Studies on Some Heavy Metal Residues in Fresh *Oreochromis niloticus*: Trails to Reduce Them by Marination and Heat Treatment

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Abstract

A total of forty fresh *Oreochromis niloticus* were collected from markets of Kalubia governorate to estimate the level of, lead (Pb) , cadmium (Cd) and mercury (Hg) residues . Heavy metal residues were estimated in raw samples then after marination with garlic, lemon and other additives after one hour and two hours then after roasting and frying of marinated samples by using Atomic Absorption Spectrophotometer (AAS) .The obtained results revealed that lead levels were within permissible limits in all raw samples while cadmium and mercury levels were higher than the permissible limits in 70% of raw flesh fish samples. Marination of fish samples with garlic, lemon and other additives for two hours decreased lead and mercury levels. Also roasting and frying of marinated samples decrease Hg and Pb levels .Meanwhile they had alittle effect on Cd level .The present results were evaluated according to the permissible limits of FAO / WHO (1992) and Egyptian Organization for Standarization and Quality Control (E.O.S.Q.C.) (1993) and (2004).

Finally it is concluded that careful periodical analysis of fish should be performed to evaluate their load of heavy metals. Good cooking and marinating of fish flesh for two hours and more should be done to avoid the hazards of heavy metal on human health. Moreover, the public health significance and suggested precautions for minimizing the level of such heavy metal residues in fish were discussed.

Introduction

Many dangerous chemical elements if released into the environment accumulate in sediment of water bodies (2) due to pollution of water with heavy metals through drainage of some industrial establishment and the complicated of this problem on sediment where plants may grow and consumed by fish (3). Heavy metals had a great public health hazards on human beings. Lead is recognized as a toxic substance which accumulates in the body due to its low rate of elimination lead toxicity causes renal tubular dysfunction, inhibit hemoglobin synthesis and produce fragile blood cell which results in anemia (4) .Cadmium is widely distributed in the environment at relatively low concentrations (5).

The use of sewage sludge as fertilizer in agriculture is considered a potential source of increase cadmium content in vegetable .Chronic exposure to cadmium lead to renal dysfunction, hypertension, lung impairment, reproductive toxicity and bone defects (6). Mercury is used as fungicides as well as mercurial fungicides used for dressing .The toxic compound of mercury accumulate mainly in liver, the alkylmercuries are more slowly metabolized and more evenly distributed in the body tissues (7).

Mercury toxicity causes redness of lips, throat and tongue, loss of teeth, swelling and redness of the skin with pink red finger tips .It affect the nervous system causing irritability (8 – 10). Human exposure to methyl mercury (MeHg) causes a variety of adverse head development delayed in children of exposed mother (11) and neurocognitive function in adults (12). Blood MeHg concentration strongly correlated with the frequency and types of sea food consumed howevere , even for pregnant women , consuming sea food has a variety of MeHg intake is known to be low (13 and 14) .

The objective of the present study is to detect the concentrations of lead, cadmium and mercury in *Oreochromis niloticus* samples which collected from the markets in Kalubia governorate as well as the effect of heat treatments (roasting and frying) on these residues after marination to ensure the safety of fish flesh samples for human consumption.

Materials and Methods

1- Collection of samples:

Forty fish samples *(Oreochromis niloticus)* were collected from markets at Kalubia governorate .Each sample was individually placed in an acid washed polyethlene bag and transported to laboratory in ice box, then prepared immediately for digestion.

2- Washing procedure: (15)

All equipment washed and cleaned using deionized water to avoid chemical contamination with trace elements or heavy metal.

3- Digestion and Analysis of Samples:

* Procedure (A):

Each fish sample was represented by two grams of fish flesh, then placed in clean screw capped tube and digested according to (16) by adding

10 ml of nitric / perchloric acid mixture (4:1). Initial digestion was performed at room temperature for 4 hours followed by careful heating at 40-45°C for one hour in a water bath, then temperature was raised to 72 °C with gentle shaking until complete digestion within 2-3 hours. The digest was allowed to cool at room temperature and diluted up to 20 ml with deionized water, then filtered through Whatmen filter paper (No.1). Lead and cadmium levels were determined in all samples by using Flam Atomic Absorption Spectrophotometer (UNICAM969 A A Spectrophotometer).

* Procedure (B):

According to (17) to determine mercury levels at minimal temperature for all fish samples. Half gram of fish flesh was digested in 10 ml solution of concentrated sulfuric acid / nitric acid in ratio 1:1 at 45 °C for 15 hours. The cold digest was diluted and filtered with filter paper (No.42), then the volume was completed to 100 ml with deionized water, then analyzed by using Flameless Atomic Absorption Spectrophotometer (A.A.S.).

4- Experimental Techniques:

Each sample was divided into 4 groups, first one raw which freshly examined for residual levels of heavy metal (lead, cadmium and mercury). Group 2 was marinated with grind mixture of garlic, green paper, lemon juice, mustard and salt and re examination after one hour and two hours. Group 3 was roasted in oven for 20-30 minutes and group 4 was fried in cotton seed oil for 10 minutes, then both groups were subjected for examination.

5- Determination of heavy metal in fish samples:

The concentrations of heavy metals (lead , cadmium and mercury) in solution of all samples (raw, after marination 1 hour , after marination 2 hours , roasted and fried samples after marination) were estimated by using Atomic Absorption Spectrophotometer , the maximum absorbance was obtained by adjusting the cathode lamps at specific slits and wave length as shown in the following table: :-

Table (1) : Specification of Spectrophotometer cathode lamps.

Metal	*Wave length*	*Slit width*
Lead	**217.0**	**0.7**
Cadmium	**228.8**	**0.7**
Mercury	**253.7**	**0.5**

The obtained results were statistically analyzed according to (18) .

RESULTES

The obtained results were tabulated in the following tables:-

Table (2): The recommended international levels of heavy metal in fish .

Metal	Permissible limits in fish	Country and references
Lead (Pb)	0.1 mg/kg 0.5ppm	Egypt : (E.O.Q.C.No230,1993) FAO/WHO (1992)
Cadmium (Cd)	0.05 ppm 0.1 mg/kg	FAO/WHO (1992) Egypt: (E.O.S.Q.C.No.2360,2004) Egypt: (E.O.S.Q.C.No.2360,1993)
Mercury (Hg)	0.5ppm 0.5 mg/kg	FAO/WHO (1992) Egypt :(E.O.S.Q.C.No.2360, 1993) as methyl mercury .

Table (3) : The concentration (ppm) of lead in *Oreochromis niloticius* flesh samples n=40 .

Treatment	Parameters			
	Min. concentration	Max. concentration	Mean	± S.E.
Raw fish	0.200	0.480	0.300	0.029*
Marination after one hours	0.200	0.370	0.282	0.049
Marination after two hour	0.190	0.270	0.242	0.030
Marinated then roasting	0.190	0.260	0.230	0.015
Marinated then frying	0.090	0.270	0.210	0.033

*Significant differences ($P \leq 0.05$) .

Table (4): The concentration (ppm) of cadmium in *Oreochromis niloticus* flesh samples (n=40).

Treatment	Parameters			
	Min. concentration	Max. concentration	Mean	± S.E.
Raw fish	0.090	0.170	0.113	0.010*
Marination after one hours	Not detected	0.150	0.010	0.020
Marination after two hour	Not detected	0.140	0.010	0.020
Marinated then roasting	Not detected	0.130	0.094	0.022
Marinated then frying	Not detected	0.130	0.092	0.015

*Significant differences (P≤ 0.05).

Table (5): The concentration (ppm) of mercury in *Oreochromis niloticus* flesh samples (n=40).

Treatment	Parameters			
	Min.	Max.	Mean	± S.E.
Marinated then roasting	0.242	0.358	0.292	0.019
Marinated then frying	0.088	0.128	0.116	0.007
Raw fish	0.450	0.720	0.622	0.034
Marination after one hours	0.312	0.600	0.439	0.050
Marination after two hour	0.310	0.422	0.369	0.020

*Significant differences ($P \leq 0.05$) .

Table (6): Acceptability of the examined *Oreochromis niloticus* samples according to their heavy metal levels.

Element	Permissible limits	Raw samples over permissible limits		Marination after 1 hr.		Marination after 2 hr.		Marinated then roasting		Marinated then frying	
		No.	%	No.	%	No	%	No	%	No	%
Lead (Pb)	(0.1)*	0	0	0	0	0	0	0	0	0	0
	(0.5)**	0	0	0	0	0	0	0	0	0	0
Cadmium (Cd)	(0.1)*	28	70	24	60	24	60	16	40	16	40
	(0.05)**	40	100	32	80	32	80	24	60	24	60
	(0.05)***	40	100	32	80	32	80	24	60	24	60
mercury (Hg)	(0. 5)*	28	70	16	40	0	0	0	0	0	0
	(0. 5)**	28	70	16	40	0	0	0	0	0	0

0.1 mg/kg (0.1)* Egypt (E.O.S.Q.C. No.2360,1993)
0.5 ppm (0.5) **: FAO / WHO (1992)
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0.05 ppm (0.05) ***: Egypt (E.O.S.Q.C. No. 2360, 2004)
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Discussion

The present study was concerned to the pollution of fish with heavy metal and the implication of this problem on the tissue burden of metals in fish especially *Oreochromis niloticus* in Egypt .

The obtained data in table, (2) showed that, levels of lead in examined raw flesh samples were ranged from 0.200 to 0.480 ppm with average mean 0.300 ± 0.029. The present results were nearly agreed with that reported by 19-22. While high levels were found by 23 - 28, while lower levels reported by 15 and 29-32 .The mean values of lead concentration (ppm) were 0.282 ± 0.049 and 0.242 ± 0.030 after marination for one hour and two hours

respectively these results showed that marination of fresh fish with garlic and other additive largely decreased concentration of lead and increasing the time of marination for two hours has good effect on lowering the residual level. Also the mean values of lead levels was 0.230 ± 0.015 and 0.210± 0.033 after roasting and frying of marinated samples respectively. Similar results were recorded by 32– 36. The reduction of lead residual levels in examined samples may be attributed to that the garlic contains chelating compounds capable of enhancing elimination of lead. This held the view pointed out by 37.

From the data illustrated in table (5) present that all samples were accepted according to permissible limits according to 38 and 39. The analysis of variance test (ANOVA) revealed that a significant variation of lead residues in examined samples of fish flesh and found between different treatments. The lower levels of lead in fish muscle samples may be result from low binding rate of lead to sulphahydryl groups in fish muscles as well as low solubility in sea water and restricted relocation of lead as mentioned by 40. Lead is a non essential element for man, with a potential toxic for all biological systems, as it accumulates in human tissues. It has haematologiacl effects as it inhibits haemoglobin synthesis (41). These effects may lead to anemia and microcythemia, thereby affect the membrane permeability of kidney, liver and brain cells, which reduce the function or completely break down of these tissue (42). The damage of central nervous system is marked and common feature particularly in children due to their low lead tolerance (43) and (44). Also sever lead poisoning has been known due to its potential carcinogenic nature, causes sterility, abortion and neonatal mortality and morbidity. Studies have demonstrated that lead toxicity lead to gametotoxic effects in both male and female, beside acut lead exposure causes gastrointestinal disturbances as constipation, diarrhea, vomiting and nausea (45). The obtained data in table, (3) it was found that cadmium levels in examined muscle samples of raw *Oreochromis niloticus* were ranged from 0.090 to 0.170 with average mean 0.113 ± 0.010. The present results are nearly in agreement with that reported by (21, 22, 26, and 46 – 48).While lower levels reported by (29, 31, 32 and 49). Highest levels were found by (3, 25, 28 and 30). The mean values of cadmium concentration (ppm) 0.010 ± 0.020 after marination for one and two hours and 0.094 ± 0.022 and 0.092 ± 0.015 after roasting and frying , these results

showed that marination of fresh fish with garlic and other additives , roasting and frying have little effect on the concentration of cadmium , similar results were recorded by (50 ,32 and 39) .

From the data obtained in table, (5) 70% of samples had cadmium over the permissible limit according to (38, 39 and 51). While after marination by one and two hours 60 % of sample showed cadmium level over the permissible limit, inmarinated roasting and frying 40 % of samples were over the permissible limit according to (39). 80 % of samples after marination with one and two hours and 60 % of samples after roasting and frying were over the permissible limit of (38 and 51). The (ANOVA) test revealed a significant variation of cadmium residues in examined samples. High incidence of cadmium may be due to contamination aquaculture by drainage of phosphate fertilizers, sewage, sludge also using plated galvanized equipments enamels and glazes (52). Chronic exposure to cadmium lead to renal dysfunction , hypertension , lung impairment , reproductive toxicity and bone defects (6) , the International Agency for Research on Cancer (IARC) classified cadmium into group I substance carcinogenic to human (53) . The obtained data in table, (4) revealed that the mercury levels in examined samples of raw *Oreochromis niloticus* were ranged from 0.450 to 0.720 with average mean 0.622 ± 0.034. The present results were nearly agreed with (47 and 54 – 59). Lower levels reported by (32 and 60). While higher levels recorded by (22, 28, 31, 47, and 61). The mean values of mercury levels were 0.439 ± 0.050 and 0.369 ± 0.020 after marination for one and two hours respectively, these results showed that marination of flesh fish with garlic and other additives largely decreased the residual level of mercury and so increasing the time of marination for two hours has a good effect on mercury level as well as roasting and frying of marinated samples. The mercury level was 0.292± 0.019 and 0.116 ± 0.007 respectively; similar results were recorded by (32 – 36). The reduction in mercury residual level in examined samples after marination may be attributed to that the garlic contain chelating compound capable of enhancing elimination. The reduction of mercury levels after roasting and frying of marinated samples may attributed to volatilized of mercury when temperature higher than 100°C (32 and 37) .

From data illustrated in table, (5) present that 70 % and 40 % in raw and after marination of one hour respectively were over the permissible

limits according to 38 and 39. All samples after two hours of marination and roasting and frying become within permissible limit of 38 and 39. It is concluded from the ANOVA results that there were high significant variation in the means of mercury between different treatments. The high level of mercury in our study may be attributed to the treatment of grain with mercury compound used as fungicides which lead to different types of human and animal poisoning and due to drainage from agriculture to aquatic environment so fish considered as the greatest source of mercury contamination of food today. On the other hand mercury is lipid soluble in its methylated form (62) , mercury is highly toxic even low concentration , methylmercury is more dangerous to man than inorganic mercury because it can not be excreted and can across the blood brain barrier to nervous system producing progressive and irreversible brain damage (63). The variation of lead, cadmium and mercury concentrations in the present study with those recorded by other investigators is logic due to the difference in size of examined fish, season, analytical procedures, locality of harvesting as well as environmental pollution (64).

From the obtained data we could concluded that the increasing the time of marination two hours and more , roasting and frying with high temperature have good effect in decreasing the levels of heavy metals also preventative measures of concern intended for minimizing the pollution of fish with such metals including : -

1- Using of treatment units, good hygienic disposal and treatment of agricultural and sewage wastes from the origin decrease the hazard caused by heavy metal.
2- Regular and period monitoring for water and sediment samples at suspected area of pollution.
3- Periodic fish analysis for heavy metals should be conducted to measure pollution level.
4- Fishing should be prevented at the polluted sites and give peoples information about the dangerous effect of consuming contaminated fish.
5- Administration of diet rich in proteins , vitamins as D and E , ascorbic acid , calcium , zinc and iron which play an important role in decreasing the absorption and toxicity of heavy metals such as lead , cadmium and mercury .

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الملخص العربي

دراسات علي بعض بقايا المعادن الثقيلة في البلطي النيلي : محاولة التقليل منها عن طريق التتبيل والمعالجة الحرارية

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تم تجميع عدد ٤٠ عينة من أسماك البلطي النيلي من أسواق محافظة القليوبية وذلك لتحديد نسبة تركيز بقايا المعادن الثقيلة مثل الرصاص والكادميوم والزنبق . تم تحديد نسبة بقايا المعادن الثقيلة في عضلات الأسماك النيئة وبعد التتبيل بالثوم والليمون والإضافات الأخرى بساعة وساعتين وبعد الشوي والقلي بواسطة استخدام جهاز الامتصاص الذري .

وقد أظهرت النتائج أن نسبة الرصاص كانت في الحدود المسموح بها دوليا ومحليا في حين أن نسبة تركيز بقايا الكادميوم والزنبق تتجاوز الحدود المسموح بها عالميا ومحليا في ٧٠ % من العينات النيئة . التتبيل بالثوم والليمون والإضافات الأخرى قلل من نسبة الرصاص والزنبق وخاصة بعد ساعتين من التتبيل وكذلك المعالجة الحرارية سواء بالشوي أو القلي للعينات المتبلة قللت نسبة الرصاص والزنبق بدرجة كبيرة جدا مع تأثير طفيف على نسبة الكادميوم وقد تم مقارنة النتائج بالحدود المسموح بها عالميا من قبل منظمة الصحة العالمية ومنظمة الفاو ومع المواصفات القياسية المصرية ومراقبه الجودة .

واخيرا فاننا نوصي بالأتي :

١ـ التحليل الدوري والمستمر لبقايا المعادن الثقيلة في الأسماك.

٢ـ التتبيل الجيد باستخدام الثوم والليمون لفترة ساعتين أو أكثر .

٣ـ الطهي الجيد للأسماك .

٤ـ معرفة الناس بالأضرار الجسيمة لبقايا المعادن وذلك للمحافظة على صحة الإنسان الذي يعتمد على الأسماك كمصدر رئيسي للغذاء .