages of oocytes (H. & E., X 300).

Fig. (18): Brain of Nile tilapia transferred to clear, Saturn - free water for 21 days showing, mild congestion and perivascular melanomacrophages cells (H. & E., X 100).

REFERENCES


Jiraungkoorskul, W.; Upatham, E.; Kruatrachue, M.; Sahaphong S.; Vichasri-Grams,


