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The Role of Zn and Cu in Muscle Tissues of *Pampus argenteus* (Euphrasen, 1788), Marketed by Karachi Fish Harbour, Pakistan

Quratulan AHMED[1,*] and Levent BAT[2]

[1]*The Marine Reference Collection and Resources Centre, University of Karachi, Karachi 75270, Pakistan*
[2]*University of Sinop, Fisheries Faculty, Department of Hydrobiology, TR57000 Sinop, Turkey*

([*]Corresponding author's e-mail: quratulanahmed_ku@yahoo.com)

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Abstract

Two essential metal contaminations were studied in a total of sixty silver pomfret *Pampus argenteus* samples. The purpose of the current study was to evaluate the levels of Zn and Cu in the muscle tissues of the commercial species *P. argenteus* in the fishing harbour of Karachi, the most important in Pakistan, and to correlate the concentration of metals with respect to fish size. Moreover, this study sought to establish the potential risk to humans from consumption of fish samples contaminated with these heavy metals. Zn concentrations in the silver pomfret were higher than Cu. The ranges of Zn and Cu concentrations were found to be from 2 to 12 and 1 to 6 mg/kg dry wt., respectively. The differences in both Zn and Cu concentrations between seasons were statistically significant. These results can be used to provide baseline information for risk assessment associated with their consumption, as the Estimated Daily Intake (EDI) for the fish tissues and Zn and Cu levels were below the daily dietary allowance recommended by international standards. Therefore, it may be concluded that these metals should not pose any health threat to the consumers resulting from the consumption of the fish. The relationship between Zn and Cu levels and fish size (both length and weight) of the fish were minimal, indicating that metal concentration in fish tissues was independent of lengths and weights.

Keywords: *Pampus argenteus*, zinc, copper, Karachi Harbour, Estimated Daily Intake

Introduction

The silver pomfret, *Pampus argenteus*, locally known as 'zobaidy', is a member of the Stromateidae family, and is widely distributed throughout the Indo-West Pacific: from the Persian Gulf to Indonesia, Japan, the west and southwest of Korea, and theeastern parts of China [1]. Silver pomfret is one of the most commercially important fish in the Northern Persian Gulf, and its stock is shared by Iran, Iraq, and Kuwait [2]. Due to the high market demand and high value, commercial fisheries in the Persian Gulf usually target this species.

Fish is a rich resource of protein and makes a major contribution to Pakistan's economy [3]. Pakistan earned an amount of Pakistani Rupee 196 million in 1996 from its export [4]. It is also found in the eastern part of China, the Korean Peninsula, and the Yellow Sea, and comes from areas where oceanic fronts occur due to mixing of warm and cold currents [5].

Among the various toxic pollutants, heavy metals have a particularly severe effect, due to the tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of the agricultural and industrial revolution, most water sources are becoming contaminated [6]. Industrial discharges containing toxic and hazardous substances, including heavy metals [7,8], contribute tremendously to the pollution of the aquatic ecosystem.

As a result of increased input from industry, agriculture, and urbanisation, the average heavy metal content in the marine environment is increasing considerably. Karachi harbour is one of the most famous fish trading centres in Pakistan; industry and agriculture near the Karachi harbour have been rapidly developed, and human activities have increased. This has become a major point of concern during the last few decades, since metal levels have reached potentially toxic levels in seafood. Fish is a healthy food because of the nutritional benefits related to its high proteins quality and desirable lipid composition.

Zn and Cu are essential heavy metals in the environment. Zn and Cu bioavailability are affected by biotic and abiotic factors, e.g., organism age and size, prior history of exposure, water hardness, pH, dissolved organic carbon, and temperature. For example Zn acts as a structural component and has specific properties that make it indispensable for life [9]; however, the danger of zinc is aggravated by its almost indefinite persistence in the environment, because it cannot be destroyed biologically, only transformed from an oxidation state or organic complex to another. Zn is a potential toxicant to fish [10], which causes disturbances of acid-base and ion regulation, disruption of gill tissue, and hypoxia [11]. Similarly, Cu is an essential part of several enzymes, and is necessary for the synthesis of haemoglobin [12]. However, a high intake of Cu has been recognized to cause adverse health problem [13].

The toxicity of these metals will depend on environmental conditions and habitat types. Thus, any risk assessment of the potential effects of Zn and Cu on organisms must take into account local environmental conditions.

Contaminated fish is an frequentroute of human exposure, and may be a potential risk to human health. Thus, levels of heavy metals in fish are of particular interest. The purpose of the current study was to evaluate the levels of Zn and Cu in muscle tissue of *P. argenteus*, and to correlate the concentration of metals with respect to fish size. Moreover, this study sought to establish the potential risk to humans due to consumption of the fish samples contaminated with these heavy metals.

Materials and methods

Collection of Samples

A total of 60 fish samples (20 each fromPre-Monsoon, Monsoon and Post-Monsoon) of the silver pomfret *P. argenteus*, used for the present study, were collected from Karachi Harbour, Pakistan, during January and December of 2012. Fish samples were transported to the Fisheries Laboratory of the Marine Reference Collection and Resources Centre, Karachi. The silver pomfret samples were washed well with bi-distilled water on arrival at the laboratory. Then, fish specimens were measured for total length (TL) by using a wooden measuring tray to the nearest 0.1 cm and weighed (W) on an electronic balance to the nearest 0.1 g.

Preparation of fish samples

Each individual fish was dissected with a corrosion-resistant stainless steel knife, the muscle tissues were isolated, and all samples were taken from the dorsolateral muscle of the right side. Each dissected tissue was placed in a polyethylene bag and provided with necessary information about identification code, date, locality, and season. The samples were placed in an icebox and transported to the laboratory. In the laboratory, the samples were stored at −21 °C, prior to heavy metal analysis.

Chemical analysis

Sample preparation and analytical determination of heavy metals was made following the recommendations given by theUNEP [14,15]. Each muscle tissue sample was homogenized and weighed. Samples were put into Erlenmeyer flasks, then placed in a vented drying oven at 105 °C for 24 h until they were of a dry constant weight. Each sample was weighed again for a dry weight measurement. Then, 10 ml of HNO_3 per g of dry wt. were added to each Erlenmeyer flask, covered with a watch glass, and the solution evaporated to dryness on a hot plate. The Erlenmeyer flasks were cooled to room temperature overnight and were replaced on the hot plate until the samples' coloured vapours gradually lost their colour in the fume hood. After allowing the samples to cool, 1 ml HNO_3 was added to bring the volume to 25 ml for muscles with distilled water. Zn and Cu concentrations in the samples were determined by using an Atomic Absorption Spectrophotometer. The samples were analysed for heavy metals using the equipment Analyst 700 with the programme win lab 32 software.

Statistical Analysis

Statistical analysis (ANOVA) was performed to test the differences between seasons, and the Tukey test was used to determine the differences [16]. Differences were regarded as significant when $p < 0.05$ level. All data prior to statistical analyses were expressed as mg/kg dry weight.

Results and discussion

The mean lengths (cm) and weights (g) of *P. argenteus* collected from Karachi Harbour during 2012 are given in **Table 1**. The concentrations of Zn and Cu detected in the fish samples have given in **Table 2**. The highest mean concentrations of Zn (9±3 mg/kg dry wt.) and Cu (4±1 mg/kg dry wt.) were recorded in the Post-Monsoon of 2012. The lowest mean concentration of Zn (5±2 mg/kg dry wt.) was determined in the Pre-Monsoon (2012), whereas the lowest mean concentration of Cu (2±0.4 mg/kg dry wt.) was recorded in the Monsoon (2012) (**Figure 1**). Statistical analysis is shown in **Table 3**. Zn and Cu showed no correlation with the total length and body weight of the silver pomfret *P. Argenteus* collected from Karachi Harbour between January and December of 2012 (**Figure 2**).

Table 1 Mean (±SD) lengths and weights of the silver pomfret *P. argenteus* collected from Karachi Harbour between January and December of 2012.

Seasons		Length (cm)	Weight (g)
Pre-M	Mean (±SD)	31±1	175±5
	(Min.-Max.)	30-32	170-182
M-soon	Mean (±SD)	32±1	179±4
	(Min.-Max.)	31-34	174-184
Post-M	Mean (±SD)	31±1	176±5
	(Min.-Max.)	31-34	171-186
Total	Mean (±SD)	31±1	177±5
	(Min.-Max.)	30-34	170-186

Table 2 Mean (±SD) of Zn and Cu concentrations (mg/kg dry wt.) in the silver pomfret *P. Argenteus* collected from Karachi Harbour between January and December of 2012. Means in the same column bearing different superscript are significantly different ($P < 0.05$).

Seasons		Zn	Cu
Pre-M	Mean (±SD)	$5±2^a$	$2±1^a$
	(Min.-Max.)	2-8	1-5
M-soon	Mean (±SD)	$8±2^b$	$2±0.4^a$
	(Min.-Max.)	3-10	2-3
Post-M	Mean (±SD)	$9±3^b$	$4±1^b$
	(Min.-Max.)	2-12	1-6
Total	Mean (±SD)	7±3	3±1
	(Min.-Max.)	2-12	1-6

Table 3 Analysis of variance (ANOVA) in the silver pomfret *P. argenteus* collected from Karachi Harbour during seasons in 2012.

Metals		Sum of squares	df	Mean square	F	Sig.
Zn	Seasons	89.136	2	44.568	12.361	.000
	No of sample	97.348	27	3.605		
	Total	186.484	29			
Cu	Seasons	20.694	2	10.347	8.825	.001
	No of sample	31.657	27	1.172		
	Total	52.352	29			

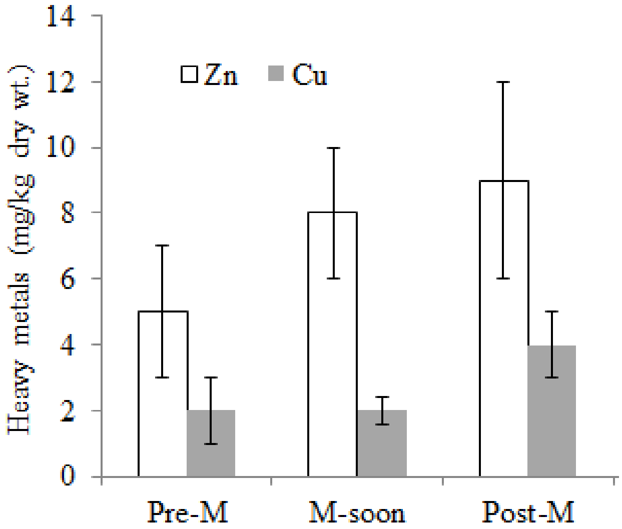

Figure 1 Zn and Cu levels in the silver pomfret *P. argenteus* collected from Karachi Harbour during Pre-Monsoon, Monsoon and Post-Monsoon in 2012.

The levels of Zn and Cu were increased significantly in the Post-Monsoon ($P < 0.05$). The seasonal fluctuation of metal concentrations in fish is affected by environmental variations and by physiological conditions (feeding or reproductive cycle). Inshore species of *P. argentus* are usually found in schools over muddy bottoms. Adults feed on ctenophores, salps, medusa, and other zooplankton groups. Western populations spawn from late winter through the summer, with peaks from April to June [17]. July is the month in the middle of the Monsoon, and October is the ending part; in the season of the Monsoon, the sea is very rough and more nutrients are available [18]. These affect the conditions of the water and the availability of metals in the marine environment. Rainbow *et al*. [19] pointed out that, as salinity increases, metal absorption decreases. In this study, concentrations of Zn and Cu varied from 2 to 12 and 1 to 6 mg/kg dry wt., respectively. Tabinda *et al*. [20] reported low values of Zn (1.143 - 1.715) and Cu (0.001 - 0.003) in *P. argenteus* from Keti Bunder, compared with the values of these metals determined in the present study. Similarly, a low value of Cu (0.340±0.009) in *P. argenteus* from the northwest coastal area of Karachi Harbour was found [21]. The high values of the metals determined in the present study appears to be due to their determination of metals on a dry wt. basis. Moreover, because of the evaporation caused by increased temperatures during the dry season, heavy metal concentration in water is generally higher compared to that of the wet season. Thus, metals accumulate more in fish during the dry season.

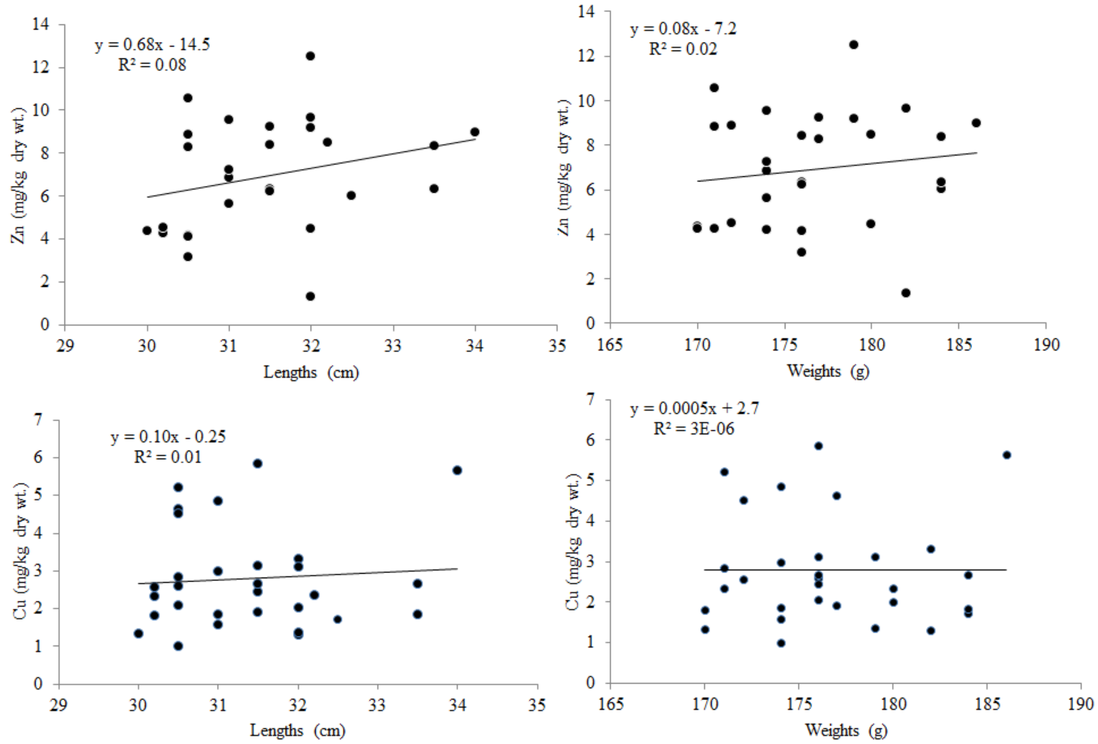

Figure 2 Relationship of Zn and Cu with length (cm) and weight (g) of the silver pomfret*Pampus argenteus*collected from Karachi Harbour between January and December of 2012.

The sizes of marine organisms have been shown to play an important role in the metal contents of tissues [18,22,23]. In this study, regression analysis was performed to assess the size dependence of Zn and Cu in *P. argenteus.* The correlation between the size (both length and weight) of the fish and the metal concentrations in the muscle tissue are presented in **Figure 2**. The length and weight is not correlated with concentrations of Zn and Cu. The relationship between heavy metal levels and size (length and weight) of the fish were minimal, indicating that metal concentration in fish tissues was independent of lengths and weights. However, a minimal increase in concentrations of Zn and Cu with an increase in body size suggests that Zn and Cu are probably accumulated at higher rates compared to their rate of excretion as the fish grows. In corroboration with the present findings, previous studies reported similar results [18,24]. Douben [24] reported that there was no relationship with fish size and Pb concentrations. Zehra *et al*. [18] reported periodic variations. Their findings of Cu and Zn concentrations in *Acanthopagurus berda* from the Baluchistan Coast, Pakistan, show that there was no significant variation with weight and length, due to rough sea. Zehra *et al*. [18] also pointed out that Cd concentration increased gradually as weight and length increased, but Pb concentration showed that smaller size fish had the same concentration. It was concluded that correlation of metal concentration with fish size may be species specific [18]. Moreover Douben [24] indicated that metal accumulation could reach a steady state after a certain age.

However Jinadasa *et al*. [25] found positive relationships between body size of the swordfish *Xiphias gladius* and the yellowfin tuna *Thunnus albacores* with Hg and Cd, and of the red snapper *Lutjanus* sp. with Cd concentration. This may be explained by the metal, once taken up into the body, being stored in particular organs, especially the liver or the kidney, and excreted at a slow ratecompared with its uptake. This also may be explained by seasonal differences.

On the contrary, Mohammadnabizadeh *et al*. [23] studied the accumulations of Cd, Ni, Pb, and Cr in the muscles, gills, kidneys, and liver of *Platycephalus indicus* and *P. argenteus* in the Hara Reserve, Iran. There was significant negative correlation between the fish size (length and weight) and heavy metal concentration in fish muscle tissues, which is generally supported in the literature [22]. Attending to the fact that metal absorption occurs mainly through the digestive system, and also that metabolic rate is higher in younger individuals than in adults, the negative relationship between metal concentration and body size was probably due to the difference of metabolic rates between younger individuals and adults [23].

The mean concentration of Zn (7±3 mg/kg dry wt.) found in *P. argenteus*was less than 50 mg/kg wet wt., well below the guideline level [26].Cu concentrations in the silver pomfret were low (mean Cu concentration was 3±1mg/kg dry wt.), and quite below the guideline level of 20 mg/kg wet wt. [26].

Tabinda *et al*. [20] indicated that the contamination of Pb and Cu in *P. argenteus* from Keti Bunder Thatta District, Sindh, Pakistan, might pose a threat for the importers due to the high per capita consumption of fish. Similarly, Mohammadnabizadeh *et al*. [23] indicated that Ni, Pb, and Cr levels in *P. indicus* and *P. argenteus*from the northeastern coast of the Persian Gulf pose a health hazard to consumers, as they exceed the permissible level in muscle tissues. However, Raza *et al*. [21] found that As, Co, Cu, Mn, and Pb levels in *P. argenteus* from the northwest coastal area of Karachi Harbour were safe for human consumption. The present study was conducted only for fish muscle, as this tissue is the most important part consumed by people. FAO estimates of fish consumption in Pakistan indicated that the adult population of Pakistan consumes 5 g of fishdaily per person [27]. This is also equivalent to 35 g/week. Internationally accepted safe levels of Zn and Cu are 7 mg and 3.5 mg per kg body weight per week for PTWI, respectively [28]. These are equivalent to 490 mg/week/70 kg body weight and 70 mg/day/70 kg body weight for Zn, and 245 mg/week/70 kg body weight and 35 mg/day/70 kg body weight for Cu. Estimated Daily Intake (EDI) for a 70 kg body weight of an adult person on the basis of the present study's results were calculated as 0.035±0.015 for Zn, and 0.015±0.005 for Cu. EDI values for the silver pomfret were below the recommended values, indicating that the health risk associated with the intake of Zn and Cu through the consumption of the studied fish samples was absent.

Conclusions

In the present study, the concentrations of Zn were found to be higher than Cu concentrations in the silver pomfret *P. argenteus*. The differences in heavy metal concentrations between seasons were statistically significant. However, these results can be used to provide baseline information for risk assessment associated with consumption, as the Estimated Daily Intake (EDI) for the fish tissues and Zn and Cu levels were below the daily dietary allowance recommended by FAO/WHO [28]. Therefore, it may be concluded that these metals should not pose any health threat to consumers resulting from the consumption of the silver pomfret *P. argenteus*.

The relationship between Zn and Cu levels and fish size (both length and weight) of the fish were minimal, indicating that metal concentration in fish tissues was independent of lengths and weights. Furthermore, fish growth and its relationship with metal concentration in the marine environment should be monitored occasionally in the field, for improved understanding of the effects of metals on fish development [22].

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