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CONCENTRATIONS OF CERTAIN HEAVY METALS IN IMPORTED FROZEN FISH IN EGYPT

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ABSTRACT

Concentrations of nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pd and Zn) were determined in imported frozen fish namely Mackerel (*Scomber scombrus*), Striped red mullet (*Mullus surmuletus*), Groster argentine (*Argentina silus*), Commen pandora (*Pagellus erythrinus*) and Atlantic horse mackerel (*Trachurus trachurus*). The average concentrations of the heavy metals analyzed exhibited the following decreasing order: Fe > Zn > Pb > Co > Cu > Cr > Mn > Ni > Cd. The concentrations of the studied heavy metals in fish tissues were lower than the Effect Range-Low (ERL) for such fish, while gill and liver exhibited elevation over the ERL for Cd only. The metal pollution index (MPI) for most studied fish fluctuated between 0.90 and 4.42 with average 2.18 for wet weight. The ingestion of heavy metals and the calculated lifetime daily intake have been reported and do not represent any actual risk due to the low concentrations of heavy metals in the studied fish tissues. These results suggest that the studied imported frozen fish might be considered as unpolluted with heavy metal.

INTRODUCTION

The past three decades have witnessed an increased awareness of problems concerning food pollution. Among them, the heavy metals are one of the most persistent and accumulative pollutants. They are natural constituents of earth crust, thus, heavy metals pollution describes elevated concentrations in different environmental compartments to a level that is detrimental to living organisms (Robert, 1991). Human beings are exposed to heavy metals via polluted air, water and food.

Cadmium has no biological function in human system and it is potentially toxic even at trace concentrations (Rbert, 1991). Kidneys are the critical target following long term exposure to cadmium, in addition to hepatotoxicity, skeletal impairment and neurotic effects (Misara *et al.*, 1998). Cadmium induced hepatic and renal injury in chronically exposed rats, likely role of hepatic cadmium-metallothionein in nephrotoxicity (Dudley *et al.*, 1982 and 1985). Cadmium intoxication can lead to oxidative damage in tissues by enhancing per-oxidation of cell membrane lipids and altering the antioxidant defense armory of the cells (Gupta *et al.*, 1991; Bagchi *et al.*, 1996). Severe contamination with cadmium had led to itai-itai disease (Yosumura *et al.*, 1980).

Accumulation of copper in liver leads to cirrhosis; in brain can lead to death of neurons with neurological symptoms; and in kidney leads to renal tubular damage (Forstner and Wittmann, 1983; Matta *et al.*, 1999).

Nickel acetate depresses circulating antibody titers (Figoni and Treagan, 1975) to T-phages and inhibits the interferon response of metal treated cells (Treagan and Furst, 1970). Nickel also inhibits the phagocytic ability and other properties of macrophages (Graham *et al.*, 1975). Delayed hypersensitivity reactions occur in guinea pigs that are exposed to nickel (Parker and Turk, 1978). Lead exerts adverse effects on the resistance of the body to disease. It also suppresses the immune system, particularly the humoral response in animals. This suppression often occurs at very low sub-clinical dosages and, therefore, may be detrimental to the health of animals and perhaps of man by mechanisms other than the typical well-documented toxicity which occurs at larger dosages. Severe contamination with Pb leads to brain damage, anemia, liver, and kidney diseases (Rippe and Berry, 1973; Goyer and Mushak, 1977).

The contamination of the near-shore marine environment and food chain by heavy industries is common in many developed and developing countries. This phenomenon is not only of major public concern, but may pose potential risks to human health through the consumption of contaminated seafood. Industrial wastes, geochemical structure and mining of metals create a potential source of heavy metal pollution in the aquatic environment (Lee and Stuebing, 1990; Gumgum *et al.*, 1994). Under certain environmental conditions, heavy metals may accumulate to a toxic concentration (Guven *et al.*, 1999), and cause ecological damage (Harms, 1975; Jefferies and Freestone, 1984; Freedman, 1989).

The present study was carried out to determine the levels of nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in muscle (tissue), liver, and gill of five imported frozen fish namely: Mackerel (*Scomber Scombrus*), Striped red mullet (*Mullus surmuletus*), Groster argentine (*Argentina silus*), Commen pandora (*Pagellus erythrinus*) and Atlantic horse mackerel (*Trachurus trachurus*). These fish species are commonly consumed as a popular diet in Egypt, specially for the average income people (Tables 1 and 2).

MATERIAL AND METHODS

Specimens of five of the imported fish namely: Mackerel (*Scomber. Scombrus*), Striped red mullet (*Mullus surmuletus*), Groster argentine (*Argentina silus*), Commen pandora (*Pagellus erythrinus*) and Atlantic horse mackerel (*Trachurus trachurus*) (Table 2) were collected from a fish market in Alexandria in January, 2003. Selection of samples was based on relative abundance and availability due to the wide array of imported species and sources of supply. From each species, eight fish were obtained, packed in ice and brought to the laboratory on the same day. Samples tissues, gill and liver of each species were dissected using clean equipment and put separately in petridishes and transferred into an oven set to 70 °C to dry. Drying continued until all the wet tissues reached to a constant weight. Dry samples (triplicate each 0.4 g) were put into digestion flasks followed by addition of 8 ml nitric acid (Merck), 3 ml perchloric acid and heated at 80 °C until all the materials were dissolved. After digestion, samples were diluted with deionized water, filtered and completed using deionized water to 25 ml. The resulting solutions were analyzed using flame atomic absorption spectrophotometer (AAS) (Perkin Elmer, Model 2380). The results were expressed in mg kg^{-1} dry wt. (UNEP/FAO/IAEA/IOC, 1984). The absorption wavelength and detection limits were as follows: 228.8 nm and 0.06 mg kg^{-1} for Cd; 240.7 nm and 0.05 mg kg^{-1} for Co; 357.9 nm and 0.06 mg kg^{-1} for Cr; 324.7 nm and 0.06 mg kg^{-1} for Cu; 248.3 nm and 0.8 mg kg^{-1} for Fe; 279.5 nm and 0.07 mg kg^{-1} for Mn; 232.0 nm and 0.09mg kg-^{1} for Ni; 217.0 nm and 0.8 mg kg^{-1} for Pb; 213.9 nm and 0.7 mg kg^{-1} for Zn, respectively.

Reagents of analytical grade were utilized for the blanks and calibration curves. Precision was checked against standard reference material, provided by the National Research Council of Canada

(DORM-1 for dogfish) and lied within the range of certified values with 94~99% recovery for all metals studied.

To prevent contamination, all used glass and plastic labware were previously washed in dilute nitric acid and deionized water.

Metal concentrations are reported as per dry weight of tissue and because metal pollution index (MPI) is measured as "per wet weight of tissue", the MPI calculation was performed by transforming all values to per wet weight values. These were calculated by dividing the dry values by 5, the wet/dry weight of most tissues.

RESULTS AND DISCUSSION

The determined levels of nine heavy metals in imported frozen fish samples are presented in Table 3. The concentrations of heavy metals followed the decreasing order of Zn > Fe > Pb > Co > Cr > Cu > Ni > Mn > Cd in Mackerel tissues; and Fe > Zn > Co > Pb > Cr > Mn > Ni > Cu > Cd in Mackerel gill; and Zn > Fe > Cu > Pb > Co > Cd > Cr > Mn > Ni in Mackerel liver. The heavy metal levels in Striped red mullet tissues were: Fe > Zn > Co > Pb > Mn > Cr > Cu > Cd > Ni; in gills Fe > Zn > Pb > Co > Cr > Mn > Cu > Ni > Cd; and in liver Fe > Zn > Cu > Pb > Cr > Co > Mn > Cd > Ni. The same heavy metal concentrations in tissues of Groster argentine were: Zn > Fe > Co > Cu > Cr > Mn > Pb > Ni > Cd; in gills Zn > Fe > Co > Pb > Cr > Mn > Cu > Ni > Cd; and in liver Fe > Zn > Cu > Co > Pb > Cr > Mn > Cd > Ni. Heavy metal concentrations in tissues of Commen pandora were: Zn > Fe > Pb > Cr > Mn > Ni > Cu > Cd > Co; in gill Fe > Zn > Pb > Mn > Co > Cr > Ni > Cu > Cd; and in liver Zn > Fe > Cd > Cu > Pb > Co > Cr > Mn > Ni. Heavy metal concentrations in tissues of Atlantic horse mackerel were: Fe > Zn > Co > Pb > Cr > Cu > Mn > Cd > Ni; in gills Fe > Zn > Pb > Co > Cr > Ni > Mn > Cu > Cd; and in liver Fe > Zn > Pb > Co > Cu > Cd > Mn > Ni > Cr.

These results showed that Ni had a low tendency to accumulate in the liver for all studied fish than accumulation of Pb. Cadmium was found to accumulate in liver for most studied fish several times more than in gills and tissues. Copper was found accumulating in liver than Pb in most studied fish except Atlantic horse mackerel. According to Tolonen (1995), in vertebrates, the highest concentrations of copper were found in liver, muscle, blood, head, and brain.

Cadmium concentrations in the studied fish ranged from 0.33 to 66 in tissues. 0.33 to 1.31 in gill and 1.64 to 29.2 in liver, with average concentration of 3.5 ± 7.42 mg kg^{-1} dry weight. Cadmium concentrations were elevated in liver collected from Mackerel and Commen Pandora, while the tissues of all studied fish samples exhibited no significant elevation of cadmium. However, cadmium accumulating in liver, gill, kidney, rather than in muscle, may be replacing zinc in some enzymes, and has a long half-life time (10-30 years) (Kotsonis and Klaassen, 1977 and 1978). In this study, cadmium concentrations in tissues were higher than the maximum acceptable limits reported by EPA (1995) but less than that reported by both CEFAS (1997) and NHMRC (1987) (Table 4).

Copper was the third most abundant element for all studied samples, and the highest concentration of copper was found in Mackerel liver 27.16 mg kg^{-1} dry weight, while the lowest one was found in Commen pandora tissue and gill 1.94 mg kg^{-1} dry weight. The highest concentration of copper reported in this study was less than the ERL (34 mg kg^{-1} dry weight) and less than the maximum acceptable limits by MAFF (1956) 100 mg kg^{-1} (Table 4).

Iron concentrations ranged from 15.18 to 41.73 in tissue; 79.67 to 284.54 in gill and 159.34 to 887.77 in liver with average value 177.3 ± 217.86 mg kg^{-1} dry weight. The highest iron concentration was found in liver of the Atlantic horse mackerel, while the lowest concentration was found in tissue of Commen pandora. Concentrations of iron in liver may be influenced by the amount of blood retained in tissue and therefore depends, in part, on the length of time between death of the fish and collection of the tissues (Thompson, 1992). As the fish in this study was imported frozen fish, this will ensure that iron from liver tissues is measured rather than from the blood.

Zinc is the second most abundant element for all studied samples, which accumulates with concentrations comparable to accumulation of iron. According to Law *et al.* (1992), the concentration of zinc in the liver ranged between 80 and 400 mg kg^{-1}. Zinc concentrations in the liver of Mackerel were much higher than in liver of other fishes. The highest concentration of zinc was 389.73 mg kg^{-1} dry weight as reported in Mackerel and the lowest one was found in Atlantic horse mackerel (12.73 mg kg^{-1} dry weight) with an average concentration of 89.95 ± 100.11 mg kg^{-1} dry weight. The highest concentration recorded in this study was lower than ERM

(410 mg kg^{-1} dry weight) and lesser than the maximum acceptable limits by NHMRC (1987) (750 mg kg^{-1}).

Lead concentrations in this study were ranging between 4.75 and 23.86 mg kg^{-1} with average concentration of 12.85 ± 5.33mg kg^{-1} dry weight, which is lower than the ERL (46.7 mg kg^{-1}). Lead was the third abundant metal accumulating in tissues following the iron and zinc. The highest concentrations of lead were found in gills followed by liver in most studied samples. The concentrations of Pb in this study were lower than the maximum acceptable limits recorded by BOE (1991) (25 mg kg^{-1}) (Table 4).

Manganese concentrations ranged between 2.57 to 10.28 mg kg^{-1} with average concentration of 5.57 ± 2.26 mg kg^{-1}. This study, showed more accumulation of manganese in gills than in both tissues and liver. According to Thompson (1992), manganese concentrations in marine mammals are normally found in concentrations lower than 28 mg kg^{-1} in any tissue.

Cobalt concentrations were fluctuating between non-detected to 17.27 mg kg^{-1}, with average concentration of 11.98 ± 3.33mg kg^{-1}. Chromium concentrations were ranging between non-detected to 10.76 mg kg^{-1} with average concentration of 7.47 ± 2.19 mg kg^{-1}. Chromium was more accumulated in gills than in tissues and liver for most studied samples. Nickel concentrations were ranged from non-detected to 8.11 mg kg^{-1}, with average concentration of 4.73 ± 2.85 mg kg^{-1}.

The overall metal content of imported frozen fishes were compared using the metal pollution index (MPI) calculated according to Usero *et al.*, (1996); Usero *et al.* (1997) where

$$MPI = (Cd \times Co \times Cr \times Cu \times Fe \times Mn \times Ni \times Pb \times Zn)^{1/9}$$

The final MPI of each site is a weight mean value, as it was obtained taking into account the total weight of fish. The calculated MPI was fluctuating between 0.9 and 4.42 with an average value of 2.18 ± 1.01. The higher MPI were recorded for liver for all studied fish, while the lowest MPI was found in fish tissues.

HUMAN HEALTH RISK ASSESSMENT

The human health risk assessment has been calculated for the studied fish, depending on the calculation made by Albering *et al.* (1999).

Ingestion of fish (IF) (mg kg^{-1} day^{-1})= $(CF \times IRF \times FI \times AF) \div BW$

Calculated lifetime daily intake (CLTDI) (mg kg^{-1} day^{-1})

$$= [(6 \times IF_{child}) \div 70] + [(64 \times IF_{adult}) \div 7]$$

Hazard index = CLTDI ÷ tolerable daily intake (TDI) Where CF = concentration of the heavy metal contaminant in fish (mg kg^{-1} wet weight); IRF = ingestion rate of fish (0.015 and 0.055 kg wet weight/day for child and adult, respectively); FI = fraction contaminated (0.5 for both child and adult); AF = absorption factor (1 for both child and adult) and BW = Body weight (15 and 70 kg for child and adult, respectively); the TDIs for heavy metals could be derived from Bockting *et al.* (1996).

The calculation of fish ingestion, lifetime daily intake (e.g. 70 years) and hazard index had been reported in Table 5. The concentrations of heavy metals recorded for imported frozen fish tissues are still low, which gave the ingestion of fish very low values. These values were multiplied by 10^{-5} mg kg^{-1} day^{-1}, which gave low calculated lifetime daily intake (CLTDI) values, and therefore gave low hazard index values for all studied frozen fish tissues. The hazard index values were lesser than 1 for all studied samples, indicating no health risk on the consumers.

CONCLUSION

This study showed that heavy metal concentrations in the imported frozen fish were so far considerably lower than the ERL and lower than the maximum permissible levels for different countries. The calculations of risk assessment showed no possible health risk from the present heavy metals due to imported fish consumption.

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Table 1. Representative relative quantities of imported frozen fish during the period 1997-2002

Year	Amounts per tons	Consumed per persons (kg/year)
1997	207	10.4
1998	176	11.7
1999	193	13.2
2001	213	14.6
2002	261	15.8

According to the Department of Statistics in NIOF, Egypt (1997-2002)

Table 2. Common and scientific names, length and exported country of frozen fish sampl

Common name	Scientific name	Length	Exported Country
Mackerel	*Scomber Scombrus*	30 - 35	Ireland
Striped red mullet	*Mullus surmuletus*	20 - 25	Spain
Groster argentine	*Argentina silus*	40-45	Netherlands
Commen pandora	*Pagellus erythrinus*	20-25	Morocco
Atlantic horse mackerel	*Trachurus trachurus*	17-20	Netherlands

Table 3. Heavy metals concentrations (mg kg^{-1} dry weight) in imported Frozen fish in Egypt.

		Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	MPI
Mackerel	Tissue	0.66	7.40	6.27	3.88	34.15	2.57	2.70	15.41	34.30	1.28
	Gill	0.66	17.27	10.76	2.91	208.66	8.99	8.11	16.91	76.39	2.46
	Liver	8.86	14.80	8.17	27.16	231.43	6.42	5.40	17.11	389.73	4.42
Striped red mullet	Tissue	0.66	12.33	5.15	4.85	41.73	7.71	ND	8.05	21.93	1.59
	Gill	1.31	14.80	10.76	4.85	284.54	6.42	2.70	15.91	66.49	2.41
	Liver	3.28	7.40	9.12	9.70	159.34	5.14	2.70	9.15	77.80	2.20
Groster argentine	Tissue	0.66	17.27	5.37	7.76	22.76	5.14	2.70	4.75	24.05	1.39
	Gill	1.31	9.87	6.97	5.82	83.47	6.42	2.70	7.85	84.52	1.26
	Liver	1.64	9.87	8.18	13.58	280.75	3.85	ND	8.92	170.46	2.96
Commen pandora	Tissue	0.66	ND	4.17	1.94	15.18	3.85	3.81	7.95	18.04	0.90
	Gill	0.33	9.87	7.27	1.94	79.67	10.28	5.40	15.91	31.83	1.55
	Liver	29.20	12.33	8.07	18.43	182.11	3.85	2.70	16.98	212.90	3.78
Atlantic horse-mackerel	Tissue	0.33	9.87	4.47	3.88	26.56	2.57	ND	7.95	12.73	1.06
	Gill	0.98	14.80	9.76	4.85	121.40	6.42	8.11	23.86	65.07	2.50
	Liver	1.97	9.87	ND	8.73	887.77	3.85	2.70	15.98	62.95	2.82
Average		3.50	11.98	7.47	8.02	177.30	5.57	4.15	12.85	89.95	2.17
Median		0.98	11.10	7.67	4.85	121.40	5.14	2.70	15.41	65.07	2.20
St. D.		7.42	3.33	2.19	6.96	217.86	2.26	2.12	5.33	100.11	1.03
ERL	Tissue	1.2	NA	81	34	NA	NA	20.9	46.7	150	
ERM	Tissue	9.6	NA	370	270	NA	NA	51.6	218.0	410	

ND = not detected; NA = not available; MPI = metal pollution index calculated for wet weight; ERL = Effect Range-Low; ERM = Effect Range-Median

Table 4: Maximum acceptable limits (mg kg^{-1}) of some heavy metals.

Metal	Concentration	Referance
Cd	0.04	EPA (1995) (safety level in fish tissue)
	5	CEFAS (1997); BOE (1991)
	10	NHMRC (1987); EEC (1979)
Cr	8	EEC (1979)
Cu	100	MAFF (1956); BOE (1991)
	350	NHMRC (1987)
Zn	250	Ministry of Food (1953)
	750	NHMRC (1987)
Pb	25	BOE (1991)
	50	Great Britain-Parliament (1979)

Table 5. Calculation of ingestion of imported frozen fish tissues (× 10^{-5}) for child and adult, and calculated lifetime daily intake (mg kg^{-1} day^{-1}) (CLTDI).

Fish name	Ingestion for	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
Mackerel	Child	8.2	92	78	49	427	32	34	193	429
	Adult	6.4	73	62	38	335	25	27	151	337
	CLTDI	0.001	0.007	0.006	0.004	0.031	0.002	0.002	0.014	0.031
Striped red mullet	Child	8.2	154	64	61	522	96	ND	101	274
	Adult	6.4	121	51	48	410	76	ND	79	215
	CLTDI	0.001	0.011	0.005	0.004	0.038	0.007	ND	0.007	0.020
Groster argentine	Child	8.2	216	67	97	285	64	34	59	301
	Adult	6.4	170	53	76	224	50	27	47	236
	CLTDI	0.001	0.016	0.005	0.007	0.021	0.005	0.002	0.004	0.022
Commen pandora	Child	8.2	ND	52	24	190	48	48	99	225
	Adult	6.4	ND	41	19	149	38	37	78	177
	CLTDI	0.001	ND	0.059	0.002	0.014	0.004	0.003	0.007	0.016
Atlantic horse-mackerel	Child	4.1	123	56	49	332	32	ND	99	159
	Adult	3.2	97	44	38	261	25	ND	78	125
	CLTDI	0.0003	0.009	0.041	0.004	0.024	0.002	ND	0.007	0.012

ND = not detected; The hazard index can be calculated by divided the CLTDI by the tolerable daily intake (TDI).