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Heavy metal and trace element contents in edible muscle of three commercial fish species, and assessment of possible risks associated with their human consumption in Saudi Arabia

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ABSTRACT

Three different highly consumed fish species from Al-Ahsa market, Saudi Arabia namely Spangled emperor (*Lethriuns nebulosus*), Red striped seabream (*Pagrus major*) and Black seabream (*Spondyliosoma cantharus*) were evaluated for their muscle contents of heavy metals (e.g., Cd and Pb) and trace elements (e.g., Cu, Zn, Fe and Mn). The possible risks associated with their human consumption were also studied. A total of 60 fresh fish samples comprising of 20 samples from each above mentioned fish were collected, and were subjected for determination of heavy metal and trace element contents by Atomic Absorption Spectrophotometry after Microwave Wet Digestion. The results showed that, accumulation patterns of the heavy metals and trace elements followed the order: Fe > Zn > Cu > Mn > Pb > Cd. There were variations among metal contents in the muscles of the three fish species; *S. cantharus* accumulated the highest levels of Cu, Zn and Mn, while the highest level of Fe could be detected in the muscles of *P. major*. The concentration of Cd and Pb remained comparable in the muscles of all three fish species. The calculated maximum daily intake (MDI) values were found as 0.0003, 0.0009, 0.0035, 0.0001, 0.0000, 0.0000 mg/day/person for Cu, Zn, Fe, Mn, Cd and Pb, respectively. Conclusively, the present study indicated that, fish muscles contain relatively less burden of heavy metals and trace elements, and no health problem can be raised from human consumption of the examined commercial fishes at Al-Ahsa market, Saudi Arabia.

Keywords

Heavy metals, *Lethriuns nebulosus*, Muscles, *Pagrus major*, *Spondyliosoma cantharus*, Trace elements

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INTRODUCTION

Most of the trace elements present in fish are essential for human at low concentrations, however, high concentrations of these elements are toxic for human (Mahboob et al., 2014). Some metals (e.g., Fe, Mn and Zn) are necessary for proper metabolic reactions in human body, and other elements (e.g., Cd and Pb) are of unknown benefits and may become toxic in the cases of chronic exposure to human (Al-Busaidi et al., 2011). Fish is considered as one of the favorite meals to the people living around the Arabian Gulf (Mahboob et al., 2014). Thus, fish plays an important role to meet the demands of human nutrition. However, the fish is able to concentrate heavy metals in their tissues (Javed and Usmani, 2011). Therefore, screening of metal contents in fish tissues is crucial to ensure that these metals are not transferring to human through fish consumption (Rahman et al., 2012). The monitoring of some metals (e.g., Cd, Pb, Cu, Zn and Fe) in food and water is obligatory, whereas monitoring of some others (e.g., Sb, Ni, Cr, Al, F and J) are suggestive (WHO, 1996, CAC/FAO, 1999; European Commission, 2000; Staniskiene et al., 2009). The Saudi Arabian Standards

Organization (SASO; 1997) suggested maximum permissible limits for several toxic heavy metals in fish species as 0.5, 1, 2, 1, 20 and 50 mg/g for Cd, Hg, Pb, As, Cu and Zn, respectively.

The contamination of Arabian Gulf with oil spill has been previously documented (NRC, National Research Council, 1985) representing 4.7% of the world's total oil pollution; moreever, the rate has increased after the Gulf war (Price and Shepparrd, 1991). The contribution of oil spill during the year 1991 to heavy metal contamination in marine environment of the Gulf in Kuwait has been investigated and significant increase in the levels of Cu and Zn has been reported (Bu-Olayan and Subrahmanyam, 1997).

Petrochemical industry wastes and refineries contribute significantly to metal pollution of marine environment of the Arabian Gulf. Accumulation of these metals in fish depends on the concentration of discharged metal and the duration of exposure. Heavy metal concentration in organic samples such as fish products are mostly assayed using Atomic Absorption Spectrometry (Kumar et al., 2012; Mahboob et al., 2014) and Inductive Coupled Plasma Mass Spectrophotometry (ICP mass) (Bashir et al., 2013; Jarapala et al., 2014). Although, heavy metal content in fish has been extensively studied (Rahman et al., 2012, Mahboob et al., 2014), periodical monitoring of metal content in edible muscles of fish is of great importance. Moreover, metal content in the commercial fish like Spangled emporer (*Lethriuns nebulosus*; Shieri), Red striped seabream (*Pagrus major*; Andak) and Black seabream (*Spondyliosoma cantharus*; Shaiam) from Saudi Arabian market has not been studied so far. Therefore, the current study was designed to determine the level of heavy metal (e.g., Cd and Pb) and trace element (e.g., Cu, Zn, Fe and Mn) accumulations in muscle tissues of these fish species collected from Al-Ahsa market in Saudi Arabia. This could provide baseline information which might contribute to the effective monitoring of both environmental quality, and the health of the organisms inhabiting in Eastern Region of Saudi Arabia.

MATERIALS AND METHODS

Sample collection: Fresh fish samples (n=60) of three different species namely Spangled emporer (*L.nebulosus*; Shieri; n=20), Red striped seabream (*P. major*; Andak; n=20) and Black seabream (*S. cantharus*; Shaiam; n=20) were collected from Al-Ahsa market, Eastern Region of Saudi Arabia. The samples were brought to laboratory on ice. The muscle samples from each fish were dissected, washed with distilled water, pooled, weighed, packed in polyethylene bags and stored at –30°C until chemical analysis. Special care was taken to prevent metal contamination of the samples that may happen by the laboratory equipments. The tissues were dissected by plastic knife to avoid contamination from cutting instrument. All laboratory wares were soaked in nitric acid for 48 h, and rinsed five times with distilled water, followed by rinsed five times with deionized water prior to use. The current study follows international and institutional guidelines for humane animal treatment and complies with the relevant legislation. Photographs of three fish species are shown in **Figure 1**. The chemicals used for the sample dissolution were of analytical grade. Local, common and scientific names of the fishes are presented in **Table 1**.

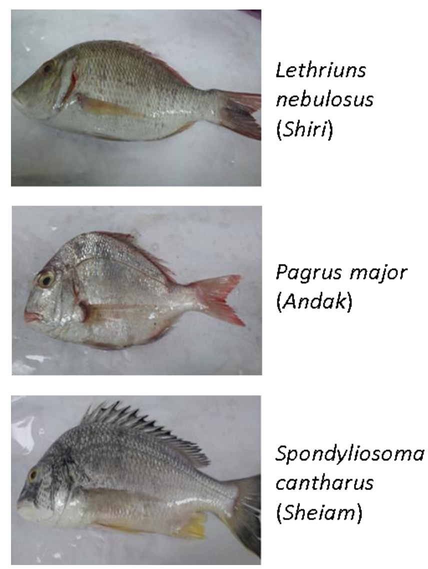

Figure 1. Photographs of three fish species collected from Al-Ahsa Market, Saudi Arabia (Italic letter: Scientific name; in Parenthesis: Local name).

Table 1. Local, common and scientific names of fish species included in the study.

Local name	Common name	Scientific name
Shiri	Spangled emporer	*Lethriuns nebulosus*
Andak	Red striped seabream	*Pagrus major*
Sheiam	Black seabream	*Spondyliosoma cantharus*

Table 2. Flame Atomic Absorption Spectrophotometer operating conditions

Condition	Cu	Zn	Fe	Mn
Wave length (nm)	324.8	213.9	239.6	257.6
Slit width (nm)	0.2	0.5	0.5	0.2
Sensitivity (μg/mL)	0.014	0.008	0.07	0.05
Flame type	Air-Acetylene			

Samples digestion: The pooled muscle samples were thawed, homogenized, and 0.5 g of sample was taken from each fish sample. Microwave method was applied for the digestion procedure of samples (Usero et al., 2003) by using Microwave digestion system (CEM, Inc. MARS Express®, Matthews, NC, USA) in accordance to USEPA method 3051. The thawed homogenate of each fish was placed in a Teflon digestion vessel with 6 mL of nitric acid (65%) and 4 mL of hydrogen peroxide (30%). The samples in the vessels were then digested using an optimized microwave method. The samples were allowed to ramp at 200°C for 15 min, and cooled down for 5 min. The digested muscle samples were diluted with deionized water to a total volume of 25 mL. The cooled mixture was then filtered using Whatman filter paper#1. After filtration, the filtrates of digested muscle samples were analyzed by atomic absorption, as per the method described by Pakshirajan et al. (2013).

Table 3. Heating programs for Cd and Pb in graphite furnace atomic absorption spectrophotometer.

Step	Temperature (°C)		Ramp(s)	Hold(s)	Argon flow rate (mL min^{-1})
	Cd	Pb			
1	150	150	5	20	250
2	200	200	5	15	250
3	500	800	10	20	250
4	1800	2000	0	5	0
5	2200	2200	1	3	250

Table 4. Recovery of trace elements and heavy metals from homogeneous mixture of muscles of the three fish species.

Elements	Concentration of metal added (mg/kg)	Concentration of metal recovered (mg/kg)	Recovery (%)
Cu	3	2.6	86.7
Zn	3	2.8	93.3
Fe	3	2.7	90
Mn	3	2.7	90
Cd	3	2.6	86.7
Pb	3	2.8	93.3

Chemical analysis: The determination of metals was carried out by using AA-6800 model flame Atomic Absorption Spectrophotometer for the determination of Cu, Zn, Fe and Mn. The maximum absorbance was obtained by adjusting the cathode lamps at the operation conditions shown in **Table 2**. GFA-EX7 graphite furnace Atomic Absorption Spectrophotometer (Shimadzu, Koyoto, Japan) was used for the determination of Pb and Cd. A high density graphite tube was used for automization and normal single hollow cathode lamps were used for irradiation. In graphite furnace Atomic Absorption Spectrophotometer, all measurements were based on integrated absorbance whereas in Atomic Absorption Spectrophotometer based on peak heights, both performed at 228.8 nm for Cd and 283.3 nm for Pb by using electrode less discharge lamps system. The furnace program for determination of Cd and Pb by graphite furnace Atomic Absorption Spectrophotometer are given in **Table 3** with the instrumental setting. Standard stock solutions of Cu, Zn, Fe, Mn, Cd and Pb were prepared with deionized water. Calibration curves were obtained for different concentrations of standard solutions prepared from 1000 mg/L commercial stock solution (Merck, Darmstadt, Germany). In order to check on the purity of the chemical used, a number of chemical blanks were run. There was no evidence of any contamination in these blanks. Argon 99.98% was used as protective gas throughout in graphite furnace Atomic Absorption Spectrophotometer.

Analytical quality control: In the present study, to check the efficiency of digestion procedures and the subsequent recovery of the metal, homogenous mixtures of three samples of fish muscles were spiked with multi-element solutions which contain standard solutions of all metals considered in the present study. The element solution was spiked in a manner to attain final concentrations of 3 µg/g. A mixture without any metal was used as control. All mixtures were then subjected to the digestion procedure. The resulting solutions were analyzed three times for metal concentrations according to the same procedures as the samples to establish confidence in the accuracy and reliability of data generated. The amount of spiked metal recovered after the digestion of the spiked samples was used to calculate percentage recovery according to Elnabris et al. (2013) as follows:

*% recovery = [(t-c)/t] 100. Where t = concentration of a metal in treatment sample, and c= concentration of a metal in control sample. Procedural blanks and standard solutions were also included for analytical quality control to assure the accuracy and reproducibility of the results. The results of recovery experiment are shown in **Table 4**.*

Assessment of daily intake: The daily intake levels of estimated heavy metals and trace elements were detected and compared with their recommended values to know whether the levels observed in the fish muscles were safe for human consumption (**Table 6**). According to FAO estimates of fish consumption in Saudi Arabia, the adult population consumes 28 g wet wt/Person/day of fish of both freshwater and marine species (Mahboob et al., 2014). The estimated daily intake (EDI) values presented in **Table 6** were detected by assuming that a 60 kg person will take 28 g of fish per day (Mahboob et al., 2014). A conversion factor of 4.8 was used to transform wet weight to dry weight (Rahman et al., 2012). The EDI values of the studied fish samples were obtained by converting dry weight metal concentrations into a wet fish weight basis for comparison.

Statistical analysis: All data were analyzed for variance using two ways analysis of variance (ANOVA). The statistical analysis was performed using the computer software package of SAS (2002).

RESULTS AND DISCUSSION

The percentage of recovery of metals was found between 86.7-93.3% as shown in **Table 4**. The results of metal recovery ensure the methodology of the study. The results of the selected trace elements (Cu, Zn, Fe and Mn) and heavy metals (Cd and Pb) analysis (mg/kg dry weight; ppm) in muscle of Spangled emporer (*L. nebulosus*; Shieri), Red striped seabream (*P. major*; Andak) and Black seabream (*S. cantharus*; Shaiam) fish are summarized in **Table 5**. The results showed that accumulation pattern of analyzed metals in all fish muscles followed the order: Fe (1.016) > Zn (0.376) > Cu (0.093) > Mn (0.036) > Pb (0.003) > Cd (0.001) mg/kg dry weight. This ranking of heavy metal accumulation in fish muscle disagree with those of obtained by Mahboob et al. (2014) in the muscle of fresh water fish collected from Wadi Hanifa of Saudi Arabia particularly for the first four metals. Among the analyzed fish samples, Cu was recorded at 0.026–0.093 mg/kg dry weight, Zn from 0.037–0.376, Fe from 0.222–1.016, Mn from 0.008–0.036, and Pb from 0.002–0.003 mg/kg dry weight whereas Cd was recorded at 0.001 mg/kg dry weight.

The values of heavy metals accumulated in the muscle of fish examined in the current study was lower than that of observed previously by Mahboob et al. (2014). The maximum concentrations of Cu, Zn and Mn were recorded in muscle of *S. cantharus* fish as compared to that of *L. nebulosus* and *P. major*. However, the maximum concentrations of Fe were found in muscle of *P. major* as compared to *L. nebulosus* and *S. cantharus*. The concentration of Cd and Pb remained comparable in muscle of all examined fish species. Copper is an essential cofactor of many enzymes and its role in hemoglobin biosynthesis is documented (Sivaperumal et al., 2007). However, a high concentration of Cu has of deleterious effects (Gorell et al., 1997). The Cu concentration in fish muscles ranged from 0.026–0.093 mg/kg dry weight with the highest concentration recorded in *S. cantharus*. The values of Cu concentration reported in the present study (**Table 5**) were found below as compared to the values reported by Mendil and Uluozlu (2007), Rahman et al. (2012) and Mahboob et al. (2014), and the maximum recommended standards (3.0 µg/g) of WHO (1985) and FEPA (2003). The permissible limit of Cu proposed by FAO and The Australian National Health and Medical Research Council's (ANHMRC) was 30 mg/kg fresh weight. The highest Cu content was observed in Frozen (45.7 µg/g) and canned anchovy fillet (7.06 mg/kg) (Mendil et al., 2005). The maximum Cu concentration in food is 20 mg/kg wet weight as reported by UK Food Standards Committee (Cronin et al., 1998), 5 mg/kg as proposed by Turkish legislation, 20 mg/kg as wet weight as proposed by Spanish legislation (Demirak et al., 2006), 10 mg/kg wet weight as proposed by Australian Food Standard Code (Alam et al., 2002).

Zinc is a trace element that can be accumulated in fatty tissues of fish (Ghosh et al., 1985). Zinc can be safe under the limit of 50 mg/kg wet weight in muscle of fish (Mahboob et al., 2014). In the current study, Zn concentration of the examined fish muscles ranged from 0.037-0.376 mg/kg dry weight, with the highest concentration recorded in *S. cantharus*. The range of Zn levels recorded in the muscles of examined fish in this study was lower than those reported by Mendil and Uluozlu (2007), who determined trace metal in fish species from lakes in Turkey and those reported by Al-Saleh and Shinwari (2002), who determined elements in four fish species from the Arabian Gulf of Saudi Arabia. The highest Zn content was observed in Frozen (566 µg/g) and canned anchovy fillet (33.8 µg/g) (Mendil et al., 2005). The amounts of Zn detected in all the fish samples were found below the standard of 1000 mg/kg, as set by the Australian National Health and Medical Research Council (Plaskett and Potter, 1979) and WHO (Cliton et al., 2008). The Fe concentration in the examined fish muscles ranged from 0.222-1.016 mg/kg dry weight, with the highest concentration recorded in *P. major*, and lowest concentration was recorded in *L. nebulosus*.

Fe levels in the examined fish muscles of the current study were lower than fish species from the Gulf of Aqaba (Wahbeh and Mahasneh, 1987; Abu Hilal and Ismail, 2008) and blue fish, *Pomatomus saltatrix* (Cross et al. (1973). The estimated Fe levels in muscle of examined fish were also lower than that of estimated in economically important fish species captured from Tuzla lagoon, Turkey (Dural et al., 2007) and commercial fish species collected from Iranian coastal

Table 5. Heavy metal and trace element concentrations in Muscles of Spangled emporer (*L. nebulosus*), Red striped sea bream (*P. major*) and Black seabream (*S. cantharus*) fish in Al-Ahsa market, Saudi Arabia (means ± standard deviation). Concentration expresses as mg/kg dry weight (ppm).

Scientific name	Metal					
	Cu	Zn	Fe	Mn	Cd	Pb
Lethriuns nebulosus	0.048 ± 0.016 Cb	0.048 ± 0.019 Bb	0.222 ± 0.137 Ac	0.008 ± 0.001 Db	0.001 ± 0.002 Ea	0.003 ± 0.020 Ea
Pagrus major	0.026 ± 0.011 Cb	0.037 ± 0.012 Bb	1.016 ± 1.199 Aa	0.012 ± 0.001 Db	0.001 ± 0.005 Ea	0.002 ± 0.013 Ea
Spondyliosoma cantharus	0.093 ± 0.082 Ca	0.376 ± 0.434 Ba	0.559 ± 0.079 Ab	0.036 ± 0.019 Da	0.001 ± 0.006 Ea	0.002 ± 0.019 Ea

Means within the same row with different capital letters are significantly differed ($p \leq 0.05$). Means within the same column with different small letters are significantly differed ($p \leq 0.05$). Note: a, is the highest value, decreased via b, c to the lowest one is e.

Table 6. Comparison of estimated daily intake of trace elements and heavy metals from studied fish species with the recommended daily dietary allowances.

Metal	Mean concentration (mg/kg dry weight)	Mean concentration (mg/kg wet weight)	Estimated daily (mg/day/person)	Recommended (mg/day/person)	Contribution	References
Cu	0.056	0.012	0.0003	3-30[a]	0	JECFA (1982)
Zn	0.154	0.032	0.0009	18-60[a]	0	JECFA (1982)
Fe	0.599	0.125	0.0035	–	0	–
Mn	0.019	0.004	0.0001	2-5[b]	0	NRC (1989)
Cd	0.001	0.0002	0.0000	0.06[c]	0	JECFA (1989)
Pb	0.002	0.0005	0.0000	0.21[c]	0	JECFA (2000)

The average per capita consumption of fish was 28g-wet wt/person/day.
Conversion factor (wet weight to dry weight): 4.8.
[a]PMTDI: provisional maximum tolerable daily intake.
[b]ESADDI: estimated safe and adequate daily dietary intake.
[c]PTDI: provisional tolerable daily intake (60 kg bwt).

water of Caspian sea (Zeynali et al., 2009). Mn is considered as essential trace elements for humans, and its role in enzymatic metabolism has been reported (Tinggi et al., 1997). Mn is used for various industrial purposes, which is the major sources of Mn pollution in aquatic environments. No maximum limit is mentioned in literature for Mn in fish tissues (Mahboob et al., 2014). The Mn concentration in the examined fish muscles of the current study ranged from 0.008–0.036 mg/kg dry weight with the highest concentration recorded in *S. cantharus*. Mn levels in the examined fish muscles of the current study were lower than the recommendation standards of WHO (1985) (0.5 µg/g) or FEPA (2003). In the current study, Mn value was lower than that described by other reports (Tariq et al., 1993; Mendil et al., 2005; Mendil and Uluozlu, 2007; Amin et al. 2011; Rahman et al., 2012; Mahboob et al., 2014).

Fe concentration of the examined fish muscle ranged from 0.222–1.016 mg/kg dry weight, with the highest concentration recorded in *P. major*. The range of Fe levels recorded in the muscles of examined fish in this study was lower than the permissible limit of 151 mg/kg dry weight recommended by ANZFA (2011). Carvalho et al. (2005) obtained Fe values of 9 (7–11) and 8 (6–10) µg/g dry weight in the muscle of *Solea vulgaris* and *Lophius piscatorius*, respectively. In the current study, Fe value was lower than that the results reported by Mahboob et al. (2014). The maximum limit of contaminants for Pb and Cd in fish and shellfish were 2 and 0.5 µg/g respectively, as proposed by Saudi Arabia (SASO, 1997). Also, the maximum limits for Cd and Pb in fish were 0.05 and 0.5 µg/g, respectively (Commission of the European Communities, 1997) whereas, European regulations proposed 0.2 µg/g for Pb and 0.05 µg/g for Cd are the maximum heavy metal limits in fish muscle (Anonymous, 2005). The maximum recommended limits of 2.0 µg/g for Pb in fish was proposed by WHO (1985) and FEPA (2003). The standard of Cd in seafood was 2.0 mg/kg, as proposed by ANHMRC, whereas Western Australian authorities recommended a concentration of 5.5 mg/kg of Cd, and a maximum level of 1 mg/kg of Cd in fish was proposed by The Spanish legislation. In addition, the maximum concentration of Pb in fish is 2 mg/kg fresh weight as proposed by UK's Lead (Pb) in Food Regulations. Cd (0.001 µg/g) and Pb (0.002-0.003 µg/g) concentrations determined in the current study were in all three fish species were found below the above described permissible limits for human consumption. In addition, Cd and Pb concentrations determined in the current study was found as comparable with those reported by Eboh et al. (2006) who demonstrated lower level of Pb in the muscle, gills and liver tissue of fish at a range of 0.001-0.002 mg kg^{-1}. In addition, Cd concentrations observed in the present study were lower than those of Khansari et al. (2005) who reported that Cd concentration in tuna fish samples from the Gulf area of Iran was average 0.0223 µg/g. The concentration of Cd and Pb observed in the present study were lower than those reported by Canli and Atli (2005) in muscle tissues of six different fish species and those reported by Mendil et al. (2005) in seven fish species obtained from some lakes in Tokat, Turkey. In the current study, Cd and Pb values were lower than that described by other reports (Rahman et al., 2012; Mahboob et al., 2014). The concentrations of Pb and Cd estimated in muscles of the examined fish in the current study were safe for human health, and were below the Saudi acceptable limits.

Public health impact of metals in the muscle of examined fish

The estimated daily intake (EDI) values were found to be 0.0003, 0.0009, 0.0035, 0.0001, 0.0000, 0.0000 mg/day/person for Cu, Zn, Fe, Mn, Cd and Pb in fish muscle, respectively (**Table 6**). As illustrated in **Table 6**, the EDI values of each metal in the studied fish muscles were less than the corresponding recommended values (JECFA, 1982, 1989, 2000; NRC, 1989; WHO, 1996). The EDI values of each metal in the studied fish muscles were less than that of previous report (Rahman et al., 2012; Mahboob et al., 2014). The contribution of the metal in toxicity was zero (**Table 6**). No health problems would be explored as a results of consumption of these fish came from these markets. In addition, the EDI values of Cd and Pb in the studied fish muscles were less than the maximum acceptable daily and weekly intake of these elements in Saudi Arabia (SASO, 1997). As per Saudi legislation, maximum acceptable weekly intake of Cd and Pb were 0.0067 and 0.083 mg/kg bwt, respectively. Previous report (Tawfik, 2013) demonstrated the EDI of metals in fish muscle of Nile Tilapia (*Oreochromis niloticus*), Gray Mullet (*Mugil cephalus*) and California Sardine (*Sardinops sagax*) collected from Riyadh market of Saudi Arabia were found as safe for human consumption.

CONCLUSION

The edible muscles of the three fish species namely Spangled emporer (*Lethriuns nebulosus*; Shieri), Red striped seabream (*Pagrus major*; Andak) and Black seabream (*Spondyliosoma cantharus*; Shaiam) from Al-Ahsa market, Saudia Arabia contain relatively less burden of heavy metals and trace elements, which is suggestive that no health problem can be occurred due to the three examined fishes.

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