

DETERMINATION OF SOME METALS IN FISH FROM CERTAIN EGYPTIAN MARKETS

Hassan, A.S.M. and Nevein S. Ahmed

The Central Laboratory of Pesticides, Agric.
Res. Center, Dokki, Giza, Egypt

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ABSTRACT: Twenty two *Tilapia nilotica* fish samples were bought from different markets at Shoubra Al-Khaima, Mostord, Torra, Wadi Hof, Helwan and Al-Hawamedia regions, during November, 2009. These fish samples contained a high level of iron (306.4 µg/g) and a low level of arsenic, 0.0818 µg/g. These high levels of metals such as 146.0 µg/g for lead may reach a hazardous state which may not occur in the most polluted fish farms at the north of Nile delta. Fish farms at Sahl Al-Hussania, which is fed by water from Bahr El-Bakar drainage, as a highly risked area, may involve only one hundredth of lead comparing with those collected from the markets. On the other hand, arsenic may be five times more than the latter. Other toxic metal such as cadmium was reached to 7.505 µg/g in fish samples from the markets. For copper, as an essential element, the highest existed level was 5.425 µg/g. Manganese and zinc, although they are essential for living organisms, they were existed by high concentrations being as 33.61 and 61.03 µg/g, respectively.

Key words: Fish, Arsenic, Lead, Cadmium, Copper, Iron, Manganese, Zinc.

INTRODUCTION

As fish is considered as an important food for a wide area of population, fish is highly traded in the markets. However, fish in the markets comes from several sources. Fish may be brought from fresh or brackish water sources. Also, fish is reared in artificial

pools and farms. Fishery from upper seas is also from the sources of obtaining fish. All of these represent a wide variety of different sources of fish sold in the markets. Fishing in the open seas brings the least contaminated fish. On the other hand, fish fished near industrial plants and other sources

of pollution is considered the most dangerous to human health. Other sources of contamination to fish may be the transportation and storing. Recently, floating dead fish collected from the River Nile after throwing a deal of insecticides is displayed on TV programs. The problem of contamination of water sources where marine organisms live may represent a serious problem, particularly metal contaminants, which are not easily removable (El-Makkawi *et al.*, 2007). The pollution of seafood by metals becomes severe due to the bioaccumulation of these metals in the organism (Gonzalez *et al.*, 1998). This metal accumulation raises the normal concentrations of metals to toxic levels exposing the human population who consume this seafood to potential danger (Klavin *et al.*, 1998).

Previously, we had studied the water and fish in private fish farms at Sahl Al-Hussania, Sharkia Governorate and in fish farms at the central laboratory of aquaculture research at Abbassa village, Abu-Hamad, Sharkia Governorate (El-Makkawi *et al.*, 2007). A present investigation was designed to determine the levels of some metals in *Tilapia nilotica* fish collected from different markets.

MATERIALS AND METHODS

Sampling

Twenty two *Tilapia nilotica* fish samples were bought randomly from different markets at several industrial districts (Shoubra Al-Khaima, Mostord, Torra, Wadi Hof, Helwan and Al-Hawamedia regions) during November, 2009. The average weight of the bought fish is about 250 grams. The fish samples were kept frozen in a deep freezer (20°C) till brought for analysis.

Sample Preparation

About 1 gram of fish sample each was accurately weighed and transferred into Kjeldahl flask and 20 mL of conc. sulphuric acid (98% w/w) and left overnight to avoid ebullition. The flask was then heated to complete the digestion process and few drops of a mixture of 1:1 v/v perchloric and sulphuric conc. acids were added to catalyze the process and remove the bright brown colour till it was colourless. The sample was transferred quantitatively into 50 mL volumetric flasks and completed with deionized water to the mark (JAOAC, 1990).

Chemicals

All the chemicals used were Analytical Grade Reagents. The element standard solutions used for creating the calibration curves were prepared from 1000 mg/L Merck stock solution of the relevant element. Hydride generation chemicals were prepared using 0.5% m/v sodium borohydride in 0.5% m/v sodium hydroxide as reductant and an acid matrix of 50% v/v (5M) hydrochloric acid as described by the instruction manual (TEIM, 2006).

Instrumentation

Thermo Elemental model: Solar M Atomic Absorption Spectrophotometer was used for all the measurements. The current, wavelength and slit band width of each element were adjusted automatically by the instrument software. Arsenic was determined using the hydride generation kit model: VP100.

RESULTS AND DISCUSSION

Heavy metals from natural and anthropogenic sources continuously enter the aquatic ecosystem where they pose serious threat because of their toxicity, long persistence,

bioaccumulation and biomagnification in the food chain (Sankar *et al.*, 2006). The presence of some heavy metals in aquatic environments and their accumulations in fish and in the other organisms has been investigated during recent years (Dural *et al.*, 2007).

In a previous study (El-Makkawi *et al.*, 2007), we found that arsenic and lead were only detected in the waters of private fish farms at Sahl Al-Hussania, Sharkia Governorate. Hence, only arsenic and lead were determined in fish collected from those fish farms. On the contrary, in the current study, the sources of fish in the markets were unknown. Only 15 elements were available for analysis. Of these only 7 elements were detected. Fortunately, of these 7 elements were arsenic and lead. The central laboratory of aquaculture research at Abbassa village, Abu-Hamad, Sharkia Governorate which contains experimental fish pools fed directly by water from Ismailia canal, was chosen as a control source for fish samples. This allows us to compare the concentrations of arsenic and lead in fish samples investigated in the 2 studies. T-test statistical analyses

were calculated between the current data versus those of the previous study. Percentage recovery tests were carried out for the seven metals by spiking a definite concentration of each element to the similarly prepared samples as those unknown samples. The range of these percentage recoveries was between 96% and 103% (Table 1).

Arsenic

Data in Table 1 show that arsenic concentrations range from 0.0818 to 0.8573 with an average of 0.3771 $\mu\text{g/g}$. In comparison to our previous study (El-Makkawi *et al.*, 2007), we found that fish samples at Hussania contained from 0.4260 to 5.349 $\mu\text{g/g}$ and at Abbassa arsenic concentration levels were between 0.5673 and 2.768 $\mu\text{g/g}$. And that, there is a statistically significant difference between the data of market fish and all of the fish farms at both Hussania and Abbassa, individually ($P \leq 0.001$) at 95% confidence. The maximum allowed concentration of arsenic in fish by the Microbiological Standards (BOE, 1991) is 1.662 $\mu\text{g/g}$ (Khaled, A, 2004). The concentrations of arsenic in fish collected from the markets in the present study are lower than the

maximum allowed limits. However, recent advances in our knowledge of arsenic carcinogenesis include the development of rat or mouse models for all human organs in which inorganic arsenic is known to cause cancer—skin, lung, urinary bladder, liver, and kidney (Meharg and Hartley-Whitaker, 2002). The toxicity of arsenic is well known historically (Berman, 1980). Arsenic combines readily with proteins due to its great affinity for sulfhydryl groups. This results in the precipitation of proteins, producing gastrointestinal irritation and irreversible inhibition of important enzyme systems. Arsenic speciation in the environment is complex, existing in both inorganic and organic forms, with interconversion between species regulated by biotic and abiotic processes (Kitchin, 2002).

Lead

For lead it was found that, the concentrations in the present study lied between 50.61 and 146.0 $\mu\text{g/g}$ and of a mean of 117.6 $\mu\text{g/g}$ of whole fish sample, Table 1. The Egyptian Organization for Standardization and Quality Control pointed that maximum allowed limit for Pb in fish must

Table 1. Concentration of Elements ($\mu\text{g/g}$) in the Market Fish Samples

Elements Samples	As (HG) ¹	Pb	Cd	Cu	Fe	Mn	Zn
1	0.5149	93.44	5.885	2.835	44.26	14.31	21.06
2	0.6193	114.8	5.070	3.955	80.65	27.96	38.69
3	0.1612	93.00	4.635	3.315	306.4	22.82	18.01
4	0.1744	50.61	7.305	2.655	71.30	18.01	ND ²
5	0.3568	90.35	7.505	4.07	225.6	26.96	26.56
6	0.4564	98.77	5.110	3.915	69.05	21.11	27.21
7	0.4922	62.11	6.255	4.535	66.55	24.06	61.03
8	0.5441	131.4	6.310	2.260	56.90	32.56	22.29
9	0.4286	146.0	5.560	5.425	299.7	23.02	33.38
10	0.2071	132.5	5.435	3.905	58.35	21.24	23.44
11	0.0818	124.5	5.055	2.995	62.35	ND	31.08
12	0.1619	134.9	5.370	3.745	87.30	26.61	13.57
13	0.1569	129.9	6.105	4.055	59.40	23.09	31.69
14	0.2222	126.0	ND ²	4.405	251.9	12.84	14.85
15	0.8573	61.34	5.175	3.715	71.60	25.76	33.46
16	0.5758	117.9	6.655	3.645	84.15	22.14	31.86
17	0.6212	145.2	6.570	3.740	64.30	31.27	ND
18	0.2887	130.3	5.905	3.555	52.55	27.03	26.29
19	0.2877	131.1	7.310	4.565	92.05	24.26	25.16
20	0.3989	131.6	7.430	4.655	86.05	14.56	30.96
21	0.3898	137.1	6.945	4.270	91.20	24.58	31.08
22	0.2989	139.6	6.890	3.980	63.75	33.61	32.96
Min.	0.0818	50.61	5.070	2.260	44.26	12.84	13.57
Max.	0.8573	146.0	7.505	5.425	306.4	33.61	61.03
Mean	0.3771	117.6	5.915	3.827	106.6	22.71	26.15
LOD ³	0.00005	0.013	0.0028	0.0045	0.0043	0.0016	0.0033
MAL ⁴		0.1	0.1	20			50
MAL ⁵	1.662	25					
MAL ⁶			5.00	100.0		2.600	
MAL ⁷			10.00	350.0	8.760		750
Recov. ⁸	99%	96%	97%	98%	103%	97%	96%

1. HG= Hydride Generation technique

2. ND= Not detected

3. LOD= Limit of detection ($\mu\text{g/mL}$)4. MAL= Maximum allowed limit ($\mu\text{g/g}$) (EOSQC, 1991, 1993)5. MAL= Maximum allowed limit ($\mu\text{g/g}$) BOE, 19916. MAL= Maximum allowed limit ($\mu\text{g/g}$) CEFAS, 19977. MAL= Maximum allowed limit ($\mu\text{g/g}$) NHMRC, 1987

8. Recov. = Percentage Recovery

not exceed 0.1 $\mu\text{g/g}$ (Labib *et al.*, 2008). The Microbiological Standards (BOE, 1991) limit of lead in fish is 25 $\mu\text{g/g}$ (Khaled, A, 2004). In Hussania fish farms the lead concentrations were at the levels of 0.1178 to 1.035 $\mu\text{g/g}$, while at Abbassa, the lead concentration values were from 0.1009 to 0.4333 $\mu\text{g/g}$ (El-Makkawi *et al.*, 2007). By comparing the two studies, there is a statistically significant difference between the data of the market fishes and all of the fishes in fish farms at both Hussania and Abbassa, individually ($P \leq 0.001$) at 95% confidence. From the preceding, one can observe that there is an extremely high range of lead concentrations (50.61- 146.0 $\mu\text{g/g}$) in fishes collected from the markets. As already mentioned before, fish at the markets is of unknown sources. And hence, the reason of these very high concentrations of lead is not clear. According to the recommendations by the WHO, the daily intake of lead should not exceed 5 $\mu\text{g/kg}$ of body weight (Berman, 1980). The average daily intake is 300 μg . Ingestion of 600 μg of lead per day may result in intoxication. Lead in the blood is mainly bound to erythrocytes. Lead is a general protoplasmic poison having cumulative, slow acting, and subtle properties. It exerts much of its

biochemical activity through sulfhydryl inhibition due to its high affinity for sulfur. Lead also interacts with carboxyl and phosphoryl groups and interferes with heme synthesis. Lead toxicity may occur by ingestion, inhalation, and through skin cuts. Organic lead compounds are absorbed into body tissues and penetrate the intact skin more rapidly than inorganic compounds. Organic lead compounds may affect nervous tissues more readily than inorganic lead compounds. About 5 to 10% of the lead ingested is absorbed into the body. The rest is discharged with the feces.

Cadmium

Cadmium was highly elevated in the fish samples from the markets. The range of concentrations of cadmium was from 5.070 to 7.505 and with an average of 5.915 $\mu\text{g/g}$, Table1. The Egyptian Organization for Standardization and Quality Control pointed that maximum allowed limit for Cd, must not exceed 0.1 $\mu\text{g/g}$ (Labib *et al.*, 2008). The Centre for Environment, Fisheries and Aquaculture Science (CEFAS, 1997) limit the cadmium level in fish to 5 $\mu\text{g/g}$ (Khaled, 2004). The National Health and Medical Research Council National for Food Standard (NHMRC, 1987) allows up to 10 $\mu\text{g/g}$ in fish

(Khaled, 2004). As can be seen, cadmium concentrations in the fishes collected from the markets is, although, about 50 to 70 times higher than that allowed by the Egyptian Organization for Standardization and Quality Control, they are within the limits allowed by other organizations (NHMRC, 1987). However, cadmium is a highly toxic metal (Berman, 1980). Cadmium inhibits the sulfhydryl containing enzymes and affects the hydroxyl, carboxyl, phosphatyl, cysteinyl, and histidyl side chains of proteins, purines, and porphyrin. It can also disrupt the pathways of oxidative phosphorylation. Cadmium competes with the absorption of some essential elements such as iron, zinc, and copper. The element may also interfere with the release of iron by transferrin. Cadmium ingestion produces symptoms such as nausea, salivation, vomiting followed by diarrhea with abdominal discomfort and pains. Cadmium is accumulated in the human body. Accumulation is continuous, because cadmium has a long biological half life. The accumulation rate of the element is 40 $\mu\text{g}/\text{day}$ (Berman, 1980). The symptomatology of chronic cadmium poisoning, due to accumulation, involves emphysema of the lung, mild liver damage, anemia, proteinuria, renal tubular

damage, some dental changes, and impairment of the sense of smell (anosmia). A characteristic disease of chronic cadmium poisoning is called Itai-itai manifested by renal dysfunction in combination with osteomalacia or severe osteoporosis. This disease is quite prevalent in Japan due to consumption of rice grown in fields close to cadmium smelters. All terrestrial and sea foods and even cigarette smoke contain cadmium. The average overall concentration of cadmium in the human body is approximately 429 $\mu\text{g}/\text{kg}$ of body weight. The concentration of cadmium in tears is below 3.0 ng/mL . Cadmium in blood may reflect current exposure, whereas in urine may indicate chronic exposure at low levels.

Copper

Table 1 shows the that the concentrations of copper in fish samples are within the range (2.260–5.425 $\mu\text{g}/\text{g}$) with the mean of 3.827 $\mu\text{g}/\text{g}$. The Egyptian Organization for Standardization and Quality Control pointed that maximum allowed limit for Cu in fish must not exceed 20 $\mu\text{g}/\text{g}$ (Labib *et al.*, 2008). Up to 350 $\mu\text{g}/\text{g}$ of Cu in fish is allowed by the National Health and Medical Research Council National for Food Standard (Khaled, 2004). Therefore, the copper concentrations found in fish

samples collected from the markets do not represent any danger to human health. Nevertheless, copper is an essential element for all living organisms (Berman, 1980). The essential role of copper became clear when its value in diets and consequently its necessity, in addition to iron, in blood formation was demonstrated in 1928. Copper is involved in the formation of several enzymes such as tyrosinase, ascorbic acid oxidase, cytochrome oxidase, monamine oxidase, and uricase. It is also essential for the cross-linking of elastin. Copper deficiencies were encountered following long-term parenteral nutrition. Toxicity by high copper intake has been reported. Salts of copper have found limited applications as germicides, fungicides, insecticides, astringents, emetics, ceramic pigments, and in the textile industry. Lethal copper poisoning was observed when copper concentrations in serum rose to 20 mg/L. The average overall concentration of copper in the human body is approximately 1.5 mg/kg of body weight. The concentrations of copper in different human samples depend on the age and dietary state of the subject. Abnormally high copper concentrations in human serum and/or urine may be characteristic of diseases such as Wilson's disease (hepatolenticular degeneration),

thalassemia (Mediterranean anemia), hemochromatosis, cirrhosis, yellow atrophy of liver, epilepsy, leukemia, hyperthyroidism, Hodgkin's disease, atherosclerosis, nephrosis, tuberculosis, and carcinomas. Lower copper concentrations occur in cystic fibrosis and kwashiorkor diseases (a syndrome produced by a severe protein deficiency in the diet and characterized by retarded growth, changes in skin and hair pigments, gastrointestinal disorders, anemia, low serum albumin, and mental apathy).

Iron

Iron levels as seen in Table 1 were ranges from 44.26 to 306.4 having an average of 106.0 $\mu\text{g/g}$. The maximum allowed limit of Fe in fish by the National Health and Medical Research Council National for Food Standard (NHMRC, 1987) is 8.76 $\mu\text{g/g}$ (Khaled, 2004). Although, iron is essential to all living organisms (Berman, 1980), the levels of iron in fish collected from the markets is much higher than the maximum allowed limits. A concentration of 306.4 $\mu\text{g/g}$ in fish is about 35 times higher than the maximum allowed limits. Even, an average of 106.0 $\mu\text{g/g}$ is about 12 times higher than the limits. Once again, as fishes obtained from the markets are of unknown sources the high levels of

iron is undefined. Iron as an essential element, functions as a catalyst and is present in amounts greater than any other non-major element. Normally, iron exists in the living body bound to protein. More than half of iron in the human body is associated with hemoglobin, 33% with non-heme iron complexes, 8.9% with myoglobin, and 0.5% with cytochrome and catalase enzymes. The heme-iron functions as oxygen carrier. Ferrous iron is absorbed more easily by the body than the ferric form. The normal iron intake for children is 10 to 20 mg/kg of body weight. Deficiency of iron causes anemia, which is widespread on a global scale. However, all soluble iron preparations ingested in excess can be toxic.

Acute iron poisoning is probably not known in adults and uncommon among young children. Serious acute poisoning in children can occur following ingestion of more than one gram of an iron salt. Two grams of iron salts can be fatal to children. Signs of iron toxicity may occur within one-half to a few hours after ingestion. Symptoms caused by an overdose initially include nausea and vomiting as results of gastrointestinal irritation and necrosis. Other symptoms may be pallor or cyanosis, lassitude,

drowsiness, hematemesis, and diarrhea.

Finally, shock and cardiovascular collapse may occur followed by death within six hours. Iron toxicity may occur through ingestion of contaminated drinking water or food. The average overall concentration of iron in the human body is approximately 60 mg/kg of body weight. The iron concentration in animals varies with the state of health, nutrition, age, sex, and species. The small intestine, acting as both absorptive and excretory organ for iron, serves as the primary control of the iron concentration in the body.

Manganese

Manganese as an essential element was also detected in fish samples. As seen in Table 1 the lowest manganese concentration was 12.84 µg/g and the highest was 33.61 µg/g with a mean of 22.71 µg/g. The limit of Mn allowed in fish by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS, 1997) is 2.6 µg/g (Khaled, 2004). All fish samples in the present study are higher than the maximum allowed limits. The elevation of the levels of Mn in fish ranges from 5 to 13 times more than the maximum allowed

limits. Although, manganese is essential element, it reaches a toxic limit in fish collected from the markets. Sources of contamination of these fish samples by manganese are still unknown. Though, manganese is necessary for normal growth, skeletal formation, and for normal reproductive functions in mammals (Berman, 1980).

Manganese activates many enzymatic reactions associated with the metabolism of organic acids, carbohydrates, nitrogen, and phosphorus. It participates in mucopolysaccharide metabolism and is connected with superoxide dismutase. A daily intake of 3 to 7 mg of manganese is considered to be sufficient for a human balanced diet. Manganese-rich foods involve fish and sea food. Deficiency of manganese is related to the dietary status. Some diseases, such as diabetes, pregnancies accompanied by nervous instability and convulsions, disorders of bony and cartilaginous growth in infants and children, rheumatoid arthritis, and certain types of sterility in males and females, may be related to manganese deficiency. The manganese status in human body may also be affected by long-term parenteral therapy. Toxicity of manganese may arise from

ingestion of contaminated foods. Symptoms of toxicity may include psychomotor instability, hallucinations, and rigidity of limb movements. The average overall concentration of manganese in the human body is approximately 286 $\mu\text{g}/\text{kg}$ of body weight.

Zinc

At last zinc was found to range from 13.57 to 61.03 with a mean of 26.15 $\mu\text{g}/\text{g}$ in fish in the present study. The Egyptian Organization for Standardization and Quality Control pointed that maximum allowed limit for Zinc in fish is 50 $\mu\text{g}/\text{g}$ (Labib *et al.*, 2008). A maximum of 750 $\mu\text{g}/\text{g}$ Zn is allowed by the National Health and Medical Research Council National for Food Standard (NHMRC, 1987) in fish (Khaled, 2004). From Table 1, it is clear that the current studied fish samples show normal levels of zinc in comparison with the maximum allowed limits by both the Egyptian and Australian organizations. Zinc has been known as an essential element since 1869 (Berman, 1980). A number of enzymes, such as alkaline phosphatase and lactic acid dehydrogenase, are zinc dependent. The element also influences the growth rate and bone development, the integrity of the

skin, and development and function of the reproductive organs. It influences wound healing. Approximately 1 to 2 mg of zinc is required daily by humans. Zinc deficiencies have been demonstrated to cause anorexia and lesions of skin and appendages. Zinc intoxication is an infrequent occurrence. Acute intoxication is manifested by nausea and vomiting. Zinc is not cumulative as are most of the toxic metals. Chronic poisoning with zinc is probably nonexistent. The average overall concentration of zinc in the human body is approximately 33 mg/kg of body weight. Zinc concentrations in different human samples may be related to the dietary status of the subject.

From all the previous one may conclude that fish sold in the markets of Egypt may involve very high levels of irrecoverable toxicants. As already has been mentioned, metals toxicity is highly persistent in the living organisms. The risk rates may reach as high as 75 to 1500 times more than the levels permitted by Egyptian Organization for Standardization and Quality Control for lead and cadmium, respectively (Labib *et al.*, 2008). Although, other organizations may permit much higher metal concentrations in fish

than the Egyptian one, iron, manganese and lead still represent a problematic case, respectively, at descending order. However, the problem may still be more dangerous for Pb, as a toxic element, than the other 2 elements. These high levels of metal contamination may derive from different sources which involve water where fish lives, fishery process, transportation, storing and most importantly terrible ways of marketing. Fish is mostly sold on street pavements and at open markets where it is exposed to all sources of pollution. We found a lack of the literatures concerning the study of contamination of fish sold in the Egyptian markets. This may be due to the difficulty of explanation of the obtained results. However, our current study may need more exploration in the future.

We may recommend here that, as already done abroad, fish is deeply frozen directly after fishing and kept in freezers till sent to the markets. Other ways of keeping fish from contamination is processing and freezing as happens in the advanced world.

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تقدير بعض المعادن فى الأسماك الموجودة ببعض الأسواق المصرية

أيمن سيد محفوظ حسن - نيفين صلاح الدين أحمد

المعمل المركزى للمبيدات بمركز البحوث الزراعية

إن الأسماك الموجودة بالأسواق يمكن أن تحتوى على مواد سامة خطيرة. ومن بين هذه المواد تكون المعادن باختلاف كونها ضرورية أم سامة. من المعادن السامة الزرنيخ والرصاص والكاديوم. ولقد قمنا بشراء ٢٢ عينة من أسماك البلطى من مختلف الأسواق المصرية ببعض المناطق الصناعية فى شبرا الخيمة و مسطرد وطرة ووادي حوف وحلوان والحوامدية خلال شهر نوفمبر عام ٢٠٠٩ ووجدنا نسب عالية جدا من كل من الرصاص والكاديوم تلاهما الزرنيخ بمستوى أقل نسبياً وإن كان ما زال يمثل خطراً على صحة المواطنين. فلقد بلغت مستويات تلك المعادن على التوالي: ١٤٦، ٧،٥٠٥، ٠،٨٥٧٣ ميكروجرام لكل جرام للثلاثة معادن على الترتيب. على الرغم من كون عناصر مثل الحديد والارصين والمنجنيز والنحاس من العناصر الضرورية للكائنات الحية إلا أنها وصلت إلى مستويات خطيرة أيضاً، فلقد وصلت فى الحديد إلى ٣٠٦،٤ ميكروجرام لكل جرام بينما وصلت فى الارصين والمنجنيز إلى ٦١،٠٣ و ٣٣،٦١ ميكروجرام لكل جرام على الترتيب وفى النحاس الذى أعتبر الأقل ملوثاً وصلت النسبة ٥،٤٢٥ ميكروجرام لكل جرام. ولقد اعتبرت هذه النسب مرتفعة جداً بالمقارنة بالمزارع السمكية الموجودة فى شمال دلتا النيل ومنها المقامة فى سهل الحسينية بمحافظة الشرقية والمعتمدة على مياه مصرف بحر البقر الذى يعتبر الأكثر تلوثاً فى تلك المنطقة كدراسة مقارنة سابقة.