

Concentration of Metals in Frozen and Canned Fish in Pakistan

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Abstract.- Fish being at the top of the aquatic food chain has the capacity to accumulate significant amount of metals in muscles that may cause health risk for consumers. Therefore, toxic and essential metals concentrations in muscles of frozen (local and imported species of fish and shrimp), canned fish (imported) samples were analyzed by Atomic Absorption Spectrophotometer. Among all frozen fish and shrimp samples analyzed, highest Cu (5.23 µg/g) concentrations were found in fish imported from Malaysia, similarly highest levels (µg/g) of Ni (3.03), Pb (2.95) and Mn (2.10) were detected in fish imported from Thailand. In canned fish samples, highest levels (µg/g) of Pb (2.50), Cu (2.18), Mn (1.62) and Ni (1.33) were detected in sardine skin imported from Thailand. Pb and Ni concentrations in majority of frozen and canned samples were greater than EC and USEPA limits.

Key words: Frozen fish, heavy metals, Pb, Cu, Ni, Mn, canned fish.

INTRODUCTION

Fish and fish products are widely consumed all over the world because it is the important constituent of healthy diet. They are source of high quality protein, valued vitamins, low saturated fat (Clarkson, 2002; Dominogo *et al.*, 2007) and omega 3 fatty acids (Ikem and Egiebor, 2005) known to prevent coronary heart disease (Oomen *et al.*, 2000). It also reduces blood clotting tendency (Ismail, 2005), thrombosis and arrhythmias (Kris-Etherton *et al.*, 2002).

Unfortunately, fishes are continually exposed to contaminants and chemicals in polluted water. Metals are categorized into three types as potentially toxic metals (Pb), probably essential metals (Ni) and essential metals (Cu, Mn) (Munoz-Olivas and Camara, 2001). Essential metals are required at low concentrations but at high concentrations they may be toxic (Oehlschlager, 2002). Over the last few decades, number of chemicals has been increased in the water bodies as a result of industrial, agricultural and domestic waste discharges (Ajani and Ayola, 2007). Pollution due to metals causes the drastic impacts on aquatic ecosystem and humans.

Fish accumulates significant amount of metals not only from aquatic pollution but also from

food processing and packaging. Copper is an essential metal which is an important part of metalloenzymes. It is also involved in hemoglobin synthesis and metabolic reactions but it becomes toxic in ionic forms. Nickel acts as activator of some enzymes at trace amounts but at higher concentrations it accumulates in lungs and causes bronchial damage. Fish accumulates substantial amount of lead in their muscles and tissues from canning process in addition to its accumulation from relevant polluted waters. It accumulates in humans where it replaces calcium in bones (Ashraf, 2006). Manganese is an essential metal that is present in all living organism at trace amounts. Excess of manganese not only damages central nervous system but also causes liver cirrhosis and Parkinson's disease (Momtaz, 2002).

There is little information regarding status of heavy metals in the frozen and canned fish. The present study aims at determining the concentration of metals in the frozen and imported canned fish in order to evaluate health risk of these products.

MATERIALS AND METHODS

Sample collection

Commercially available frozen and canned fish were purchased during the Oct 2011 to March 2012. Samples were placed in icebox at 4°C during transportation and stored in the laboratory at -20°C (Al-Busaidi *et al.*, 2011).

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0030-9923/2014/0006-1521 \$ 8.00/0

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Procedure

Metal concentrations were determined in edible portion (muscle) of fish (*Pangasius hypophthalmus*, *Sardina pilchardus*, *Thunnus alalunga*) and shrimp (*Penaeus vannamei*) samples. Dry weights of samples were calculated after oven drying them at 90°C.

Samples were ground, homogenized and transferred to digestion vessels for acid digestion. Dried samples (2g) were digested in digestion flasks with 10 ml of 65% concentrated HNO₃ for 30 min at 25-180°C and then for 15 min at 180°C till clear solution was attained (Al-Busaidi *et al.*, 2011). Digested samples were filtered by 0.45 µm membrane filter (Sastre *et al.*, 2002) and analyzed for determination of lead (Pb), copper (Cu), nickel (Ni) and Manganese (Mn) using Flame Atomic Absorption Spectrophotometer, Shimadzu AA-7000F.

Dietary metal intake was obtained using the formula:

$$\text{Dietary metal intake } (\mu\text{g/kg/day}) = \text{Metals levels } (\mu\text{g/g}) \times \text{daily intake (kg)}.$$

Metal level is the amount of metal present in fish samples and daily intake is the average food quantity consumed by a person daily. Dietary metal intakes were calculated by considering fish intake about 2.2 kg per capita per year (National policy and strategy for fisheries and aquaculture development in Pakistan, 2007).

Statistical analysis

Relationship between metal concentrations in fish samples were evaluated by linear regression analysis using SPSS 16.0. 't' test was performed to analyze statistical differences among mean values (Tabinda *et al.*, 2010).

RESULTS AND DISCUSSION

Lead

Among frozen imported fish samples, highest level of lead (µg/g dry weight) was detected in fish from Thailand (2.95), followed by fish from Malaysia (2.20) and fish from Pakistan (1.50) as shown in Table I. Lead concentrations exceeded EC

permissible value (0.2 µg/g) in all fish samples. These concentrations were 15, 11 and 7.5 times higher than EC limit in samples from Thailand, Malaysia and Pakistan, respectively.

Among imported canned fish analyzed, highest concentration of Pb was determined in fish imported from South Wales and England while lowest concentration was found in fish imported from Thailand. Lead concentration (µg/g dry weight) in canned fish samples ranged between 1.00-1.94. Order of lead concentrations in canned fish samples imported from different countries was South Wales, England > Spain > Greenford, England > Thailand (Table II). Lead concentrations in all canned fish samples were above the EU permissible level (0.2µg/g). Average Pb concentrations in fish imported from South Wales, England, Spain, Greenford, England and Thailand were 10.2, 6.2, 5.7 and 5.0, respectively times greater than the EU permissible limit.

Lead concentrations were insignificantly higher ($p > 0.05$) in frozen fish samples (2.08µg/g) as compared to the canned fish samples (1.33µg/g). Average concentrations of lead in frozen samples and canned samples were 10.4 and 6.6 times greater than EU tolerable limit.

Lead concentrations in canned sardine and tuna were higher in sardine (1.36µg/g) than the tuna (1.25µg/g). Sardine mostly feed on zooplanktons and phytoplankton (filter feeding). They normally live 12 to 13 years but some can live up to 20 to 25 years (Ashraf *et al.*, 2006).

Estimated daily intake value of Pb in frozen (fish and shrimp) and canned samples was about 12 and 7 µg/day) which was within the Provisional Tolerable Weekly Intake for the consumer (250 µg/day equivalent to 1750 µg/week/kg body weight) proposed by WHO (1993).

Copper

Among frozen fish samples analyzed, highest Cu concentrations (µg/g) were recorded in fish imported from Thailand (5.23) followed by fish collected from Pakistan (0.3) and from Malaysia (0.01) (Table I). Cu concentration in all fish samples were within USEPA allowable limit (120µg/g).

Cu concentration (µg/g) ranged between 0.12-1.3 among various imported fish samples.

Table I.- Metals concentrations in $\mu\text{g/g}$ (Mean \pm SD) in the frozen and canned species[§].

Species types	Species	Country	Tissue	Mean values \pm SD ($\mu\text{g/g}$ dry weight) of metal concentration ⁺			
				Pb	Cu	Ni	Mn
Frozen fish	<i>Pangasius hypophthalmus</i>	Malaysia	Muscle	2.20 \pm 2.03 ⁺	0.01 \pm 0.03	3.03 \pm 2.45	0.94 \pm 0.88
		Thailand	Muscle	2.95 \pm 2.30	5.23 \pm 2.67	0.99 \pm 0.35	2.10 \pm 2.05
		Pakistan	Muscle	1.50 \pm 1.25	0.37 \pm 0.21	0.56 \pm 0.21	0.35 \pm 0.21
Frozen shrimp	<i>Penaeus vannamei</i>	South East Asian countries	Muscle	1.68 \pm 1.25	0.30 \pm 0.12	0.53 \pm 0.42	1.40 \pm 1.03
Canned fish	<i>Sardina pilchardus</i>	South Wales, England	Skin	2.05 \pm 1.98	1.57 \pm 1.20	0.30 \pm 0.21	0.15 \pm 0.12
			Muscle	1.94 \pm 1.84	0.74 \pm 0.54	0.25 \pm 0.22	0.00
		Thailand	Skin	2.50 \pm 2.43	2.18 \pm 2.14	1.33 \pm 1.01	1.62 \pm 1.02
			Muscle	1.00 \pm 0.48	1.30 \pm 1.05	0.95 \pm 0.10	0.90 \pm 0.42
		Greenford, England	Skin	2.00 \pm 1.89	0.51 \pm 0.45	0.10 \pm 0.02	0.45 \pm 0.27
Canned fish	<i>Thunnus alalunga</i>	Spain	Muscle	1.15 \pm 1.23	0.20 \pm 0.15	0.37 \pm 0.15	1.00 \pm 0.98
			Skin	0.15 \pm 0.40	0.04 \pm 0.10	ND	ND
			Muscle	1.25 \pm 1.15	0.12 \pm 0.05	0.5 \pm 0.42	0.34 \pm 0.24

Nd, not detectable

[§]Results are expressed as $\mu\text{g g}^{-1}$ dry weight.⁺Mean values \pm standard deviation.**Table II.- Linear regression equation and Correlation coefficient (R^2) between metal concentrations in frozen fish and shrimp and canned fish species.**

Species type	Species	Linear regression equation	Correlation coefficient (R^2)
Frozen fish and shrimp	Pb and Cu	$y = 3.314x - 5.425$	0.739
	Ni and Cu	$y = -0.104x + 1.432$	0.049
	Ni and Mn	$y = -0.060x + 1.274$	0.009
	Cu and Mn	$y = 0.236x + 0.847$	0.644
	Pb and Mn	$y = 0.918x - 0.716$	0.652
	Ni and Pb	$y = 0.532x + 0.168$	0.085
	Canned fish	Pb and Cu	$y = 0.696x - 0.215$
Ni and Cu		$y = 0.428x + 0.118$	0.548
Ni and Mn		$y = 1.074x + 0.047$	0.714
Cu and Mn		$y = 0.400x + 0.223$	0.296
Pb and Mn		$y = 0.254x + 0.174$	0.113
Ni and Pb		$y = 0.226x + 0.134$	0.145

Concerning imported canned fish, highest concentration of Cu was detected in fish imported from Thailand whereas its lowest concentration was determined in fish imported from Spain. Copper concentration in samples of different countries was in the order: Thailand > South Wales, England > Greenford, England > Spain (Table II). Cu levels in all imported canned fish samples were within the maximum copper consumption limit 120 $\mu\text{g/g}$

(USEPA, 2002).

Cu concentration in frozen fish samples (1.47 $\mu\text{g/g}$) was significantly higher ($p < 0.05$) than the canned fish (0.5 $\mu\text{g/g}$). Cu concentrations in all frozen and canned fish samples were within the USEPA Permissible limit (120 $\mu\text{g/g}$).

Among canned sardine and tuna, higher Cu concentrations were detected in sardine (0.74 $\mu\text{g/g}$) than the tuna (0.5 $\mu\text{g/g}$). Concentrations of copper in

canned sardine and tuna were within the USEPA copper consumption value.

Estimated dietary intake value of Cu in frozen and canned samples was 8.12 and 3.24 μg per day respectively, and was safe within the acceptable limit (2000-3000 $\mu\text{g}/\text{day}$) recommended by NRC (Huang, 2003).

Nickel

Highest nickel concentrations were found in frozen fish samples imported from Malaysia (3.03 $\mu\text{g}/\text{g}$) followed by fish samples imported from Thailand (0.99 $\mu\text{g}/\text{g}$) and fish collected from Pakistan (0.56 $\mu\text{g}/\text{g}$) (Table I). Nickel concentration in fish samples imported from Malaysia was 3 times greater than its permissible limits (1 $\mu\text{g}/\text{g}$) recommended by USEPA, (2002). Concentrations of fish imported from Thailand and from Pakistan were within USEPA tolerable limit.

Regarding to, canned fish, concentrations of Ni were highest in fish imported from Thailand whereas its lowest concentrations were determined in fish imported from South Wales, England. Nickel concentration ($\mu\text{g}/\text{g}$) ranged between 0.25 and 0.95. Order of imported canned fish was Thailand > Spain > Greenford, England > South Wales, England (Table II). Nickel concentrations in all canned fish samples were within the permissible limits (1 $\mu\text{g}/\text{g}$) recommended by USEPA (2002).

Nickel concentrations were significantly higher ($p < 0.05$) in frozen fish samples (1.27 $\mu\text{g}/\text{g}$) than the canned fish samples (0.76 $\mu\text{g}/\text{g}$). Islam *et al.* (2010) reported similar trend, they reported that frozen samples were contained higher nickel concentration (0.43 $\mu\text{g}/\text{g}$) than canned samples (0.24 $\mu\text{g}/\text{g}$).

Concentrations of Ni in frozen fish samples were 1.2 times greater than its USEPA tolerable limit. Average concentration of all canned fish samples were within the permissible value given by USEPA.

Among canned sardine and tuna samples, higher nickel contents were detected in sardine (0.52 $\mu\text{g}/\text{g}$) than the tuna (0.5 $\mu\text{g}/\text{g}$). Ashraf *et al.* (2006) reported similar trend, for sardine (1.33 $\mu\text{g}/\text{g}$) and tuna (0.41 $\mu\text{g}/\text{g}$). Ni concentrations in canned sardine and tuna were within the USEPA tolerable limits.

Maximum daily intake ($\mu\text{g}/\text{day}$) of Ni estimated in frozen and canned fish was 7.02 and 2.84 μg per day respectively. It was within the allowable intake value (300 $\mu\text{g}/\text{day}$) proposed by WHO (1993).

Manganese

Imported frozen fish samples of manganese highest concentrations ($\mu\text{g}/\text{g}$) were determined in frozen fish imported from Thailand (2.10) followed by fish imported from Malaysia (0.94) and Pakistan (0.35) as shown in Table I.

Highest concentration of Mn was determined in canned fish imported from Greenford, England while it was lower in canned fish imported from South Wales, England. The mean Mn concentrations were ranged from 0.00-1.00 $\mu\text{g}/\text{g}$. Order of imported fish samples was Greenford, England > Thailand > Spain > South Wales, as shown in Table II.

Mn concentrations were insignificantly higher ($p > 0.05$) in frozen fish samples (1.19 $\mu\text{g}/\text{g}$) than the canned fish (0.56 $\mu\text{g}/\text{g}$). Islam *et al.* (2010) reported similar results where frozen samples contained higher manganese concentration (1.5 $\mu\text{g}/\text{g}$) than canned samples (0.3 $\mu\text{g}/\text{g}$).

In canned sardine and tuna samples, manganese concentrations were twice in sardine (0.6 $\mu\text{g}/\text{g}$) as compared to tuna (0.3 $\mu\text{g}/\text{g}$).

The estimated Mn daily consumption value in frozen and canned samples was 6.58 and 3.08 μg per day, respectively, which was within the WHO acceptable limit (2000 to 5000 $\mu\text{g}/\text{day}$) (Chen and Chen, 2001).

Correlation between metals concentrations in frozen (fish and shrimp) and canned fish samples

In one hand, highly significant ($p < 0.01$) correlations were observed between Pb and Cu ($r = 0.73$), Ni and Mn ($r = 0.71$) in frozen and canned samples, respectively. Also, the correlation between Pb and Mn ($r = 0.65$) and Cu and Mn ($r = 0.64$) frozen samples as well as Cu and Ni ($r = 0.54$) in canned samples were significant ($p < 0.05$) as shown in Figures 1 and 2.

On the other hand, there were no significant correlations between the other metals as shown in Figures 1 and 2.

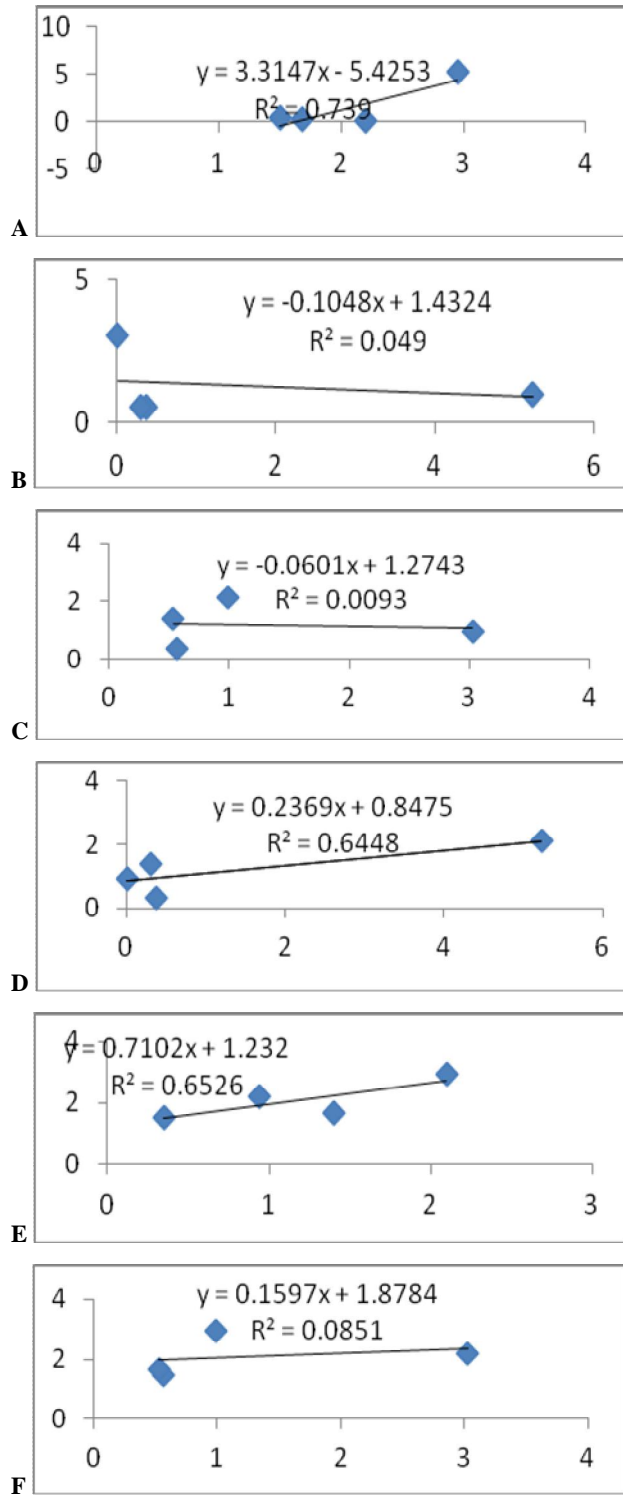


Fig. 1. Linear regression analysis between Pb and Cu (A), Ni and Cu (B), Ni and Mn (C), Cu and Mn (D), Pb and Mn (E) and Pb and Ni (E) in frozen fish and shrimp.

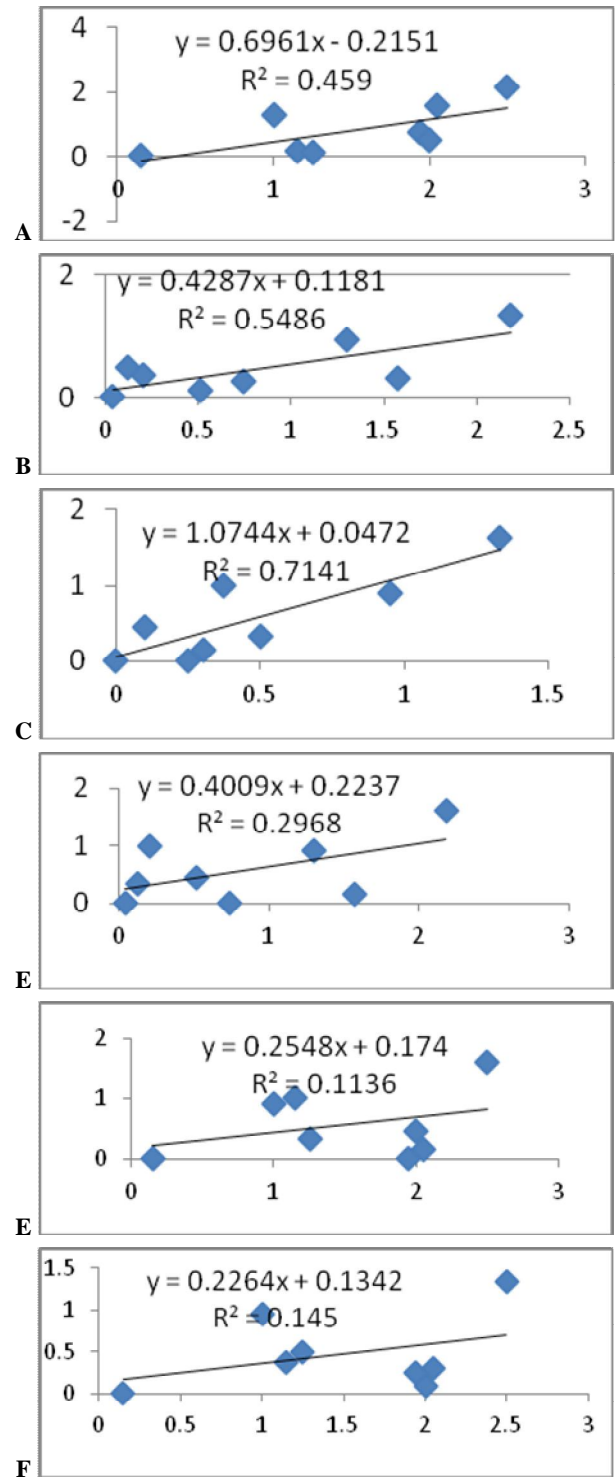


Fig. 2. Linear regression analysis between Pb and Cu (A), Ni and Cu (B), Ni and Mn (C), Cu and Mn (D), Pb and Mn (E) and Pb and Ni (E) in canned fish.

CONCLUSIONS

Pb and Ni concentrations in majority of frozen (fish and shrimp) and canned fish samples were greater than the tolerable limits proposed by EC and USEPA that is, might pose health risk for the public. Cu and Ni concentration was significantly higher ($p < 0.05$) in frozen fish and shrimp as compared to canned fish. Dietary metal intake was higher in frozen fish and shrimp than canned fish. DMI of all metals was within the permissible limits proposed by different authorities and do not cause any risk for the local consumers due to their low intake. But high contamination of Pb and Ni in majority of frozen and canned samples may cause risk for the importers due to high per capita intake of fish and shrimp.

ACKNOWLEDGEMENTS

Authors are grateful to Department of Chemistry, GCU, Lahore for their help in metal analysis during the research.

Conflict of interest declaration

The authors declare that no conflicts of interest, and financial or other, exists. The work described has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to this journal.

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- (Received 7 October 2013, revised 16 August 2014)