

## **EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA (*ORECHROMIS NILOTICUS*)**

**Mohamed El- Sayed Farag**

*Genetics Department, Central Laboratory for Aquaculture Research, Abbassa, Egypt. Agricultural Research center.*

### **ABSTRACT**

One of the main factors which affect the quality of fish is the water quality. Therefore, the present study aims to investigate the impacts of different water sources on the protein electrophoreses of *Oreochromis niloticus* fish reared in three different sources at EL –Abbassa, Sharkia governorate, Egypt. The first source was irrigation water from Ismailia canal, which is the branch of the river Nile; the second from rice field water treated with herbicide Whip-super (field concentration 0.0008 mg/l) and the third was drainage water. Water samples were taken randomly from different sources throughout experimental time and fish samples were taken after 3 months for determination of gonadosomatic, hepatosomatic index and muscle protein fractions.

The results of the 96 hour half-lethal concentration (96 hrs LC50) of the herbicide Whip-super was 0.08 ppm for fingerlings  $1 \pm 0.3$  g of Nile tilapia; *Oreochromis niloticus* and fish exhibited erratic swimming movements, mucus secretion accumulated on the gills and the fish exhibited a respiratory disorder with surfaced swimming, opening their mouth with rapid and frequent exhalation. The field concentration of this herbicide was 0.0008 ppm. Analysis of variance for average water temperature, dissolved oxygen (DO), PH value, water nitrate content and total ammonia revealed that differences among the three tested water sources were significantly high. High levels were in rice water and agriculture drainage water. The differences in concentration of Fe, Cu, Zn and Pb was highly significant and not significant in Cd concentration. On the other hand, the high concentrations of Fe and Cd in agriculture drainage water were  $2.742 \pm 0.72$  and  $0.0016 \pm 0.0001$  mg/l, respectively. While, high concentration of Cu, Zn and Pb in rice water were  $0.46 \pm 0.17$ ,  $0.278 \pm 0.07$  and  $0.0773 \pm 0.02$  mg/l, respectively. High significant showed in male gonadosomatic index and female hepatosomatic index while, non significant difference was found in male hepatosomatic index. The correlation between gonadosomatic index and hepatosomatic index in males and females showed low significance in males and high significance in female.

The densitometric analysis of SDS-PAGE of W.S.P. from muscles showed that the total number of bands ranged from 2-6 bands. The three water sources shared one common band at molecular weight 208 KD and 210 KD and rice water had two specific bands (number 3 & 7). Also, Agriculture drainage water had two specific bands at band number 4&10. However, new band in rice water occurred at relative front of 0.223. This new band might

be attributed to an increased synthesis of acute phase proteins which act as a buffer or as protective proteins against rapid intracellular spread of reactive ions and hence toxicity with heavy metals.

From the present results, it can be concluded that conditions at irrigation water were appropriate for fish rearing and fish are safe for human consumption.

**Keywords:** Nile tilapia, *Oreochromis niloticus*, physical and chemical qualities of water sources, Herbicide, Whip-super toxicity and protein fractionation.

## INTRODUCTION

Water contaminants have a high potential risk for the health of populations. Protection from toxic effects of environmental water pollutants primarily involves considering the mechanism of low level toxicity and likely biological effects in organisms that live in these polluted waters, (Almeida *et al.*, 2002). Aquaculture is the main source of fish production in Egypt; it presents about 61% of the total production (GAFRD, 2006). Fishes are important members of aquatic ecosystem and an important source of food for human. For many reasons, fish often used as test animals in aquatic environmental researches (Katz *et al.*, 1969). Tilapia species have become very important and are cultured in Egyptian fish farms throughout the country. Their economic importance is constantly increasing for their fast growth, disease resistance, different feeding habits and palatability (Dagzie, 1982).

One of the main targets of the Egyptian government is to compensate for the deficiency in meat production by increasing high quality fish production. This production should not only be for the local consumption but also for export. However, different sources of water as agriculture drainage water are usually used in the fish farms in Egypt because of the shortage of water sources. These different water sources have different physical, chemical and biological characteristics (Pulatsu *et al.* 2004) which correspondingly affect the quality of the cultured fish (Ali, 2007). Einemaki and Badawy (2005 & 2006) found that the concentrations of different heavy metals exceeded the maximum permissible concentrations in the tissue of both *Mugil cephalus* and *Ctenopharyngodon idella* raised in water environment which received water from different sources. It is known that fish can store about 58-93% of pesticides in their tissues

There are many techniques which have been used to identify and characterize different genera, species, and individuals and develop finger-prints for different

fishes. Isozyme polymorphism and protein banding patterns in addition to the molecular study have been described. Smith (1990) reported that, gel electrophoresis is a powerful technique for fish-stock identification. The protein electrophoresis was successfully employed to study the variations among marine fish populations.

Abdel-Gawad et al. (1997) recorded that the electrophoretic study gave evidence of the great genetic divergence between *Solea vulgaris* and *Solea aegyptiaca* from Abu-Kir Bay (Alexindria). The high value of genetic distance of *Solea aegyptiaca* populations from Abu-Kir Bay and Qarun Lake (Fayowm) showed a markedly genetic variability, which may be induced as a result of the environmental differences between these two habitats for many years and exhibited contrasting selection pressures on fish genome for inducing genetic variations to reflect adaptation to local conditions. This degree of biogenetic variations indicated that the solea fish from Qarun Lake became a distinct population. Frati *et al.* (1992) reported that the environmental pollution may affect genetic variation in population inhabiting polluted sites. The same effect of differences in genetic variations and define the electrophoretic pattern of fish protein (*O. niloticus*) in Lakes Maryout and Nozha may be induced by the difference between the two habitats in heavy metal concentrations (El-Demerdash. and Elagamy, 1999). Kamel (1999), using SDS-PAGE of soluble muscle proteins to study genetic variations among three strains of *Oreochromis niloticus* collected from different locations in Egypt and found that Maryout strain is genetically distinct from both Abbassa and Zawia strain (Kafer El-Saeikh). Roark and Brown (1996) suggested using the genetic structure and variability as a bioindicators of heavy metal contamination. This could represented promising tool for genetic monitoring of environmental stressors on the allozymic patterns according to Virgilio, *et al.* (2003).

The objective of the present investigation is to study the effect of herbicide Whip-super (field concentration 0.0008 mg/l) and different water quality on protein fractionation of *Oreochromis niloticus*.

**MATERIALS AND METHODS****1 - Description of the study area:**

A group of apparently healthy Nile tilapia (*Oreochromis niloticus*) fingerlings having a body weight of  $1 \pm 0.3$  g was brought from the hatchery of the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia. These group fingerlings was transformed in aerated holding tanks and then accommodated into aerated fiber glass tanks  $1\text{m}^3$ . The dissolved oxygen was kept at 5.7 mg/l, the temperature at  $26 \pm 2$  C° and the pH at  $7.2 \pm 0.2$  acclimatized under laboratory conditions for acclimation for one day.

A number of 300 fingerlings were used in glass aquaria of 100-liter capacity to study the 96 hour half-lethal concentration (96 hrs LC50) of the herbicide Whip-super (Whip-super-75 EW) Ethyl ® -2-(4-((6-chloro-2-benzoxazolyl) oxy) phenoxy) propionate is aqueous solution containing phenoxy propionate Bara Ethyl 7.5 % as active material produced by Co., Agrevo-Berlin\_Germany. The Whip-super herbicide is an aqueous solution used to kill any weeds in rice fields in fresh water according to Behreus and Karbeur (1953) in order to compare it with the filed concentration.

The rest of fingerlings were randomly assigned into three groups in order to be raised in three different water sources. The first group of fingerlings were stocked into three earthen pond ( $1000\text{ m}^3$ ) each irrigated by fresh water received from Ismailia canal.

The second group of fingerlings were cultured in another three similar earthen pond however supplied with agriculture drainage water from El-Wadi drain.

A third group of the same fingerlings were transported to a rice filed irrigated with fresh water treated by herbicide (Whip-super) field concentration (0.0008 mg/l) in Ibrahymia city 50 Km away from Abbassa. After acclimation in cloth hapa in the irrigation water could for one day, the healthy fingerlings were stocked in the rice filed.

Stocking density of the three types of water sources was two fingerlings /  $\text{m}^3$  a normal diet 25% protein was used for feeding the fingerlings at feeding rate of 3% of the body weight for three months.

EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA  
(*ORECHROMIS NILOTICUS*)

**2 - Sampling procedures:**

Water samples were taken randomly through 3 months from the three sources of water for analytical procedures to measure the physico-chemical parameters and heavy metals residues in water; iron (Fe) Copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb). Fish samples were taken from the three sources of water after three months for muscle protein electrophoreses, gonadosomatic and hepatosomatic index.

**3 - Analytical methods:**

Physico-chemical parameters of water were measured as follows:

**PH:**

PH was measured using glass electrode pH meter (Digital Mini-pH meter, Model 55).

**Temperature and dissolved oxygen (DO):**

Temperature and dissolved oxygen were measured at the site of sampling using an oxygen meter (model 58 YSI).

**Total ammonia and deionized ammonia:**

Total ammonia concentration was measured by Hach comparison apparatus following the method reported by **APHA, (1985)**.

**Nitrate NO<sub>3</sub>-N:**

Nitrate-nitrogen was measured by phenoldisulphonic acid method according to Boyd (1984). The spectrophotometer model used was Milton roy 21D and the resulting color was measured at 410 nm wave length.

**Heavy metals:**

The heavy metals; Fe, Cu, Zn, Cd and Pb in water were measured using atomic absorption spectrophotometer (Thermo 6600, thermo electron corporation, Cambridge, UK).

**4- Water –Soluble Protein (WSP) Electrophoresis: -**

Samples of 0.5 g of fish muscle representative to the three different water habitats were prepared for electrophoretic analysis by Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) as performed according to the

method of Studier (1973). Each sample was mixed with 0.5 ml of sample buffer and homogenized and centrifuged for 5-10 minutes at 12000 r.p.m. A volume of 50  $\mu$ l of extraction was added to the same volume of 2x Laemmli buffer. Mercaptoethanol was added to each tube (10%v/v, for each sample). All samples were then boiled in a water bath for 10 minutes at 100 °C and loaded on the gel after adding one drop of bromophenol blue (0.025%). The next step was to pour 4.2 liters of the run buffer into a running tank to be precooled by flowing tap water through cooling tubes. A new 800 ml of the run buffer were added to cover the gels. Gels were run at 175V for 10 minutes and then were raised to 200 V for two hours. Gels were placed in 100 ml of Coomassie Brilliant Blue R 250 staining solution for one hour, removing the staining solution from tray, 200ml-destining solution was added. Gel was agitated for three times become clear. The gel was photographed and diagrammatically illustrated.

#### **5 - Statistical analysis of data:**

Statistical analysis was performed using the Analysis of variance (ANOVA) one way classification and Duncan's multiple Range Test, to determine differences between treatment means at significance level of  $P < 0.05$ . All statistics were carried out using Statistical Analysis program (SAS, 2000).

## **RESULTS AND DISCUSSION**

### **physico-chemical analysis of water**

Analysis of variance for overall average physico-chemical parameters of water were shown in Table (2).

#### **Temperature:**

Water temperature is one of the most influencing environmental factors affecting pond dynamic and both the metabolism and growth of fish Herzing and Winkler (1986). Boyd (1990) mentioned that, water temperature in fish ponds is related to solar radiation and air temperature. In the present study, analysis of variance for average water temperature in Table (2) revealed that rice field water showed significantly higher average temperature ( $23.1 \pm 1.007$  °C) compared to ponds receiving irrigation water ( $21.16 \pm 1.091$  °C) and agriculture water sources

EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA  
(*ORECHROMIS NILOTICUS*)

( $20.2 \pm 1.33$  °C). Generally water temperature in agriculture drainage pond, rice water and irrigation water was in favorable range for fish culture described by Boyd (1990).

**Dissolved oxygen (DO):**

Average DO concentration (mg/l) as affected with the water supply was significantly different. The rice field water had significantly ( $p < 0.05$ ) higher dissolved oxygen ( $6.6 \pm 0.49$  mg/l) compared to those with irrigation water ( $5.765 \pm 0.35$  mg/l), and ponds with agriculture water ( $4.86 \pm 0.78$  mg/l) Table (2).

The higher value of DO in the rice filed water may be due to the abundance of phytoplankton that increased photosynthetic activity leading to production of large amount of DO. The recorded dissolved oxygen in the three groups of water was favorable for fish culture (Boyd, 1990, Abdel-Tawwab *et al.*, 2007 and Ali, 2007).

**PH:**

There was significant differences in between the three tested groups of water ( $p < 0.05$ ). PH value reading in Table (2), revealed a higher value for agriculture drainage water ( $8.82 \pm 0.44$ ) against that of irrigation ponds water ( $7.85 \pm 0.35$ ) and rice field water ( $8.125 \pm 73$ ). The overall mean PH values were significantly higher in the agriculture drainage water compared to irrigation water and rice field water. This may be because the agriculture drainage water had higher nitrogen and phosphorus concentrations which contained more abundance of phytoplankton than the irrigation water. The present result agreed with those of Saeed (2000) and Ali (2007).

**Nitrate  $\text{NO}_3\text{-N}$ :**

The different water sources showed significant effect on water nitrate content. Ponds of agriculture drainage water showed higher nitrate content ( $0.19 \pm 0.03$  mg/l) than those that received irrigation water ( $0.127 \pm 0.06$  mg/l) and rice field water  $0.042 \pm 0.008$  mg/l. This may be due to that agriculture water was rich in nitrate content. Moreover, the high level of ammonia in agriculture water may had been nitrified to nitrate (Gross *et al.* 2000).

**Total ammonia (NH<sub>3</sub> + NH<sub>4</sub>):**

Analysis of variance for overall average of total ammonia and unionized ammonia in Table (2), showed that the differences among the three tested group of water were significant ( $p < 0.05$ ). Rice field water showed lower value of total ammonia which was  $1.23 \pm 0.12$  mg/l, pond receiving irrigation water showed higher value ( $3.42 \pm 0.71$  mg/l) and those receive agriculture drainage water recorded  $2.26 \pm 0.52$  mg/l.

The lower ammonia concentration in the rice field water may be due to rice culture and transformation of a large part of nitrogen into fish protein by phytoplankton (Vymazal, 1995).

**The 96-hour half lethal concentration and clinical signs:**

After exposure of Nile tilapia; *Oreochromis niloticus* to different concentrations of the Whip-super herbicide, the 96- hour half lethal concentration (96-hr LC50) was found to be 0.08 ppm for *Oreochromis niloticus*. During determination of the 96-hr LC50, fish exhibited erratic swimming movements. The mucus secretion appeared to increase and accumulated on the gills and the fish exhibited a respiratory disorder with surfaced swimming, opening their mouth with rapid and frequent exhalation. The erratic swimming and frequent surface movements can be attributed to hypercontraction of the muscles due to cholinesterase inhibition as previously reported by Ferguson (1989). Also, Post (1987) reported that surfacing and floating of the fish on the surface of water may be due to impairment in gas bladders, while Atallah *et al.* (1997) attributed such changes to the extraordinary need for oxygen which could be attributed to coating of the gills with profuse mucus together with congestion and hyperplastic epithelium of the secondary lamellae. These results agreed with those of Mousa (2004) who reported that wide variety of pollutants at low pH as well as stress from handling, stimulate increasing secretion by mucus glands in the gills and skin. Toxins might be responsible for sluggish movement due to its effect on brain and nerves. These results agreed with Salem *et al.* (1989). Also, it may be responsible for skin darkening as its reflex appeared in fish against any toxins and this agreed with StosKopf (1993). Exophthalmia might be developed due to the direct effect of



EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA  
(*ORECHROMIS NILOTICUS*)

biologically active metabolites or toxic substance on the endothelial lining of blood vessels leading to escape of blood fluids into beds and cavities like eye (Olufemi & Roberts, 1986).

**Heavy metals in water:**

In differences concentration of Fe, Cu, Zn and Pb was significantly high in three water sources and not significant different for Cd concentration was detected as shown in Table (3). Agriculture drainage water was characterized by higher concentrations of Fe and Cd ( $2.742 \pm 0.72$  and  $0.0016 \pm 0.0001$  mg/l, respectively), while rice field water maintained higher levels of Cu, Zn, and Pb than the other two water sources ( $0.46 \pm 0.17$ ,  $0.278 \pm 0.07$  and  $0.0773 \pm 0.02$  mg/l, respectively). On the other hand, low concentrations of Fe, Cu, Zn and Pb were maintained in irrigation water ( $0.163 \pm 0.013$ ,  $0.0051 \pm 0.0006$ ,  $0.0787 \pm 0.2$ ,  $0.0125 \pm 0.0005$  and  $0.0014 \pm 0.0001$  mg/l, respectively). This finding agreed with Malcolm, (1995) who stated that toxicity of heavy metals is usually reduced as pH increase because at higher pH metals bind to form hydroxide and carbonate complexes which are considered less toxic to fish rather than the metal ion. Moreover, concentration of Fe in the three water groups ( $2.7415 \pm 0.72$  mg/l) in ponds receiving agriculture water and rice field water ( $0.5283 \pm 0.198$  mg/l) were higher in the present study than the permissible levels (0.3 mg/l) according to USEPA (1986).

**Gonado and hepatosomatic index of male and female**

There was significant differences among values of hepatosomatic and gonadosomatic index in males of the three types of water. While, in females, significant differences in mean value of gonadosomatic index in the three types of water was noticeable and no significance difference was observed regarding hepatosomatic index in the three types of water as shown in Table (3). On the other hand, the correlation was no significance between gonadosomatic and hepatosomatic index in males and females in the three types of water.

Fish raised in irrigation water were characterized by low gonadosomatic index were observed in females ( $0.595 \pm 0.25$  mg %). Also, both males and females had low hepatosomatic index ( $0.69 \pm 0.2$  and  $280.60 \pm 0.1$  mg %, respectively). On

the other hand, higher mean values of gonadosomatic index in females ( $1.54 \pm 0.68$  mg %) and hepatosomatic index in males ( $1.30 \pm 0.011$  mg %) was detected for fish raised in rice field. While, significant increase was noted in female hepatosomatic index in draining water was noticeable  $0.92 \pm 0.13$  mg%. These results agreed with Hanson *et al.* (2007) who indicated that the pesticides (lindane, pentachlorophenol and propoxur) had adverse effects on the general growth and reproduction of fish as shown by gonadosomatic indices of *Oreochromis niloticus*, *Clarias gariepinus*, and *Chrysichthys nigrodigitatus*. While, Barakate, (2004) found that, the duration between periods of spawning was increased from 30-36 to 55-75 days in control and contaminated groups, respectively, and the average value of eggs per spawn was decreased from 162-195 in the control groups to 120-170 in the contaminated ones

#### **Water –Soluble Protein (WSP) Electrophoresis: -**

Densitometric analysis of SDS-PAGE of W.S.P. from muscles of *Oreochromis niloticus* showed band numbers, relative front and molecular weight of each individual in Table (5). The protein banding pattern of the five samples collected from three water sources are given in Figures (1-3).

Banding patterns of proteins drawn by the electrophoresis techniques are the satisfactory methods which helped to formulate a better idea about the physiological and genetical effects and the action of chemicals such as heavy metals. In the present study, densitometric analysis of SDS-PAGE of W.S.P. from muscles of *Oreochromis niloticus* showed that the total number of bands from 2 to 3 in irrigation water, from 5 to 6 in rice water and from 2 to 5 in agriculture drainage water. The three water sources shared two common bands at molecular weight 208 KD and 210 KD. These bands could be used as a marker bands for *Oreochromis niloticus*. This agreed well with Kamel (1999) reported that the number of bands ranged from 11-14 for the three parental strains Abbassa, Zawia and Maryout. The three strains shared five common bands at mobility of 0.01, 0.05, 0.16, 0.22 and 0.33. These bands could be used as marker bands for *O. niloticus* nevertheless. Also, this disagreed with Ibrahim (2004) who reported that some protein bands were missing while others appeared as new bands when *O. niloticus* were exposed to different concentrations of copper and lead. Abdel-Tawwab, *et al.* (1988)

EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA  
(*ORECHROMIS NILOTICUS*)

recorded that no differences could be scored among fish populations collected from different locations in Egypt on the basis of water-soluble muscle protein. It is well established that different results obtained might be due to different experimental conditions.

On the other hand, Rice filed water had two specific bands at bands number 3 & 7 and molecular weight of 183 and 103 KDa. These new bands might be a result of the high concentration stress of Cu, Zn and Pb in rice filed water. Also, Agriculture drainage water had two specific bands at band numbers 4&10 and molecular weights of 176 and 35 KDa. This observation agree partially with the results of Farag *et al.* (2007) who reported that the electrophoretic patterns of muscles of common carp *Cyprinus carpio* after exposure to 1/2 field and field concentrations of the herbicide (basegran and whebsuper) for 90 days disclosed the presence of new fractions which appeared at relative front of 0.055 & 0.15. These new bands might be a result of the genotoxic effects of herbicide and some unexpressed genes that might have been induced to be expressed to face such effect. This also agree with Rashed, *et al.* (1992) who reported that the change detected in the muscle protein electrophoresis of *Oreochromis* species after exposure to organophosphorus insecticides might be a result of pesticide stress, and some unexpressed genes might have come to be expressed to counteract such effect. They showed also, that time of exposure and concentration of compound enhanced the protein content. El-Demerdash and Elagamy (1999) reported that *O. niloticus* from Lake Maryout contained higher concentrations of cadmium than those from Nozha with marked difference in electrophoretic patterns of protein in both sites.

A new band in rice water was observed at relative front 0.223 and molecular weight 165.262 KDa. This new band in this study might be attributed to an increased synthesis of acute phase proteins which act as a buffer or as protective proteins against rapid intracellular spread of reactive ions and hence toxicity with heavy metals. This suggestion was in accordance with that of Thanai (1994) and Rizkalla, *et al.* (1997) who studied that the genotoxic effects of different doses of organophosphorus compound (Hinosan) and carbamate (Sevin) for 30 days on the

electrophoretograms of sarcoplasmic proteins of common carp (*Cyprinus carpio*) with regard to the number, mobility and density of fractions. Hinosan exposed specimen's yielded more bands than control. On the other hand, in sevin treated specimens, new bands appeared. Also, some bands disappeared and other appeared. In addition, Manna and Mukherjee (1986) reported some variation in protein bands number, density, and mobility. Straus and Griffin (2001) reported that toxicity of copper is reduced logarithmically as a linear function of total alkalinity, so it can be extremely toxic to fish in water of low alkalinity.

Concurrent the electrophoretic investigation showed complete disappearance of fraction number 2, 6 and 8 at relative fronts of 0.081, 0.321 and 0.557 for fish of agriculture drainage water group. The reduction in protein bands sometimes occurred due to the increase of protein breakdown as a result of toxicants to compensate the increase in energy demands. Our results agreed with Rizkalla, *et al.* (2005) who noted the disappearance of three fractions (3&12) when *Oreochromis aureus* was treated with 3 mg aflatoxin B1 for 90 days and fraction 9 when it treated with 3 mg aflatoxin B1+ selenium for 120 days and fraction 3 when fish were treated with 3 mg aflatoxin B1+ vitamin E for 90days. While, Ahmed (1990) studied the effect of insecticide tamaron on muscle proteins electrophoresis of *O. niloticus* and *O. aureus*. The control samples exhibited six bands with different densities and intensities. Band 4 was missing after exposure to different concentrations of tamaron for different exposure times. The opposite trend was observed in the two species (*S. galeellius* and *T. zilli*) where a new band was detected (band 7 in *S. galeellius* and band 1 in *T. zilli*). These new bands might be a result of the pesticide stress. Some unexpressed genes might have come to be expressed to face such effect. Also, Farag (2001) reported that densitometric analysis of SDS-PAGE of W.S.P. from muscles of *Oreochromis niloticus* showed that disappearance of two common bands at relative mobility 0.03 and 0.27 from both Altal Alkaber and Manzalla when iron, lead, zinc, copper and nitrite increased in these two locations. The missing of these bands might be due to the effect of heavy metals stress in water of this location. On the other hand, Sharf-Eldeen and Abdel-Hamid (2002) mentioned that the exposed fish to copper resulted in the

EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA  
(*ORECHROMIS NILOTICUS*)

disappearance of some protein fractions and changed relative electrophoretic mobility which might indicate genetic damage.

**Dendogram analysis:**

The dendogram was obtained from sorting the electrophoretic patterns of proteins. It is interesting to note from Figure (3) and Table (5) that irrigation water fish was quite different from both draining water fish and rice water fish, where they were divided into three groups, one group to rice water fish (G2), the second group for draining water fish (G3) and the third group was divided to two main subgroups, the first divided to two subgroups had irrigation water fish (G1) and draining water fish (3), and the second had rice water fish (2) and draining water fish (3). This result agreed well with high concentrations of Fe and Cd in agriculture drainage water and also, high concentration of Cu, Zn and Pb in rice water, while, low concentrations of Fe, Cu, Zn, Pb and Cd in irrigation water. Moreover, the results agreed well with the band numbers ranged from 2-3 in irrigation water, ranged from 5-6 in rice water and ranged from 2-5 in agriculture drainage water.

From the present results, it can be concluded that. Conditions at irrigation water were appropriate for fish rearing.

**Table (1): Chemical analysis of diet on dry matter basis.**

| <b>Items</b>                  | <b>(%)</b>    |
|-------------------------------|---------------|
| <b>Moisture</b>               | <b>10.34</b>  |
| <b>Crude protein</b>          | <b>25.51</b>  |
| <b>Ether extract</b>          | <b>6.08</b>   |
| <b>Ash</b>                    | <b>13.13</b>  |
| <b>Crude fiber</b>            | <b>5.59</b>   |
| <b>Carbohydrates</b>          | <b>39.35</b>  |
| <b>Gross energy (KCal/kg)</b> | <b>4537.7</b> |

Table (2): Mean  $\pm$  standard deviation of physical parameters of the different water sources.

|                                 |          | Temperature | O <sub>2</sub> | ph     | Nitrate (mg/l) | N(NH <sub>4</sub> ) (mg/l) |
|---------------------------------|----------|-------------|----------------|--------|----------------|----------------------------|
| Irrigation water pond           | Mean     | 21.16       | 5.765          | 7.85   | 0.127          | 3.42                       |
|                                 | $\pm$ SD | 1.091b      | 0.35b          | 0.35b  | 0.06b          | 0.71a                      |
| Rice water                      | Mean     | 23.1        | 6.6            | 8.125  | 0.042          | 1.23                       |
|                                 | $\pm$ SD | 1.007a      | 0.49a          | 0.73b  | 0.008c         | 0.12c                      |
| Agriculture drainage water pond | Mean     | 20.2        | 4.86           | 8.82   | 0.19           | 2.263                      |
|                                 | $\pm$ SD | 1.33b       | 0.78c          | 0.44a  | 0.03a          | 0.52b                      |
| F - value                       |          | 16.459      | 23.446         | 8.8451 | 33.417         | 45.721                     |
| Significant                     |          | ***         | ***            | **     | ***            | ***                        |

Table 3: Mean  $\pm$  standard deviation of chemical parameters of the different water sources.

|                            |          | Fe     | Cu      | Zn     | Pb      | Cd       |
|----------------------------|----------|--------|---------|--------|---------|----------|
| Irrigation water           | Mean     | 0.163  | 0.0051  | 0.0787 | 0.0125  | 0.0014   |
|                            | $\pm$ SD | 0.013b | 0.0006b | 0.02c  | 0.0005b | 0.0001b  |
| Rice water                 | Mean     | 0.5283 | 0.46    | 0.2783 | 0.0773  | 0.0015   |
|                            | $\pm$ SD | 0.198b | 0.17a   | 0.07a  | 0.02a   | 0.0002ab |
| Agriculture drainage water | Mean     | 2.7417 | 0.0085  | 0.1589 | 0.018   | 0.0016   |
|                            | $\pm$ SD | 0.72a  | 0.003b  | 0.07b  | 0.001b  | 0.0001a  |
| F - value                  |          | 63.121 | 44.974  | 17.11  | 96.376  | 2.5      |
| Significant                |          | ***    | ***     | ***    | ***     | Ns       |

Ns = Non significant

EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA  
(*ORECHROMIS NILOTICUS*)

Table (4): Effect of water sources on gonado and hepatosomatic index of male and female Nile tilapia (*Oreochromis niloticus*).

| Treatment        |      | Hepatosomatic index |        | Gonadosomatic index |       |
|------------------|------|---------------------|--------|---------------------|-------|
|                  |      | Female              | Male   | Female              | Male  |
| Irrigation water | Mean | 0.60                | 0.69   | 0.595               | 0.27  |
|                  | SD±  | 0.12b               | 0.28c  | 0.25b               | 0.18a |
| Rice water       | Mean | 0.73                | 1.30   | 1.54                | 0.16  |
|                  | SD±  | 0.18b               | 0.011a | 0.68a               | 0.02b |
| Draining water   | Mean | 0.92                | 0.91   | 0.53                | 0.10  |
|                  | SD±  | 0.13a               | 0.04b  | 0.17b               | 0.09b |
| F – value        |      | 12.51               | 36.91  | 17.24               | 5.23  |
| Significant      |      | ***                 | ***    | ***                 | *     |

MOHAMED EL- SAYED FARAG

Table (5): Densitometric analysis of SDS-PAGE of W.S.P from muscles of Nile tilapia (*Oreochromis niloticus*) collected from three different water sources showing band number, relative front and calibrated molecular weight.

| Lane Number | Relative Front | Mol. Wt. KDa | Irrigation water (G1) |     |     |     |     | Rice water (G2) |     |     |     | Agriculture drainage water (G3) |     |     |     |     |    |
|-------------|----------------|--------------|-----------------------|-----|-----|-----|-----|-----------------|-----|-----|-----|---------------------------------|-----|-----|-----|-----|----|
|             |                | Marker       | 1                     | 2   | 3   | 4   | 5   | 6               | 7   | 8   | 9   | 10                              | 11  | 12  | 13  | 14  | 15 |
| 1           | 0.060          | 210          |                       |     |     | 210 | 210 |                 |     |     |     | 210                             | 210 | 210 | 210 | 210 |    |
| 2           | 0.081          | 208          | 208                   | 208 | 208 |     |     | 208             | 208 | 208 | 208 |                                 |     |     |     |     |    |
| 3           | 0.149          | 183          |                       |     |     |     |     | 183             | 183 | 183 | 183 |                                 |     |     |     |     |    |
| 4           | 0.150          | 176          |                       |     |     |     |     |                 |     |     |     | 176                             |     | 176 | 176 | 176 |    |
| 5           | 0.223          | 165          |                       |     |     |     |     | 165             |     |     |     |                                 |     |     |     |     |    |
| 6           | 0.321          | 144          | 144                   | 144 | 144 |     |     | 144             | 144 | 144 | 144 |                                 |     |     |     |     |    |
| 7           | 0.397          | 103          |                       |     |     |     |     | 103             | 103 | 103 | 103 |                                 |     |     |     |     |    |
| 8           | 0.557          | 84           | 84                    | 84  | 84  |     |     | 84              | 84  | 84  | 84  |                                 |     |     |     |     |    |
| 9           | 0.654          | 78           |                       |     |     | 78  |     |                 |     |     |     | 78                              |     | 78  | 78  | 78  | 78 |
| 10          | 0.702          | 35           |                       |     |     |     |     |                 |     |     |     | 35                              | 35  | 35  | 35  | 35  |    |
| 11          | 0.819          | 21           |                       |     |     | 21  | 21  |                 |     |     |     | 21                              | 21  | 21  | 21  | 21  | 21 |



EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA (*ORECHROMIS NILOTICUS*)

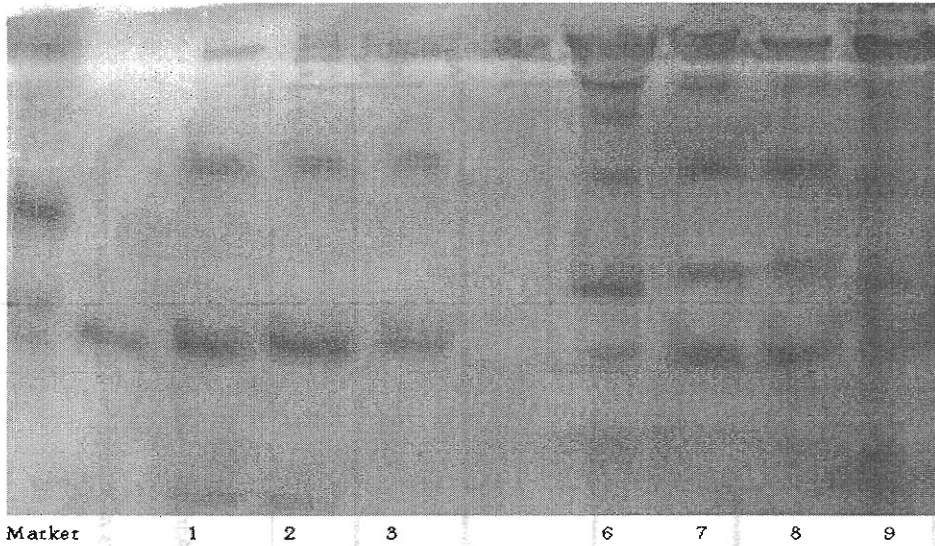


Fig. (1): Variation in electrophoretic patterns for muscle protein of Nile tilapia (*Oreochromis niloticus*) collected from three different water sources.

1, 2 & 3 – Muscle from irrigation water

6,7,8&9 – Muscle from rice water

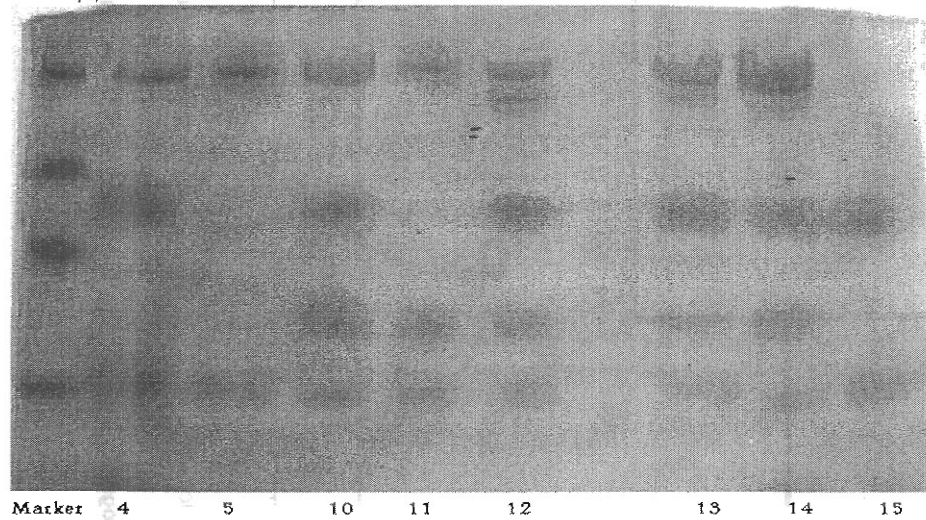


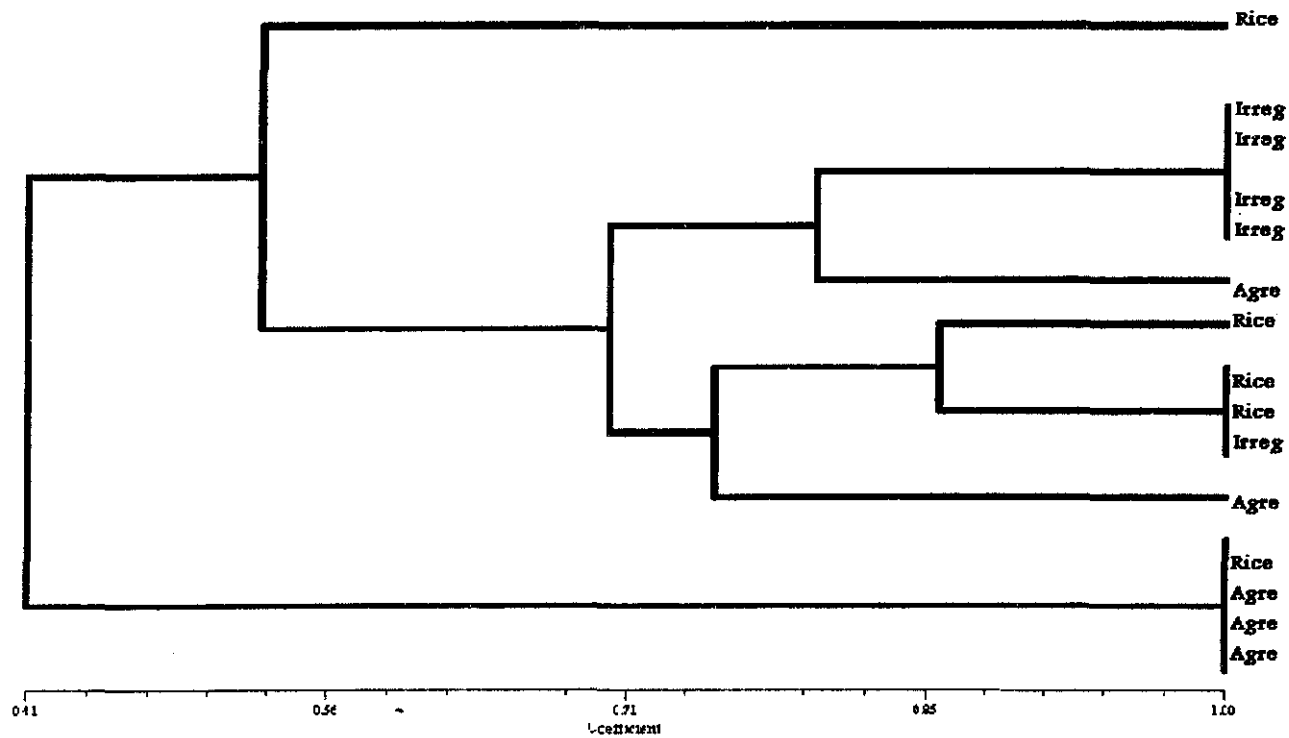
Fig. (2): Variation in electrophoretic patterns for muscle protein of Nile tilapia (*Oreochromis niloticus*) collected from three different water sources.

4 & 5 – Muscle from irrigation water

10 – Muscle from rice water

11,12,13,14 & 15 – Muscle from agriculture drainage water

MOHAMED EL- SAYED FARAG



Rice = Rice field water

Irreg = Irrigation water

Agre = Agriculture drainage water

Fig (3): Phenogram demonstration the relationship of Nile tilapia (*Oreochromis niloticus*) from three water sources.

## REFERENCES

- Abd-Gawad, A.M.; Abderrassoul, H.A.; Bakhoum, SH.A. and Ragheb, E. 1997. Genetic distance on electrophoretic isoesterases of two sole species from Mediterranean sea and Lake Qarun, Egypt. Bull. Nat. Ins. Of Oceanogr. And Fish . A.R.E., Vol. (23): 187-202.
- Abdel-Tawwab, M., A. E. Abdelghany, and M. H. Ahmad. 2007. Effect of diet supplementation on water quality, phytoplankton community structure, and the growth of Nile tilapia, *Oreochromis niloticus* (L.), common carp, *Cyprinus carpio* L., and silver carp, *Hypophthalmichthys molitrix* V. polycultured in fertilized earthen ponds. J. Appl. Aquac., 19. 1: 1-24.
- Abdel-Tawwab, F. M.; Rashed, M.A. and Abdel-Hamid, A.A. 1988. Phylogenetic relationships in some species of genus tilapia in Egypt. Proc. 2nd Conf. Agric. Develop. Res., Ain Shams Univ.
- Ahmed, E. G. 1990. Effect of Environmental Pollutants on The Genetic System in Fish. M.Sc. Department of Genetics Faculty of Agriculture Ain Shams University.
- Ali, A. N. 2007. Ecological study on some water characteristics used in fish farms and their relation to fish productivity. Ph. D. Thesis. Al-Azhar Unive. Fac Sci.. Chemistry dept. Egypt.
- Almeida, J.A., Diniz, Y.S., Marques, S.F., Faine, L., A., Ribas, B.O., Burneiko, R.C. and Novelli, E.L.,(2002): The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ. Int. 27 (8): 673-679.
- P. H. A. 1985. Standard methods for examination of water and waste water. Amrican puplic health association. Washington. D. D., USA.
- Atallah, O. A.; A. M. Ali; A. S. Ibrahim and S. F. Sakr (1997): Prevalence of pathologic changes associated with the fin – rot – indicating bacterial disease in freshwater fish. Alex. J. Vet. Sci., 13(6): 629-644.
- Barakat, K. K. (2004): Effect of some water pollutants on the biology of the Nile Bolti, *Oreochromis niloticus*. Pakistan. J. Biological Scinces. 7. 3: 305-308.
- Behreus, A. S. and L. Karbeur (1953): Determination of LD50. Arch. Exp. Path. Pharm., 28: 177-183.
- Boyd, C. E. 1990. Water Quality in ponds for aquaculture. Agriculture Experiment Station, Auburn Univ., Alabama, U.S.A: 482.
- Boyd, C. E. 1984. Water Quality in Warm water Fishponds. Alabama Agriculture Experiment Station, Auburn Unive., Auburn, Alabama, USA.
- Dagzie, S. 1982. Species combination in tilapia culture. Auaculture, 27:295-299.
- El-Demerdash, F.M. and Elagamy, E.I. (1999): Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. Int.J.Environ.Heal.Res. 9: 173-186
- El nemeki, F. A and T. E. Badawy. 2006. Impact of different water sources and seasonal variations

- on the heavy metals concentration in Grass carp. *Ctenopharyngod idella*. African. J. Biol Sci. 2: 2:67-74.
- El nemeki, F. A and T. E. Badawy. 2005. Effect of water quality and seasonal variations on heavy metals concentrations in *Mugil cephalus* tissues, at El-Abbassa fish farm Egypt. *Aln shams Science. Bulletin.*43 :33-44.
- Farag, M. E. 2001. Genetical and Physiological studies on fish Collected from polluted Locations. Thesis PH. D. Fac. Sci., Zagazig University.
- Farag, M. E., Hussain, A. E.; Metwally, A. A. M. and Fouad, I. M. 2007. Muscle proteins profile in Common carp (*Cyprinus carpio*) expose to herbicides (whebsuper and Basegran. Egypt. J. Aquat. Biol.& Fish., Vol. 11, No. 3: 1203-1215 (2007) ISSN 1110-6131.
- Ferguson, H. W. (1989): Textbook of Systemic Pathology of fish, 1st ed., Iowa State Univ. Press, Amer. Iowa 50010, Canada.
- Fratl,F., Fanciulli,P.P, and Posthuma,L.(1992): Allozyme variation in reference and metal exposed natural populations of *Orchesella cincta* (Insecta:Collembola).*Bio.Ch.Sys.Eco.* 20 (4): 297-310
- G. A. F. R. D. (General Authority for Fish Sources Development). 2006. Fishery Annual Report, Cairo, Egypt.
- Gross, A., C. E. Boyd and C.W. Wood. 2000. Nitrogen transformations and balance in channel catfish ponds. *Aquac. Eng.* 24: 1-14.
- Hanson R, Dodoo DK, Essumang DK, Blay J Jr. and Yankson K. (2007): The effect of some selected pesticides on the growth and reproduction of fresh water *Oreochromis niloticus*, *Chrysicthys nigrodigitatus* and *Clarias gariepinus*.*Bull Environ Contam Toxicol.* 2007 Nov;79(5):544-7. Epub 2007 Oct 18.
- Herzing, A. and Winkler, A. 1986. The influence of temperature on the embryonic development of three cyprinoid fishes, *Abramis brama*, *Chalcal burnus*, *Chalcoides mento* and *Vimba vimba*. *J. Fish Biol.* 28: 171-181.
- Ibrahim, A.A. (2004): Impact of water pollution on genetic characters of fish. M.V.sc. Thesis. Department of Animal Wealth Development. Faculty of Veterinary Medicine, Suz Canal University.
- Kamel, A. E. 1999. Genetic studies on Nile Tilapia (*Oreochromis niloticus*) in Egypt. Ph. D. Department of Zoology, Girls College for Arts, Science and Education, Ain Shams University.
- Katz, M., Pederson, G., L., Yoshinaka, M. & Sjolseth, D. 1969. Water pollution (effect of pollution on fish life). *J.W.P.C.F.*, 41, 994-1015.
- Malcolm, J. 1995. Text book of environmental biology of fishes. First edition. Printed in Great Britain by T. J. Press (Padstow) LTD. Padstow. Cornwall.

EFFECT OF DIFFERENT WATER SOURCES ON MUSCLE FRACTIONS OF NILE TILAPIA (*ORECHROMIS NILOTICUS*)

- Manna, G.K. and Mukherjee, P.K. (1986): Effect of organophosphate insecticide Malathion on chromosomes, cell division and total muscle protein of cichlid fish, tilapia. In perspectives in cytology and genetics, 5:225-235 (Manna.G.K. and Sinha, U., Eds).
- Mousa, M. A. A. (2004): Toxicological studies on the effect of machete herbicide on some fish species. Egypt. J. Appl. Sci., 19(5): 1-11.
- Olufemi, B. E. and Roberts, R. J. (1986): Induction of clinical aspergillomycosis by feeding contaminated diet to tilapia (*Oreochromis niloticus*). J. Fish Disease, (9) 123-128.
- Post, G. (1987): Text-Book of Fish Health. T. F. H. Publication, INC. Revised and expanded edition.
- Pulatsu, S; F. Rad; G. Koksai; F. Aydm; A. C. K. Benli and A. Topcu. 2004. The Impact of Rainbow Trout Farm Effluents on Water Quality of Karasu Stream, Turkey. Turkish J. fisheries and aquatic Sci. 4: 09-15
- Rashed, M.A.; Ibrahim, S.A.; El-Seoudy, A.A.; Abdel-Tawab, F.M. and Ahmed, E.E. 1992. Effect of pollutant with organophosphorous insecticide Tamaron on muscle protein electrophoresis to some Tilapia species. Egypt. J. App. Sci., 7(11): 498-510.
- Rizkalla, E.H.; El-Gamal, A.A.; Mahmoud, A.A.; Shawky, A.S. and Ramadan, A.A. 1997. Effect of some Pesticides on the sarcoplasmic protein fractionation of common carp (*Cyprinus carpio*). Egypt. J. Agric. Res., 75(1): 225-245.
- Rizkalla, E. H., Shalaby A. M. E and Farag, M. E. 2005. Influence of dietary aflatoxin contamination on plasma protein profile of blue Tilapia fish (*Oreochromis aureus*) and the role of selenium and vitamin E. Egypt. J. Basic Appl. Physiol., 4(1): 49-69.
- Roark, S. and Brown, K. (1996): Effect of metal contamination from mine tailings on allozyme distributions of populations of Great Palms fishes. Environ. Toxicol. Chem. 15 (6): 821-27.
- Salem, A. ; Refai, M. ; Eissa, I. ; Marzouk, M. and Moustafa, M. (1989): Mycological investigations on cultured tilapia in Egypt. Alex. Vet. Sci., 5(2) 625-636.
- Saeed, M. S. 2000. A study on factors affecting fish production from certain fish farms in the delta. M. Sc. Thesis. Ins. Environmental studies and Research. Ain Shams Univ. Egypt.
- S. A. S. 2000. Statistical Analysis Systems. program Ver. 6. 12, SAS institute incorporation. Cary. NC 27513. USA.
- Sharf-Eldeen, K and Abdel-Hamid, N. (2002): Sublethal effects of copper sulphate, malathion and paraquat on protein pattern of *Oreochromis niloticus*. Egypt. J. Aquat. Biol. & Fish. 6 (2): 167-182.
- Smith, P.J. 1990. Proteins electrophoresis for identification of Australian fish stocks. Aust. J. Mre. Feeshwat. Res. (41)6, pp 823-833.
- Stoskopf, M. K. (1993): Fish Medicine W. B. Sannder Company. Harcourt Brace Jovanevish, Montreal, Tokyo.

- Straus, D.L. and Griffin, B.R. (2001): Effect of alkalinity on acute toxicity of copper sulfate to blue tilapia. *Aquacult. Conf. Proc.* 2001. PP.617.
- Studier, F.W. 1973. Analysis of bacteriophage Tz early RNAs and proteins on slab gels. *J. Mol. Biol.* (79): 237.
- Thanae M.A.S. 1994. Biochemical Study on Heavy Metals Residues in Some Fishes. (Ph.D.) Thesis, Biochemistry and Clinical Biochemistry Department, Faculty of Veterinary Medicine, Alexandria University.
- U. S. E. P. A. United States Environmental Protection Agency. 1986. Quality Criteria for Water. EPA 440/5-86-001.
- Virgilio, M., Baroncini, N., Trombini, C. and Abbiati, M. (2003): the relationships between sediments and tissue contamination and allozymic patterns in *Hediste diversicolor* (Polychaeta Nereididae) in the Pialassa lagoons (north Adriatic Sea). *Oceanologica Acta.* 26 (1): 85-92
- Vymazan, J. 1995. Algae and element cycling in wet lands. Duke Univ. school of the environment. Duke wet land center Durham, North Carolina. CRC Press . Inc.

## تأثير مصادر المياه المختلفة على التفريد الكهربى لبروتين عضلات أسماك البلطى النيلية

محمد السيد فرج

قسم الوراثة المعمل المركز لبحوث الأسماك، العباسية، أبو حماد، شرقية، مصر.

ان الهدف الرئيسى للحكومة المصرية ليس فقط زيادة إنتاج الأسماك من المصادر المختلفة خاصة من الأستزراع السمكى ولكن أيضا تحسين جودة الأسماك المنتجة. أحد العوامل الرئيسية التى تؤثر على جودة الأسماك هو جودة المياه، لذلك يهدف البحث الحالى إلى دراسة تأثير مصادر مختلفة من المياه على التفريد الكهربى لبروتين عضلات أسماك البلطى النيلية للمربى فى مياه حقول الأرز بمصادر مياه عذبة معاملة بجرعة حقلية بمبيد الحشائش الريب سوبر وأحواض ترابية مستزرعة بمصدرين مختلفين من مصادر المياه بمنطقة العباسية بمحافظة الشرقية بمصر. حيث تستخدم المعاملة الأولى المياه العذبة من ترعة الاسماعلية المتفرعة من نهر النيل، بينما تستخدم المعاملة الأخرى مياه الصرف الزراعى. عينات المياه كانت تأخذ عشوائيا من الأحواض المختلفة وحقل الأرز لتقدير الخصائص الفيزيائية والكيميائية فى الأحواض وحقل الأرز خاصة درجة الحرارة، الأس الهيدروجينى PH، الامونيا الكلية، والأكسجين، والنيتريت. كما أخذت عينات الأسماك مرة واحدة بعد ٩٠ يوم لعمل التفريد الكهربائى لبروتين عضلات الاسماك ووزن الكبد والخصية.

أظهرت النتائج أن هناك اختلافات وزيادة معنوية فى قياسات درجة الحرارة، الأس الهيدروجينى PH، الامونيا الكلية، والأكسجين، والنيتريت فى الثلاث مصادر المياه وزيادة فى مستوى القياسات فى حقول الأرز والأحواض التى تستخدم مياه الصرف الزراعى مقارنة بالأحواض التى تستخدم مياه الرى. كما وجد اختلافات معنوية فى تركيز العناصر الثقيلة ( الحديد، الزنك، النحاس، والرصاص) ولا يوجد اختلافات معنوية فى تركيز عنصر الكاديوم.

وفى هذه الدراسة تم تعيين الجرعة المميئة للنصف من المبيد محل الدراسة لسمة البلطى النيلية خلال ٩٦ ساعة وكانت ٠.٠٨ مجم/لتر، وقد أخذت الجرعة الحقلية من هذا المبيد والتى تساوى ١٠٠/١ من الجرعة المميئة للنصف (٠.٠٠٠٨ مجم/لتر) لدراسة تأثيرهما على وزن المبيض والكبد والتفريد الكهربى لبروتين العضلات فى السمكة محل الدراسة وأظهرت الأسماك أثناء التعرض للمبيد سلوكيات غير متزنة مثل العم على سطح الماء وزيادة افراز المواد المخاطية والتى تركزت فوق الخياشيم مع اضطرابات فى عملية التنفس.

وأظهرت النتائج أن هناك زيادة فى وزن الخصية والمبيض بالنسبة لوزن الجسم فى الذكر والانثى وزيادة فى وزن الكبد بالنسبة لوزن الجسم فى الانثى فقط ولا يوجد معنوية فى العلاقة بين وزن الكبد والمبيض بالنسبة لمزن الجسم فى الذكر والانثى. بينما نتائج التفريد الكهربى لوحظ وجود طرط جينيه مشتركه وأختفاء أخرى ووجود طرط مميزه لبعض الأسماك التى تعيش فى حقول الأرز عند الوزن الجزيئى ٠.٢٢٣ كيلو دلتون مع احتمال وجود بعض الجنات التى تظهر لمقاومة التلوث بالعناصر الثقيلة مثل النحاس والزنك والرصاص المرتفعة الناتجة من التسميد والمبيدات العشبية بحقول الارز.