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# BIOLOGICAL EVALUATION OF RATS FED ON MARYOUT LAKE-FISH *(Oreochromis niloticus)*

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## ABSTRACT

The purpose of this research is to determine the concentration levels of heavy metals, i.e., Cd, Pb, Hg, Cu, Zn and Mn in *(oreochromis niloticus)* of rats fed on these fish at different locations of Maryout-lake. The biological evaluation of rats fed on these fish was also carried out. The obtained results indicated that, Maryout lake-fish and its water contained higher heavy metals especially, Cd, Pb and Hg than the river Nile-fish and its water samples.

There are no significant differences of heavy metals concentration in both rats serum and liver, which fed on control and Nile fish, whereas rats fed on Maryout lake-fish showed significant increase. Biological evaluation indicated that no significant differences were found in both liver and kidney functions of rats, fed on diets contained Nile fish or casein meanwhile, urea and cereatinine were increased in rats fed on Maryout-fish for 60 days.

Both total protein and lipids in serum did not affect in all groups of rats, neither for different sources of fish nor experimental period, but cholesterol was increased. A significant decrease in haemoglobin parameter of rats at the end of feeding period of Maryout lake-fish had been achieved. Growth rate, feed consumption and feed efficiency ratio didn't affect, except for rats fed on diet containing Maryout-fish for 60 days.

## INTRODUCTION

Contamination of food stuff resulting from heavy metals in the environment is one of the most important problems. These heavy metals are toxic to human beings. Many researchers studied the influence of these heavy metals on experimental animals (Gallo *et al.*, 1998; El-Zoghbi and El-Kady 1999 and Hassan *et al.*, 2002 a-b). They reported that heavy metals were bioaccumulated in animal organs with different rates, in addition, they are toxic and harmful for the experimental animals.

In Egypt, several studies on the level of heavy metals in the river Nile and the Egyptian-fish lakes have been carried out (Ibrahim, 1996; Salem, 1997; Daoud *et al* 1999; Ali, 2000 and Rashed, 2001). They found that there are correlations between bioaccumulation of heavy metals in fish flesh and various industrial localities in Egypt. The continuing discharge of many industrial wastes, as well as, agrochemicals in water streams might increase the levels of heavy metals, and subsequently, in surface and ground water (Mohamed, 1998). Copper sulphate is one of the most widely-used algicides for the control of phytoplankton in lakes, ponds and reservoirs (Karan *et al.*, 1998). Moreover, the sewage and domestic-discharge water are the main responsible factors for the pollution of heavy metals (Soltan, 1995). There is

a correlation between accumulation of heavy metals in tissue fishes and their concentrations in water (Daoud *et al.,* 1999; Emam and Hamide, 2001).

The concentration level of heavy metals (Cd, Pb, Cu and Zn) in fish samples and water of "Maryout lake" were affected by variations in the discharge rates of dumped wastes and industrial pollution (Ghazaly, 1992). Fish of Maryout lake contains high concentration of lead and mercury (El-Demerdash and El-Agamy, 1999). While, Abou Donia, (1990) reported that fish samples contained Cu and Zn were in the permissible limits (20 and 50 mg/kg), but Cd, Pb and Hg exceeded the permissible limits (0.5, 2.0 and 0.5 mg/kg).

Accumulation of heavy metals in the fish flesh increased with increasing fish size, weight and age (Allam *et al.,* 1993 and Rashed, 2001). Moreover, seasonal variations affect the distribution of heavy metals concentration in fish and water samples (Gomaa, 1995). Bioaccumulation of heavy metals was the highest in gills followed by liver and the lowest value was found in fish flesh (Aboul Naga and Allam, 1996 and Atta *et al.,* 1997).

Many investigators reported that liver-enzymes activity of rats was decreased due to the presence of heavy metals in their diets (El-Massarawy, 1997; El-Demerdash and El-Agamy, 1999). However, Hassan *et al.,* (2000 a, b) found that cadmium accumulated in rabbit organs was higher in the kidneys than in the liver, Cd moderately affected the growth performances and decreased haemoglobin concentration. Meanwhile, the activity of ALT increased the elevation of cereatinine and urea concentration.

The purpose of this investigation is to determine the concentration levels of heavy metals (Hg, Cd, Pb, Cu, Zn and Mn) in *Tilapia* fish at different locations of Maryout lake. In addition, the biological evaluation of these polluted fish was carried out. Bioaccumulation effect of these heavy metals on the liver and kidney functions of rats was also considered.

## MATERIALS AND METHODS

**Materials:**

Fish *(Oreochromis niloticus)* and water samples were collected from different areas of Maryout lake and the river Nile from El-Wasta locations in July 2002.

**Animals and experimental design:**

Thirty male albino rats, an average weight 72.2 ± 3.1g. were used. Animals were divided into three main groups, each contained 10 rats. Food and water were provided *ad libitum* for 30 and 60 days. Feed intake and body weight were recorded twice weekly.

**Preparation of diets:**

Fish samples used in the diets were dried in an electric oven at 60°C for 36 hours to be semi-dry. Three kinds of diet were prepared. The first one was used as a negative control diets and it consisted of 17.5% casein (as 15.0% protein), corn starch 65.0%, corn oil 10.0%, cellulose 2.5%, salt 4.0%

and vitamin mixtures 1.0%. Salt and vitamin mixtures were prepared according to AOAC (2000). The second and the third diets were designed as the same negative control diet with the addition of 20% semi-dry Nile and /or Maryout-fish samples (as 15.0% protein) instead of casein, respectively.

At the end of experiment, the rats were scarified, blood was collected and placed into a dry-clean glass tube until clotted at room temp., then serum was separated by centrifugation at 3000 r.p.m. for 15 min. Plasma was kept at -18°C until analysis.

**Methods:**

Heavy metals (Cd, Hg, Pb, Cu, Zn and Mn) were determined by using spectrophotometric analysis according to the method described by Anon (1982). Measurements were carried out by Atomic Absorption Spectrophotometer (Perkin Elmer Model 2380). Haemoglobin was determined according to the method adopted by Drabkin and Austin (1985). While hematocrit (Packed cell volume) was estimated as a percentage according to the method of Dacie and Lewis (1984). Total protein and lipids were determined in serum according to the method of Henry (1964) and Knight *et al.* (1972). Urea, cereatinine and cholesterol were determined in the blood serum of animals according to Patton and Crouch, (1977) and Allain *et al.*, (1974), respectively.

Collected plasma samples were analyzed for liver functions, aspartate transaminase enzyme (AST) and alanine aminotransferase (ALT) were estimated according to Reitman and Frankel (1957).

Feed-conversion ratio was calculated according to Yossef and Abd El-Aziz (1995). Statistical analysis of the obtained data was estimated as the method described by Snedecor and Chocran (1980).

## RESULTS AND DISCUSSION

Heavy metals content in tissue fish (µg/kg.) and water in different locations in both the river Nile and Maryout-lake (ppm) are tabulated in table (1). The obtained results showed that Maryout lake-fish contained higher heavy metals than the Nile-fish samples. These metals are affected by variations in discharge rate of dumped wastes in Maryout lake (Ghazaly, 1992; El-Demerdash and El-Agmy (1999). Also, heavy metals in either Nile or Maryout lake water samples showed the same trend of results, i.e. heavy metals in Maryout water were higher than that in the Nile water. It could be noticed that there was correlation between accumulation of heavy metals in tissue fish and their concentrations in the water. This trend of results was in accordance with those found by Emam and Hamide (2001) and Daoud *et al.*, (1999). It is worthy to mention that the studied metals were at the permissible limits according to FAO/WHO (1992). Meanwhile, fishes of Maryout lake contained higher amounts of Hg, Cd, and Pb than the permissible limits, i.e. 0.5, 0.5 and 2.0 µg/kg., respectively.

**Table (1): Heavy metals content in fresh-tissue fish and water of river Nile and Maryout lake.**

| Metals / Samples | Cd | Pb | Hg | Cu | Zn | Mn |
|---|---|---|---|---|---|---|
| Nile fish (µ/kg) | 0.32 | 1.12 | 0.31 | 0.15 | 5.5 | 1.23 |
| Maryout fish (µ/kg) | 0.55 | 2.02 | 0.59 | 0.38 | 8.61 | 1.42 |
| Nile water (p.p.m.) | 0.047 | 0.32 | 0.02 | 0.05 | 0.57 | 0.23 |
| Maryout water (p.p.m.) | 0.081 | 0.53 | 0.06 | 0.07 | 0.88 | 0.31 |
| L.S.D. 0.05 | 0.011 | 0.056 | 0.014 | 0.009 | 0.221 | 0.05 |

Results of bioaccumulation of heavy metals in serum of rats fed on fish samples are found in table (2). The level concentrations of some heavy metals (Cd, Pb, Hg and Cu) in rats serum fed on Maryout fish showed significant increase at $P \leq 0.05$ when compared with rats serum fed on both the river-Nile fish and casein diet. While, Zn and Mn levels in serum of all rats groups did not significantly differ. The level of heavy metal concentrations in serum of rats group was increased with the increment of the experimental period.

**Table (2): Bioaccumulation of heavy metals in serum of rats fed on diets containing tissue-fish samples (ppm).**

| Diets | Control | | Nile fish | | Maryout fish | |
|---|---|---|---|---|---|---|
| Period / Metals | 30 days | 60 days | 30 days | 60 days | 30 days | 60 days |
| Cd | 0.312 | 0.315 | 0.347 | 0.450 | 0.640* | 0.750* |
| Pb | 0.302 | 0.313 | 0.340 | 0.394 | 0.807* | 1.28* |
| Hg | 0.031 | 0.037 | 0.040 | 0.040 | 0.067* | 0.080* |
| Cu | 0.521 | 0.530 | 0.60 | 0.66 | 1.00* | 1.30* |
| Zn | 0.121 | 0.122 | 0.133 | 0.143 | 0.143 | 0.157 |
| Mn | 0.862 | 0.871 | 0.95 | 1.12 | 1.11 | 1.06 |

*** Indicate statistical significant differences at $P \leq 0.05$**

Bioaccumulation of heavy metals in liver of rats fed on different diets are shown in table (3). The amounts of these metals were found to be Pb (6.70-9.36), Cd (6.03-8.33), Hg (5.07-8.0), Cu (1.80-2.77), Zn (5.23-5.97) and Mn (16.0-16.7) µg/kg (fresh weight basis). Statistical analysis of these data showed that, there are no significant differences ($P \leq 0.05$) between rats fed on casein diet and those fed on diet of Nile-fish samples in either for 30 or 60 days. Meanwhile, rats fed on diet containing Maryout fish for 60 days only showed a significant increase of these metals in rat's liver. This observation might be due to the higher concentration of heavy metals in both water and tissue fish of Maryout lake than the river Nile as shown in table (1). In addition to, Maryout lake samples of water and tissue fish were more polluted with industrial wastes (Ghazali, 1992).

**Table (3): Bioaccumulation of heavy metals in liver of rats fed on diets containing tissue-fish samples (µg/kg. fresh weight).**

| Diets | Control | | Nile fish | | Maryout fish | |
|---|---|---|---|---|---|---|
| Period / Metals | 30 days | 60 days | 30 days | 60 days | 30 days | 60 days |
| Cd | 6.10 | 6.03 | 6.20 | 6.33 | 7.0 | 8.33* |
| Pb | 6.70 | 6.90 | 7.37 | 7.67 | 7.73 | 9.36* |
| Hg | 5.60 | 5.07 | 5.67 | 5.71 | 5.87 | 8.00* |
| Cu | 1.80 | 1.80 | 1.80 | 2.27 | 2.17 | 2.77* |
| Zn | 5.23 | 5.67 | 5.77 | 5.33 | 5.43 | 5.97 |
| Mn | 16.0 | 16.0 | 16.7 | 16.2 | 16.2 | 16.7 |

* Indicate statistical significant differences at P ≤ 0.05

Results of biochemical parameters of rats fed on diets containing different fish samples are illustrated in table (4). Serum total protein ranged from 5.98 to 6.32 g/dl. The highest value was found in rats fed on Maryout fish for 60 days and the lowest level was noticed in the group of animals fed on casein diet. On the other hand, serum total lipids of experimental rats ranged from 6.56 to 6.83 g/dl. The lowest value was noticed in rats fed on control diet and the highest level was found in rats fed on Maryout fish for 60 days. Statistical analysis of these results showed that there are no significant differences (P≤ 0.05) in both serum total protein, and serum total lipids of all groups of rats.

Haemoglobin level of all animals ranged from 12.5 to 14.1 g/dl. There is only significant decrement (P≤ 0.05) in rats fed on Maryout fish for 60 days. This might be due to the effect of high concentration of heavy metals on haemoglobin level. But, rats fed on either Nile fish or casein didn't affect. This trend of results was in agreement with those found by Hassan *et al.* (2002-a) who reported that Cd caused a decrease in haemoglobin concentration. Hematocrit (PVC) percentages didn't affect by either source of fish, or by the experimental period. These percentages ranged from 41.6 to 44.6%. Serum cholesterol level recorded an increase at the end of experimental period, i.e. after 60 days. There is only a significant (P≤0.05) increase in serum cholesterol of rats fed on Maryout fish.

It could be concluded that, rats fed on Maryout fish for 60 days showed significant increase in cholesterol and significant decrease at P≤ 0.05 in haemoglobin, while rats fed on other diets had no significant effect.

**Table (4): Biochemical parameters of rats fed on diets containing tissue-fish samples (ppm).**

| Diets | Control | | Nile fish | | Maryout fish | |
|---|---|---|---|---|---|---|
| Period / Parameters | 30 days | 60 days | 30 days | 60 days | 30 days | 60 days |
| Haemoglobin | 13.9 | 14.1 | 13.3 | 13.6 | 13.2 | 12.5* |
| Hematocrit PVC (%) | 44.6 | 44.4 | 43.9 | 43.1 | 41.6 | 41.9 |
| Total protein (g/dl) | 6.01 | 5.98 | 6.0 | 6.21 | 6.32 | 6.29 |
| Total lipids (g/dl) | 6.62 | 6.60 | 6.56 | 6.69 | 6.81 | 6.83 |
| Cholesterol (mg/dl) | 139.1 | 143.6 | 146.5 | 149.1 | 151.3 | 174.6* |

* Indicate statistical significant differences at P ≤ 0.05

Liver and kidney functions of rats fed on diet containing fish flesh are found in table (5). Liver function (represented by aminotransferase AST and ALT enzyme activities) was slightly deceased in rats group fed on flesh of *Tilapia* fish from Maryout lake in either 30 or 60 days. This slight decrease may be resulted from the toxic metals (Cd, Pb and Hg) which are present in the tissue-fish samples. These results are in accordance with those found by El-Massarawy (1997) and Ali (2001), who reported that there is an interaction between the heavy meals i.e. Zn and Cu have protective effect against lead toxicity.

There were no significant differences ($P \leq 0.05$) in serum enzyme activities (ALT and AST) of rats fed in either diets containing fish samples or casein diet. In contrast, there is significant increase in kidney functions (urea and cereatinine) in the serum of rats fed on tissue fish of Maryout lake after 60 days, but groups of rats fed on other diets had no significant effect. The same trend of results was mentioned by Hassan *et al.* (2002-b) who found that cadmium accumulated in rabbit organs was higher in kidney than liver and elevation in cereatinine and urea concentration of rabbit serum.

**Table (5): Liver and kidneys functions of rats fed on diet containing tissue-fish samples.**

| Die | Control | | Nile fish | | Maryout fish | |
|---|---|---|---|---|---|---|
| Period / Parameters | 30 days | 60 days | 30 days | 60 days | 30 days | 60 days |
| AST (µ/L.) | 32.2 | 32.7 | 32.9 | 31.8 | 31.7 | 29.6 |
| ALT (µ/L.) | 20.1 | 20.8 | 19.9 | 19.4 | 19.5 | 18.3 |
| Serum urea (mg/dL) | 25.1 | 24.9 | 25.0 | 26.2 | 26.3 | 28.2* |
| Serum cereatinine mg/dL | 0.661 | 0.653 | 0.692 | 0.735 | 0.794 | 0.885* |
| AST/ALT | 1.60 | 1.57 | 1.65 | 1.64 | 1.63 | 1.56 |

*** Indicate statistical significant differences at $P \leq 0.05$**

Biological parameters of rats fed on different diet are tabulated in table (6). There was no significant difference ($P \leq 0.05$) in body-weight gain, except only for rats fed on diet containing Maryout-fish samples for 60 days which showed statistically significant decrease. This observation may be attributed to decreasing fed absorption or partial loss of appetite as a result of diet pollution with heavy metals. This trend of results was in agreement with those found by Hassan *et al.* (2002 b).

Feed consumption and feed efficiency ratio showed the same trend of results. Also, feeding on diet containing Maryout fish markedly declined the feed intake as compared with other diets. This reduction could explain the significant decrease in weight gain. These results are in agreement with those noticed by Hassan *et al.* (2002 b).

Finally, it could be concluded that Maryout lake-fishes are containing higher levels of heavy metal concentrations than river Nile fishes. These studied heavy metals are toxic and harmful for either in experimental animals or human being.

**Table (6): Biological parameters of rats fed on diet containing tissue fish samples.**

| Diets | Control | | Nile fish | | Maryout fish | |
|---|---|---|---|---|---|---|
| Period / Parameters | 30 days | 60 days | 30 days | 60 days | 30 days | 60 days |
| Initial body weight (g) | 75.3 | 75.3 | 72.2 | 72.2 | 74.9 | 74.9 |
| Final body weight (g) | 145.6 | 203.9 | 140.3 | 196.6 | 137.9 | 191.3 |
| Total weight gain (g) | 70.3 | 128.6 | 68.1 | 124.4 | 63.0 | 116.4 |
| Weight gain (%) | 93.4 | 170.8 | 94.3 | 172.3 | 84.1 | 155.4* |
| Feed intake (g) daily | 14.13 | 13.61 | 14.30 | 13.67 | 14.22 | 13.02* |
| Food efficiency ratio (FER) | 6.03 | 6.35 | 6.30 | 6.59 | 6.77 | 7.12* |

* Indicate statistical significant differences at $P \leq 0.05$

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## التقييم البيولوجي للفئران المغذاة على أسماك بلطي بحيرة مريوط

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يهدف هذا البحث إلى تقدير العناصر الثقيلة (كادميوم ، رصاص، زئبق ، نحاس ، زنك ، منجنيز) الموجودة فى أنسجة أسماك بلطي بحيرة مريوط ودراسة التقييم البيولوجي للفئران المغذاة عليها لمدة ٣٠ ، ٦٠ يوم. وقد دلت النتائج على احتواء أسماك بحيرة مريوط على تركيزات عالية من العناصر الثقيلة وخاصة الكادميوم والرصاص والزئبق بالمقارنة بأسماك وميـاه النيل.

لا توجد أي فروق معنوية في مستوي تركيز العناصر الثقيلة في كل من مصل وكبد الفئران التي تغذت على أسماك النيل أو الكازين ، بينما لوحظ زيادة معنوية في الفئران التي تغذت على أسماك بحيرة مريوط.

وقد دل التقييم البيولوجي على عدم وجود فروق معنوية في كل من وظائف الكبد والكلي للفئران فيما عدا زيادة مستوي اليوريا والكرياتنين فى مصل الفئران المغذاة لمدة ٦٠ يوم على وجبة بها أسماك بحيرة مريوط.

لم يلاحظ أى فروق معنوية في البروتينات أو الليبيدات الكلية في جميع حيوانات التجربة سواء باختلاف مصدر الأسماك ، أو مدة التجربة بينما لوحظ إرتفاع مستوي الكولستيرول ونقص تركيز الهيموجلوبين معنويا في الحيوانات المغذاة لمدة ٦٠ يوم على وجبة بها أسماك بحيرة مريوط.

لم تتأثر معدلات النمو والاستهلاك اليومي ومعامل تحويل الغذاء في حيوانات التجربة فيما عدا المجموعة التي احتوي غذاؤها على أسماك بحيرة مريوط لمدة ٦٠ يوم.