

Heavy metal content in coral reef-associated fish collected from the central Red Sea, Saudi Arabia

by

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Abstract

The concentration of heavy metals in marine fish is important for assessing the health risks associated with fish consumption. In this study, the concentration of metals such as copper, lead and manganese were analyzed in the muscle tissue of five coral reef-associated fish species collected from the central Red Sea, Saudi Arabia. The maximum copper content of $0.183 \mu\text{g g}^{-1}$ (ww) was detected in fish samples. Fish samples also showed maximum lead and manganese values of 0.030 and $0.064 \mu\text{g g}^{-1}$ (ww), respectively. The metal concentration in fish tissue samples did not vary significantly between the fish species. In conclusion, the results obtained indicate that the content of heavy metals in the coral reef-associated fishes is below the values determined by various agencies for seafood safety.

Key words: seafood, marine fish, marine pollution, bioaccumulation, metal pollution index

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1. Introduction

Anthropogenic stressors are one of the significant causes of marine ecosystem degradation throughout the world (Lotze et al. 2006; Wu et al. 2017). Contaminants entering the coastal environment as a result of various human activities affect marine organisms (Richmond et al. 2018). The major sources of pollution are mainly activities such as construction works (slits, paint, cement, solvents etc.), waste dumping in coastal regions, surface runoff, antifouling paint, tourism, sewage treatment plants and industrial discharges (Halpern et al. 2008; Sheppard et al. 2010; Wilhelmsson et al. 2013; Cabral et al. 2019). Heavy metals are considered one of the major pollutants due to their toxic effects and bioaccumulation in marine organisms (Zeng et al. 2012). At low concentrations, most heavy metals are considered essential for normal physiological functions of organisms (Celik & Oehlerschlager 2004). However, higher concentrations of metals such as mercury, cadmium and lead can affect marine organisms and are considered one of the significant health risk factors for human populations (Castro-Gonzalez & Mendez-Armenta 2008; Qiu et al. 2011). For instance, copper and manganese are essential elements to organisms for many physiological functions (Tinggi et al. 1997; Choi et al. 2018). However, accumulation of these metals above the required level is considered toxic and has negative effects on organisms. Lead is a toxic metal to all living organisms, including humans, and has no biological functions (Tamele & Loureiro 2020).

Marine fish constitute a major nutritional source for people living near coastal zones throughout the world (Wong et al. 2001; Saha et al. 2016; You et al. 2018). The health benefits of eating fish have been discussed by many previous researchers (Torpy 2006; Larsen et al. 2011). In brief, fish are rich in protein containing all important amino acids (Larsen et al. 2011), omega-3 fatty acids and bioactive compounds (Chiesa et al. 2016). Many minerals and essential nutrients are also present in marine fish (Ahmad et al. 2015). Fish also have the ability to accumulate toxic elements from the environment in their tissues (Jeziarska & Witeska, 2006). Heavy metal contamination of marine fish is a growing problem throughout the world due to environmental pollution (Rahman et al. 2012). As food is the main pathway for heavy metals to enter the human population, assessment of the heavy metal content in common fish species is important for understanding the health risks (Kumar et al. 2012; Liu et al. 2015). The most common non-essential heavy metals in marine fish of particular health concern are cadmium, lead and mercury (Tamele & Loureiro

2020). In general, heavy metal distribution in fish may depend on species, age, tissue type, sampling time and physiological factors (Catsiki & Stroglyoudi 1999; Canli & Atli 2003). Metal accumulation in fish also depends on factors such as metal concentration in the environment, water temperature, pH, salinity and hardness (Jeziarska & Witeska, 2006).

The coastal Red Sea area supports a diverse and almost unique coral reef community that provides habitat for a large group of marine biota, including commercially important fish species. Coral reefs in the Red Sea are under constant threats from pollution, mainly heavy metals, petroleum chemicals and herbicides (Ali et al. 2011b). The pollutants can affect coral species and the associated organisms. Therefore, it is important to assess the concentration of heavy metals in marine fishes associated with coral reefs to know the bioaccumulation status. In Saudi Arabia, the heavy metal content in commercially important fish has previously been determined to assess health risks (Al-Saleh & Shinwari 2002; Tharwat & Al-Owafeir 2003; Said et al. 2014; Younis et al. 2015; Hakami 2016). Although these studies reported metal concentrations within the acceptable limits, continuous monitoring of heavy metal concentrations in marine fish is essential to assess the safety of fish consumption. Therefore, in this study, the concentration of metals such as lead, cadmium and manganese were determined in five common edible coral reef-associated fish species collected from the central Red Sea, Saudi Arabia. The results obtained in this study will further advance our knowledge on the heavy metal content of the marine fishes from the Red Sea environment and the safety of seafood.

2. Materials and methods

2.1. Sample collection

Five fish species, *Lethrinus mahsena* (sky emperor), *Acanthurus gahhm* (black surgeonfish), *Siganus rivulatus* (marbled spinefoot), *Scarus ferrugineus* (rusty parrotfish) and *Hipposcarus harid* (longnose parrotfish) were collected from the central Red Sea region of Saudi Arabia in December 2019. These fish species were selected based on their abundance at the sampling site. They are also marketed in the Jeddah fish market. The size (length), feeding behavior and habitat details of the fish collected during this study are presented in Table 1. Samples were collected by a fisherman using nets from the central Red Sea region (about 60 km south of Jeddah) of Saudi Arabia (21°27'22.5"N; 39°07'15.9"E; Figure 1). The collected



Table 1

List of fish species collected in this study along with size and feeding habits. Feeding behavior and habitat details were retrieved from fishbase (<https://www.fishbase.de/search.php>).

Species name	Size (length, cm)	Feeding behavior	habitat
<i>Lethrinus mahsena</i>	22.66 ± 1.75	carnivorous	coral reefs, seagrass
<i>Acanthurus gahhm</i>	31.3 ± 0.60	omnivorous	reefs
<i>iganus rivulatus</i>	21.6 ± 0.79	herbivorous	coral reefs, other subtidal hard and soft substrates
<i>Scarus ferrugineus</i>	32.6 ± 1.35	herbivorous	coral reefs
<i>Hipposcarus harid</i>	24.36 ± 0.70	herbivorous	coral reefs

samples were kept in an icebox and transported to the laboratory (approximate transport duration: 1 h). In the laboratory, the size of the fish was measured and three individuals of the same size group from each species were selected for heavy metal analysis. The whole fish samples were stored at -80°C until further analysis.

2.2. Processing of samples

The fish samples were processed for heavy metal analysis according to the protocols previously described (Idris et al. 2015) with some modifications. In brief, the collected fish samples were kept on a chopping board (ceramic) and dissected using a ceramic knife. Approximately 20 g of dorsal muscle tissue from each fish species was removed (in replicate, $n = 3$ for each fish), washed in distilled water and dried (24 h) in an oven at 65°C to measure the wet-to-dry weight ratio. After that, the tissue samples were powdered using a blender. The powdered tissue samples were again dried in an oven at 105°C for 24 h. These dried tissue samples (1.0 g) were placed in an acid-cleaned Teflon vial and digested with 10 ml of concentrated HNO_3 and 4 ml of H_2O_2 at room

temperature for 3 h. The digested samples were kept in a sand bath at 80°C for 7 h. The samples were then dried by heating the vessel without the lid at 100°C for ~ 3 h. The digestion procedure was repeated several times to obtain a small amount of white residue. The dry residue was then collected, dissolved in 2% HNO_3 (80 ml) and filtered through a filter paper (No. 42). Finally, the solution was diluted to 100 ml with 2% HNO_3 and used to measure the metal concentration.

2.3. Reagents

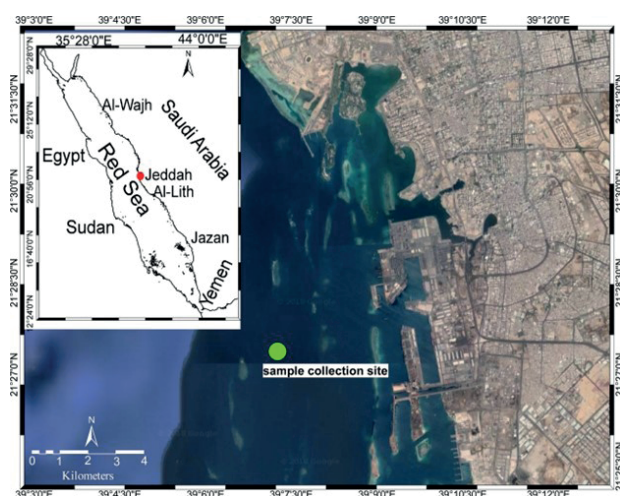
Sigma-Aldrich analytical grade chemicals and reagents were used for the analysis of the heavy metals. AccuStandard reference standards such as copper ICP standard (ICP-15N-1), lead ICP standard (ICP-29N-5) and manganese ICP standard (ICP-33N-1) were used for preparation of standard solution of the heavy metals analyzed in this study. Milli-Q (Millipore) water was used for dilution and preparation of reagents.

2.4. Measurement of metal concentration

The concentration of metals such as copper, lead and manganese was measured using ICP-AES (inductively coupled plasma atomic emission spectrometry) model 6010D. The wavelengths used for the measurement were 324.754, 220.353 and 257.610 respectively for Cu, Pb and Mn (Martin et al. 1994). The concentration of each metal was calculated based on tissue water content and presented as $\mu\text{g g}^{-1}$ wet weight (ww) of fish tissue. The minimum detection level for the metals was determined as $0.001 \mu\text{g g}^{-1}$.

2.5. Quality control

The glassware used for processing and analysis of the fish samples were initially cleaned in dilute HNO_3 , followed by a rinse using double distilled water. Blank (using Milli-Q water) and spiked fish tissue samples were also prepared using the same procedure for fish tissue samples. Standard solutions for each

**Figure 1**

Map showing the fish sample collection area

metal (AccuStandard) were prepared and added to the tissue samples. The recovery rate of the metals was calculated according to the method previously described (Clesceri et al. 1999). The recovery rates of metals from the reference materials were 96, 89 and 103% respectively for copper, lead and manganese. The precision of the sample preparation procedure was checked by preparing three samples (for each individual) and analyzed for heavy metals. Standard deviations for these three analytical replicates were below 5%. The limit of detection was calculated from the calibration curve of the standard solution prepared with different concentrations. The limit of detection was defined as the metal concentration equal to three times standard deviation of the blank solution divided by the slope of the calibration curve (Payehghadr et al. 2013).

2.6. Analysis of data

One-way ANOVA (analysis of variance) was used to understand the differences ($P < 0.05 = \text{significant}$) in metal content between the fish species. The data were checked for homogeneity using Levene's test. The data were homogeneous and used for ANOVA without transformation. The estimated daily intake (EDI) of heavy metals was also calculated from the results. This index indicates the estimated daily intake of a selected metal through consumption of fish and was calculated by multiplying the metal concentration in fish by the average daily fish consumption by adults in Saudi Arabia using the formula previously reported (Ahmed et al. 2015). The average daily fish consumption rate (21.37 g) for an individual in Saudi Arabia was adapted from Burger et al. (2014):

$$EDI = DFC \times MC$$

where DFC is the daily fish intake rate and MC is the metal concentration in fish tissue.

3. Results

The copper content in muscle tissues varied between 0.137 and 0.183 $\mu\text{g g}^{-1}$ ww among the fish species used in this study (Figure 2). The lowest concentration was observed in *H. harid* and the highest in *L. mahsena*. *S. ferrugineus* also showed higher concentration of copper – 0.181 $\mu\text{g g}^{-1}$ ww, which is almost the same as that of *L. mahsena*. The two other species, *A. gahhm* and *S. rivulatus*, showed copper concentrations of 0.165 and 0.179 $\mu\text{g g}^{-1}$ ww, respectively. Further, the copper concentration in fish

samples did not show significant variation between the fish species ($F = 1.174$; $df = 4, 10$; $P = 0.378$, Table 2).

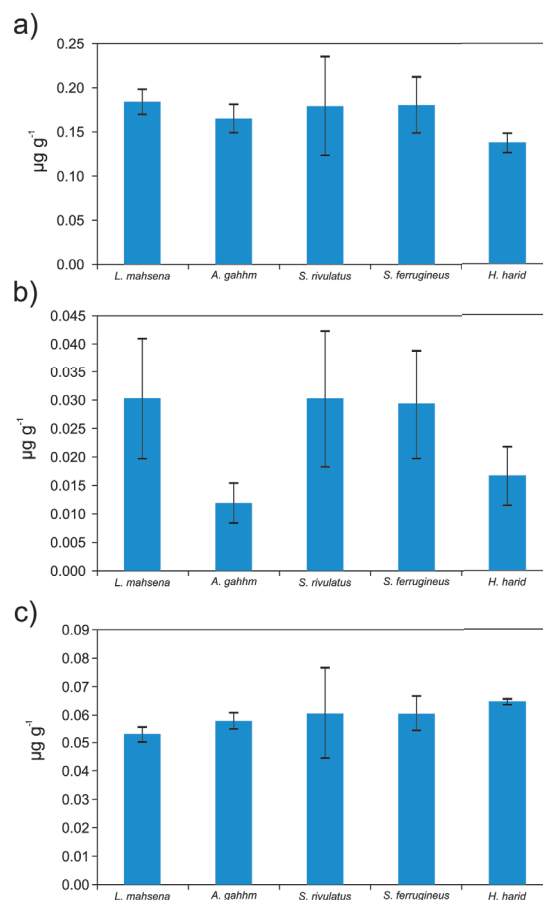


Figure 2

Concentrations of metals ($\mu\text{g g}^{-1}$ ww) in fish species collected from the Red Sea: a) copper, b) lead, c) manganese. Error bars indicate the SD of the mean ($n = 3$).

Table 2

One-way ANOVA results for the concentration of copper, lead and manganese in fish samples collected in this study.

Metal	Source of variation	df	MS	F	P
copper	between species	4	0.001	1.174	0.378
	within species	10	0.000		
	Total	14			
lead	between species	4	0.000	2.948	0.075
	within species	10	0.000		
	Total	14			
manganese	between species	4	0.000	0.851	0.524
	within species	10	0.000		
	Total	14			



Lead concentration in fish muscle tissue showed a range between 0.016 and 0.030 $\mu\text{g g}^{-1}$ ww (Figure 2). The highest value was recorded in *L. mahsena* and *S. rivulatus*. The value of 0.029 was recorded in the tissue of *S. ferrugineus* and 0.012 $\mu\text{g g}^{-1}$ ww in *A. gahhm* tissue. The lowest value (0.016 $\mu\text{g g}^{-1}$ ww) of lead in muscle tissues was determined for *H. harid*. The concentration of lead did not vary significantly among the five fish species ($F = 2.94$; $df = 4, 10$; $P = 0.075$, Table 2).

The manganese concentration in the fish muscle tissue samples collected in this study varied between 0.052 and 0.064 $\mu\text{g g}^{-1}$ ww (Figure 2). The manganese content was low in the muscle of *L. mahsena* (0.052 $\mu\text{g g}^{-1}$ ww) and high in *H. harid* muscle (0.064 $\mu\text{g g}^{-1}$ ww). The muscle tissue of *S. rivulatus* and *S. ferrugineus* showed a manganese concentration of 0.606 and 0.607 $\mu\text{g g}^{-1}$ ww, respectively. The manganese content in the muscles of *A. gahhm* was 0.058 $\mu\text{g g}^{-1}$ ww. The concentrations of manganese in the fish muscle tissues also did not vary between the species ($F = 0.85$; $df = 4, 10$; $P = 0.524$, Table 2).

The estimated daily intake (EDI) value for lead was 0.49 $\mu\text{g day}^{-1}$ ww. The calculated EDI for copper and manganese were 3.61 and 1.26 $\mu\text{g day}^{-1}$ ww, respectively (Figure 3).

4. Discussion

This study indicated that the heavy metal content in the muscle tissues differed between the fish species, but the differences were not statistically significant.

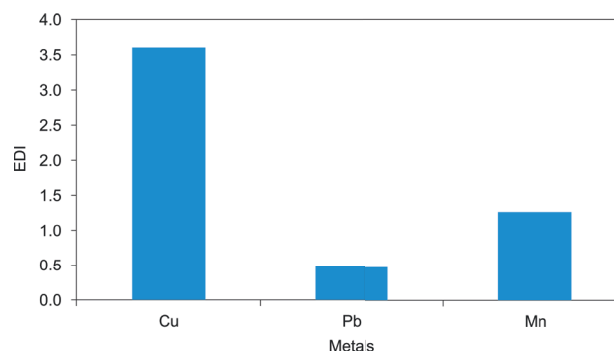


Figure 3

Estimated daily intake (EDI) of the marine fishes collected in this study.

The differences may be due to the feeding behavior and habitats of the fish species collected in this study. The results of this study also indicated that the concentration of selected heavy metals in the coral reef-associated fishes was not high compared to the values reported in the literature from the Red Sea (Table 3). The observation of lower metal concentrations in this study may be due to the fish species studied and the location where the samples were collected. Bioaccumulation of metals in fish is influenced by many factors such as species, age, sex, feeding behavior and location (Zhao et al. 2012; El-Moselhy et al. 2014). In addition, sample preparation protocols and analytical methods are not consistent across previous studies (e.g. El-Moselhy et al. 2014; Idris et al. 2015).

Table 3

Comparison of concentration of heavy metals in fish collected from the Red Sea and other regions (all values are in $\mu\text{g g}^{-1}$ wet weight).

Study area	Sampling year	Sample type	Hg	Cu	Pb	Mn	Reference
Jeddah, Central Red Sea, Saudi Arabia	2019	muscle	***-0.005	0.137-0.183	0.016-0.03	0.052-0.064	present study
Jazan, Red Sea, Saudi Arabia	--	muscle	--	--	0.15-0.38	0.07-0.12	Idris et al. 2015
Jeddah, Red Sea, Saudi Arabia	--	muscle	--	--	1.03-3.40	--	Ali et al. 2011a
Red Sea, Egypt	2010-2011	muscle	--	0.17-0.77	0.21-0.88	0.1-0.93	El-Moselhy et al. 2014
Red Sea, Egypt	--	whole fish	--	--	0.05-1.30	--	Emra et al. 1993
Gulf of Aqaba, Red Sea	--	whole fish	--	--	4.80	1.63	Abu Hilal and Ismail, 2008.
Gadani ship breaking area, Pakistan, Arabian Sea	--	muscle	--	--	1.15-4.04	1.3-10.59	Kakar et al. 2020
Iskenderun Bay, Turkey	2016	muscle	0.019	--	0.096	0.149	Kaya and Turkoglu, 2017
Aliaga Bay, Mediterranean Sea	2014	muscle	--	0.3	0.08	--	Pazi et al. 2017
Gaza fishing harbor, Mediterranean Sea	2013-2014	muscle	--	3.01-17.47	0.73-3.24	0.26-1.26	Zaqoot et al. 2017
Corsica Island, Mediterranean Sea	2012	muscle	--	0.349	0.084	0.072	Gobert et al. 2017
Southeast coast of India, Indian Ocean	2010-2011	muscle	0.24	4.56	0.22	0.35	Thiyagarajan et al. 2012.
U.A.E coast, Arabian Gulf	2018	whole fish	0.04-0.18	1.2-24	--	--	Alizada et al. 2020

Among the studied metals, lead tends to accumulate more in the bones than in the soft tissues of fish and therefore its biomagnification potential in the marine food web is low compared to other metals (Flegal 1986; Tukker et al. 2001). Therefore, the estimation of lead concentrations in tissues may not indicate the actual bioaccumulation in fish. Fish muscle is primarily consumed by humans, so the level of lead content in the tissues will help to understand the intake of lead through seafood sources. The relatively low Pb content in *A. gahhm* (omnivorous) and *H. harid* (herbivorous) indicated that the differences observed between the species may not be due to feeding habits alone. Moore and Ramamoorthy (1981) reported that the low mobility of Pb compounds in the cells was one of the reasons for its low content in muscle tissues. Sediment is one of the major sources of Pb and fish living near the bottom may accumulate it in higher concentrations than others (Sarkar et al. 2016). Therefore, the habitat of the fish species collected in this study may be one of the possible reasons for interspecific differences in Pb content.

Copper concentration in the fishes also did not reflect the feeding habits as higher concentrations were recorded both in carnivorous and herbivorous species. The content of low molecular weight metallothionein-like proteins in the fish species may also explain species-specific variation in the copper concentration in fish muscle tissue, in addition to other factors (Kumar et al. 2012). Though not toxic like other metals, manganese concentrations observed in the fish species were below the levels reported for the fish caught in the Red Sea (Table 3). However, the manganese values observed in this study were higher than the values (0.008 and 0.036 $\mu\text{g g}^{-1}$) reported by El-Bahr and Abdelghany (2015) in fish samples collected from a fish market in Saudi Arabia. This difference may be due to the bioaccumulation tendency of different fish species.

The estimated daily intake (EDI) value of heavy metals is used to assess the safety of food. The calculated EDI indicated that the fish species collected from the Saudi Arabian Red Sea are safe for consumption. The maximum intake levels for various heavy metals and toxins through consumption of food have been regulated by agencies such as WHO, FAO, GCC standardization organization (GSO) etc. to maintain food safety. The mean copper values observed in this study were below the range previously reported for human consumption. For instance, the WHO recommended intake levels for copper are 3500 $\mu\text{g Kg}^{-1}$ body weight week⁻¹ (FAO/WHO, 2004). The intake of copper above this recommended level may affect internal organs

especially liver and kidney damage (Turkmen et al. 2009). The recommended weekly intake for lead is 0.025 mg Kg^{-1} body weight week⁻¹ (FAO/WHO, 2004). Furthermore, the present study showed that lead concentrations in fish samples are below the FAO and European Community (0.3 mg kg^{-1} wet weight) standards for food safety (Obaidat et al. 2015). Similarly, the observed lead concentrations are below the values value (0.3 mg kg^{-1}) of the GSO guidelines (GSO 2013). Further, the Mn concentrations of the fish species analyzed in this study were below the maximum permissible limit of 0.5 $\mu\text{g g}^{-1}$ stipulated by WHO (WHO 1985).

5. Conclusions

The results of this study provide information on the current status of concentrations of essential (Cu, Mn) and non-essential (Pb) metals in five marine fish species collected from the coral-reef habitat of the Red Sea. This study indicated that the concentrations of selected heavy metals in fish samples were below the values recommended for human intake. Therefore, consumption of fish collected from the coral reef habitats in the central Red Sea region would not pose any health risks from the heavy metals. Further, monitoring of heavy metal content in other common edible fish species from the central Red Sea region may provide more details for assessing the safety of seafood marketed in Jeddah.

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