

## METALS RESIDUES, HISTOLOGICAL ALTERATIONS AND COOKING METHODS OF FISH CULTURED IN WASTEWATER PONDS

SAMYA I. A. HASSANIN

*Central Laboratory for Aquaculture Research, Agricultural Research Center, Egypt*

---

### **Abstract**

In this study, field investigation was done by collection of Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus* L.) from fish ponds which obtained its water supply from Bahr El-Baquer drain, to evaluate the fish fed with domestic and industrial wastewater with respect to the potentially some toxic metals. Cadmium (Cd), lead (Pb), mercury (Hg), zinc (Zn), iron (Fe) and manganese (Mn) contents were determined in fresh gills, muscles including skin, livers and ovaries of Nile tilapia and grey mullet as well as histological alterations were recorded in these organs. Cooking methods; deep-frying, grilling, baking in the oven and the microwave oven were evaluated for proximate chemical composition and residues of Cd, Pb, Hg, Zn, Fe and Mn in fish fillets.

Marked histological alterations were observed in the examined organs of two fish species, also high concentrations of Cd, Pb, Zn, Fe and Mn were determined in the livers followed by gills. The fish fillets revealed non significant increases in Cd and Hg after all cooking methods. The fillets cooked by baking or microwave showed significant decrease in the lead content. A significant decrease in Zn and Mn levels of all fillets samples cooked by different methods were observed. The protein and ash contents were gradually increased during different treatment for two fish species, while the highest level of fat was seen in the fried fillets. From the results obtained in the present study, it may be recommended that, the best methods for cooking fish fillets (tilapia and grey mullet) are baking and grilling. Consuming fish fillets is better than the whole fish to minimize exposure to heavy metals.

**Keywords:** fish tissues, metals residues, histological alterations, cooking methods.

### **INTRODUCTION**

Pollution of aquatic environments with heavy metals has seriously increased worldwide attention and under certain environmental conditions fish may concentrate large amounts of some metals from the water in their tissues (Mansour and Sidky, 2002). Some metals such as zinc, iron are essential in trace amounts for normal growth and development; however, others such as cadmium, lead and mercury are potentially harmful to most organisms even in very low concentrations. Heavy metals and more specifically mercury have been reported as hazardous environmental pollutants able to accumulate along the aquatic food chain with severe risk for animal and human health. However, considerable controversy surrounds the interpretation of

the relationship between pathological changes in Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus L.*) and prolonged exposure to water pollutants. It was reported that metals are taken up through different organs of the fish and induced morphological, histological and biochemical alterations in the tissues which may critically influence fish quality (Olojo *et al.*, 2005 and Fadel and Gaber, 2007).

Tilapia and mullet are common cultural fish, they are widely distributed in all water resources. Fish is usually cooked by various processes, such as grilling, frying or baking before consumption. On the other hand, the use of the microwave oven for cooking has increased greatly during the past few decades (Arias *et al.*, 2003). It is important to determine the concentrations of heavy metals in commercial fish in order to estimate the intake of these metals by humans (Cid *et al.*, 2001). Many studies have been published on the determination of heavy metals in raw fish (Yazkan *et al.*, 2002), but these studies are inadequate for estimating the intake of metals by humans after different cooking methods. Recently, agricultural and industrial developments as well as increase in the population have substantially increased the contamination of Bahr El-Baquar water resource. Thus, the present study aimed to evaluate the fish reared in wastewater via determination of pathological alterations and metals contents in some organs of Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus L.*), and to evaluate the effects of different cooking methods on the concentration of these metals and the chemical composition of two fish species fillets after cooking.

## MATERIALS AND METHODS

### Collection of Samples:

Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus L.*), weighed 150-200 gm each, of both sexes were collected from fish ponds which obtained its water supply from Bahr El-Baquar drain 4 times during the period of June 2006 - June 2007. In every time the total number of fish was 100 from each species.

Fish were kept in cold ice boxes and transported immediately to the laboratory of quality control and processing at Central Laboratory for Aquaculture Research. Fish were washed with tap water then examined for morphological and postmortem lesions. Thereafter, scales, tails, all fins, head and viscera were removed, fish cut to skinned fillets with ribs using a sharp knife. Tissue specimens were taken from the gills, muscles including skin, livers and ovaries for histological examination and determination of cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn), iron (Fe) and manganese (Mn). Moreover, the skinned fillets of each species were randomly divided into 5 equal groups (1.400 Kg. each), then each group was analyzed in the same way.

**Histological examination:**

Tissue specimens from fresh Nile tilapia and grey mullet were taken (gills, muscles including skin, livers and ovaries) and fixed in 10 % buffered neutral formalin for 2 days. They were processed to obtain five micron thick paraffin sections then stained with hematoxylin and eosin, (H&E). (Bancroft *et al.*, 1996) and examined under light microscope.

**Samples preparation and cooking methods:**

Fresh Nile tilapia and grey mullet fillets were washed twice with distilled water to remove adhering blood and slime and cut equals to each other. Thereafter, the mean core temperature immediately after cooking was 76-92 °C in the thickest portion of the fillet for all cooking methods (Weber *et al.*, 2008). The internal fillet temperature was monitored using a digital calibrated thermometer (Traceable Long-Stem, VWR, Friendswood, TX, USA).

According to common household methods the skinned fillets samples from both fish species were cooked as follows: The first group was control (raw) fillets samples. Second group: Fillets samples were powdered with wheat flour 72% extraction using frying pan made of aluminum. Fillets immersed in sunflower oil as the medium for deep-frying which heated to 180 °C for 5-10 min and removed when a golden brown color appeared on surface. Third group: Fish fillets were previously powdered with bran then grilled at 180 °C for 10 min on each side using a grilling plate made of tin. Forth group: The fillets baked in the household oven at 180 °C for 20 min on an aluminum pan, being turned once after 5 min. Fifth group: The fish fillets were placed in a glass baking dish and cooked in microwave oven (a Litton Menu-master system 70/50) operating at 2450 MHZ for 10 min, then the fillets immediately removed. All cooked Fillets samples were cooled at room temperature and kept in the refrigerator at about 5 °C. Samples of raw and cooked fillets of Nile tilapia and grey mullet were separately homogenized in a stainless-steel hand grinder and analyzed to determine metals residues and proximate chemical composition.

**Analytical techniques:**

1-The residual analysis of Cd, Pb, Hg, Zn, Fe and Mn were determined in all fresh (gills, muscles including skin, whole livers and ovaries) and in skinned fillets samples from Nile tilapia and grey mullet following cooking. The selected metals were measured using an atomic absorption spectrophotometry (Perkin-Elmer 2380) according to the procedures recommended by AOAC (2002).

2- Proximate chemical composition: Homogeneous mixtures of minced fillets weighed 5g were dried at 105°C to constant weight for moisture determination, Total protein was determined by kjeldahl's method using a conversion factor (6.25xN), fat was determined using Soxhlet system, ash content was determined by ignition at 550

°C in an electric furnace according to the methods described in AOAC (2002). The total carbohydrate was calculated using difference of the value of each nutrient. The total carbohydrates % = 100 - (moisture% + total protein % + fat % + ash %) according to Egan *et al.* (1981).

#### **Statistical analysis:**

Three replicates of each trial were performed to evaluate Cd, Pb, Hg, Zn, Fe and Mn residues in fresh gills, muscles including skin, liver and ovaries as well as proximate chemical compositions (total protein, fat, ash and carbohydrates) and residues of metals in fresh raw and cooked fish fillets. 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**

#### **Morphological and postmortem examinations:**

Some Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus L.*) revealed normal morphology while the other showed, excessive mucus covering the body with mild skin darkening and focal scales loss in some tilapia samples (Fig.1 & 2). In the two fish species, congestion of gills and ovaries, enlargement of gall bladder were noticed. The changes may be attributed to the irritants in industrial and agricultural wastewater which induced excess mucus secretion, congestion and aggregation of melanine pigment. These findings were similar to those mentioned by Mohana (1996).

#### **Histological examination:**

**The gills** of Nile tilapia showed mild congestion and edema of the primary lamellae. Severe hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary lamellae were observed (Fig.3). The gill arch, especially at the bases of the gill filaments, showed numerous mononuclear leukocytic infiltration, edema and congestion (Fig.4). Grey mullet showed similar picture as mentioned before in addition to telangiectasis and hemorrhages in the secondary lamellae. Partial sloughing of secondary gill lamellae was seen (Fig.5). The apex of gill filaments showed congestion, hyperactivation of the mucous and chloride cells with epithelial vacuolation of the secondary lamellae (Fig.6). The histological alterations attributed to prolonged exposure to heavy metals lead to respiratory, osmoregulatory and circulatory impairment these findings were demonstrated by Fernandes *et al.* (2008). Moreover Alvarado *et al.* (2006) reported that, the dramatic increase of chloride cells in the gills that produces epithelial thickening of the filament epithelium enhances migration of chloride cells up to the edge of the secondary lamellae and provokes the hypertrophy and fusion of secondary lamellae.

These could be considered as unspecific biomarker responses of heavy metals exposure and disturbed health of fish.

The skin, of Nile tilapia, showed hyperplasia, hyperactivation of the mucous cells and spongiosis of epidermis with dermal edema (Fig.7). Focal epidermal cell necrosis was evident. In grey mullet the epidermis showed hyperplasia with hypertrophy of alarm and mucous cells, aggregation of melanomacrophages and dermal edema (Fig.8). The proliferation of melanomacrophages could play a role in the clearance of the tissues from the accumulated heavy metals. The muscles, of Nile tilapia and grey mullet, showed marked intermuscular edema and focal hyaline degeneration as well as zenker's necrosis of muscles fibers with few leukocytic cells infiltration (Figs.9 &10). It was reported that, metals toxicity is attributed to its high affinity with thiols on which it binds from any protein or peptide with cysteine or even methionine groups, and to increase permeability of cell membrane and impaired mitochondrial ATP production causing specific patterns of cell damages Mohana (1996) and Ribeiro *et al.* (2008).

The liver showed vacuolar degeneration of the hepatocytes around the congested hepatic sinusoids and central vein. Nuclear pyknosis in the majority of hepatic cells could be seen. Some pancreatic acini suffered degeneration and devoid of zymogenic granules (Fig.11). The picture in grey mullet liver as mentioned above in addition to, focal coagulative necrosis and some hepatocytes revealed granular eosinophilic cytoplasm with karyorrhexis of nuclei (Fig.12). These findings was apparent as the liver consider the organ of detoxification, excretion and binding proteins such as metallothionein (MTs).The metal-binding proteins were present in the nuclei, sinusoids, extracellular space and the acinar cells of hepatopancreas suggested that the increase in the cell damages (De Smet and Blust, 2001). Similar results were observed by Van Dyk (2003) and Mela *et al.* (2007).

The ovaries of Nile tilapia showed edema and degeneration of some ovarian follicles. The majority of oocytes were atretic (Fig.13). In grey mullet the ovarian stroma showed hemorrhage, hemosiderin deposits and mild edema. Degeneration, necrosis and atretic follicles were also observed (Fig.14). These findings were seen where, the ovaries are known to be the target organ of heavy metals accumulation, high metabolic activities and impairment of steroid sex hormone. The obtained results are in agreement with Shanbhag and Saidapur (1996). Our results suggest a relationship between element contents in fish organs and their pathological changes ( McVicar, 1997).

#### **Residual analysis of metals:**

The concentrations of metals expressed in (ppm) on dry weight in gill, muscles including skin, liver and ovaries of Nile tilapia and grey mullet are recorded in (Table 1). In which the highest bioaccumulation were observed in the organs

mainly implicated in metals metabolism. For both fish species the cadmium (Cd) in tissues was high in the liver > gills > muscles with skin > ovaries; lead (Pb) residue in the liver > gills > muscles with skin > ovaries; mercury (Hg) in the muscles with skin > ovaries > liver > gills; zinc (Zn) in liver > ovaries > muscles with skin > gills; iron (Fe) in liver > gills > ovaries > muscles with skin; manganese (Mn) in gills > liver > ovaries > muscles including skin.

The differences in metal levels between two fish species may be related to feeding habits, specific metabolism of the metal in the fish body, the differences in physiological functions of organs considered, age of the fish and availability of the metal in the environment (Watanabe *et al.*, 2003). The highest concentrations of Cd, Pb, Zn and Fe were found in liver and the highest Mn level was in gills these attributed to the tendency of liver and the gills as target tissues to accumulate pollutants at different levels from their environment. Other studies carried out with different fish species have shown that heavy metals accumulate mainly in metabolic organs such as liver that stores metals to detoxicate them by producing specific binding proteins known as metallothioneins (MTs) (De Smet and Blust, 2001).

The metal levels were higher in the gill than the muscle tissues of two fish species. Metals concentration in the gills could be due to the element complexation with the mucus is impossible to be completely removed from the lamellae, before tissue is prepared for analysis. Also, the adsorption of metals onto the gill surface, as the first target for pollutants in water, could be an important influence in the total metal levels of the gills (Hülya *et al.*, 2004). In Cd exposed fish, (MTs) was demonstrated mainly in mucocytes, chloride cells of the main branchial epithelium and the respiratory epithelium of secondary lamellae Alvarado *et al.* (2007).

Results presented in this study indicated that all metals level in the tissues of Nile tilapia were lower than those determined in grey mullet tissues. The highest Hg accumulation was determined in muscles including the skin and ovaries of grey mullet. However, muscle with skin and ovaries were commonly analyzed because they are the main fish part consumed by humans and were implicated in health risk. Muscles of fish are considered to be sensitive and selective biomonitors of Hg pollution of the aquatic ecosystems (Szefer *et al.*, 2003). Chicourel *et al.* (2001) reported that, there was no significant statistical difference in Hg levels in the samples taken from regions near the head or from central and tail parts, indicating homogeneous distribution of the metal in muscles throughout the body.

The Zn concentrations appeared considerably higher in the liver than in other tissues. The low concentrations of Zn in the muscles than liver of the examined fish species may reflect the low levels of binding proteins (MTs) in the muscle (Allen-Gil and Martynov, 1995). The muscles tended to accumulate less Fe and Mn levels which

were lowest in the muscle with skin than other organs for Nile tilapia (*Oreochromis niloticus*) and grey mullet (*Mugil cephalus L.*). Similar results were reported from a number of fish species where the muscle is not an active tissue for the accumulation of Mn (Karadede and ünlü, 2000). The results showed that the accumulation levels of the essential metals (Zn, Fe and Mn) are generally higher and more homeostatic than the non-essential metals Cd, Pb and Hg (heavy metals) in two fish species. The Cd, Pb, Zn and Fe concentrates largely in the liver, followed by gills and lower in the muscle tissues, indicating that the concentrations of some metals could be decreased by trimming and gutting fish before consumption. Muscle tissues were containing more Hg, which binds to muscle proteins. Thus, trimming and gutting can actually result in a greater average concentration of Hg in the remaining fillet tissues compared with the concentration in the whole untrimmed fish proteins (Gutenmann and Lisk, 1991).

The results obtained from Table (3) and (4) showed insignificant increases in Cd and Hg after cooking by all cooking methods. Also insignificant increases of Pb contents were found after deep- frying and grilling for tilapia and mullet fillets compared with the raw fillets. The Fe content in fillets cooked by baking was significant increase  $P < 0.05$  which were 80.72 and 119.37 ppm for Nile tilapia and grey mullet, respectively compared with the raw tilapia and mullet fillets which were 76.09 and 114.14 ppm, respectively. These findings were similar to those mentioned by (Gokoglu *et al.*, 2004).

Table 1. The residual analysis of metals in fresh Nile tilapia tissues.

Nile tilapia tissues	Metals (ppm)					
	Cadmium	Lead	Mercury	Zinc	Iron	Manganese
Gills	0.391 ±	0.483 ±	0.009 ±	7.8 ±	141.5 ±	22.41 ±
	0.015 <sup>b</sup>	0.015 <sup>b</sup>	0.002 <sup>bc</sup>	0.53 <sup>c</sup>	2.7 <sup>b</sup>	0.51 <sup>a</sup>
Muscles with skin	0.317 ±	0.415 ±	0.023 ±	11.4 ±	76.09 ±	6.95 ±
	0.013 <sup>b</sup>	0.012 <sup>b</sup>	0.004 <sup>a</sup>	0.72 <sup>b</sup>	1.8 <sup>d</sup>	0.31 <sup>d</sup>
Livers	0.523 ±	0.623 ±	0.015 ±	16.9 ±	219.34 ±	14.01 ±
	0.022 <sup>a</sup>	0.017 <sup>a</sup>	0.003 <sup>b</sup>	0.81 <sup>a</sup>	2.5 <sup>a</sup>	0.55 <sup>b</sup>
Ovaries	0.212 ±	0.155 ±	0.020 ±	15.2 ±	94.35 ±	9.04 ±
	0.014 <sup>c</sup>	0.020 <sup>c</sup>	0.005 <sup>a</sup>	0.65 <sup>ab</sup>	1.5 <sup>c</sup>	0.42 <sup>c</sup>

<sup>a-d</sup> Means within a column with the same superscript are significantly different ( $p < 0.05$ ).

Values are expressed as Mean ± SE.

Table 2. The residual analysis of metals in fresh grey mullet tissues.

Grey mullet tissues	Metals (ppm)					
	Cadmium	Lead	Mercury	Zinc	Iron	Manganese
Gills	0.411 ±	0.611 ±	0.017 ±	13.5 ±	212.25 ±	34.74 ±
	0.021 <sup>b</sup>	0.019 <sup>b</sup>	0.005 <sup>cd</sup>	0.65 <sup>d</sup>	3.1 <sup>b</sup>	0.56 <sup>a</sup>
Muscles with skin	0.326 ±	0.534 ±	0.053 ±	19.2 ±	114.14 ±	10.41 ±
	0.015 <sup>c</sup>	0.021 <sup>c</sup>	0.003 <sup>a</sup>	0.58 <sup>c</sup>	2.6 <sup>d</sup>	0.38 <sup>d</sup>
Livers	0.573 ±	0.805 ±	0.021 ±	28.1 ±	319.01 ±	21.11 ±
	0.017 <sup>a</sup>	0.017 <sup>a</sup>	0.004 <sup>c</sup>	0.71 <sup>a</sup>	3.7 <sup>a</sup>	0.45 <sup>b</sup>
Ovaries	0.213 ±	0.192 ±	0.035 ±	25.4 ±	141.52 ±	13.12 ±
	0.013 <sup>d</sup>	0.017 <sup>d</sup>	0.006 <sup>b</sup>	0.83 <sup>b</sup>	2.5 <sup>c</sup>	0.31 <sup>c</sup>

<sup>a-d</sup> Means within a column with the same superscript are significantly different ( $p < 0.05$ ).

Values are expressed as Mean ± SE.

Table 3. The residual analysis of metals in raw and cooked fillets from Nile tilapia (on dry weight).

Cooking methods	Metals (ppm)					
	Cadmium	Lead	Mercury	Zinc	Iron	Manganese
Raw	0.317 ±	0.415 ±	0.023 ±	11.4 ±	76.09 ±	6.95 ±
	0.013 <sup>a</sup>	0.012 <sup>a</sup>	0.004 <sup>a</sup>	0.72 <sup>a</sup>	1.8 <sup>a</sup>	0.31 <sup>a</sup>
Deep-frying	0.396 ±	0.440 ±	0.030 ±	9.25 ±	73.79 ±	5.44 ±
	0.05 <sup>a</sup>	0.04 <sup>a</sup>	0.002 <sup>a</sup>	0.17 <sup>b</sup>	2.1 <sup>a</sup>	0.34 <sup>b</sup>
Grilling	0.370 ±	0.460 ±	0.025 ±	8.52 ±	75.40 ±	5.23 ±
	0.07 <sup>a</sup>	0.04 <sup>a</sup>	0.001 <sup>a</sup>	0.13 <sup>b</sup>	2.5 <sup>a</sup>	0.45 <sup>b</sup>
Baking	0.380 ±	0.277 ±	0.025 ±	9.03 ±	80.72 ±	5.02 ±
	0.05 <sup>a</sup>	0.05 <sup>b</sup>	0.002 <sup>a</sup>	0.15 <sup>b</sup>	3.0 <sup>a</sup>	0.33 <sup>b</sup>
Microwave	0.355 ±	0.244 ±	0.023 ±	8.01 ±	70.31 ±	5.61 ±
	0.06 <sup>a</sup>	0.02 <sup>b</sup>	0.001 <sup>a</sup>	0.11 <sup>b</sup>	2.4 <sup>a</sup>	0.26 <sup>b</sup>

<sup>a-b</sup> Means within a column with the same superscript are significantly different ( $p < 0.05$ ).

Values are expressed as Mean ± SE.



Table 4. The residual analysis of metals in raw and cooked fillets from grey mullet (on dry weight).

Cooking methods	Metals (ppm)					
	Cadmium	Lead	Mercury	Zinc	Iron	Manganese
Raw	0.326 ±	0.534 ±	0.053 ±	19.2 ±	114.14 ±	10.41 ±
	0.015 <sup>a</sup>	0.021 <sup>a</sup>	0.003 <sup>a</sup>	0.58 <sup>a</sup>	2.6 <sup>a</sup>	0.38 <sup>b</sup>
Deep-frying	0.433 ±	0.541 ±	0.072 ±	15.53 ±	110.96 ±	8.85 ±
	0.03 <sup>a</sup>	0.04 <sup>a</sup>	0.003 <sup>a</sup>	0.51 <sup>b</sup>	2.5 <sup>a</sup>	0.32 <sup>b</sup>
Grilling	0.403 ±	0.558 ±	0.057 ±	13.96 ±	112.38 ±	8.31 ±
	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.002 <sup>a</sup>	0.33 <sup>b</sup>	3.1 <sup>a</sup>	0.41 <sup>b</sup>
Baking	0.415 ±	0.309 ±	0.058 ±	14.49 ±	119.37 ±	8.11 ±
	0.06 <sup>a</sup>	0.04 <sup>b</sup>	0.001 <sup>a</sup>	0.51 <sup>b</sup>	2.7 <sup>a</sup>	0.35 <sup>b</sup>
Microwave	0.381 ±	0.261 ±	0.053 ±	13.12 ±	107.25 ±	9.10 ±
	0.04 <sup>a</sup>	0.04 <sup>b</sup>	0.003 <sup>a</sup>	0.61 <sup>b</sup>	2.0 <sup>a</sup>	0.27 <sup>b</sup>

<sup>a-b</sup> Means within a column with the same superscript are significantly different ( $p < 0.05$ ).

Values are expressed as Mean ± SE.

Ersoy *et al.* (2006) mentioned that, the increase in metals may be related to decrease in the moisture content that occur during cooking. Also, the cooking in the microwave oven increased Cd level significantly in fish fillets. Chicourel *et al.* (2001) reported that, frying, baking and using microwave oven did not affect original Hg levels present in fish tissues. While Burger *et al.* (2003) revealed that Hg levels increased after deep-frying of fish fillets due to the breading and absorption of oil compared with the raw fillets.

A significant decrease in Pb content of fillets was found after cooking by baking and microwave oven methods, which were 0.277 and 0.244 ppm, respectively for tilapia fillets and were 0.309 and 0.261 ppm, for mullet fillets respectively. The reduction in Pb depends on cooking conditions, such as time, temperature and medium of cooking. Similarly, Atta *et al.* (1997) showed decrease in the concentrations of Pb and Zn in Nile tilapia after baking. On the other hand, results showed that, the Zn and Mn levels in fillets were significantly decreased after all the cooking methods. Similar results were recorded by Gokoglu *et al.* (2004). Moreover, Ersoy *et al.* (2006) reported that, the heavy metals content in all fish parts decreased on baking. Metals under study in the edible parts of the investigated two fish species

were in the safety permissible levels for human use. Fish preparation (trimming, eviscerating, beheading, filleting and washing) with cooking procedures (deep-frying, grilling, baking, microwave oven) can modify the amount of metals ingested by fish consumers. Grilling or cooking the fish as the fat drips away, may reduce the levels of these metals. The mercury can not be removed by cooking or cleaning the fish. The concentrations of metals are below the limits values for fish proposed by FAO (1983).

**Proximate composition:**

The proximate composition of raw and cooked Nile tilapia and grey mullet fillets are given in Table (5). The moisture content in raw Nile tilapia and grey mullet fillets, reached to 82.82 % and 81.72 %, respectively which were somewhat higher than the value which reported by Ez-El-Rigal (2007) for cultural Nile tilapia fillets and by El-Sebaiy and Metwalli (1989) in grey mullet, these limits may be related to disturbance in osmoregulation and edema in fish tissues which was observed during histological examination.

The moisture content in all cooking methods was significantly decreased ( $p < 0.05$ ) than that in raw fillets. Minimum moisture value was characteristic of deep-fried fillets which were 73.97% and 73.61 % for Nile tilapia and grey mullet respectively and maximum moisture content was found for microwave - cooked fillets 77.97 % and 78.60%. for Nile tilapia and grey mullet respectively.

The crude protein percentages basis on dry weight in raw Nile tilapia fillets and grey mullet were 85.66 % and 77.00 %, respectively, the values were lower than the levels reported by Khalil *et al.* (1980). The decrement of protein percentages in raw fillets may be due to histological alterations induced by metals on hepatic parenchyma that lead to disturbance in protein metabolism and the activities of proteases enzymes which induce proteolysis intended to increase the role of proteins in the energy production during heavy metals stress (De Smet and Blust, 2001). From obtained results the protein percentages were significantly increased ( $p < 0.05$ ) after all cooking methods. Maximum protein content was in the baking fillets 89.51% for tilapia and was 82.37 % for grey mullet. These limits related to the moisture loss, which lead to increases in the percentage of all the other nutrients, in fact protein and ash percentages, presented a higher increase than fat (Castrillón *et al.* 1997).

The fat content of raw tilapia and mullet fillets were 5.50 % and 12.43 %, respectively. The fat content was significant increased ( $p < 0.05$ ) in deep-frying only and was significant highest in grey mullet fillets than tilapia fillets. These levels due to fillets which cooked in oil medium contain additional lipid material, as well as the oil

penetrates the fillets after water is partially lost by evaporation. These results coincide with those reported by Echarte *et al.* (2001) and Türkkan *et al.* (2008). In grilling, baking and microwave methods the fat percentages were significantly decreased ( $p < 0.05$ ) in two fish species fillets. These results are in agreement with Abd-Allah *et al.* (1985) who revealed that grilling of fish caused a remarkable loss in total lipids. Also, the losses of lipids were probably related to autoxidation and its diffusion with the drip separated during grilling and baking, although the amount lost depends on the temperature and time of cooking (Saldanha and Bragagnolo, 2008). In fact, it has been established by Waters (1988) that the link between water and fat in fish is an inverse relation, not only among different species but also for the same species at different times of the year. Furthermore, Varela and Ruiz-Roso (1992) declared that the gain or loss of fat during frying was strongly related to the initial lipid content of fish. Moreover, they observed that as water is lost by evaporation during the frying process, fat comes to compensate this loss.

The average ash contents were 6.45 % and 8.68 % for raw fillets of tilapia and mullet, respectively. The result revealed high percentages of ash level in raw mullet fillets compared to tilapia fillets. A significant increased in the ash levels after deep-frying, grilling, baking and microwave for two fish species). Due to the moisture was loss during cooking, it induces an apparent concentration of ash percentages from fillets. These changes were similar to those found by Weber *et al.*, (2008). Castrillón *et al.* (1997) found that fish preparation (beheading, eviscerating, scales, tails, all fins and backbone-removing) diminishes the ash content compared to ash in the whole fish and therefore increases the percentages of the other nutrients. Data in Table 5 showed that, the carbohydrate levels were significantly decreased ( $P < 0.05$ ) after all cooking methods used compared with the carbohydrate contents in raw fillets of tilapia and mullet.

The results in the present study showed that precautions need to be taken in order to prevent future water pollution. It is possible to reduce the metals in fish parts by choosing a suitable method of cooking. Therefore cooking fish fillets is better than the whole fish to minimize exposure to heavy metals. It may be recommended to cook fish (Nile tilapia and grey mullet) by baking followed by grilling.

Table 5. The Proximate compositions of raw and cooked Nile tilapia and grey mullet, (\*% on dry weight basis).

Constituent	Moisture %	Protein * %	Fat * %	Ash * %	Carbohydrate * %
<b>Raw</b>					
Nile tilapia	82.82± 0.52 <sup>a</sup>	85.66 ± 0.55 <sup>ab</sup>	5.50 ± 0.13 <sup>c</sup>	6.45 ± 0.12 <sup>d</sup>	2.39 ± 0.02 <sup>a</sup>
Grey mullet	81.72 ± 0.73 <sup>a</sup>	77.00 ± 0.51 <sup>c</sup>	12.43 ± 0.21 <sup>a</sup>	8.68 ± 0.13 <sup>b</sup>	1.89 ± 0.05 <sup>ab</sup>
<b>Deep-frying</b>					
Nile tilapia	73.97 ± 0.61 <sup>c</sup>	86.17 ± 0.24 <sup>ab</sup>	5.61± 0.25 <sup>c</sup>	7.15 ± 0.13 <sup>c</sup>	1.07 ± 0.03 <sup>b</sup>
Grey mullet	73.61 ± 0.54 <sup>c</sup>	77.16 ± 0.56 <sup>c</sup>	12.41 ± 0.22 <sup>a</sup>	9.94 ± 0.15 <sup>a</sup>	0.49 ± 0.03 <sup>bc</sup>
<b>Grilling</b>					
Nile tilapia	76.36 ± 0.75 <sup>bc</sup>	89.05 ± 0.32 <sup>a</sup>	2.66 ± 0.15 <sup>d</sup>	6.98 ± 0.14 <sup>cd</sup>	1.31 ± 0.04 <sup>ab</sup>
Grey mullet	77.17± 0.61 <sup>bc</sup>	81.21 ± 0.65 <sup>b</sup>	8.23 ± 0.21 <sup>b</sup>	9.90 ± 0.12 <sup>a</sup>	0.66 ± 0.03 <sup>b</sup>
<b>Baking</b>					
Nile tilapia	76.79 ± 0.72 <sup>bc</sup>	89.51 ± 0.26 <sup>a</sup>	2.17 ± 0.16 <sup>d</sup>	6.94 ± 0.10 <sup>cd</sup>	1.38 ± 0.04 <sup>ab</sup>
Grey mullet	77.56 ± 0.73 <sup>bc</sup>	82.37 ± 0.47 <sup>b</sup>	7.11 ± 0.20 <sup>b</sup>	9.81± 0.13 <sup>a</sup>	0.71± 0.02 <sup>b</sup>
<b>Microwave</b>					
Nile tilapia	77.97 ± 0.55 <sup>b</sup>	88.2 ± 0.32 <sup>a</sup>	3.45 ± 0.14 <sup>d</sup>	6.81 ± 0.11 <sup>d</sup>	1.54± 0.04 <sup>ab</sup>
Grey mullet	78.60 ± 0.64 <sup>b</sup>	79.86 ± 0.65 <sup>bc</sup>	9.58± 0.20 <sup>b</sup>	9.72 ± 0.14 <sup>a</sup>	0.84 ± 0.05 <sup>b</sup>

<sup>a-d</sup> Means within a column with the same superscript are significantly different (P<0.05).

Values are expressed as Mean ± SE.

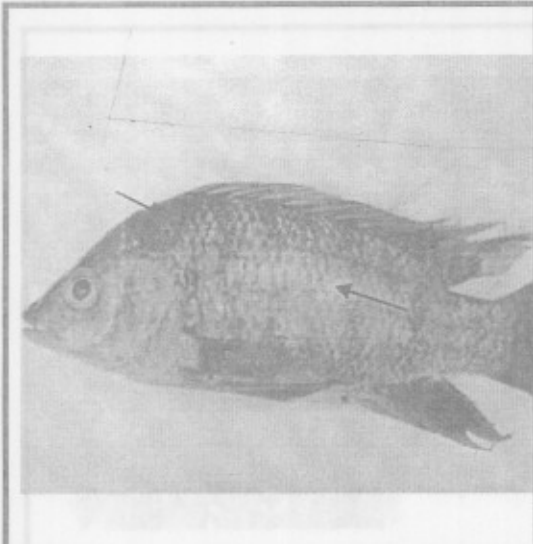


Fig. 1. Nile tilapia showing skin darkening and focal scales loss.

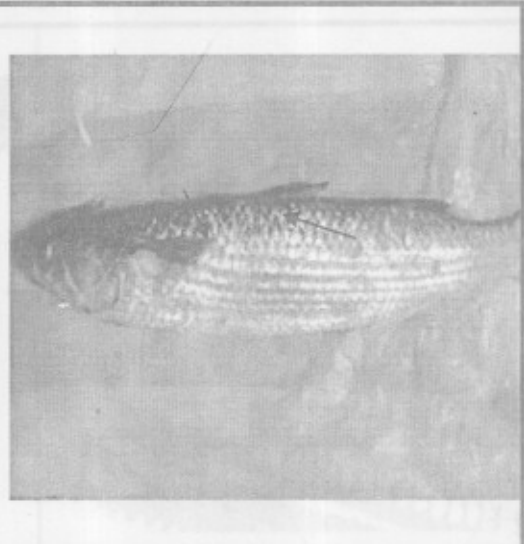


Fig. 2. Grey mullet, showing mild skin darkening.

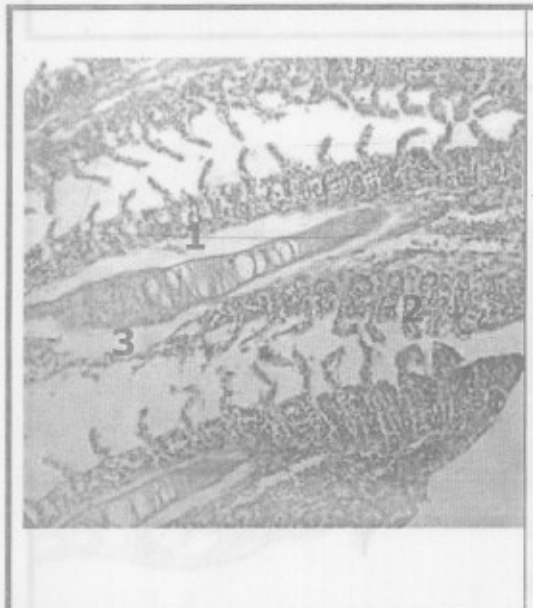


Fig. 3. Gill of Nile tilapia showing edema in primary lamellae<sup>(1)</sup> as well as hyperplasia, fusion<sup>(2)</sup> and focal desquamation of the secondary lamellae<sup>(3)</sup> (H.&E., X 250).

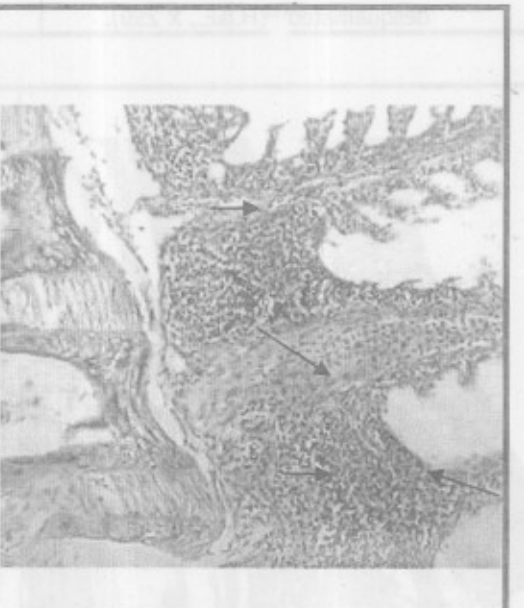
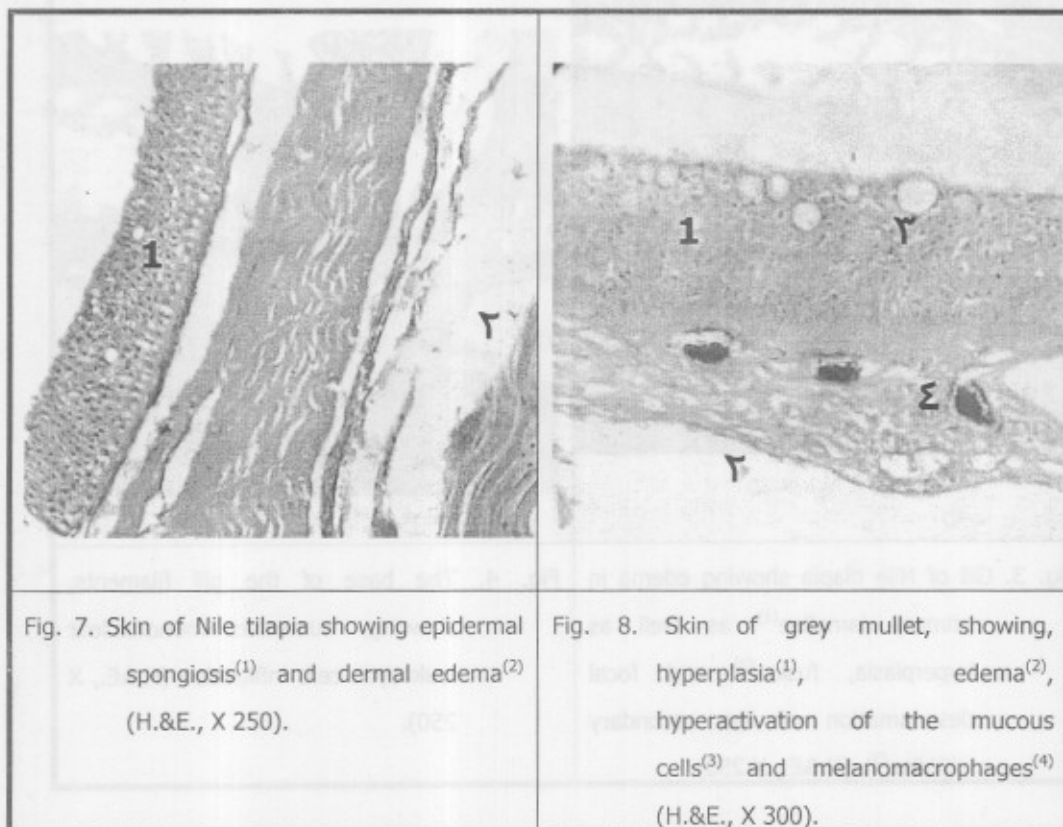
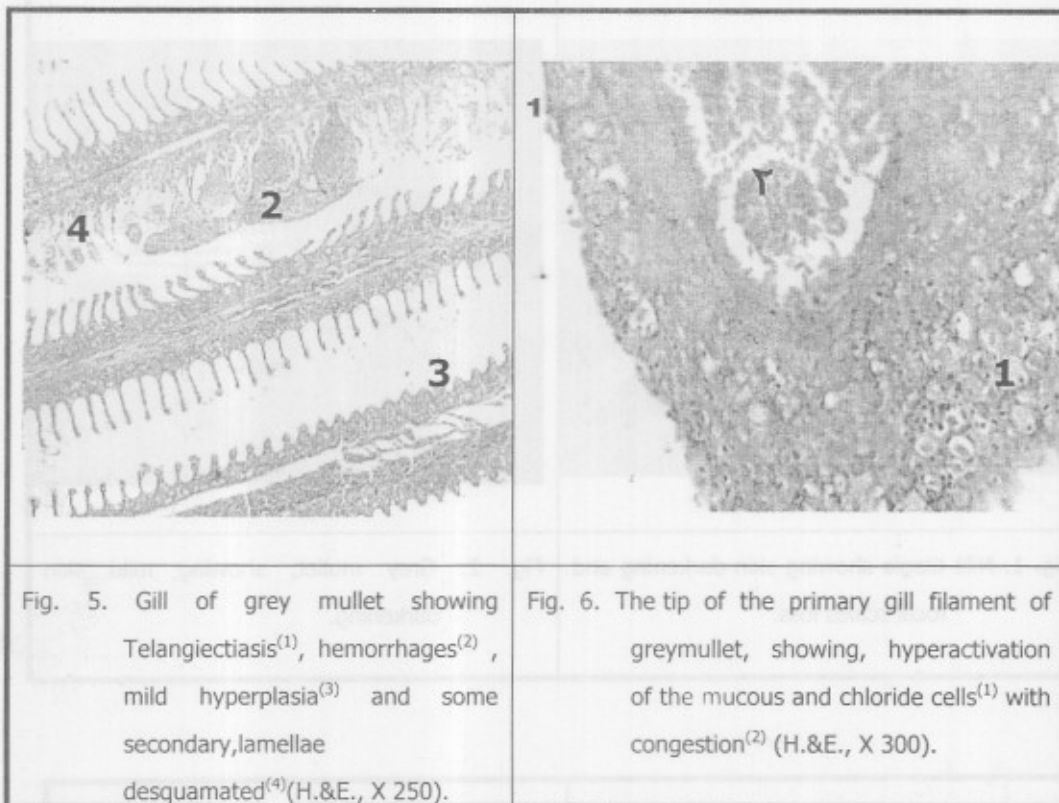


Fig. 4. The base of the gill filaments, showing, numerous mononuclear leukocytic cells infiltration (H.&E., X 250).



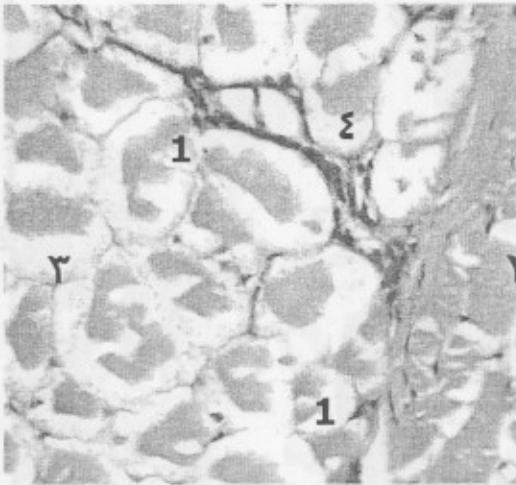


Fig. (9): Muscles of Nile tilapia, showing Zenker's necrosis<sup>(1)</sup>, hyaline degeneration<sup>(2)</sup>, severe edema<sup>(3)</sup> and few leukocytic cells infiltrations<sup>(4)</sup> (H.&E., X 250).

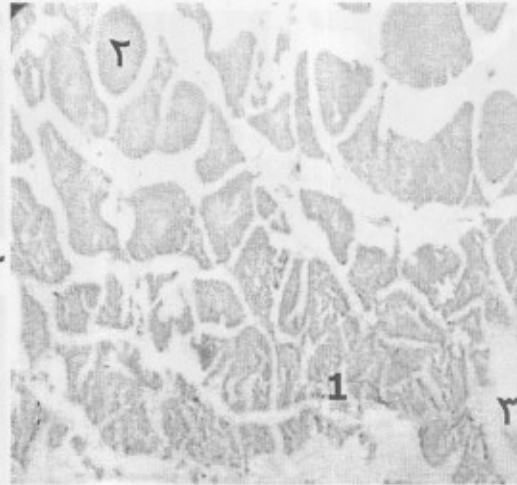


Fig. 10. Muscles of grey mullet, showing, Zenker's necrosis<sup>(1)</sup>, hyaline degeneration<sup>(2)</sup> and edema<sup>(3)</sup> (H.&E., X 250).

REFERENCES

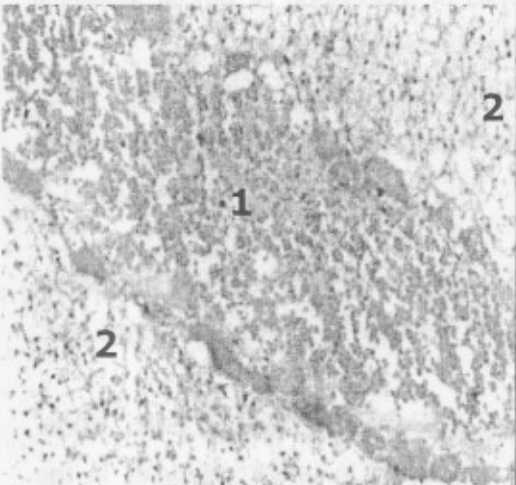


Fig. 11. Liver of Nile tilapia, showing severe congestion<sup>(1)</sup>, vacuolar degeneration and nuclear pyknoses of the hepatocytes<sup>(2)</sup> (H.&E., X 150).

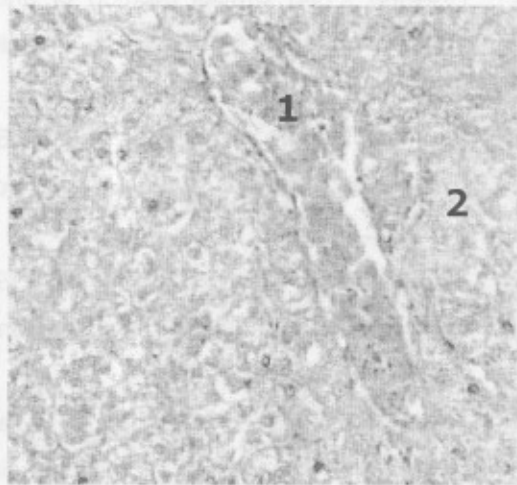


Fig. 12. Liver of grey mullet, showing congested hepatic vein<sup>(1)</sup> and focal coagulative necrosis<sup>(2)</sup> (H.&E., X 250).



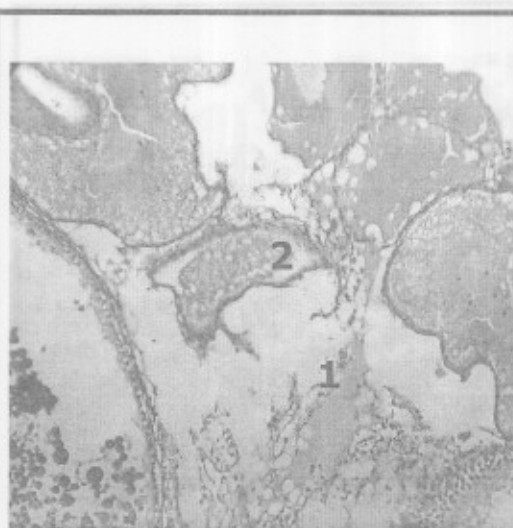


Fig. 13. Ovary of Nile tilapia showing, edema<sup>(1)</sup> and degeneration of some ovarian follicles<sup>(2)</sup>. (H.&E., X 250).

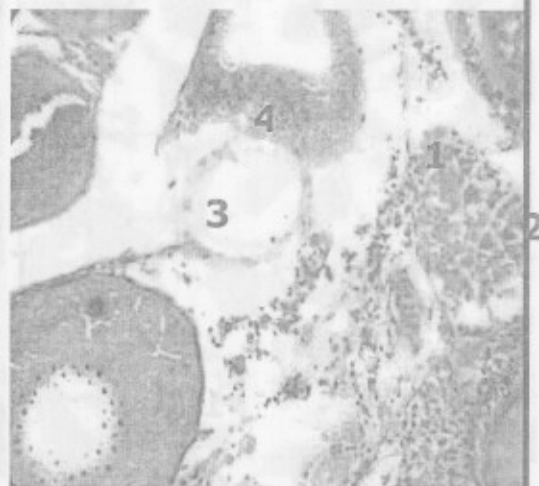


Fig. 14. Ovary of grey mullet showing, stromal hemorrhages<sup>(1)</sup>, hemosiderin deposits<sup>(2)</sup>, edema<sup>(3)</sup>, degeneration and necrosis of some follicles<sup>(4)</sup> (H.&E., X 250).

## REFERENCES

1. A.O.A.C. 2002. Official method of analysis of association of official analytical chemists. Chapter 4 P. 40 vol. 1, 17 edition Bancroft, G.D.; USA.
2. Abd-Allah, M. A., Y. H. Foda, M. H. El-Kalyoubi and L. D. El-Mahdy. 1985. Changes in triglycerides fatty acids of Egyptian Karouss and Teeban fish cooked by different methods. *Annals. Agric. Sci., Fac. Agric. , Ain-Shams Univ.*, 30 (2) 1321-32.
3. Allen-Gil, S. M. and V. G. Martynov. 1995. Heavy metals burdens in nine species of freshwater and anadromous fish from the Pechora River, Northern Russia. *Sci. Total Environ.*, 653 - 9.
4. Alvarado, N. E., I. Quesada, K. Hylland, I. Marigómez and M. Soto. 2006. Quantitative changes in metallothionein expression in target cell-types in the gills of turbot (*Scophthalmus maximus*) exposed to Cd, Cu, Zn and after a depuration treatment. *Aquat. Toxicol.*, 77(1): 64-77.
5. Alvarado, N. E., I. Cancio, K. Hylland, I. Marigómez and M. Soto. 2007. Immunolocalization of metallothioneins in different tissues of turbot (*Scophthalmus maximus*) exposed to Cd. *Histol. Histopathol.*, 22 (7) 719-28.



6. Arias, M. T., E. A. Pontes, M. C. Fernandez and F. J. Muniz. 2003. Freezing/defrosting/frying of sardine fillets. Influence of slow and quick defrosting on protein quality. *J. Sci. of Food and Agriculture*, 83: 602–608.
7. Atta, M. B., L. A. El-Sabaie, M. A. Noaman and H. E. Kassab. 1997. The effect of cooking on the concentration of heavy metals in fish (*Tilapia nilotica*). *Food Chemistry*, 58: 1–4.
8. Bancroft, G. D., A. Stevens and D. R. Turner. 1996. *Theory and Practice of Histopathological Technique*. 4<sup>th</sup> edition, Churchill Livingstone, New York, London, San Francisco and Tokyo.
9. Burger, J., C. Dixon, C. Boring and M. Gochfeld. 2003. Effect of deep-frying fish on risk from mercury. *J. Toxicol. Environ. Health*, 66, (9), 817-28.
10. Castrillón A. M., P. Navarro and E. Alvàrez-Pontes. 1997. Changes in chemical composition and nutritional quality of fried Sardine (*Clupea pilchardus*) produced by frozen storage and microwave reheating. *J. Sci. of Food and Agriculture*, 75: 125-32.
11. Chicourel, E. L., A. M. Sakuma, O. Zenebon and A. T. Filho. 2001. Inefficacy of cooking methods on mercury reduction from Shark. *Arch Latinoam Nutr.*, 51 (3) 288-92.
12. Cid, B. P., C. Boia, L. Pombo and E. Rebelo. 2001. Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electro-thermal atomic absorption spectrometry. *Food Chemistry*, 75: 93–100.
13. De Smet, H. and R. Blust. 2001. Stress responses and changes in protein metabolism in carp (*Cyprinus carpio L.*) during cadmium exposure. *Ecotoxicol. Environ. Saf.*, 48 (3) 255-62.
14. Echarte, M., M. A. Zulet and I. Astiasaran. 2001. Oxidation process affecting fatty acids and cholesterol in fried and roasted salmon. *J. Agric. Food Chemistry*, 49 (11) 5662-67.
15. Egan, H., R. S. Kirk and R. Sayer. 1981. *Pearson's chemical analysis of foods* 8<sup>th</sup> ed., Churchill livingstone. Edinburgh.
16. El-Sebaiy, L. A. and S. M. Metwalli. 1989. Changes in some chemical characteristics and lipid composition of salted fermented Bouri fish muscle (*Mugil cephalus L.*). *Food Chemistry*, 31: 41-50.
17. Ersoy, B., Y. Yanar, A. Küçükgülmez and M. Çelik. 2006. Effects of four cooking methods on the heavy metal concentrations of seabass fillets (*Dicentrarchus labrax Linnæus*). *Food Chemistry*, 99 (4) 748-751.
18. Ez-El-Rigal, A. 2007. Quality evaluation of some fishes in Egyptian markets. *Egypt. J. Agric. Res.*, 85 (3), 1107-16.

19. Fadel, N. G. and H. S. Gaber, 2007. Effect of exposure to pollutants on different organs of two fish species in Rossetta branch at River Nile.Egypt. J. Comp. Path. and Clinic. Path., Vol. 20 No. 1, 364-89.
20. FAO .1983. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fish Circ;464:5-100
21. Fernandes, C., A. F. Fernandes, M. Ferreira and M. A. Salgado. 2008. Oxidative stress response in gill and liver of (*Liza saliens*), from the Esmoriz-Paramos Coastal Lagoon, Portugal. Archives of Environ. Contamin. and Toxicol., 55 (2).
22. Gutenmann, W. H. and D. J. Lisk. 1991. Higher average mercury concentration in fish fillets after skinning and fat removal. J. Food Safety, 11(2) 99 -103.
23. Gokoglu, N., P. Yerlikaya and E. Cengiz. 2004. Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). Food Chemistry, 84, 19-22.
24. Hülya, K., S. A. Oymakb and E. ünlüa. 2004. Heavy metals in mullet (*Liza abu*), and catfish (*Silurus triostegus*), from the Atatu`rk Dam Lake (Euphrates), Turkey. Environ. Interna., 30, 183 - 88.
25. Khalil, M. E., E. K. Moustafa and H. O. Osman. 1980. Composition of bolti (*Tilapia nilotica*) muscle proteins. Food Chemistry, Vol. 5, (2) 175-184.
26. Karadede, H and ünlü E. 2000. Concentrations of some heavy metals in water, sediment and fish species from The Atatu`rk Dam Lake (Euphrates), Turkey. Chemosphere, 41:1371- 6.
27. Mansour, S. A. and M. M. Sidky. 2002. Ecotoxicological studies: 3. Heavy metals contaminating water and fish from Fayoum Gov., Egypt. Food Chemistry, 78: 15-22.
28. McVicar, A. H. 1997. The development of marine environmental monitoring using fish diseases. Parassitologia, 39:177-81.
29. Mela, M., R., M. A. F. Ventura, D. F. Carvalho, C. E. V. Pelletier and C. A. O. Ribeiro. 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish (*Hoplias malabaricus*). Ecotoxicol. Environ. Saf., 68: 426 - 35.
30. Mohana, E. E. 1996. Pathological studies on fish experimentally intoxicated by certain heavy metals. Ph.D. Thesis, Dept. of Path. and Parasit., Fac. of Veter. Med., Alex. Univ.
31. Olojo, E. A., K. B . Olurin, G. Mbaka and A. D. Oluwemim. 2005. Histopathology of the gills and liver tissues of the African catfish (*Clarias gariepinus*) exposed to lead. African J. of Biochemistry, 4 (1) 117 - 22.

32. Ribeiro, C. A., M. Nathalie, P. Gonzalez, D. Yannick, B. Jean-Paul, A. Boudoub and J. C. Massabuau. 2008. Effects of dietary methylmercury on zebrafish skeletal muscle fibers. *Environ. Toxicol. and Pharmacol.*, 25: 304 - 9.
33. SAS. 2000. SAS User's Guide: statistics, SAS Institute INC., Cary, NC.
34. Saldanha, T. and N. Bragagnolo. 2008. Relation between types of packaging, frozen storage and grilling on cholesterol and fatty acids oxidation in Atlantic hake fillets (*Merluccius hubbsi*). *Food Chemistry*, 108: 619-27.
35. Shanbhag, B. A. and S. K. Saidapur. 1996. Atretic follicles and corpora lutea in the ovaries of fishes: Structure -function correlations and significance. In *Fish Morphology – Horizon of New Research*. J.S.D. Munshi and H.M. Dutta (Eds). Science Publishers, Inc. USA., 147-68.
36. Szefer, P., M. D. Wieloszewska, J. Warzocha, A. G. Wesolowska and T. Ciesielski 2003. Distribution and relationships of mercury, lead, cadmium, copper and zinc in Perch (*Perca Fluviatilis*) from the Pomeranian Bay and Szczecin Lagoon, southern Baltic. *J. Food Chemistry*, 81: 73 - 83.
37. Türkkan, A., Cakli S. and Kilinc, B. 2008. Effects of cooking methods on the proximate composition and fatty acid composition of seabass (*Dicentrarchus labrax Linnaeus*). *Food and Bioproducts Processing*, 11, 1-4.
38. Van Dyk, J.C. 2003. Histological changes in the liver of (*Oreochromis mossambicus*) (*Cichlidae*) after exposure to cadmium and zinc. M. Sc. Dissertation, Rand Afrikaans University, South Africa.
39. Varela, G. and B. Ruiz-Rosso 1992. Some effects of deep frying on the dietary fat intake. *Nutr. Rev.*, 50, 256 - 62.
40. Watanabe, K. H., F. W. Desimone, A. Thiyagarajah, W. R. Hartley and A.E. Hindrichs. 2003. Fish tissue quality in the lower Mississippi River and health risks from fish consumption. *Sci. Total Environ.* 302 (1-3) 109 -26.
41. Waters, M. E. 1988. Chemical composition and frozen storage of Weakfish (*Cynoscion regalis*). *Marine Fish Rev.*, 50 (2), 27-33.
42. Weber, J., V. Bochi, C. Ribeiro, A. Victorio and T. Emanuelli. 2008. Effect of different cooking methods on the oxidation, peroxide and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food chemistry*, 106, 140-146.
43. Yazkan, M., F. Özdemir and M. Gölükcu. 2002. Antalya koşullarında avlanan bazı balık türlerinde Cu, Zn, Pb ve Cd içerikleri. *Turkish J. Veterinary and Animal Sciences*, 26, 309 -13.

## متبقيات المعادن والتشويرات النسيجية وطرق طهي الأسماك المستزرعة فى أحواض مياه الصرف

سامية إبراهيم على حسنين

المعمل المركزي لبحوث الثروة السمكية بالعباسة- مركز البحوث الزراعية - مصر

أجريت دراسة حقلية وذلك بتجميع اسماك البلطى النيلى والبورى من أحواض سمك فى منطقة بحر البقر بهدف تقييم الأسماك التي تم تغذيتها على مياه صرف المخلفات الزراعية والصناعية. تم تقدير بعض العناصر (الكادميوم والرصاص والزنك والحديد والمنجنيز) وإجراء الفحص المجهرى لأنسجة الخياشيم والجد والعضلات والكبد والمبايض لسمك البلطى والبورى الطازج. وتمت دراسة تأثير طرق الطهى المختلفة (القلى العميق والشوى على الصاج والشوى فى الفرن والطهى فى الميكروويف) على التركيب الكيميائى ومستوى متبقيات العناصر (الكادميوم والرصاص والزنك والحديد والمنجنيز) لشرائح الأسماك المطهية وأسفرت النتائج عن: وجود تغيرات هستولوجية واضحة مع متبقيات العناصر حيث كان أعلاها فى كبد سمك البورى وبتبعه الخياشيم. كما تعكس النتائج أن طرق الطهى المختلفة للشرائح أحدثت زيادة غير معنوية للكادميوم، والزنك مقارنة بشرائح الأسماك الخام. وجد نقص معنوى فى الزنك و المنجنيز لشرائح سمك البلطى والبورى فى كل طرق الطهى، وأيضاً وجد نقص فى الرصاص فى الشرائح المطهية بالشوى فى الفرن، والطهى بالميكروويف، من حيث التركيب الكيميائى حدثت زيادة تدريجية فى محتوى شرائح الأسماك المختلفة من البروتين والرماد فى كل طرق الطهى، وكان أعلى معدل زيادة فى الدهن للشرائح المطهية بالقلى بينما حدث انخفاض تدريجى لمحتوى الرطوبة للشرائح المطهية بالطرق المختلفة مقارنة بشرائح الأسماك الخام.

يتضح من هذه الدراسة أن أفضل طرق استهلاك اسماك البلطى والبورى كانت المطهيه بطريقة الشوى فى الفرن ثم الشوى على الصاج على الترتيب. وأن تناول شرائح الأسماك المطهيه أفضل من طهى السمكة كاملة وذلك للإقلال من اثر الملوثات البيئية التى تتعرض لها الأسماك المستزرعة فى أحواض مياه الصرف.