Vet.Med.J.,Giza. Vol. 50, No.4. (2002):547-557.

DETERMINATION OF SOME HEAVY METAL RESIDUES IN SALTED AND SMOKED FISHES

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Received: 2. 3. 2002

Accepted: 5. 6. 2002

SUMMARY

Fish contamination with heavy metals and its relationship to public health have a considerable concern in the last few years, so the concentrations of some heavy metals in salted and smoked fishes were determined. A total of 60 ready to eat samples, 30 each of salted sardine and smoked herring samples were randomly purchased from different shops at Alexandria Governorate - Egypt, and analysed for their flesh content of lead (Pb), cadmium (Cd), mercury (Hg) and copper (Cu) by using Atomic Absorption Spectrophotometer (A.A.S.). Results revealed that the mean values ± S.E. of Pb, Cd, Hg and Cu residues in fish flesh were 0.275 ±0.044; 0.081 ±0.003; 0.222 ±0.026 and 6.992 ±0.472 p.p.m (wet weight), respectively in salted sardine and 0.145 ±0.017; 0.070 ± 0.003; 0.158 ±0.018 and 7.877 ±0.484 p.p.m (wet weight), respectively in smoked herring fishes. The present results were evaluated according to the permissible limits of FAO/WHO (1992) and E.S.S. No. 2360 issued by Egyptian Organization for Standardization and Quality Control "E.O.S.Q.C." (1993) and also compared with the limits of some other countries. The preventive measures which lead to have safe salted and smoked fishes fit for human consumption were discussed. Also, careful periodical analysis of fresh, salted and smoked fishes should be performed to evaluate their load of heavy metals to avoid their hazards to human.

INTRODUCTION

Fish is one of the important foods for human consumption due to its high protein quality, its essential amino-acid, unsaturated fatty acids, trace elements and minerals content that are not easily synthesized in the mammalian body. Salted and smoked fishes are a popular food items. Pollution

with heavy metals is a problem of magnitude and ecological significance because they are not biodegradable and are not easily eliminated from the ecosystem (Linde et al., 1996). Lead, cadmium and mercury are non essential elements and are considered as toxic metals due to their competition with the essential metal for binding sites and also their interference with sulfhydryl groups and structural protein (Ahmed et al., 1993). Where-as copper is an essential metal and important in the maintenance of normal physiological function of the body, but in large concentrations cause toxic effects that tend to be more complicated than that of the non-essential metals (Sorensen, 1991). In this connection, it is worth recalling that the content in food of toxic metals such as Cd, Hg and Pb in some cases can reach threshold levels (Nielsen, 1974). Meanwhile much attention had been paid to the possible danger of metal poisoning in human as a result of consumption of contaminated fishes (Daoud et al., 1999). The toxic elements such as lead (Pb), cadmium (Cd) and mercury (Hg) are translocated through the food chain to man and animals. The nature of toxicity depends on the chemical form of the element, the dosage, the route, the frequency and duration of administration (Gough and Shacklette, 1976; Underwood, 1977).

The purpose of this study is to determine lead, cadmium, mercury and copper concentrations in salted sardine and smoked herring fishes sold in Alexandria Governorate to ensure their safety for human consumption.

MATERIALS AND METHODS

Collection of Samples:

A total of 60 ready to eat fish samples, 30 each of salted sardine and smoked herring of various sizes, were randomly purchased from different shops at Alexandria Governorate. Each fish sample was individually placed in an acid-washed polyethylene bag and transported to the laboratory, then prepared immediately for digestion.

Digestion and Analysis of Samples:

Procedure (A): Each fish sample was represented by two grams of flesh dissected from the axial muscle behind the head after removal of the scales at this region, then placed in a clean screw-capped tube and digested according to Al-Ghais (1995) by addition of 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 75°C with gentle shaking untile digestion was completed 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 whatman filter paper No. 1. Blank solution was prepared to check the possible traces of metals that may present in the acids or deionized water used

in digestion and dilution of the samples. Lead, cadmium and copper levels were determined in all examined samples by using flame Atomic Absorption Spectrophotometer (UNICAM 969 A A Spectrometer) in Toxicology Unit of the Animal Health Research Institute, Ministry of Agriculture Dokki, Giza, Egypt.

Procedure (B):

The procedure described by Diaz et al. (1994) was carried out for mercury determination at minimal temperature for all fish samples. Half gram of macerated fish muscle 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. Five milliliters stannous chloride solution were added to reduce mercury to elemental form and then analysed by using flamless Atomic Absorption Spectrophotometer "A.A.S." equipped with mercury hydried system "M.H.S.". Cold Vapour Technique for measuring the mercury levels in examined fish samples was undertaken in Central Laboratory of the Faculty of Veterinary Medicine, Zagazig University.

Analytical quality control and background corrections were performed for all elements. Blank was also run with each batch of five samples. Standard mineral solutions were used to calibrate the "A.A.S." with each batch of samples. The samples were run in duplicate and the average was calculated.

The obtained results were statistically analysed according to the method of Selvin (1996).

RESULTS

The heavy metal concentrations in salted sardine and smoked herring fishes were statistically analysed and summarized in table (1). The recommeneded international levels of heavy metals in fishes were summarized in table (2). The frequency of polluted samples exceeding the maximum permissible limits given by E.O.S.Q.C. (1993) were tabulated in table (3).

Table (1): Heavy metals concentrations p.p.m. (wet weight) in salted and smoked fishes.

Type of examined samples	Metal	Min.	Max.	$\overline{X}$	S.D.	± S.E.	C.V.
Salted fish (Sardine)	Pb	0.041	0.723	0.275	0.242	0.044	88.00
	Cd	0.040	0.129	0.081	0.019	0.003	23.45
	Hg	0.065	0.632	0.222	0.144	0.026	64.86
	Cu	5.020	14.220	6.992	2.587	0.472	36.98
Smoked fish (Herring)	Pb	0.030	0.371	0.145	0.095	0.017	65.51
	Cd	0.020	0.098	0.070	0.022	0.003	31.42
	Hg	0.071	0.564	0.158	0.100	0.018	63.29
	Cu	5.414	14.210	7.877	2.653	0.484	33.68

No. of examined samples = 30.
p.p.m.: Part per million.
Pb: Lead; Cd: Cadmium; Hg: Mercury; Cu: Copper.
Min.: Minimum levels.
Max.: Maximum levels.
$\overline{X}$: Mean values.
S.D.: Standard Deviation.
± S.E.: Standard Error.
C.V.: Coefficient of Variation.

Table (2): Recommended international levels of heavy metals in fishes.

Metal	Permissible limits in fishes	Country and References
Lead (Pb)	0.1 mg/Kg. 0.5 P.P. m 2.0 mg/Kg 4.0 µg/g 5.0 µg/g 10 µg/g	Egypt: ("E.S. S." No. 2360, 1993). FAO/WHO (1992). England: (MAFF, 1979). Newzealand: In: Julshamn (1983). Spain : Boletin Oficial del Estado (1991). In: Schuhmacher and Domingo (1996). Canada: Julshamn (1983).
Cadmium (Cd)	0.05 p.p.m 0.1 mg/Kg 1.0 mg/Kg	FAO/WHO (1992). Egypt: ("E.S.S." No. 2360, 1993). Spain: Boletin Oficial del Estado (1991). In: Schuhmacher and Domingo (1996).
Mercury (Hg)	0.5 p.pm 0.5mg/Kg 1.0 µg/g	FAO/WHO (1992). Egypt: ("E.S.S." No. 2360, 1993). as methyl mercury. Spain: Boletin Oficial del Estado (1991) In: Schuhmacher and Domingo (1996).
Copper (Cu)	20.0 p.p.m 20.0 µg/g	Food Stuffs, Cosmetics and Disinfectants (1972) Spain :Boletin Oficial del Estado (1991) In: Schuhmacher and Domingo (1996).

N.B: p.p.m = µg/g = mg/Kg.

Table (3): Frequency distribution of some heavy metals in polluted fish samples.

Metal	Maximum permissible limits (mg/Kg) ("E.S.S." No. 2360, 1993).	Samples exceeding the permissible limits ("E.S.S." No. 2360, 1993).			
		Salted Sardine		Salted Herring	
		No.	%	No.	%
Lead (Pb)	0.1	14	46.67	11	36.67
Cadmium (Cd)	0.1	2	6.67	0	0
Mercury (Hg) (Methyl mercury)	0.5	3	10	1	3.33

("E.S.S." No. 2360) : Egyptian Specification Standard No. 2360 issued by Egyptian Organization for Standardization and Quality Control (1993).

DISCUSSION

Heavy metals make up one of the most important groups of pollutants, so it is necessary to monitor the levels of heavy metal residues to evaluate the acceptability to human consumption.

Lead (Pb):

Lead is carcinogenic in nature and its biological half-life in bone is about 27 years (Shibamoto and Bjeldanes, 1993). The human daily intake of lead is 0.3 mg (Casarett and Doull, 1975), while the maximum provisional weekly intake from lead for human is 0.05 mg/Kg body weight ("E.S.S." No. 2360, 1993).

The results illustrated in table (1) indicated that lead concentrations in salted and smoked fishes were ranged from 0.041 to 0.723 and 0.030 to 0.371 with mean values ± S.E. of 0.275 ±0.044 and 0.145 ±0.017 p.p.m (wet weight), respectively. Nearly similar findings were recorded in muscles of *Mugil Cephalus* (Sallam, 1997) and in salted and smoked fishes (Sallam and El-Gazzar, 1997). In Egypt, high lead values were reported in salted fishes (Nassar et al., 1996 and Zaki, 1998) and in fresh water fishes (El-Safy, 1996; Seddek et al., 1996; Ahmed and El-Boushy, 1998; Daoud et al., 1999). Meanwhile much high levels were estimated in raw fishes at many areas of the world such as: England (MAFF, 1979), Canada and New Zealand (Julshman, 1983), Spain (Boletin Oficial del Estado, 1991), FAO/WHO (1992) in table (2). Low lead levels in muscles of various

species of raw fishes were reported by El-Mowafi (1995); Glueck and Hahn (1995). By comparing the lead levels in the present study with the maximum permissible limits of Egyptian Standard Specification ("E.S.S." No. 2360, 1993)., it was evident that 14 (46.67%) of salted sardine samples and 11 (36.67%) of smoked fish samples exceeded the limits of Pb (Table 3). High lead levels in the present study may be due to industrial and agriculture discharges which are the primary source of lead poisoninging in fishes.

Cadmium (Cd):

Cadmium is toxic in any chemical form, the human daily intake of cadmium is 0.018-0.2 mg (Casarett and Doull, 1975), while the maximum provisional tolerable weekly intake from cadmium for human is 0.0067 - 0.0083 mg/Kg body weight ("E.S.S." No. 2360, 1993).

The results illustrated in table (1) pointed out that the concentrations of cadmium in examined salted and smoked fishes ranged from 0.040 to 0.129 and from 0.020 to 0.098 with mean values ±S. E. of 0.081 ±0.003 and 0.070 ±0.003 p.p.m (wet weight), respectively. The recorded results were within the permissible limits intended by Boletin Oficial del Estado (1991) in Spain and ("E.S.S." No. 2360, 1993) of Egypt (Table 2). Also the obtained results were nearly similar to those recorded by Ahmed and El-Boushy (1998). High levels of cadmium were detected in *Tilapia sp.*, (El-Safy, 1996), in salted fishes "Sardine" (Nassar et al., 1996; Sallam and El-Gazzar, 1997; Zaki, 1998). Low cadmium levels were obtained by Sheo et al. (1991) in fresh water fishes, El-Mowafi (1995) in *Tilapia sp.* Glueck and Hahn (1995) in fresh water fishes, Sallam (1997) in *Tilapia sp.*; Sallam and El-Gazzar (1997) in smoked fishes and Daoud et al. (1999) in fresh water fishes.

From the obtained data in table (3), it was evident that 2 (6.67%) samples of salted sardine exceeded the limits of "E.S.S." No. 2360 (1993) while smoked herring samples were within the limits. In the present study, the low cadmium levels in fish muscles could be attributed to the elevated concentrations of cystine and methionine in fishes than other protein. Absence of sulfhydryl groups in these sulpher-rich amino acids probably play a role in decreasing cadmium binding in skeletal muscles. This held the view reported by Beveridge (1947).

Mercury (Hg):

It is an extremely toxic metal in all its forms, it is accumulative poison because of the affinity of tissues to it (Underwood, 1977). Consumption of Hg contaminated fish provoked Minamata disease in human with symptoms of neurological damage, loss of vision, paralysis and death, it is also passed through human placenta causing chromo-

somal disorders and tetratogenicity (Sorensen, 1991). The human daily intake of Hg is 0.02 mg (Casarett and Doull, 1975), while the human weekly intake of Hg is 0.0033 mg/Kg body weight ("E.S.S." No. 2360, 1993).

The concentrations of mercury in examined salted and smoked fish samples ranged from 0.065 to 0.632 and 0.071 to 0.564 with mean values ±S.E. of 0.222 ±0.026 and 0.158 ±0.018 p.p.m (wet weight) respectively (Table 1). The obtained results (Table 2) were within the permissible limits reported by Boletin Oficial del Estado (1991), FAO/WHO (1992) and "E.S.S." No. 2360 (1993). On the other hand, low mercury values were detected in different types of fishes by Sheo et al. (1991); El-Mowafi (1995) in Nile *Tilapia*; Glueck and Hahn (1995) in fresh water fishes; El-Safy (1996) in cat fish; Nassar et al. (1996) in salted fish "Sardine"; Sallam and El-Gazzar (1997) in smoked fish; Ahmed and El-Boushy (1998) in Clarias lazera. Where-as, high mercury values were recorded by Sallam (1997) in *Clarias lazera*, Sallam and El-Gazzar (1997); Zaki (1998) in salted fish "Sardine" and Daoud et al. (1999) in fresh water fishes. From the obtained data in table (3), it was found that three samples (10%) of salted sardine and one sample (3.33%) of smoked herring were exceeding the limits of "E.S.S." No. 2360 (1993).

High mercury levels in small number of examined fish samples may be attributed to high affinity of mercury to muscle tissues of fish (Lovett et al., 1972). The average (88.9%) of total mercury in fish musculature was in the form of methyl mercury (Bishop and Neary, 1974) which are lipid soluble and easily absorbed and distributed through biological system (Manahan ,1989).

Copper (Cu):

Copper is an essential element for all animals, it is widely distributed and always present in food. Copper is common contaminant of the aquatic environment. It is found in natural water as a trace metal (< 5 µg/ml) and as a result of industrial processes by high quantity (EIFAC, T_{27}, 1973). The human daily intake of copper is 3.2 mg (Casarett and Doull, 1975), while the maximum provisional tolerable daily intake from copper for human is 0.05 - 0.5 mg/Kg body weight ("E.S.S." No. 2360, 1993).

Copper concentrations in examined salted and smoked fish samples (Table 1) ranged from 5.020 to 14.220 and 5.414 to 14.210 with mean values ±S.E. of 6.992 ±0.472 and 7.877 ±0.484 p.p.m (wet weight) respectively. The obtained results were within the permissible limits of Food Stuffs, Cosmetics and Disinfectanats (1972) and Boletin Oficial del Estado (1991) in Spain (Table 2). Nearly similar findings were recorded in salted

fish "*Aleastes nurse*" from Assiut City (Nassar et al., 1996), in flesh of *Chelolabroscus* from Spain (Legorburu et al., 1988) and in muscles of *Orechromis niloticus* from Helwan (Daoud et al., 1999). Meanwhile, low copper values were recorded by Oehlenschager (1990) in red fish fillets, Abo-Salem et al. (1992) in *Tilapia nilotica*, Abd El-Kader et al. (1993) in *Mugil Sp.* and Seddek et al. (1996) in Nile fish sp. High copper values were detected by Window et al. (1973) in North Atlantic fin fish, Mohamed (1993) in Nile fishes from Assiut City; Tantawy (1997) in *Tilapia sp.* and Zaki (1998) in salted sardine.

In conclusion, one can safely say that mercury, cadmium and lead are toxic and not essential to human and constitute public health hazardous. These minerals accumulate in fishes and therefore their levels must be continually monitored especially in ready to eat ones. In this regard copper and zinc are similar in that they all have electrons in the third orbital that form bonds and in large concentrations are considered toxic for the public health. These minerals are commonly referred to as transition elements Gordon (1986).

The variations of Pb, Cd, Hg and Cu concentrations among the results and those recorded by other investigators are considered logical due to the differences in fish sp., sizes, localities, the analytical procedures, season, salinity, habitats as well as the environmental pollution. This held the finding reported by Tariq et al. (1994) who concluded that metal concentrations in fishes were site and species specific. Therefore the preventive measures of significant concern intended for minimizing the pollution of fish with such metals include:

1. The use of uncontaminated fish, good quality salt and hygienical processing of salt lead to safe salted fish for human consumption and consequently protect consumer health.
2. Minimize the use of phosphate and sludge for land fertilization as possible and preventing industrial and agricultural discharges into the surface water.
3. Regular analysis of fish for heavy metal pollution and their load should be evaluated according to the international guidelines as a fruitful advise.

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