

Product Quality and Safety

Heavy metals analysis in some freshwater and marine fish species from markets of Lahore, Pakistan

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(Manuscript received 28 November 2018; accepted for publication 19 March 2019)

Abstract. Muscle portion of 72 fish samples of eight selected species, four from marine (*Pampus Chinensis*, *Otolithes ruber*, *Trachipteridae* and *Oncorhynchus tshawytscha*) and four from fresh water (*Labeo rohita*, *Catla catla*, *Rita rita* and *Walago atu*) fishes were collected from three superstores in Lahore, Pakistan. The prepared fish samples were analyzed for determination of Chromium (Cr), Lead (Pb) and Cadmium (Cd) concentrations by using the inductively coupled plasma (PerkinElmer, Model: Optima 7000DV ICP-OES). It was found that Cd (0.294 ± 0.001 mg/kg) concentration was the highest in *Labeo rohita* collected from Metro Superstore while the lowest level (0.023 ± 0.000 mg/kg) was in the *Oncorhynchus tshawytscha* of the same superstore. Species wise concentration of Cd varies significantly. Concentration of Cr (0.172 ± 0.001 mg/kg) was the highest in *Oncorhynchus tshawytscha* collected from Main Fish Market of Lahore while the lowest level (0.002 ± 0.001 mg/kg) was in *Labeo rohita* collected from Hyperstar superstore. Concentration of Pb (0.057 ± 0.000 mg/kg) was the highest in *Oncorhynchus tshawytscha* collected from Metro Superstore while the lowest (0.012 ± 0.001 mg/kg) in *Otolithes ruber* of the same superstore. Species wise concentration of Pb varies non-significantly. Origin wise t-test shows that: a) Cd concentrations vary significantly, while Cr and Pb concentrations vary non-significantly when marine and fresh water species were compared; b) The range of Pb (0.011-0.057 mg/kg) and Cr (0.002-0.172 mg/kg) was within limits and of Cd (0.023-0.297 mg/kg) was higher than the WHO international standards (0.123 mg/kg for Pb, 0.050 mg/kg for Cd and 0.1 mg/kg for Cr) in most of the fish species of main Fish Market Lahore, Hyperstar and Metro store.

Keywords: freshwater and marine fish, Pb, Cd, Cr, Lahore, commercial markets

Introduction

Fishes are an important constituent of aquatic web and considered as one of the main and excellent protein sources in human food. Worldwide people obtain about 25% of their animal protein from fish. Demand for fish and fish derived products is increasing day by day with the growing human population and significant need for food supply (Bahnasawy et al., 2009). According to the American Heart Association eating fish at least twice a week in order to reach the daily intake of omega 3-fatty acid is an essential constituent to prevent coronary heart diseases (Martin et al., 2009).

Heavy metals are high density, non-biodegradable metallic elements including highly toxic metals (lead, mercury, cadmium, etc.), toxic metals (nickel and cobalt, etc.) and essential metals (zinc, aluminum, manganese, copper, beryllium, etc.). These are also called trace elements because they are present in small amount in the biological system (Kori et al., 2017). Trace elements enter the aquatic environment by lithogenic and anthropogenic activities (Tavakoly Sany et al., 2013). Contingency associated to these metals toxicity is directed to their concentration and they tend to exert somehow auspicious as well as more injurious effects on plants, animals along with humans (Martin et al., 2009). Some of these metals are more toxic to living organism even at their diminutive concentration, whereas others are biologically imperative and become noxious as they are ingested in excess because

they combine with body's biomolecules such as proteins and enzymes multiplying their structure and hindering them from the bio reaction of their function (El-Zokam et al., 2012).

The consumption of fish and fish derived products has generally increased in the world during recent decades. It has been predicted that fish consumption in developing countries will be expected to increase from 57% (in 1997) to 98.6% (in 2020). Like other countries, fish also becomes part of the main menu for Pakistani people and it is exceeding without realizing the level of heavy metal content in fish (Chatta et al., 2017). The per capita consumption is 2.0kg per annum, which is quite low as compared to the European countries where it is 20kg per annum. In Lahore, there is only one main wholesale fish market for the sale of marine and freshwater fish species. The main source of fish supply in the market and superstore in Lahore are privately owned fish farms and rivers while marine fish is brought from Karachi.

All organisms including human beings are not able to destroy heavy metals (Vinodhini and Narayanan, 2008). Heavy metals do not degrade, instead they tend to accumulate in different body organs such as liver muscles and bones. Therefore, heavy metals are amongst the most dangerous pollutants. High level of lead can cause serious effect in animals. It can be the reason of neurotoxicity in vertebrates (Tavakoly Sany et al., 2013). It can also affect children significantly especially their intellectual ability concentration and behavior disturbed adversely, if prolonged (Paudel et al., 2016).

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Chromium (Cr) may cause low immune system, ulcer, skin problems, lung cancer and in some cases death (Afshan et al., 2004). Cadmium (Cd) causes kidney damage, chronic toxicity, including impaired reproductive capacity, kidney and hepatic dysfunction (Abdel-Mohsien and Mahmoud, 2015).

The aim of this study is to determine the level of some heavy metals concentration in freshwater and marine commercial fish species available in markets of Lahore, Pakistan and to make quality assessment of the fishes based on the permissible limits allowed for the fish and fish derivative stuffs.

Material and methods

Sample collection and preparation

The study was carried out in winter 2015-2016 to investigate the Chromium (Cr), Lead (Pb) and Cadmium (Cd) concentrations in the marine and freshwater fish species available in the local market of Lahore city (Main fish market, Hyperstar and Metro Store). The Lahore fish wholesale market is the largest market of freshwater fish in Pakistan. It supplies around 100-120t of fish daily all over the country during

the winter season and also supplies the superstores in the city. For the purpose of the study, eight fish species - four marine: *Pampus Chinensis*, *Otolithes ruber*, *Trachipteridae* and *Oncorhynchus tshawytscha*, and four freshwater: *Labeo rohita*, *Catla catla*, *Rita rita* and *Walago atu* were selected due to their greater consumption by people than other fishes.

A total of 72 fish samples were analysed in triplicate for heavy metal concentrations. An individual sample was washed with distilled water before placing in the ice box then packed into polyethylene bags and carried to the Chemistry Laboratory of Fish Quality Control Labs at the Manawa Fisheries Complex. Samples were kept in freezer at -4°C until further chemical analysis. The samples were dissected and the muscle portion was used for the analysis. 10g of fish muscle from each sampled fish was taken on wet body weight basis into a 100mL beaker and 10mL of freshly prepared Hydrogen peroxide and Nitric Acid Solution (1:1 V/V) were added for wet digestion. For the initial reaction the beaker was covered with watch glass and set aside for 1 hour. Then the beaker was placed on hot plate at 160°C for 2 hours or more until the crystal clear solution was obtained and the volume was reduced to 2-2.5mL. This solution was filtered by Millipore

Table 1. Heavy metal concentrations in tested fish species from different fish markets of Lahore (Mean±SD)

Location	Origin	Fish species	Heavy metals concentrations (mg/kg)		
			Cd	Cr	Pb
Main fish market	Fresh water	<i>Labeo rohita</i>	0.274±0.001	0.022±0.001	0.039±0.001
		<i>Catla catla</i>	0.130±0.001	0.006±0.000	0.029±0.001
		<i>Rita rita</i>	0.050±0.001	0.097±0.001	0.052±0.001
		<i>Walago atu</i>	0.052±0.000	0.111±0.001	0.026±0.001
	Marine	<i>Pampus Chinensis</i>	0.056±0.001	0.137±0.001	0.020±0.000
		<i>Otolithes ruber</i>	0.039±0.000	0.095±0.000	0.042±0.001
		<i>Trachipteridae</i>	0.094±0.000	0.024±0.001	0.049±0.001
		<i>Oncorhynchus tshawytscha</i>	0.212±0.000	0.172±0.001	0.038±0.001
Hyperstar	Fresh water	<i>Labeo rohita</i>	0.142±0.001	0.002±0.001	0.037±0.001
		<i>Catla catla</i>	0.120±0.001	0.075±0.001	0.037±0.001
		<i>Rita rita</i>	0.088±0.001	0.141±0.001	0.028±0.001
		<i>Walago atu</i>	0.042±0.001	0.144±0.001	0.038±0.000
	Marine	<i>Pampus Chinensis</i>	0.054±0.001	0.037±0.001	0.045±0.001
		<i>Otolithes ruber</i>	0.037±0.000	0.066±0.001	0.043±0.001
		<i>Trachipteridae</i>	0.042±0.001	0.066±0.002	0.027±0.001
		<i>Oncorhynchus tshawytscha</i>	0.095±0.001	0.141±0.001	0.031±0.001
Metro	Fresh water	<i>Labeo rohita</i>	0.297±0.001	0.135±0.001	0.037±0.001
		<i>Catla catla</i>	0.196±0.001	0.006±0.000	0.035±0.002
		<i>Rita rita</i>	0.111±0.001	0.092±0.001	0.024±0.001
		<i>Walago atu</i>	0.096±0.000	0.087±0.000	0.025±0.001
	Marine	<i>Pampus Chinensis</i>	0.124±0.001	0.085±0.001	0.016±0.001
		<i>Otolithes ruber</i>	0.093±0.001	0.052±0.001	0.012±0.001
		<i>Trachipteridae</i>	0.062±0.001	0.042±0.001	0.020±0.000
		<i>Oncorhynchus tshawytscha</i>	0.023±0.000	0.127±0.000	0.057±0.000

Table 2. Species wise and location wise ANOVA results of heavy metals concentrations with minimum and maximum ranges

Category	Metal	Source	Mean	SD (\pm)	Range		F-test	Sig
					Min	Max		
By species	Cd n=9	<i>Labeo rohita</i>	.238	.073	.141	.297	15.758	0.000
		<i>Catla catla</i>	.149	.036	.120	.196		
		<i>Rita rita</i>	.083	.027	.049	.112		
		<i>Walago atu</i>	.063	.025	.041	.096		
		<i>Pampus Chinensis</i>	.078	.035	.053	.125		
		<i>Otolithes ruber</i>	.056	.028	.037	.094		
		<i>Trachipteridae</i>	.066	.023	.041	.094		
		<i>Oncorhynchus tshawytscha</i>	.110	.083	.023	.212		
	Cr n=9	<i>Labeo rohita</i>	.053	.062	.002	.136	12.631	0.000
		<i>Catla catla</i>	.029	.034	.006	.075		
		<i>Rita rita</i>	.110	.023	.092	.142		
		<i>Walago atu</i>	.114	.025	.087	.145		
		<i>Pampus Chinensis</i>	.086	.043	.036	.137		
		<i>Otolithes ruber</i>	.071	.019	.052	.095		
		<i>Trachipteridae</i>	.044	.018	.024	.067		
		<i>Oncorhynchus tshawytscha</i>	.146	.020	.127	.172		
	Pb n=9	<i>Labeo rohita</i>	.038	.001	.036	.040	1.701	0.125
		<i>Catla catla</i>	.034	.003	.029	.037		
		<i>Rita rita</i>	.035	.013	.024	.053		
		<i>Walago atu</i>	.030	.006	.025	.038		
		<i>Pampus Chinensis</i>	.027	.013	.016	.045		
		<i>Otolithes ruber</i>	.032	.015	.011	.043		
		<i>Trachipteridae</i>	.032	.013	.020	.049		
		<i>Oncorhynchus tshawytscha</i>	.042	.012	.031	.057		
By location	Cd n=24	Main Market	.113	.083	.039	.275	2.981	0.057
		Hyperstar	.077	.038	.037	.142		
		Metro	.125	.081	.023	.297		
	Cr n=24	Main Market	.083	.057	.006	.172	0.085	0.919
		Hyperstar	.084	.051	.002	.145		
		Metro	.078	.041	.006	.136		
	Pb n=24	Main Market	.037	.011	.020	.053	4.488	0.015
		Hyperstar	.036	.006	.026	.045		
		Metro	.028	.014	.011	.057		

*p<0.05

membrane filter (0.45 μ m, type HV) into the volumetric flask and diluted up to 25mL by using deionized water (Chatta et al., 2017).

Heavy metals content analysis

The prepared fish samples were analyzed for determination of concentration of three non-essential heavy metals Cr, Pb and Cd by using the inductively coupled plasma (PerkinElmer, model: Optima 7000DV ICP-OES).

Fish quality assessment

The assessment of the muscles quality of the studied fish

as food for humans by the content of Cd, Cr and Pb was made by comparing the results obtained with the norms of different organizations: Philippines DAO, 1990-34, US (EPA) and WHO.

Statistical analysis

The data collected from heavy metal analysis was statistically analyzed by mean, standard deviation, analysis of variance (ANOVA) and t test by using statistical package SPSS 22 (statistical package for social sciences).

Results and discussion

The mean concentration of the heavy metals (mg/kg±SD) of various fish species available in local markets of Lahore is given in Table 1. *Cadmium (Cd)*. The highest mean concentration of Cd (0.294±0.001 mg/kg) was observed in *Labeo rohita* collected from Metro superstore while the lowest (0.023±0.000 mg/kg) in the *Oncorhynchus tshawytscha* of the same superstore. Species wise concentration of cadmium was found significant by using ANOVA (Table 2). Origin wise t-test results (Table 3) showed that Cd is significant when comparing marine and fresh water species. The mean concentration of Cd in *Labeo rohita* collected from Metro superstore was 0.297±0.001 mg/kg that is higher than the described value of WHO. It is also found higher than the findings of Chatta et al. (2017) where they found concentration from fish forms and local fish market of Lahore in the range 0.0258±0.0029 - 0.0080±0.0045 mg/kg. Cd concentration in common carp and rainbow trout was observed 0.040 and 0.200 mg/kg, respectively, by Stoyanova et al. (2016). Qadir et al. (2009) recorded Cd concentration in muscles of *Labeo rohita* fish collected from Chenab river tributaries 0.89±0.06µg/g, which was much higher than the present study.

Chromium (Cr). The highest mean concentration of Cr (0.172±0.001 mg/kg) was found in *Oncorhynchus tshawytscha* of main fish market of Lahore and the lowest concentration (0.002±0.001 mg/kg) was established in *Labeo rohita* collected from Hyperstar super store. ANOVA results (Table 2) showed that the concentration of chromium was found significant species wise and non-significant location wise. Origin wise t-test results (Table 3) showed that chromium is non-significant when comparing marine and fresh water species.

The range of chromium 0.002-0.172 mg/kg is in admissible limit defined by various international standards. The current findings are contrary to the findings of Chatta et al. (2017) where they found slightly higher concentration of chromium in fish samples collected from fish farms and retailer's shops of Lahore. Qadir et al. (2009) found much higher concentration of Cr 3.630±0.401 mg/kg. Unluckily, there is no comprehensive guidance and standards for host metal residue in fish in which range they are permissible or not. However, more precise and elaborated work is desired to evaluate the current situation of heavy metal pollution in the metropolitan city of Lahore.

Lead (Pb). The highest concentration of Lead (0.057±0.000 mg/kg) was determined in *Oncorhynchus tshawytscha* of Metro super store and the lowest concentration (0.012±0.001 mg/kg) was determined in *Otolithes ruber* of the same superstore. ANOVA (Table 2) results showed that the concentration of lead was found non-significant species wise and significance location wise. Origin wise t-test results (Table 3) are non-significant for lead when comparing between freshwater and marine species. The results obtained for lead concentration were contrary to the results established by Chatta et al. (2017). In the current study, lead concentration is found in admissible limit as described by international standards in samples from different markets of Lahore.

Table 3. Origin wise t-test results of heavy metals concentrations with mean and standard deviation (n=36)

Metal	Type	Mean	Std. Deviation	T-test	Sig
Cd	Fresh Water	.133	.081	3.492	0.001*
	Marine	.078	.051		
Cr	Fresh Water	.077	.053	-0.882	0.381
	Marine	.087	.046		
Pb	Fresh Water	.034	.008	0.293	0.770
	Marine	.033	.014		

*p<0.05

Table 4. Maximum acceptable levels of heavy metals in fish muscles according to international standards and range of concentrations found in the muscle of fishes from Lahore

International organizations	Parameters		
	Pb (mg/kg)	Cd (mg/kg)	Cr (mg/kg)
Philippines DAO, 1990-34	0.050	0.010	-
US (EPA)	0.065	0.0043	-
WHO	0.123	0.050	0.1
Range of metals in the present study	0.011-0.057	0.023-0.297	0.002-0.172

Comparison of the results obtained with acceptable limits and norms. The range of Cd (0.023-0.297 mg/kg) was found higher than the safe limits given in various international organization (Table 4). The range of Cr (0.002-0.172 mg/kg) showed that all 72 samples from all markets of Lahore are found within safe limits when compared with international standards (Table 4). The range of Pb (0.012-0.057 mg/kg) showed that the values are within safe limits when compared with most important international standards.

Conclusion

Heavy metals (Cd, Cr, Pb) concentration of both marine (*Pampus Chinensis*, *Otolithes ruber*, *Trachipteridae* and *Oncorhynchus tshawytscha*) and fresh water fish species (*Labeo rohita*, *Catla catla*, *Rita rita* and *Walago atu*) sold in markets of the metropolitan city of Lahore varied significantly by fish species (Cd 0.023-0.297 mg/kg; Cr 0.002-0.172 mg/kg; Pb 0.011-0.057 mg/kg). Maximum concentrations of all heavy metals in all fish species exceeded the permissible limits set by WHO, US (EPA) and Philippines DAO, 1990-34. Hence, the

prolonged consumption of these fish containing the established quantities of heavy metals may pose health hazards to humans. More precise and focused studies are required to be conducted to get proper insight of this grave issue.

Acknowledgement

The authors are highly thankful to Department of Fisheries Punjab Lahore, Pakistan for support during this research work.

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