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AN ATTEMPT FOR REDUCING LEAD CONTENT IN TILAPIA AND MUGIL DURING PREPARING AND COOKING OF FISH

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ABSTRACT

This study aimed to evaluate the effect of fish treatment with ethylene diamine tetra acetic acid (EDTA) and/or cooking methods on the Pb content in fish flesh. Therefore, samples of mullet (*Mugil cephalus*) from Mediterranean sea, lake Al-Manzalah and fish farms as well as of tilapia (*Oreochromis niloticus)* from the River Nile and fish farms in Damietta governorate were bought from the local market. Each fish species and according to its sampling location was divided into four sections for the treatment with EDTA solutions by soaking fish in the solutions for half an hour, then typed as the following: the first section was not soaked, the second, the third and the fourth sections were soaked in three concentrations (0.07, 0.14, and 0.21 ppm EDTA, respectively). A part of each fish species, sampling location, and treatment concentration was left fresh without cooking (control), whereas another parts were cooked using wheat flour for frying and wheat bran for grilling. The samples were analyzed for lead. The obtained results revealed that there were no significant ($P \geq 0.05$) differences in lead concentrations in the tilapia fish muscles due to either sampling locations, cooking methods, nor to the treatment (EDTA) levels. Also, frying led to decrease the Pb level than in the fresh samples. Moreover, the elevation of EDTA levels up to 0.14 ppm was responsible for increasing the Pb levels in the fish muscles. But, the highest EDTA level (0.21 ppm) led to lowering the Pb concentration comparing with the control. Frying the fish (whether from farms or from the Nile) was causative for remarkable decrease in Pb levels, particularly in muscles from the Nile fish samples. However, the fresh samples contained higher Pb levels in Nile fish than in the farm fish; so the Pb decrease was more noticeable by frying the Nile samples than frying the farm samples. The gradual increase of EDTA concentration (except 0.14 ppm) led to gradual decrease in the level of pollution with Pb in muscles of the farm fish, but the opposite was true for the Nile fish which reflected higher muscular contents of Pb by raising the EDTA levels up to 0.07 and 0.14 ppm only. However, the farm fish were highly contaminated with Pb than the Nile fish without EDTA treatment.

There were significant differences in Pb concentration of mugil muscles due to the interaction between cooking methods and treatment concentrations. Since, the grilled samples reflected higher Pb values than the fresh samples at the graded levels of EDTA. Significant differences were calculated among Pb concentrations in mugil flesh due to sampling locations, cooking method, EDTA concentrations, and their interactions. Marine samples contained significantly higher Pb than farm and lake samples. Also, grilled samples had significantly higher Pb than the fresh samples. However, the highest EDTA level (0.21 ppm) significantly raised Pb content of mugil muscles than the other tested levels. There were significant ($P \leq 0.0001$) differences in Pb concentration of mugil muscles due to the interaction between sampling locations and treatment concentrations. Since, the highest Pb level was reported in marine samples at 0.00 ppm EDTA, followed by marine samples at 0.21 ppm EDTA, lake samples at 0.21 ppm EDTA and marine samples at 0.14 ppm EDTA, respectively. Meanwhile, the lowest Pb values were given by lake samples at 0.07 ppm EDTA, followed by lake samples at 0.00 ppm EDTA, lake samples at 0.14 ppm

EDTA, farm samples at 0.21 ppm EDTA, farm samples at 0.00 ppm EDTA and farm samples at 0.14 ppm EDTA, respectively. There were significant differences ($P \leq 0.0034$) differences in Pb concentration of mugil muscles due to the interaction between cooking methods and treatment concentrations. Since, the grilled samples reflected higher Pb values than the fresh samples at the graded levels of EDTA.

The interactions among sampling location, cooking methods, and treatment concentrations were significant ($P \leq 0.0001$) and show that the lowest Pb values in mugil flesh was found in lake samples, whether fresh at 0.07, 0.14, and 0.21 ppm EDTA or grilled at 0.00 and 0.07 ppm EDTA. Whereas the highest Pb values were reported for the lake grilled samples at 0.21 ppm EDTA, followed by marine grilled samples at 0.00, 0.21, and 0.14 ppm EDTA, and farm grilled samples at 0.07ppm EDTA, respectively. Among main variable, there were significant differences in Pb concentrations in fish muscles only due to fish species and fish sampling locations, since tilapia reflected higher levels than mugil and samples from farm, marine and Nile contained more Pb than from lake. Significant differences for Pb concentrations in fish muscles were found for the interactions among sampling locations X cooking methods, cooking methods X EDTA concentrations, and sampling locations X cooking methods X EDTA concentrations. There were significant differences in Pb concentrations in fish muscles due to the interaction between fish sampling locations and cooking methods. The Nile fresh samples were more contaminated with Pb (0.205 ppm) than the farm fresh (0.109 ppm) than the marine fresh samples (0.086 ppm), respectively. Frying and grilling effects differed depending on the sampling location of the fish. There were significant ($P \leq 0.014$) differences in Pb concentrations in fish muscles due to the interaction between fish cooking methods and treatment concentrations.

Pb levels varied from 0.00 ppm to 0.502 ppm. Generally, increasing EDTA levels was accompanied with lowering the Pb contents in fish flesh. However, the variables effect of fish species and cooking methods as well as the effects of the interactions sampling positions X cooking methods, cooking methods X EDTA concentrations, and sampling positions X cooking methods X EDTA concentrations were significant. In conclusion, it seems that there are significant effects on Pb concentrations in fish flesh due to fish species, sources, and cooking methods as well as due to treating fish with EDTA solutions.

Keywords: Nile tilapia – Mugil – EDTA – Cooking.

INTRODUCTION

According to GAFRD (2007) total fish production in Egypt was 1,008,008 tons where 635,517 tons were produced through aquaculture. Aquaculture represents 63.0% of the total fish production (Ahram, 2010). The mullet species represent about 30% of the harvest and considered as important consummated species. The expansion in Nile tilapia was also associated with the production of all male tilapia and since then Nile tilapia has become the most important aquaculture species with a total harvest of about 390 280 tons, more than 55% of the total aquaculture harvest in 2009 (FAO, 2011).

Although fish are considered as an important source of animal protein, fish can also be a source of threaten to human health by transporting the toxic materials directly to the consumers. The major class of toxic chemicals includes the heavy metals. Fish can cause poisoning, especially when the fish are caught from polluted areas (Abdelhamid *et al*., 2007). There are

issues with fish contaminated with heavy metals (such as lead). In aquatic ecosystem, heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts (Abo El Ella *et al.*, 2005). Pollutant could be accumulated in fish by the rate of 10.000 times more than its concentration in the water or at least ten times that found in the surrounding water (Abou-EL-Ezz and Abdel-Razeq, 1991). Lead is nonessential metal which widely distributed in the aquatic environment mainly as a result of human activities, such as mining, refining of ores, the use of phosphate fertilizers, gasoline, containing lead from boats. This metal accumulate and concentrate from the water in different parts of fish especially those exposed to the water. Pb water pollution causes many diseases to plant fish and human beings, so the pollution of rivers by Pb has attracted attention for considerable time (Rashed and Awadallah, 1994).

Ethylene diamine tetra acetic acid "EDTA" is used to bind metals in the practice. It prevents free radicals from injuring blood vessel walls (Loren, 1996). Therefore, the aim of the present study was to evaluate the effects of treating tilapia and mugil fish from different locations in fresh or prepared (grilled or fried) forms with graded levels of EDTA aqueous solutions.

MATERIALS AND METHODS

Fish samples:

Mullet (*Mugil cephalus*) from Mediterranean sea, lake Al-Manzalah and fish farms as well as tilapia (*Oreochromis niloticus*) from the River Nile and fish farms in Damietta governorate were bought from the local market at the end of January and during February, 2011. The fish were cleaned by removing the scales, gills and eviscerated by removing all guts. Each fish species and according to its sampling location was divided into four sections for the treatment with ethylene diamine tetra acetic acid (EDTA) solutions by soaking fish in the solutions for half an hour, then typed as the following: the first section was not soaked, the second, the third and the fourth sections were soaked in three concentrations (0.07, 0.14, and 0.21 ppm EDTA, respectively). The fish were filled with garlic and spices. A part of each fish species, sampling location, and treatment concentration was left fresh without cooking (control), whereas another parts were cooked using wheat flour for frying and wheat bran for grilling.

Fish analysis:

Samples of fish muscles were wet digested for Pb determination according to Dybern (1983) using an Atomic Absorption Spectrometer (AAS, model 2380, from Perkin Elemer Company).

Statistical analysis:

The obtained numerical data were statistically analyzed using SAS (2001) for analysis of variance. Differences between comparisons among treatment means were made by using Duncan multiple range test (Duncan, 1955).

RESULTS

Tilapia fish:

Effect of sampling location, cooking method, and treatment concentrations:

The next Tables (1-5) present different effects of sampling location, cooking type, and treatment concentrations on lead (Pb) concentrations in the tilapia fish muscles as means ± standard errors). Table (1) showed that there were no significant (P ≥ 0.05) differences in lead concentrations in the tilapia fish muscles due to either sampling locations, cooking methods, nor to the treatment (EDTA) levels. Yet, the Nile samples had slightly higher Pb levels than those of farms (0.167±0.200 vs. 0.147±0.033 ppm). Also, frying led to decrease the Pb level than in the fresh samples, being 0.136±0.073 and 0.178±0.187 ppm, respectively. Moreover, the elevation of EDTA levels up to 0.14 ppm was responsible for increasing the Pb levels in the fish muscles, being 0.141±0.031, 0.196±0.268, and 0.178±0.056 ppm for 0.0, 0.07, and 0.14 ppm EDTA, respectively. But, the highest EDTA level (0.21 ppm) led to lowering the Pb concentration (0.115±0.090 ppm) comparing with the control (0.141±0.031 ppm). . .

Table (1): Effect of sampling location, cooking method, and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the tilapia fish muscles (means ± standard errors).

Items Item	Concentration of lead
Sampling Locations	
Farm	0.147±0.033
Nile	0.167±0.200
Cooking Method	
Fresh	0.178±0.187
Frying	0.136±0.073
Concentration of EDTA, ppm	
0.00	0.141±0.031
0.07	0.196±0.268
0.14	0.178±0.056
0.21	0.115±0.090

Effect of sampling location and cooking type:

Table (2) reflected insignificant (P ≥ 0.05) differences in Pb concentrations in tilapia fish muscles due to sampling location nor to cooking type; yet, frying the fish (whether from farms or from the Nile) was causative for remarkable decrease in Pb levels, particularly in muscles from the Nile fish samples (0.130±0.099 vs. 0.205±0.270 ppm in frying and fresh Nile fish and 0.143±0.040 vs. 0.152±0.028 ppm in frying and fresh farm fish). However, the fresh samples contained higher Pb levels in Nile fish than in the farm fish (Tables 1 and 2); so the Pb decrease was more noticeable by frying the Nile samples (0.130±0.099 vs. 0.205±0.270 ppm) than frying the farm samples (0.143±0.040 vs. 0.152±0.028 ppm).

Table (2): Effect of sampling location and cooking method on lead concentration (ppm, fresh weight basis) in the tilapia fish muscles (means ± standard errors).

Sampling Locations	Cooking Method	Concentration of lead
Farm	Fresh	0.152±0.028
Farm	Frying	0.143±0.040
Nile	Fresh	0.205±0.270
Nile	Frying	0.130±0.099

Effect of sampling location and treatment concentrations:

Also, there were no significant (P ≥ 0.05) differences in Pb concentrations in tilapia fish muscles due to sampling location nor to treatment concentrations (Table 3); yet, there were some changes, since the gradual increase of EDTA concentration (except 0.14 ppm) led to gradual decrease in the level of pollution with Pb in muscles of the farm fish, but the opposite was true for the Nile fish which reflected higher muscular contents of Pb by raising the EDTA levels up to 0.07 and 0.14 ppm only, being 0.251±0.398 and 0.184±0.078 ppm vs. 0.117±0.026 and 0.118±0.137 ppm Pb for 0.00 and 0.21 ppm EDTA. However, the farm fish (0.165±0.009 ppm) were highly contaminated with Pb than the Nile fish (0.117±0.026 ppm) without EDTA treatment (Table 3).

Table (3): Effect of sampling location and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the tilapia fish muscles (means ± standard errors).

Sampling Locations	Concentration of EDTA	Concentration of lead
Farm	0.00	0.165±0.009
Farm	0.07	0.141±0.036
Farm	0.14	0.172±0.034
Farm	0.21	0.112±0.011
Nile	0.00	0.117±0.026
Nile	0.07	0.251±0.398
Nile	0.14	0.184±0.078
Nile	0.21	0.118±0.137

Effect of cooking type and treatment concentrations:

Table (4) showed no significant (P ≥ 0.05) differences in Pb concentrations in tilapia fish muscles due to cooking type nor to treatment concentrations; yet, 0.21 ppm EDTA remarkably decreased Pb level to 0.061±0.070 ppm; otherwise, 0.07 and 0.14 ppm EDTA elevated Pb level to 0.337±0.333 and 0.191±0.050 compared with 0.125±0.037 ppm at 0.00 ppm EDTA for the fresh samples. But in the fried samples, only the 0.07 ppm EDTA decreased the Pb level to 0.055±0.063 ppm, comparing with the 0.00 ppm EDTA which reflected higher Pb level in fish muscles (0.156±0.019 ppm), the other two EDTA concentrations (0.14 and 0.21 ppm) gave higher Pb levels, being 0.164±0.066 and 0.169±0.078 ppm, respectively (Table 4). Since there was a significant (P ≥ 0.03) difference in the interaction between

cooking method and EDTA concentration on the Pb concentrations in tilapia fish muscles.

Table (4): Effect of cooking method and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the tilapia fish muscles (means± standard errors).

Cooking Method	Concentration of EDTA	Concentration of lead
Fresh	0.00	0.125±0.037
Fresh	0.07	0.337±0.333
Fresh	0.14	0.191±0.050
Fresh	0.21	0.061±0.070
Frying	0.00	0.156±0.019
Frying	0.07	0.055±0.063
Frying	0.14	0.164±0.066
Frying	0.21	0.169±0.078

Effect of sampling location, cooking type, and treatment concentrations:

No significant (P ≥ 0.05) differences in Pb concentrations in tilapia fish muscles were recorded due to sampling location, cooking type nor to treatment concentrations (Table 5); yet, Nile fresh sample at 0.07 and 0.14 ppm EDTA reflected the highest Pb content (being 0.502±0.473 and 0.226±0.000 ppm, respectively) among all tested samples. Since there was a significant (P≤0.03) difference in the interaction between cooking methods and EDTA concentrations as well as a significant (P≤0.07) difference in the interaction among sampling location, cooking method and EDTA concentration on the Pb concentrations in tilapia fish muscles. Moreover, Nile fresh samples at 0.21 ppm EDTA and Nile fried samples at 0.07 ppm EDTA were free from Pb contamination.

Table (5): Effect of sampling location, cooking method, and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the tilapia fish muscles (means±standard errors).

Sampling Locations	Cooking Method	Concentration of EDTA	Concentration of lead
Farm	Fresh	0.00	0.157±0.000
Farm	Fresh	0.07	0.172±0.000
Farm	Fresh	0.14	0.157±0.051
Farm	Fresh	0.21	0.122±0.000
Farm	Frying	0.00	0.172±0.000
Farm	Frying	0.07	0.109±0.000
Farm	Frying	0.14	0.186±0.000
Farm	Frying	0.21	0.102±0.000
Nile	Fresh	0.00	0.094±0.000
Nile	Fresh	0.07	0.502±0.473
Nile	Fresh	0.14	0.226±0.000
Nile	Fresh	0.21	0.000±0.000
Nile	Frying	0.00	0.139±0.000
Nile	Frying	0.07	0.000±0.000
Nile	Frying	0.14	0.142±0.105
Nile	Frying	0.21	0.237±0.000

Mugil fish:

Effect of sampling location, cooking type, and treatment concentrations:

Significant differences were calculated among Pb concentrations in mugil flesh due to sampling locations, cooking method, EDTA concentrations, and their interactions (Table 6). Marine samples contained significantly higher Pb (0.120±0.051 ppm) than farm and lake samples (0.082±0.030 and 0.046±0.086 ppm, respectively). Also, grilled samples had significantly higher Pb than the fresh samples, being 0.110±0.076 and 0.055±0.042 ppm, respectively. However, the highest EDTA level (0.21 ppm) significantly raised Pb content of mugil muscles than the other tested levels.

Table (6): Effect of sampling location, cooking method, and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the mugil fish muscles (means ± standard errors).

Item	Concentration of lead
Sampling Locations	
Farm	**0.082±0.030b**
Marine	**0.120±0.051a**
Lake	**0.046±0.086c**
Cooking Method	
Fresh	**0.055±0.042b**
Grilled	**0.110±0.076a**
Concentration of EDTA	
0.00	**0.085±0.069b**
0.07	**0.063±0.055b**
0.14	**0.074±0.052b**
0.21	**0.110±0.084a**

a-c: means in the same category with the same letter are not significantly (P ≥ 0.05) different.

Effect of sampling location and cooking type:

As given in Table (7), there were no significant (P ≥ 0.05) differences in Pb concentration of mugil muscles due to the interaction between sampling location and cooking type; yet, the grilled samples reflected higher Pb values (0.096±0.032, 0.154±0.048 and 0.080±0.109 ppm) than the fresh samples (0.067±0.019, 0.086±0.026 and 0.013±0.038 ppm) in farm, marine and lake sampling locations, respectively.

Table (7): Effect of sampling location and cooking method on lead concentration (ppm,fresh weight basis) in the mugil fish muscles (means ± standard errors).

Sampling Locations	Cooking Method	Concentration of lead
Farm	**Fresh**	**0.067±0.019**
Farm	**Grilled**	**0.096±0.032**
Marine	**Fresh**	**0.086±0.026**
Marine	**Grilled**	**0.154±0.048**
Lake	**Fresh**	**0.013±0.038**
Lake	**Grilled**	**0.080±0.109**

Effect of sampling location and treatment concentrations:

There were significant (P ≤ 0.0001) differences in Pb concentration of mugil muscles due to the interaction between sampling locations and treatment concentrations. Since, the highest Pb level was reported in marine samples at 0.00 ppm EDTA (0.154±0.059 ppm), followed by marine samples at 0.21 ppm EDTA (0.138±0.033 ppm), lake samples at 0.21 ppm EDTA (0.120±0.146 ppm) and marine samples at 0.14 ppm EDTA (0.103±0.064 ppm), respectively. Meanwhile, the lowest Pb values were given by lake samples at 0.07 ppm EDTA (0.000±0.000 ppm), followed by lake samples at 0.00 ppm EDTA (0.027±0.053 ppm), lake samples at 0.14 ppm EDTA (0.039±0.045 ppm), farm samples at 0.21 ppm EDTA (0.070±0.009 ppm), farm samples at 0.00 ppm EDTA (0.073±0.010 ppm) and farm samples at 0.14 ppm EDTA (0.080±0.031 ppm), respectively (Table 8).

Table (8): Effect of sampling location and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the mugil fish muscles (means ± standard errors).

Sampling Locations	Concentration of EDTA	Concentration of lead
Farm	0.00	0.073±0.010
Farm	0.07	0.103±0.049
Farm	0.14	0.080±0.031
Farm	0.21	0.070±0.009
Marine	0.00	0.154±0.059
Marine	0.07	0.085±0.024
Marine	0.14	0.103±0.064
Marine	0.21	0.138±0.033
Lake	0.00	0.027±0.053
Lake	0.07	0.000±0.000
Lake	0.14	0.039±0.045
Lake	0.21	0.120±0.146

Effect of cooking type and treatment concentrations:

There were significant (P ≤ 0.0034) differences in Pb concentration of mugil muscles due to the interaction between cooking methods and treatment concentrations. Since, the grilled samples reflected higher Pb values (0.096±0.092, 0.077±0.067, 0.106±0.044, and 0.162±0.081 ppm) than the fresh samples (0.074±0.041, 0.049±0.041, 0.042±0.039, and 0.057±0.049 ppm) at the graded levels of EDTA (0.00, 0.07, 0.14, and 0.21 ppm, respectively) as given in Table (9).

Table (9): Effect of cooking method and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the mugil fish muscles (means ± standard errors).

Cooking Method	Concentration of EDTA	Concentration of lead
Fresh	0.00	0.074±0.041
Fresh	0.07	0.049±0.041
Fresh	0.14	0.042±0.039
Fresh	0.21	0.057±0.049
Grilled	0.00	0.096±0.092
Grilled	0.07	0.077±0.067
Grilled	0.14	0.106±0.044
Grilled	0.21	0.162±0.081

Effect of sampling location, cooking method, and treatment concentrations:

The interaction among sampling location, cooking method, and treatment concentrations was significant (P ≤ 0.0001). Table (10) show that the lowest Pb values in mugil flesh was found in lake samples, whether fresh at 0.07, 0.14, and 0.21 ppm EDTA or grilled at 0.00 and 0.07 ppm EDTA, being 0.000±0.000 ppm. Whereas the highest Pb values were reported for the lake grilled samples at 0.21 ppm EDTA (0.241±0.079 ppm), followed by marine grilled samples at 0.00, 0.21, and 0.14 ppm EDTA (0.205±0.000, 0.166±0.000, and 0.158±0.000 ppm), and farm grilled samples at 0.07ppm EDTA (0.142±0.000 ppm), respectively.

Table (10): Effect of sampling location, cooking method, and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the mugil fish muscles (means ± standard errors).

Sampling Locations	Cooking Method	Concentration of EDTA	Concentration of lead
Farm	Fresh	0.00	0.065±0.000
Farm	Fresh	0.07	0.063±0.030
Farm	Fresh	0.14	0.078±0.037
Farm	Fresh	0.21	0.062±0.000
Farm	Grilled	0.00	0.082±0.000
Farm	Grilled	0.07	0.142±0.000
Farm	Grilled	0.14	0.083±0.039
Farm	Grilled	0.21	0.078±0.000
Marine	Fresh	0.00	0.103±0.000
Marine	Fresh	0.07	0.082±0.000
Marine	Fresh	0.14	0.047±0.000
Marine	Fresh	0.21	0.110±0.000
Marine	Grilled	0.00	0.205±0.000
Marine	Grilled	0.07	0.088±0.042
Marine	Grilled	0.14	0.158±0.000
Marine	Grilled	0.21	0.166±0.000
Lake	Fresh	0.00	0.053±0.076
Lake	Fresh	0.07	0.000±0.000
Lake	Fresh	0.14	0.000±0.000
Lake	Fresh	0.21	0.000±0.000
Lake	Grilled	0.00	0.000±0.000
Lake	Grilled	0.07	0.000±0.000
Lake	Grilled	0.14	0.078±0.000
Lake	Grilled	0.21	0.241±0.079

Tilapia and Mugil fish:

Effect of species, sampling location, cooking method, and treatment concentrations:

Among main variable, there were significant differences in Pb concentrations in fish muscles only due to fish species and fish sampling locations, since tilapia reflected higher levels (0.157±0.141 ppm) than mugil (0.085±0.065 ppm) and samples from farm, marine and Nile contained more Pb than from lake, being 0.114±0.045, 0.120±0.051, 0.167±0.200, and 0.053±0.086 ppm, respectively (Table 11). Significant differences for Pb

concentrations in fish muscles were found for the interactions among sampling locations X cooking methods, cooking methods X EDTA concentrations, and sampling locations X cooking methods X EDTA concentrations at P ≥ 0.0517, 0.014, and 0.0003, respectively. Otherwise, there were no significant (P ≥ 0.05) differences.

Table (11): Effect of fish species, sampling location, cooking method, and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Item	Concentration of lead
Fish Species	
Tilapia	**0.157±0.141a**
Mugil	**0.085±0.065b**
Sampling Position	
Farm	**0.114±0.045a**
Marine	**0.120±0.051a**
Nile	**0.167±0.200a**
Lake	**0.053±0.086b**
Cooking Method	
Fresh	**0.107±0.134**
Fried	**0.136±0.073**
Grilled	**0.110±0.076**
Concentration of EDTA	
0.00	**0.112±0.057**
0.07	**0.116±0.181**
0.14	**0.115±0.074**
0.21	**0.112±0.084**

a-b: means in the same category have the same letter are not significantly (P ≥ 0.05) different.

Effect of fish species and sampling location:

Table (12) present that there were no significant (P ≥ 0.05) differences in Pb concentrations in fish muscles due to the interaction between fish species and fish sampling locations. The Pb levels took the range of 0.053 (in mugil fish from lake) to 0.167 ppm (in tilapia fish from Nile)**.**

Table (12): Effect of fish species and sampling locations on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Fish Species	Sampling Position	Concentration of lead
Tilapia	**Farm**	**0.147±0.033**
Tilapia	**Nile**	**0.167±0.200**
Mugil	**Farm**	**0.082±0.030**
Mugil	**Marine**	**0.120±0.051**
Mugil	**Lake**	**0.053±0.086**

Effect of fish species and cooking methods:

Table (13) present that there were no significant (P ≥ 0.05) differences in Pb concentrations in fish muscles due to the interaction between fish species and cooking methods. The Pb levels took the range of 0.060 (in mugil fresh fish) to 0.178 ppm (in tilapia fresh fish).

Table (13): Effect of fish species and cooking methods on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Fish Species	Cooking Method	Concentration of lead
Tilapia	Fresh	0.178±0.187
Tilapia	Fried	0.136±0.073
Mugil	Fresh	0.060±0.041
Mugil	Grilled	0.110±0.076

Effect of fish sampling locations and cooking methods:

Table (14) present that there were significant (P ≤ 0.0517) differences in Pb concentrations in fish muscles due to the interaction between fish sampling locations and cooking methods. The Pb levels took the range of 0.027 (in lake fresh fish) to 0.205 ppm (in Nile fresh fish. The Nile fresh samples were more contaminated with Pb (0.205 ppm) than the farm fresh (0.109 ppm) than the marine fresh samples (0.086 ppm), respectively. Frying (positive and negative effects in Nile and farm samples, respectively) and grilling (positive in farm and negative in marine and lake samples, respectively) effects differed depending on the sampling location of the fish.

Table (14): Effect of fish sampling location and cooking method on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Sampling Position	Cooking Method	Concentration of lead
Farm	Fresh	0.109±0.049
Farm	Fried	0.143±0.040
Farm	Grilled	0.096±0.032
Marine	Fresh	0.086±0.026
Marine	Grilled	0.154±0.048
Nile	Fresh	0.205±0.270
Nile	Fried	0.130±0.099
Lake	Fresh	0.027±0.049
Lake	Grilled	0.080±0.109

Effect of fish species and treatment concentrations:

Table (15) present that there were no significant (P ≥ 0.05) differences in Pb concentrations in fish muscles due to the interaction between fish species and treatment concentrations. The Pb levels took the range of 0.063 (in mugil fish at 0.07 ppm EDTA) to 0.196 ppm (in tilapia fish at 0.07 ppm EDTA).

Table (15): Effect of fish species and treatment concentrations (ppm) on lead concentrations (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Fish Species	Concentration of EDTA	Concentration of lead
Tilapia	0.00	0.141±0.031
Tilapia	0.07	0.196±0.268
Tilapia	0.14	0.178±0.056
Tilapia	0.21	0.115±0.090
Mugil	0.00	0.094±0.064
Mugil	0.07	0.063±0.055
Mugil	0.14	0.074±0.052
Mugil	0.21	0.110±0.084

Effect of fish sampling location and treatment concentrations:

Table (16) present that there were no significant (P ≥ 0.05) differences in Pb concentrations in fish muscles due to the interaction between fish sampling location and treatment concentrations. The Pb levels took the range of 0.000 (in lake fish at 0.07 ppm EDTA) to 0.251 ppm (in Nile fish at 0.07 ppm EDTA).

Table (16): Effect of fish sampling location and treatment concentrations (ppm) on lead concentration in the fish muscles (ppm, fresh weight basis, means ± standard errors).

Sampling Position	Concentration of EDTA	Concentration of lead
Farm	0.00	0.119±0.049
Farm	0.07	0.122±0.045
Farm	0.14	0.126±0.057
Farm	0.21	0.091±0.024
Marine	0.00	0.154±0.059
Marine	0.07	0.085±0.024
Marine	0.14	0.103±0.064
Marine	0.21	0.138±0.033
Nile	0.00	0.117±0.026
Nile	0.07	0.251±0.398
Nile	0.14	0.184±0.078
Nile	0.21	0.118±0.137
Lake	0.00	0.053±0.062
Lake	0.07	0.000±0.000
Lake	0.14	0.039±0.045
Lake	0.21	0.120±0.146

Effect of fish cooking methods and treatment concentrations:

Table (17) present that there were significant (P ≤ 0.014) differences in Pb concentrations in fish muscles due to the interaction between fish cooking methods and treatment concentrations. The Pb levels took the range of 0.055 (in fried fish at 0.07 ppm EDTA) to 0.169 ppm (in fried fish at 0.21 ppm EDTA).

Table (17): Effect of fish cooking method and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Cooking Method	Concentration of EDTA	Concentration of lead
Fresh	0.00	0.105±0.032
Fresh	0.07	0.164±0.245
Fresh	0.14	0.101±0.087
Fresh	0.21	0.059±0.055
Fried	0.00	0.156±0.019
Fried	0.07	0.055±0.063
Fried	0.14	0.164±0.066
Fried	0.21	0.169±0.078
Grilled	0.00	0.096±0.092
Grilled	0.07	0.077±0.067
Grilled	0.14	0.106±0.044
Grilled	0.21	0.162±0.081

Effect of fish species, sampling location, and cooking methods:

Table (18) present that there were no significant (P ≥ 0.05) differences in Pb concentrations in fish muscles due to the interaction among fish species, sampling location and cooking methods. The Pb levels took the range of 0.027 (in mugil – lake – frsh – fish) to 0.205 ppm (in tilapia –Nile – fresh - fish).

Table (18): Effect of fish species, sampling location and cooking method on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Species	Position	Cooking Method	Concentrate of lead (Pb)
Tilapia	Farm	Fresh	0.152±0.028
Tilapia	Farm	Fried	0.143±0.040
Tilapia	Nile	Fresh	0.205±0.270
Tilapia	Nile	Fried	0.130±0.099
Mugil	Farm	Fresh	0.067±0.019
Mugil	Farm	Grilled	0.096±0.032
Mugil	Marin	Fresh	0.086±0.026
Mugil	Marin	Grilled	0.154±0.048
Mugil	Lake	Fresh	0.027±0.049
Mugil	Lake	Grilled	0.080±0.109

Effect of fish species, sampling location and treatment concentrations:

Table (19) present that there were no significant (P ≥ 0.05) differences in Pb concentrations in fish muscles due to the interaction among fish species, sampling location and treatment concentrations. The Pb levels took the range of 0.000 (in mugil – lake fish at 0.07 ppm EDTA) to 0.251 ppm (in tilapia – Nile fish at 0.07 ppm EDTA).

Table (19): Effect of fish species, sampling location and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Fish Species	Sampling Locations	Concentration of EDTA	Concentration of lead
Tilapia	Farm	0.00	0.165±0.009
Tilapia	Farm	0.07	0.141±0.036
Tilapia	Farm	0.14	0.172±0.034
Tilapia	Farm	0.21	0.112±0.011
Tilapia	Nile	0.00	0.117±0.026
Tilapia	Nile	0.07	0.251±0.398
Tilapia	Nile	0.14	0.184±0.078
Tilapia	Nile	0.21	0.118±0.137
Mugil	Farm	0.00	0.073±0.010
Mugil	Farm	0.07	0.103±0.049
Mugil	Farm	0.14	0.080±0.031
Mugil	Farm	0.21	0.070±0.009
Mugil	Marine	0.00	0.154±0.059
Mugil	Marine	0.07	0.085±0.024
Mugil	Marine	0.14	0.103±0.064
Mugil	Marine	0.21	0.138±0.033
Mugil	Lake	0.00	0.053±0.062
Mugil	Lake	0.07	0.000±0.000
Mugil	Lake	0.14	0.039±0.045
Mugil	Lake	0.21	0.120±0.146

Effect of fish sampling location, cooking type, and treatment concentrations:

There were significant ($P \leq 0.0003$) differences in Pb concentrations which were attributed to the interaction among fish sampling location, cooking methods, and treatment concentrations. Table (20) shows that Pb levels varied from 0.00 ppm (in Nile – fresh samples at 0.21 ppm EDTA, Nile – fried samples at 0.07 ppm EDTA, lake – fresh samples at 0.07, 0.14 and 0.21 ppm EDTA, and in lake – grilled samples at 0.00 and 0.07 ppm EDTA, respectively) to 0.502 ppm (in Nile – fresh samples at 0.07 ppm EDTA).

Table (20): Effect of fish sampling location, cooking type, and treatment concentrations (ppm) on lead concentration (ppm, fresh weight basis)in the fish muscles (means ± standard errors)

Sampling Locations	Cooking Methods	Concentration of EDTA	Concentration of lead
Farm	Fresh	0.00	0.125±0.037
Farm	Fresh	0.07	0.337±0.333
Farm	Fresh	0.14	0.191±0.050
Farm	Fresh	0.21	0.061±0.070
Farm	Fried	0.00	0.156±0.019
Farm	Fried	0.07	0.055±0.063
Farm	Fried	0.14	0.164±0.066
Farm	Fried	0.21	0.169±0.078
Marine	Fresh	0.00	0.091±0.021
Marine	Fresh	0.07	0.049±0.041
Marine	Fresh	0.14	0.042±0.039
Marine	Fresh	0.21	0.057±0.049
Marine	Grilled	0.00	0.096±0.092
Marine	Grilled	0.07	0.077±0.067
Marine	Grilled	0.14	0.106±0.044
Marine	Grilled	0.21	0.162±0.081
Farm	Fresh	0.00	0.111±0.053
Farm	Fresh	0.07	0.118±0.065
Farm	Fresh	0.14	0.117±0.058
Farm	Fresh	0.21	0.092±0.034
Farm	Fried	0.00	0.172±0.000
Farm	Fried	0.07	0.109±0.000
Farm	Fried	0.14	0.186±0.000
Farm	Fried	0.21	0.102±0.000
Farm	Grilled	0.00	0.082±0.000
Farm	Grilled	0.07	0.142±0.000
Farm	Grilled	0.14	0.083±0.039
Farm	Grilled	0.21	0.078±0.000
Marine	Fresh	0.00	0.103±0.000
Marine	Fresh	0.07	0.082±0.000
Marine	Fresh	0.14	0.047±0.000
Marine	Fresh	0.21	0.110±0.000
Marine	Grilled	0.00	0.205±0.000
Marine	Grilled	0.07	0.088±0.042
Marine	Grilled	0.14	0.158±0.000
Marine	Grilled	0.21	0.166±0.000
Nile	Fresh	0.00	0.094±0.000
Nile	Fresh	0.07	0.502±0.473
Nile	Fresh	0.14	0.226±0.000
Nile	Fresh	0.21	0.000±0.000
Nile	Fried	0.00	0.139±0.000
Nile	Fried	0.07	0.000±0.000
Nile	Fried	0.14	0.142±0.105
Nile	Fried	0.21	0.237±0.000
Lake	Fresh	0.00	0.107±0.000
Lake	Fresh	0.07	0.000±0.000
Lake	Fresh	0.14	0.000±0.000
Lake	Fresh	0.21	0.000±0.000
Lake	Grilled	0.00	0.000±0.000
Lake	Grilled	0.07	0.000±0.000
Lake	Grilled	0.14	0.078±0.000
Lake	Grilled	0.21	0.241±0.079

Generally, increasing EDTA levels was accompanied with lowering the Pb contents in fish flesh. However, the variables effect of fish species and cooking methods as well as the effects of the interactions sampling positions X cooking methods, cooking methods X EDTA concentrations, and sampling positions X cooking methods X EDTA concentrations were significant.

Effect of fish species, sampling location, cooking type, and treatment concentrations:

Table (21) present that there were no significant ($P \geq 0.05$) differences in Pb concentrations in fish muscles due to the interactions among fish species, sampling location, cooking methods and treatment concentrations.

Table (21): Effect of fish species, sampling location, cooking type, and treatment concentrations (ppm) on lead concentrations (ppm, fresh weight basis) in the fish muscles (means ± standard errors).

Fish Species	Sampling locations	Cooking Method	Concentration of EDTA	Concentration of lead
Tilapia	Farm	Fresh	0.00	0.157±0.000
Tilapia	Farm	Fresh	0.07	0.172±0.000
Tilapia	Farm	Fresh	0.14	0.157±0.051
Tilapia	Farm	Fresh	0.21	0.122±0.000
Tilapia	Farm	Fried	0.00	0.172±0.000
Tilapia	Farm	Fried	0.07	0.109±0.000
Tilapia	Farm	Fried	0.14	0.186±0.000
Tilapia	Farm	Fried	0.21	0.102±0.000
Tilapia	Nile	Fresh	0.00	0.094±0.000
Tilapia	Nile	Fresh	0.07	0.502±0.473
Tilapia	Nile	Fresh	0.14	0.226±0.000
Tilapia	Nile	Fresh	0.21	0.000±0.000
Tilapia	Nile	Fried	0.00	0.139±0.000
Tilapia	Nile	Fried	0.07	0.000±0.000
Tilapia	Nile	Fried	0.14	0.142±0.105
Tilapia	Nile	Fried	0.21	0.237±0.000
Mugil	Farm	Fresh	0.00	0.065±0.000
Mugil	Farm	Fresh	0.07	0.063±0.030
Mugil	Farm	Fresh	0.14	0.078±0.037
Mugil	Farm	Fresh	0.21	0.062±0.000
Mugil	Farm	Grilled	0.00	0.082±0.000
Mugil	Farm	Grilled	0.07	0.142±0.000
Mugil	Farm	Grilled	0.14	0.083±0.039
Mugil	Farm	Grilled	0.21	0.078±0.000
Mugil	Nile	Fresh	0.00	0.103±0.000
Mugil	Nile	Fresh	0.07	0.082±0.000
Mugil	Nile	Fresh	0.14	0.047±0.000
Mugil	Nile	Fresh	0.21	0.110±0.000
Mugil	Nile	Grilled	0.00	0.205±0.000
Mugil	Nile	Grilled	0.07	0.088±0.042
Mugil	Nile	Grilled	0.14	0.158±0.000±
Mugil	Nile	Grilled	0.21	0.166±0.000
Mugil	Lake	Fresh	0.00	0.107±0.000
Mugil	Lake	Fresh	0.07	0.000±0.000
Mugil	Lake	Fresh	0.14	0.000±0.000
Mugil	Lake	Fresh	0.21	0.000±0.000
Mugil	Lake	Grilled	0.00	0.000±0.000
Mugil	Lake	Grilled	0.07	0.000±0.000
Mugil	Lake	Grilled	0.14	0.078±0.000
Mugil	Lake	Grilled	0.21	0.241±0.079

The Pb levels took the range of 0.000 (in tilapia – Nile – fresh fish at 0.21 ppm EDTA, tilapia – Nile – fried fish at 0.07 ppm EDTA, and mugil – lake – fresh fish at 0.07, 0.14, 0.021 ppm EDTA and mugil – lake grilled fish at 0.00 and 0.07 ppm EDTA) to 0.502 ppm (in tilapia – Nile – fresh fish at 0.07 ppm EDTA).

DISCUSSION

From the foregoing results, it could be concluded that there were no significant differences in Pb concentrations in tilapia fish muscles due to sampling location, cooking type nor to treatment concentrations; yet, the tilapia from Nile samples had slightly higher Pb levels than those of farms. Also, frying led to decrease the Pb level than in the fresh samples. Moreover, the elevation of EDTA levels up to 0.14 ppm was responsible for increasing the Pb levels in the fish muscles. But, the highest EDTA level (0.21 ppm) led to lowering the Pb concentration comparing with the control. However, the fresh samples contained higher Pb levels in Nile fish than in the farm fish. The farm fish were highly contaminated with Pb than the Nile fish without EDTA treatment. Nile fresh sample at 0.07 and 0.14 ppm EDTA reflected the highest Pb content among all tested samples. Since there was a significant difference in the interaction between cooking methods and EDTA concentrations as well as a significant difference in the interaction among sampling location, cooking method and EDTA concentration on the Pb concentrations in tilapia fish muscles. Moreover, Nile fresh samples at 0.21 ppm EDTA and Nile fried samples at 0.07 ppm EDTA were free from Pb contamination.

On the other hand, significant differences were calculated among Pb concentrations in mugil flesh due to sampling locations, cooking method, EDTA concentrations, and their interactions. Marine samples contained significantly higher Pb than farm and lake samples. Also, grilled samples had significantly higher Pb than the fresh samples. However, the highest EDTA level (0.21 ppm) significantly raised Pb content of mugil muscles than the other tested levels. Also, there were significant differences in Pb concentration of mugil muscles due to the interaction between cooking methods and treatment concentrations. Since, the grilled samples reflected higher Pb values than the fresh samples at the graded levels of EDTA. There were no significant differences in Pb concentration of mugil muscles due to the interaction between sampling location and cooking type; yet, the grilled samples reflected higher Pb values than the fresh samples in farm, marine and lake sampling locations, respectively. But there were significant differences in Pb concentration of mugil muscles due to the interaction between sampling locations and treatment concentrations. Since, the highest Pb level was reported in marine samples at 0.00 ppm EDTA, followed by marine samples at 0.21 ppm EDTA, lake samples at 0.21 ppm EDTA and marine samples at 0.14 ppm EDTA, respectively. Meanwhile, the lowest Pb values were given by lake samples at 0.07 ppm EDTA, followed by lake samples at 0.00 ppm EDTA, lake samples at 0.14 ppm EDTA, farm samples

at 0.21 ppm EDTA, farm samples at 0.00 ppm EDTA and farm samples at 0.14 ppm EDTA, respectively. The interactions among sampling location, cooking method, and treatment concentrations were significant and show that the lowest Pb values in mugil flesh was found in lake samples, whether fresh at 0.07, 0.14, and 0.21 ppm EDTA or grilled at 0.00 and 0.07 ppm EDTA. Whereas the highest Pb values were reported for the lake grilled samples at 0.21 ppm EDTA, followed by marine grilled samples at 0.00, 0.21, and 0.14 ppm EDTA, and farm grilled samples at 0.07ppm EDTA, respectively.

Among main variable, there were significant differences in Pb concentrations in fish muscles only due to fish species and fish sampling locations, since tilapia reflected higher levels than mugil and samples from farm, marine and Nile contained more Pb than from lake. Significant differences for Pb concentrations in fish muscles were found for the interactions among sampling locations X cooking methods, cooking methods X EDTA concentrations, and sampling locations X cooking methods X EDTA concentrations. Otherwise, there were no significant differences. There were no significant differences in Pb concentrations in fish muscles due to the interaction between fish species and fish sampling locations. There were no significant differences in Pb concentrations in fish muscles due to the interaction between fish species and cooking methods. There were significant differences in Pb concentrations in fish muscles due to the interaction between fish sampling locations and cooking methods. The Pb levels took the range of 0.027 to 0.205 ppm. The Nile fresh samples were more contaminated with Pb (0.205 ppm) than the farm fresh (0.109 ppm) than the marine fresh samples (0.086 ppm), respectively. Frying and grilling effects differed depending on the sampling location of the fish. There were no significant differences in Pb concentrations in fish muscles due to the interaction between fish species and treatment concentrations. The Pb levels took the range of 0.063 to 0.196 ppm. There were no significant differences in Pb concentrations in fish muscles due to the interaction between fish sampling location and treatment concentrations. The Pb levels took the range of 0.000 to 0.251 ppm. There were significant differences in Pb concentrations in fish muscles due to the interaction between fish cooking methods and treatment concentrations. The Pb levels took the range of 0.055 to 0.169 ppm. There were no significant differences in Pb concentrations in fish muscles due to the interaction among fish species, sampling location and cooking methods. The Pb levels took the range of 0.027 to 0.205 ppm. There were no significant differences in Pb concentrations in fish muscles due to the interaction among fish species, sampling location and treatment concentrations. The Pb levels took the range of 0.000 to 0.251 ppm. There were significant differences in Pb concentrations which were attributed to the interaction among fish sampling location, cooking methods, and treatment concentrations. Pb levels varied from 0.00 ppm to 0.502 ppm. Generally, increasing EDTA levels was accompanied with lowering the Pb contents in fish flesh. However, the variables effect of fish species and cooking methods as well as the effects of the interactions sampling positions X cooking methods, cooking methods X EDTA concentrations, and sampling positions X cooking methods X EDTA

concentrations were significant. There were no significant differences in Pb concentrations in fish muscles due to the interactions among fish species, sampling location, cooking methods and treatment concentrations. The Pb levels took the range of 0.000 to 0.502 ppm.

For interpretation of the obtained results, it may through some lights on the corresponding literature. Generally, fish are an important part of a healthy diet. They are a lean, low caloric source of protein. Evard (2011) mentioned that for many poor people, fish provides important nutrients, including protein. The FAO estimates that fish accounts for 22% of the sub-Sahara people's protein diet. For the poorest African countries, the share is more than 50%. Global fish consumption hit a record high at an average of 17 Kg/capita. Africa's /capita consumption was only half as much (8.5 Kg/capita). Fish taken from polluted waters might be hazardous to health. Eating fish containing chemical pollutants may cause birth defects, liver damage, cancer, and other serious health problems. Water pollution is an alteration of the physical, chemical, biological, bacteriological, or radiological properties of water that result in an impairment of designated uses. Pollution may be accidental (sometimes with grave consequences) but is most often caused by the uncontrolled disposal of sewage and other liquid wastes resulting from domestic uses of water, industrial wastes containing a variety of pollutants, agricultural effluents from animal husbandry and drainage of irrigation water, and urban run-off. Pollution of the water of this territory may be detrimental to public health and welfare, and may adversely affect livestock, wildlife, fish and aquatic life, and may progressively obstruct agricultural, industrial, recreational and other beneficial uses of water. Saving of our water resources from different types of water pollutants is very important to get good and healthy environment. Also, from the public health point of view, the wisdom still right, that prophylaxis from drastic effects of water pollutants, is more useful than treatments (Abdelghany, 2009).

However, many heavy metals are frequently occurred in Egyptian water, earth, plants and animals including fish, whether of freshwater or saltwater, and differ in their concentrations from species to another species of fish, and from season to other as well as from location to other (Abdelhamid and Gawish, 1998 and Abdelahmid *et al.*, 2000).

There was variations in Pb levels due to sampling locations (attributed to the activities in these locations) and species (Hassaan and Nadia, 2000). Hussein and Mekkawy (2001) cited that all studies agree that Pb concentration of fish muscles show the least accumulation than those of different organs. Also, usually marine species, accumulate less Pb than freshwater species. In a worldwide study screening thirty-four commonly consumed sea food's, mean Pb level in edible tissues (mostly skeletal muscles) are uniform among various species, with only a few species averaging over 0.6 ppm. No species exceed 1 ppm and Pb levels average 0.49 in fresh fish muscles. Science this metal is eliminated to reach a point of equilibrium with tissues. Also, this reduction in Pb level may be due to the chelation of the dissolved metal with the excreted organic matter which may in turn reduce its accumulation in body organs.

Early clinical features of lead toxicity are non-specific and an occupational history is particularly valuable. Lead in the body comprises 2% in the blood (t1/2 35 days) and 95% in bone and dentine (t1/2 20–30 years). Blood lead may remain elevated for years after cessation from long exposure, due to redistribution from bone. Blood lead concentration is the most widely used marker for inorganic lead exposure. Symptomatic patients with blood lead concentration >2.4 mmol showed receive sodium calcium-EDTA i.v. (Gordon *et al.,* 2002). On the other hand, Sitohy *et al.* (2009) found that ethylene diamine tertra acetic acid (EDTA, as chemical chelator) and rice husk and orange peel (as natural substances) were capable for reducing Pb concentrations in water as well as in muscles, gills, liver, and kidneys of the *O. niloticus* fingerlings.

Lead-induced oxidative stress contributes to the pathogenesis of lead poisoning for disrupting the delicate prooxidant/antioxidant balance that exists within mammalian cells. Production of reactive oxygen species (ROS) is increased after lead treatment in *in vitro* studies. *In vivo* studies suggest that lead exposure causes generation of ROS and alteration of antioxidant defense systems in animals and occupationally exposed workers. The mechanisms for lead-induced oxidative stress include the effect of lead on membrane, DNA, and antioxidant defense systems of cells. From low to high doses of lead exposure, there are different responses of lead-induced oxidative stress in various target sites including lung, blood vessels, testes, sperm, liver, and brain in epidemiological as well as animal studies. Therefore, reducing the possibility of lead interacting with critical biomolecules and inducing oxidative damage, or bolstering the cell's antioxidant defenses might be associated with the beneficial role of antioxidant nutrients through exogenous supplementation of antioxidant molecules. Although many researchers have investigated the benefit of antioxidants in preventing lead toxicity, the mechanisms of antioxidant nutrients being effective via rebalancing the impaired prooxidant/antioxidant ratio are not completely clear. Antioxidant nutrients including, vitamin E, vitamin C, vitamin B6, b-carotene, zinc, and selenium, are addressed in this review to discuss their beneficial role in lead-induced oxidative stress (Hsu and Guo, 2002).

Mzimela *et al.* (2002) mentioned that long-term exposure to Pb resulted in significant increases in blood glucose, haemoglobin content and the acid-base status of the fish. Also, Ayyat et al. (2003) found that live body weight and daily body gain of Nile tilapia fish decreased significantly with increasing dietary Pb levels. Moreover, Salem (2003) reported that Pb caused a significant reduction in the average weight gain, specific growth rate, survival rate, feed conversion, protein efficiency ratio and carcass dry matter and protein percentages, besides increased residual Pb concentration in the fish tissues. .

The lead (Pb) chelator, meso-2,3-dimercaptosuccinic acid (DMSA) may be effective in reversing some of the adverse effects of Pb exposure. Pb-induced behavioral deficits observed in fish are due to disruptions in the integrative functioning of the medulla, cerebellum, and optic tectum. Pb exposure increased serotonin (5-HT) content in all three brain regions without

an effect on 5-hydroxy-3-indoleacetic acid (5- HIAA). Pb exposure followed by no Pb in the diet increased 5-HT and 5-HIAA content in all three brain regions. Treatment with DMSA may be more effective than removal of Pb from the diet in reversing Pb-induced alterations in 5-HT (Rademacher *et al*., 2003). Also, Sharaf Eldeen *et al.* (2003) came to the conclusion that the addition of EDTA (0.15-0.31 ppm in rearing water) to the ½ LC50 of mercury, eliminated the severe effects of Hg. Moreover, Al Nagaawy and Shalaby (2009) tested tea waste and peanut shells in removing Pb from waste water and found that peanut shells had better removing efficiency than tea waste. They added that removing efficiency increased as contact period increased. It differed also according to investigated materials concentrations and water salinity.

Glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH) and pyruvate kinase (PK) are key metabolic enzymes. G6PDH has been used as a biomarker of pollution- induced carcinogenesis in fish. LDH has been used as marker of lesions in toxicology and clinical chemistry, and PK catalyses the conversion of phosphoenol pyruvate to pyruvate, with regeneration of ATP. The effect of different concentrations of lead nitrate on the activity of these enzymes in two different early ontogenetic stages (embryonic and free embryonic stage) of the African catfish *Clarias gariepinus* was investigated. There was a significant decrease in activities of all three enzymes from 30 h-PFS to 96 h-PFS, followed by a significant increase in G6PDH and LDH. PK showed insignificant fluctuations in activity. Different patterns of enzyme activities were recorded due to exposure to different lead nitrate concentrations (100 lg/l, 300 lg/l and 500 lg/l). In the pre-hatching stage (30 h-PFS) the activity of the three enzymes increased at exposure to 100 lg/l lead nitrate and then decreased with increasing dose. In the post-hatching stages (48 h- PFS–168 h-PFS) G6PDH activity increased and LDH activity decreased with increasing lead concentrations. Unlike G6PDH and LDH, the PK enzyme fluctuated during the post-hatching stages and did not reveal a specific trend of response (increase or decrease) with increasing lead concentrations. Therefore, the measurement of G6PDH and LDH activities, but not PK activity, could be useful biomarkers of intoxication to reveal the embryo toxic potential of lead nitrate in fish embryos. The post-hatching stages of the African catfish are more sensitive than the pre-hatching stage (30 h-PFS) is, probably due to the protective capacity provided by the hardened chorion. The interaction and the main effects of age and lead doses were found to be highly significant, referring to the great impact of lead on these enzyme systems with increasing early development (Osman *et al*., 2007).

The possible nephrotoxic effects of waterborne lead exposure (as Pb(NO3)2) were investigated in the freshwater rainbow trout (*Oncorhynchus mykiss*). Kidney lead accumulation was time-dependent, increasing upon exposure to 0.57±0.01 mg dissolved Pb L−1 for up to 96 h with a significantly higher burden occurring in the posterior kidney compared to the anterior segment. Urine analyses in trout exposed to 1.20±0.09 mg dissolved Pb L−1 revealed a significant increase in urinary lead excretion rate throughout 96 h of exposure. Urine flow rate and glomerular filtration rate (GFR) were not

impacted with the exception of a significant decrease in GFR from 84 to 96 h in lead-exposed trout. Urine pH decreased significantly over time in lead-exposed fish. Correspondingly, urine ammonia excretion rate showed a marked increase from 48 h onwards. In experimental fish, urine glucose excretion was significantly greater by 96 h while urine lactate, urea and protein excretion were not significantly altered by lead exposure. The urine excretion rate of Ca2+ increased significantly by approximately 43% after only 24 h of lead exposure, and was maintained at a higher rate than controls for up to 96 h. Magnesium excretion increased in a time-dependent fashion, reaching a two- to three-fold rise by 96 h. In contrast, rates of Na+ and Cl− excretion were decreased in experimental fish by approximately 30% by 48 h, this trend continuing for the duration of lead-exposure. There were no changes in any of these parameters in similarly treated control fish. Clearance ratio analyses indicated progressive decreases in the net reabsorption efficiencies of the renal system for Ca2+, Mg2+, Pb, and glucose, suggesting that the active tubular transport mechanisms for these substances were inhibited by lead exposure, while Na+, K+, Cl−, lactate, and protein reabsorptions were unaffected. Net ammonia secretion increased. We conclude that changes in renal function both reflect and help to minimize some of the associated disturbances in systemic physiology. Lead-induced ionoregulatory toxicity in rainbow trout, particularly the disturbance of Ca2+ homeostasis, is not exclusively a branchial phenomenon, but is in part a result of disruption of ionoregulatory mechanisms at the kidney. This action of lead outside the gills is critical to consider when developing guidelines for water quality (Patel *et al.*, 2006).

An investigation on the effect of the heavy metal, lead (Pb) on the gill and liver of the African catfish *Clarias gariepinus* was carried out in the laboratory. One hundred and sixty (160) fingerlings of the fish were exposed to continuous exposure to sub-lethal concentrations (0.006 mg/l and 0.008 mg/l) of lead for a period of three weeks. The results showed that the degree of distortion of the gills and liver was proportional to the exposure periods and concentration of the metals was found to be dose and time dependent (Olojo *et al.*, 2005).

The wide spectrum of damage to the health of children from environmental lead (Pb) is a matter of global concern. The neurotoxicity of long-term low-level exposure to lead has a special relevance in children. The aim of this pilot study was to establish the social variables predictive of higher lead levels in young Egyptian children (Boseila *et al.,* 2004).

However, Pb concentration was found between 0.20 and 5.81 mg/Kg wet weight basis in muscles of four common Slovak fish species. Levels of Pb exceeded the maximum allowable concentration in Slovakia by Codex Alimentarius for safe human consumption (0.20 mg/Kg) in the majority of samples (97.2%) (Andreji *et al.*, 2005).

Abdelhamid *et al*. (2007) reported that sampling locations and seasons affected also significantly fish contents of Pb. The same trends of the significant influences were recorded for the bioaccumulation factor (BAF) of the heavy metal in the fish tested. So, fish samples collected from Marsa Matroh and Port Saied were the highest containing for Pb. Summer fish

samples contained the highest levels of Pb. However, Alexandria fish reflected the highest BAF of Pb (1664.26%). Winter fish samples gave the most Pb – BAF (1003.12%). Generally, male fish accumulated more Pb (594.25%) than the females. There were significant correlations between: Pb content and its BAF in fish, Fe content and each of Pb content and its BAF in fish, Fe-BAF and each of Pb, Fe and Pb – BAF in fish, Pb and Cd contents in fish, Fish length and each of Pb and Pb – BAF in fish, Fish depth and each of Pb, Pb – BAF, and fish length, Fish weight and each of Pb, Pb – BAF, length and depth of fish, and TBC and each of Pb content and fish length, depth and weight. T-test between *Shigella* incidence rate and Pb content in fish showed significances. From the foregoing results, it could be concluded that there is pollution with heavy metals (particularly with iron and lead) and pathogenic bacteria (specially *E. coli*) in all fish feeds, rearing water and sediments of the tested locations (mainly in summer season). Also, there is no difference between fish of natural resources and those of aquaculture concerning metals pollution and bacterial contamination. So, it is a legal must to take considerations from the responsible authorities for treating all kinds of waste waters before reaching water bodies to protect aquatic life and consumers.

Lead is a well known neurotoxic metal leads to impairment of neurodevelopment in children is the most critical effect. Exposure in uterus, during breast feeding and in the early childhood may all be responsible for effects. Lead accumulates in skeleton and its mobilization from bones during pregnancy and lactation causes exposure to foetus and breast fed infant. The life time exposure of women before pregnancy is important. Cognitive effects in children are associated with lead levels in blood of about 100-150 µg L-1 (Pb-B either in pregnant woman, cord blood and child). There are indications that Pb is even harmful at blood concentrations considerably below 100 µg L-1 and there may be no threshold for these effects. Major decrease of Pb-B over the last decades, phasing out leaded petrol, reducing other sources of exposure. Lowest average Pb-B in European countries is 20 µg L-1 (De Temmerman, 2006).

Moreover, Eweedah *et al.* (2006) reported that the concentrations of Pb in edible muscle of different freshwater fish of Egypt are often above the maximum permissible limits according to FAO standards. The child intake of fish with these levels is generally above the maximum allowable concentrations, which means human health risk with current Egyptian dietary intakes of fish. Chronic lead (Pb) toxicity tests with *Brachionus calyciflorus, Chironomus tentans,* and *Lymnaea stagnalis* were performed in artificial freshwaters.

Due to increasing concern about the intake of contaminants in foods, a study was performed to monitor the exposure of the Korean population to heavy metal contaminants (lead) from typical diets, and to estimate the health risk. A food list representing typical dietary practices of Koreans was developed, based on the results of the 1998 National Health and Nutrition Survey and the 1999 Seasonal Nutrition Survey, which included a nationwide sample of 4000 and 3000 households, respectively, including everyone 1 year and older. Foods were prepared for consumption (table-ready) according to representative recipes and typical cooking methods, and were

chemically analyzed to measure the levels of heavy metals. Then, the dietary intake of each heavy metal was estimated based on the mean food intake of the population, and the associated risk was evaluated by comparing intakes with the provisional tolerable weekly intakes (PTWIs). Although seaweeds and fishes were highest in heavy metal content, the contribution of foods to total heavy metal intake was more influenced by the amount of food consumed. Nevertheless, the estimated dietary intakes of lead (24.4 mg/person/day) from the 116 foods tested were well within the safe limits (under 30% of PTWIs). It appears that there is no imminent health risk due to heavy metals examined in this study for the total diet of the Korean population (Lee *et al*., 2006).

Lead (Pb) concentrations in vacuum packaged smoked fish species that are commercially sold on the Ankara market were evaluated. A total of 73 smoked fish fillet samples were purchased from Ankara hypermarkets between 2004- 2005. Trace metal concentrations were measured by GFAAS. The range found for Pb was 0.001-0.791 mg kg-1 dry weight. Pb levels in 27 fish samples (36.9%) exceeded the Turkish acceptable limit of 0.2 mg kg-1. However, at even the highest heavy metal concentrations measured, the estimated weekly intakes of Pb for a 60 kg adult consuming 400 g of fish per week would be below the provisional tolerable weekly intakes recommended by the Joint FAO/WHO Expert Committee of 25 µg kg-1 body weight for Pb (Şireli *et al.*, 2006).

Abou El-Ella *et al.* (2007) reported that the progressive Pb bioaccumulation in different tissue organs of grass carp was evident and relatively proportional to the time of exposure and concentration of the metal in water. Pb in liver and kidney was heavily bioaccumulated indicating their relative critical importance for the detoxification and release mechanisms. Ali et al. (2007) and Lotfy (2007) reported higher Pb concentrations in Wadi El Rayan and southern region of Manzala Lakes' water, respectively than the permissible level. Selected toxic (lead) metal was determined by means of differential pulse stripping anodic voltammetry (DPSAV) in some different brands and kinds of fishery products purchased from the popular supermarkets of Turkey. Among the fishery products, the highest concentration of lead was found in the frozen anchovy (314.2 lg/kg). Canned tuna fish (Brand A) had the lowest lead (76.1 lg/kg). The concentrations of toxic element in the selected products were high and often exceeded legal limits set by health authorities. Therefore these products must be monitored more often (Çelik and Oehlenschläger, 2007).

Shaker and Saeed (2007) found that Pb concentration in tissues of tilapia fish inhabiting Lake Edku was ranging between 0.08 and 53.81 µg/g dry weight of Tilapia zillii, 0.11-31.36 µg/g dry weight for Oreochromis aureus, and 0.11-4.27 µg/g dry weight for *O. niloticus.* There were remarkable variations in Pb levels among fish species and organs as well as among collection seasons.

Saeed (2007) added that Pb concentration in tissue/organ of the catfish was higher in sall size fish than in large size fish. Again, there were significant variation among sampling seasons as well as among tissues in Pb levels (the highest in ovaries, gills, kidney and liver but the lowest in muscles). Pb

concentrations in gills were higher than the corresponding legal limits indicating that the fish in Lake Edku are suffering from pollution with heavy metal (Saeed, 2007 and Shaker and Saeed, 2007). Also, Karak *et al.* (2010) found that Pb concentrations in fish muscles were affected by each of fish species and sampling locations, although they still within the permissible levels of Syrian standards.

Zaki (2007) mentioned that water pollution is one of the major problems in the world, especially in the developing countries including Egypt. The course of hazards markedly increased during the last decades after the propagation of many industrial and culture projects with increased world population. Aquatic fauna, the main recourses for fish production is affected mainly by heavy metals pollution, especially from chemical and biological effluents. The impact of heavy metals on fishes is biomarkers for water pollution in rivers and seas. Aquatic fauna, especially fishes, are exposed today to chronic and so called pollutants that do not cause heavy mortalities but survive and accumulate various amount of microbial agents or chemical residues of heavy metals, thus have an unpleasant taste or are potentially dangerous. It has been estimated that some activities have augmented the flow of heavy metals in the sea fourfold increasingly stringent controls and changes in process technology have led to reduction in discharges in industrial countries but the problem in some developing countries remain unclear.

El-Kassas (2009) isolated a heavy metal resistant strain of marine fungus from a polluted sea spot in the Mediterranean, Alexandria. It was *Fusarium solani* which effectively uptake Pb. The fungus exhibited both adsorption of the metal on the fungal cell wall and its accumulation within the cells. It removed 92-97% of the Pb concentrations. Pb may be adsorbed extracellular at the mycelia. So, marine fungi are integral members.

Bentonite was used as an adsorbent agent with levels (0, 1 and 2%) to alleviate the toxic effects of dietary lead oxide with levels (0, 100 and 1000 ppm) on growth performance and survival rate, carcass composition and its residues in fish muscles, and blood hematological and biochemical parameters of mono-sex Nile tilapia *Oreochromis niloticus* for 16 weeks. So, the factorial design analysis (3X3) was used in the present study. The obtained results showed that contaminated diets with lead oxide led to significantly ($P \leq 0.05$) decreased of growth performance (final weight, weight gain, average daily gain, specific growth rate and feed intake), carcass composition (crude protein), blood hematological (hemoglobin, red blood cells, packed cell volume, MCHC, blood platelets, white blood cells and the percentage of lymphocytes), blood biochemical (total protein, albumin, globulin, albumin/globulin ratio and total cholesterol) compared with control group. While, survival rate and blood indices (MCH) insignificantly ($P \geq 0.05$) decreased. But, feed conversion ratio impaired significantly compared with control group. However, dry matter, ether extract, ash, bioaccumulation of total lead in fish muscles, MCV, the percentage of monocytes, neutrophils, eosinophils and aspartate aminotransferase, alanine aminotransferase and uric acid concentrations were increased significantly ($P \leq 0.05$) compared with the control group. As well as, these drastic effects were increased by

increasing level of dietary lead oxide. On the other side, dietary supplemented by bentonite as an adsorbent agent reduced the toxic effects of lead oxide on mentioned measured Parameters. Consequently, it could be recommended that the safety and useful addition of bentonite with levels 1 or 2% to alleviate the toxic effects of dietary contaminated by lead oxide of *O niloticus* fish (Farrag *et al.*, 2009).

Moreover, Khalid *et al.* (2009) found Pb in crayfish from farms and lakes of Alexandria and Behera governorates as high as 1.948 µg/g wet weight, with highest levels in exoskeleton, hindgut and hemolymph. Also, Khorshed (2009) detected Pb in marine and fresh water fish samples by a contamination percentage of 73.6 of the total number of analyzed samples. The maximum limit (ML)of Pb exceeded in 1.9 % of all samples analyzed. In marine fish samples analyzed, 67.9 % were contaminated. However, no exceeding of the levels of Pb above its ML. One sample of twenty five freshwater fish samples exceeded the ML. Shy added that the estimated weekly intake of Pb for 60 Kg adult consuming 60.2 g/week of marine fish and 37.1 g/week of freshwater fish was below the respective tolerable weekly intake (25 µg/Kg body weight).

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محاولة خفض محتوى الرصاص فى أسماك البلطى والبورى أثناء الإعداد والطهى

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كان الهدف من هذه الدراسة هو معرفة تأثير كل من ملح الإيثيلين ثنائي الأمين رباعي حمض الخليك (EDTA) وطرق الإعداد والطهي للأسماك من محافظة دمياط على محتواها من الرصاص.

فاستخدمت محاليل مائية لهذا الملح بتركيزات متدرجة (٠,٠٧، ٠,١٤، ٠,٢١ جزء في المليون) لنقع عينات من أسماك البلطي النيلي (من نهر النيل ومن مزارع في محافظة دمياط) والبوري (من البحر المتوسط ومن بحيرة المنزلة) لمدة نصف ساعة (بجانب عينات سمكية لم تُنقع كمقارنة)، ثم تم حشو الأسماك بالثوم والتوابل. جزء من هذه الأسماك تُرك طازجا بدون طهي، وجزء غُمس في دقيق القمح لقليه، وجزء غُطى بالردة للشواء.

ثم تم أخذ عينات من عضلات الأسماك (طبقا لنوع السمك، مصدر السمك، طريقة الطهي للسمك، تركيز ملح الـ EDTA) للتحليل لمحتواها من عنصر الرصاص بعد تحضيرها للتقدير على جهاز مقياس الطيف الذرى (AAS)، وتم تحليل النتائج المتحصل عليها إحصائيا، وكانت أهمها:

١)وجود تأثير معنوي للتداخل بين طرق الطهي وتركيز ملح الـ EDTA، وكذلك بين مصدر عينات السمك وطرق الطهي وتركيز ملح الـ EDTA على محتوى رصاص عضلات أسماك البلطي، فعكست تركيزات

٠,٠٧ و ٠,١٤ جزء في المليون من ملح الـ EDTA أقصى تركيزات لرصاص عضلات السمك (٠,٥٠٢ و ٠,٢٢٦ جزء في المليون، على الترتيب)، بينما خلى السمك من الرصاص في العينات الطازجة المعاملة بتركيز ٠,٢١ جزء في المليون من ملح الـ EDTA، وكذلك العينات المقلية والمعاملة بتركيز ٠,٠٧ جزء في المليون من ملح الـ EDTA.

٢)وُجدت فروق معنوية في تركيزات رصاص أسماك البوري، والتي ترجع لكل من مصدر السمك وطريقة طهيه وتركيز ملح الـ EDTA، فاحتوت الأسماك البحرية على تركيزات أعلى من أسماك كل من المزارع والبحيرة، والشواء زاد تركيزات الرصاص عنه في العينات الطازجة، وكان لزيادة تركيز الملح (٠,٢١ جزء في المليون) أثر سيء ، إذ أدى لظهور أعلى تركيز رصاص في عضلات السمك.

٣)كان للتداخل بين كل من (مصدر السمك وتركيز ملح الـ EDTA)، (طريقة الطهي وتركيز ملح الـ EDTA)، (مصدر السمك و طريقة الطهي وتركيز ملح الـ EDTA) تأثيرات معنوية على تركيز الرصاص في عضلات أسماك البوري. فأقل قيم رصاص في السمك وُجدت في عينات البحيرة الطازجة المعاملة بأي من تركيزات ملح الـ EDTA الثلاثة، أو في العينات المشوية والمعاملة بأقل تركيز للملح (صفر و ٠,٠٧ جزء في المليون من الـ EDTA)، بينما أعلى تركيزات لرصاص الأسماك وجد في عينات البحيرة المشوية والمعاملة بأعلى تركيز ٠,٢١ جزء في المليون EDTA يليها عينات البحر المشوية والمعاملة بتركيزات صفر، ٠,٠,٢١، ٠,١٤ جزء في المليون EDTA على الترتيب.

٤)بأخذ نوعى السمك في الاعتبار، وجد تأثير معنوي لكل من نوع السمك ومصدره على محتواه من الرصاص، بالبلطي احتوى على تركيزات أعلى معنويا عن البوري (الضعف تقريبا كمتوسط عام، ٠,١٥٧ و ٠,٠٨٥ جزء في المليون، على الترتيب)، واحتوت أسماك البحيرة (البوري) أقل متوسط لتركيز الرصاص (٠,٥٣ جزء في المليون) يليه أسماك المزارع (بلطي ٠,١١٤ جزء في المليون) ثم الأسماك البحرية (بوري ٠,١٢٠ جزء في المليون) ثم الأسماك النيلية (بلطي ٠,١٦٧ جزء في المليون).

٥)للتداخل تأثير معنوي على تركيز الرصاص فى عضلات الأسماك عند النظر لنوعى السمك معا، من حيث تداخل (طريقة الطهي وتركيزات ملح الـ EDTA) و (المصدر وطريقة الطهي وتركيزات ملح الـ EDTA).

وفى الخلاصة يمكن القول بأن لنوع السمك ومصدره وطريقة طهيه، بالإضافة لمعاملة الأسماك بمحاليل ملح الـ EDTA تأثيرات معنوية على محتوى عضلات الأسماك من عنصر الرصاص السام.

قام بتحكيم البحث

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