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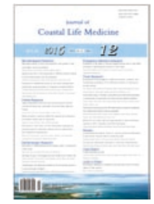

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Metal contents in common edible fish species and evaluation of potential health risks to consumers

Naglaa Farag Soliman*, Samir Mahmoud Nasr

Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria, Egypt

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ABSTRACT

Objective: To conduct a health risk assessment of some heavy metals attributed to consumption of common edible fish species available for consumers.
Methods: Concentrations of Cd, Cr, Cu, Fe, Mn, Pb and Zn were determined in muscles, gills, livers, bones and skins of six common edible fish species, namely *Oreochromis niloticus, Mugil cephalus, Sardinella aurita, Mullus barbatus, Boops boops, Pagrus pagrus*. Concentrations of heavy metals were determined by atomic absorption spectrophotometer and expressed as µg/g of wet tissue.
Results: Results showed that iron and zinc were the most abundant among all fish tissues under investigation. The data obtained in the present work were compared well with the counterpart data reported internationally. The estimated values of all metals in muscles of fish in this study were below the permissible limits. Moreover, the potential health risks of metals to human via consumption of seafood were assessed by estimating daily intake and target heath quotient. Generally, risk values for the measured metals do not pose unacceptable risks at mean ingestion rate for muscles.
Conclusions: It can be concluded that the investigated metals in edible parts of the examined species have no health problems for consumers.

1. Introduction

Fish is highly nutritious due to its high protein content and the presence of omega-3 fatty acids, amino acids, fats and vitamins. Fish also contains several trace metals, such as Zn, Fe, Ca, Cd, Pb and Cu[1]. Moreover, the intake of fish is beneficial to children's growth and development and against some diseases such as rheumatoid arthritis, psychiatric disorders and lung disease[2]. In contrast to the potential health benefits of dietary fish intake, the chemical pollutants contained in these products have emerged as an issue of concern, particularly for frequent fish consumers[3]. In this regard, heavy metals have the tendency to accumulate in various organs of aquatic organisms, especially fish which in turn may enter into the human metabolism through consumption causing serious health problems[4]. Metals such as Zn, Fe, Cu and Mn are essential elements. They may produce toxic effects when their levels exceed certain limits in organisms[5]. Nwaedozie reported that Zn contamination affects the hepatic distribution of other trace metals in fish[6]. Zn, Cu and Mn, which are essential elements, compete for the same site in animals. This will undoubtedly affect tissue metals concentrations as well as certain important physiological processes. Therefore, many consumers regard any presence of these metals in fish as a hazard to health[7]. Fish are integral component of human diet. They need to be carefully screened to ensure that unnecessarily high levels of heavy metals will not be transferred to human population through consumption of contaminated fish[8]. Recently, many attentions are focused on the concentrations of heavy metals in fish in order to check for those hazards for human health[9]. The objectives of this study were to determine the concentrations of Cd, Cr, Cu, Fe, Mn, Pb and Zn in the muscles, gills, livers, bones and skins of six common edible fish species in Alexandria, Egypt, and to conduct a health risk assessment of metals attributed to consumption of common edible freshwater and marine fish species available in Alexandria markets.

*Corresponding author: Dr. Naglaa F. Soliman, Department of Environmental Studies, Institute of Graduate Studies and Research, 163 El Horreya Avenue, Shatby 21526, P.O. Box 832, Alexandria, Egypt.
Tel: (+20) 01004830165
E-mail: naglaa_farag2007@yahoo.com

2. Materials and methods

2.1. Analytical method and instrumentation

Six species of commonly consumed fish were purchased from local markets in Alexandria, Egypt, namely *Oreochromis niloticus* (*O. niloticus*), *Mugil cephalus* (*M. cephalus*), *Sardinella aurita* (*S. aurita*), *Mullus barbatus* (*M. barbatus*), *Boops boops* (*B. boops*), *Pagrus pagrus* (*P. pagrus*), in February 2014. The samples were placed in ice, brought to the laboratory, washed, separated by species and then stored frozen at -20 °C prior to analysis. The fish samples were thawed to room temperature. Bones and other tissue samples were taken for the metal analysis (*i.e.*, liver, gills, skin, bones and muscle samples). The body parts were removed with plastic knives, homogenized and weighed. Small amounts (1–2 g) of the homogenized samples were digested in a quartz Erlenmeyer flask with 11 mL of a mixture of HNO_3–$HClO_4$ (8:3) using a hotplate heated to 120 °C. Additional aliquots of nitric acid were added until a completely colorless solution was obtained. After evaporation, the residue was dissolved in 2 mL of water, and finally, the volume was made up to 25 mL with deionised water. Metal concentrations in the samples were measured by a GBC SensAA flame atomic absorption spectrophotometer. All instrumental settings were those recommended in the manufacturer's manual book. Standard solutions were prepared from 1 000 mg/L stock solution of each metal. Instruments were calibrated by using sets of at least five standards covering the range of concentrations encountered in the literature. All calibration curves showed good linearity ($r > 0.99$).

2.2. Analytical quality control

Reagent blanks, parallel replicates, and a standard reference material (IAEA-142/TM from IAEA's certified reference materials, Vienna, Austria) were incorporated in each digestion batch for quality control and quality assurance. Precision and accuracy of the method were checked by analyzing ten replicate samples of the reference materials. The recovery of the investigated metals ranged from 90% to 104%. Precision expressed as relative standard deviation was lower than 7% for all the measured metals. The detection limit of the method for each metal was 0.009, 0.110, 0.029, 0.048, 0.056, 0.009, 0.037 µg/g for Cd, Cr, Cu, Fe, Mn, Pb, and Zn, respectively.

2.3. Calculation of the daily and weekly intakes of metals

The daily and weekly intakes were calculated on the basis of the concentration of heavy metals in the muscles in order to determine the extent of exposure through fish consumptions.

The estimated daily intake (EDI) of metals for adults in (µg/kg/day) was determined by the following equation:

$$EDI = \frac{C_{metal} \times W_{fish}}{B_w}$$

where, C_{metal} is the concentration of heavy metals in studied fish samples (µg/g, on fresh weight basis); W_{fish} represents the daily average consumption of fish in this region (g/day); and B_w is the body weight of an adult (kg). The weekly intakes were calculated accordingly.

2.4. Health risk assessment

The associated health risks from consumptions of fish by local inhabitants were assessed based on the target hazard quotient (THQ).

The method of estimating risk using THQ was provided in the United States Environment Protection Agency region III risk-based concentration table and it is described by the following equation[10]:

$$THQ = \frac{EF_r \times ED \times FI \times MC}{RfD \times BW \times AT}$$

where, THQ refers to target hazard quotient; EF_r is the exposure frequency (365 days/year); ED is the exposure duration over a lifetime (70 years), FI is the mean ingestion rate (36 g/person/day); MC is the metal concentration in fish edible tissues (µg/g, on fresh weight), and AT is the average life time (70 years × 365 days/year); RfD is the oral reference dose for Fe, Cu, Mn, Zn, Cr and Cd suggested by the United States Environment Protection Agency 0.700, 0.040, 0.140, 0.300, 0.003 and 0.001 µg/g/day, respectively[11], 0.004 µg/g/day for Pb[12]; BW is the average body weight (70 kg); AT is the average time for non-carcinogens (365 days/year × number of exposure years, assuming 70 years in this study). The total THQ (TTHQ) of metals for individual species is the sum of the following composition:

TTHQ (individual species) = THQ (toxicant 1) + THQ (toxicant 2) + THQ (toxicant *n*).

3. Results

3.1. Contents of heavy metals in selected fishes

This paper presents the assessment outcome of Cd, Cr, Cu, Fe, Mn, Pb and Zn levels in muscles, gills, livers, bones and skins of six common edible fish species, namely *O. niloticus, M. cephalus, S. aurita, M. barbatus, B. boops, P. pagrus*. Concentrations of heavy metals under investigation are presented in Figure 1. There were differences in the concentrations of the studied metals between different organs and between species. Fe, Zn and Mn were the most abundant in all the examined organs. In the liver samples, Fe > Zn > Mn> Cu > Cr > Cd > Pb; in the gills, Fe > Zn > Mn > Cu > Cr > Pb > Cd; in the muscles samples, Fe > Zn > Cr > Cu >Mn > Cd = Pb; in the skin, Zn > Fe > Cr > Mn > Cu > Pb > Cd; whereas in bones, Mn > Fe > Zn > Cu > Cr > Pb > Cd.

Concentrations of Cd in bones, gills, liver, skin and muscles of the selected fish samples ranged from 0.01 to 0.11, 0.01 to 0.04, 0.02 to 0.35, 0.01 to 0.05 and 0.02 to 0.08 µg/g, with the average levels of 0.04, 0.03, 0.18, 0.03 and 0.05 µg/g, respectively. Measured levels of Cd were higher in the liver tissues.

The measured concentrations of Cr in the present study ranged from 0.57 to 3.8, 1.7 to 2.21, 0.19 to 2.46, 0.47 to 1.29, 9.24 to 0.56 µg/g, with average levels of 1.42, 1.91, 0.64, 0.72, 0.34 µg/g for skin, muscles, liver, gills and bones. It was more accumulated in the muscles and skin, while the least concentration was observed in the bones.

The measured levels of Cu in the present study ranged from 0.73 to 1.55, 0.73 to 1.55, 0.64 to 1.45, 0.46 to 1.04 and 0.34 to 0.56 µg/g with mean values of 1.04, 1.01, 0.88, 0.79, and 0.45 µg/g for gills, muscles, skin, liver and bones, respectively. The metal was found to be more accumulated in the gills and muscles, but least accumulated in the bones.

As illustrated in Figure 1, Fe was detected in an amount that fluctuated between 11.8 and 64.95, 15.52 and 24.87, 81.92 and 403.17, 57.69 and 568.21, and 11.54 and 114.92 µg/g wet weight in skin, muscles, liver, gills and bones, respectively. The highest concentration of Fe was recorded in fish's gills, where *O. niloticus* had the highest iron content (568.21 µg/g) while *M. barbatus* recorded the lowest value (57.69 µg/g).

Concentrations of Mn in bones, gills, liver, skin and muscles samples ranged from 18.04 to 114.92, 2.38 to 19.70, 0.63 to 1.31 and 0.38 to 1.06 µg/g, with the average levels of 78.61, 9.84, 1.11, 0.98 and 0.58 µg/g, respectively. The mean levels of Mn followed the decreasing order: bones > gills > liver > skin > muscles.

In this study, concentration of Pb in skin, muscles, liver, gills and bones ranged from 0.03 to 0.08, 0.02 to 0.08, 0.07 to 0.60, 0.04 to 0.10 and 0.01 to 0.30 µg/g with average values of 0.06, 0.05, 0.17, 0.07 and 0.12 µg/g, respectively. Measured levels of Pb were found to be higher in liver and bones.

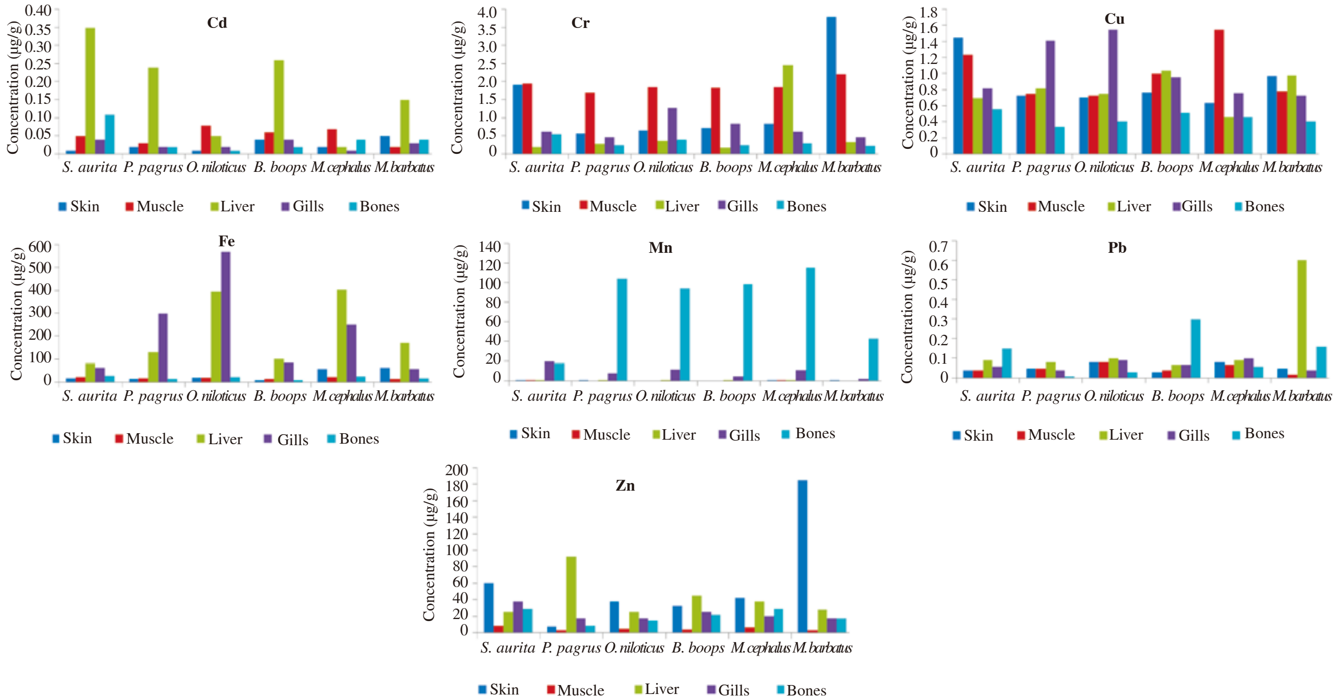

Figure 1. The concentrations of heavy metals (µg/g) in fish samples from the study area.

In the present study, the minimum to the maximum concentrations of Zn were found to be 8.13 to 184.56, 3.3 to 9.1, 25.97 to 92.49, 17.48 to 38.47 and 9.11 to 29.7 µg/g with average levels of 61.14, 5.44, 42.61, 23.04, 20.40 µg/g in skin, muscles, liver, gills and skin, respectively. Mean levels of Zn followed the decreasing order: skin > liver > gills > bones > muscles. Thus, it was more accumulated in the skin and liver. Zn concentrations in muscles were the highest in *S. aurita* and lowest in *M. barbatus*.

3.2. The daily and weekly intakes of metals

The EDI of metals for adults in the Alexandria region via consumptions of fish are presented in Table 1. The trends of EDIs for metals in fish muscles were in the order of Fe > Zn > Cr > Cu > Mn > Cd > Pb. Table 2 shows the estimated weekly intakes (EWIs) for metals caused by the consumptions of fish muscles.

Table 1

EDIs of metals via consumptions of fish muscles in Alexandria, Egypt. µg/kg/day.

Species	Dietary intake of metals						
	Cd	Cr	Cu	Fe	Mn	Pb	Zn
S. aurita	0.026	1.010	0.640	12.800	0.550	0.021	4.680
P. pagrus	0.015	0.880	0.390	9.070	0.200	0.026	1.820
O. niloticus	0.041	0.960	0.380	10.810	0.210	0.041	2.790
B. boops	0.031	0.950	0.510	8.880	0.280	0.021	2.350
M. cephalus	0.036	0.960	0.800	12.280	0.370	0.036	3.460
M. barbatus	0.010	1.140	0.400	7.990	0.210	0.010	1.700

Table 2

EWIs of metals via consumptions of fish muscles in Alexandria, Egypt. µg/kg/week.

Species	EWIs						
	Cd	Cr	Cu	Fe	Mn	Pb	Zn
S. aurita	0.18	7.06	4.47	89.61	5.82	0.14	32.79
P. pagrus	0.11	6.13	2.70	63.48	1.37	0.18	12.72
O. niloticus	0.29	6.70	2.63	75.66	1.44	0.29	19.53
B. boops	0.22	6.63	3.60	62.15	1.98	0.74	16.77
M. cephalus	0.25	6.70	5.58	85.93	2.56	0.25	24.21
M. barbatus	0.07	7.96	2.81	55.92	1.44	0.07	11.89

3.3. Health risk assessment

Table 3 shows the associated health risks from consumptions of fish by local inhabitants by caculating THQs and TTHQs.

Table 3

THQs and TTHQs for individual metals by consumptions of fish muscles.

Species	THQs							TTHQ
	Cd	Cr	Cu	Fe	Mn	Pb	Zn	
S. aurita	0.026	0.336	0.016	0.018	0.004	0.005	0.016	0.421
P. pagrus	0.015	0.291	0.010	0.013	0.001	0.006	0.010	0.343
O. niloticus	0.041	0.319	0.009	0.015	0.001	0.010	0.009	0.406
B. boops	0.031	0.315	0.013	0.013	0.002	0.005	0.008	0.387
M. cephalus	0.036	0.319	0.020	0.018	0.003	0.009	0.012	0.415
M. barbatus	0.010	0.379	0.010	0.011	0.001	0.003	0.006	0.420

4. Discussion

4.1. Heavy metals' concentrations in various fish organs

4.1.1. The contents of Cd

Cd is widely known to be a highly toxic non-essential heavy element. It doesn't have an essential role in biological process in living organisms. Thus, even at its low concentration, Cd could be harmful to living organisms[13].

The distribution pattern of Cd in the present study was the decreasing order of liver > muscles > bones > gills > skin. The liver seemed to be the organ which accumulates the highest level of Cd. This is in agreement with WHO–IPCS Environmental Criteria of Cd (1987) which reported that Cd is stored in the body in various tissues. The main site of accumulation of Cd in aquatic organisms is the kidney and liver[14]. Mean levels of Cd in the muscles was relatively lower than the permissible limits suggested by Food and Agriculture Organization (FAO)[15] (0.5 µg/g) and Turkish permissible concentration (0.1 µg/g)[16], Saudi Arabian allowable limits (0.5 µg/g).

Cd levels in the present study were generally in similar ranges with the literature, where it has been reported as 0.02–0.24 µg/g for muscles of fish from the Black Sea coasts[17]; 0.3–0.12 µg/g for muscles and 0.02–0.35 µg/g for livers of fish from the Tuzla Lagoon, Mediterranean Sea region[18]; 0.13–0.47 µg/g for livers of fish from the Marmara, Aegean and Mediterranean Seas[19]; 0.09–0.27, 0.30–1.49, 0.27–0.66 µg/g and 0.03–0.52, 0.07–0.41, 0.07–0.10 µg/g for muscle, liver and gill tissues respectively for fish from the Egyptian Mediterranean Sea[20,21].

4.1.2. The contents of Cr

Cr is an essential trace metal. The biologically usable form of chromium plays an essential role in glucose metabolism. It has been estimated that the average human requires nearly 1 µg/day[22]. The present Cr concentrations were comparable to those reported from Masoud *et al*.[23] with 0.23–1.26, 1.02–5.71 and 0.39–3.22 µg/g for fish from Alexandria coastal waters, Egypt, for muscle, liver and gill tissues, respectively.

The maximum guideline, 12–13 µg/g, stipulated by the United States Food and Drug Administration was higher than the concentrations of Cr measured in all the fish samples used in this study[24]. On the other hand, the Western Australian Food and Drug Regulations restrict Cr levels to a concentration of 5.5 µg/g, which is also higher than the values found in the present study. The present study revealed that the concentrations of Cr in both liver and bone were not consistently higher than in muscle.

4.1.3. The contents of Cu

Copper is an essential metal in fish and is regulated in the muscle tissue with high molecular weight proteins (metallothionein-like) [25]. Turkish food codex, Saudi Arabian allowable limits and FAO/WHO (2001) established limits for Cu in fish as 20, 20 and 30 µg/g, respectively. The concentrations of Cu in these samples were far below these values, therefore regular consumptions of fish with such low amounts of Cu could not lead to any serious health risk so far as Cu is concerned. Cu levels in the present study are in good agreement with previous recorded values of fish species where the values ranged from 0.35 to 12.00 µg/g for livers of fish from the Tuzla Lagoon[18]; 0.32 to 6.48, 5.29 to 14.90 µg/g for fish from Marmara, Aegean and Mediterranean Seas for muscles and livers, respectively[19]; 0.74 to 2.24 for fish from the Iskenderun Bay for muscles[26]; 0.70 to 27, 3.10 to 323 µg/g for fish from the Lake Budi[27]; 7.32 to 13.22, 9.13 to 10.83, 12.97 to 14.92, 68.84 to 184.85 µg/g for muscles, skins, gills, livers , respectively, for fish from Lake Qaroun[28].

4.1.4. The contents of Fe

Iron is an essential element of most life forms and normal human physiology. In humans, it is an essential component of protein involved in oxygen transports from the lungs to the tissue[29]. Fe is also essential for the regulation of cell growth and differentiation[30].

Iron concentrations in the present study were generally in agreement with the literature where iron levels mainly ranged between 30 and 160 µg/g for muscles of fish from the Black Sea coasts[17]; 105 and 442 µg/g for livers of fish from Marmara, Aegean and Mediterranean Seas[19]; 68.6 and 163 µg/g for muscles of fish from the Black and Aegean Seas[31]. The estimated maximum guideline for Fe is 50 µg/g[32]. Thus, the concentration of Fe in muscles was far below the stipulated limit. Comparatively the iron levels detected from this study was below the regulatory food standards, therefore they presented no risk to the popular consumers of the fishes from the study area.

4.1.5. The contents of Mn

Mn is a metal with low toxicity but has considerable biological significances and seems to accumulate in fish species[25]. On the average basis, Mn levels were found to be lower than the recommended WHO limits (1 µg/g)[33].

Our data are within the ranges reported in the literature where values in the range of 0.69–3.56 mg/kg are recorded for fish from the Black Sea coasts[17], 1.30–3.10 µg/g for fish from the Iskenderun Bay, 0.14–3.36 µg/g for fish from Indian fish markets[34], 0.18–2.78 µg/g for fish from the Aegean and Mediterranean Seas[35], ND-2.19 µg/g for fish from the French coast of the Eastern English Channel and Southern Bight of the North Sea for muscles[36]; 0.72–7.33 µg/g for fish from the Turkish Seas[37], 0.55–5.40 µg/g for fish from the Marmara, Aegean and Mediterranean Seas[19], 0.47–9.90 µg/g for fish from the Aegean and Mediterranean Seas[35], ND–11.1 µg/g for fish from the French coast of the Eastern English Channel and Southern Bight of the North Sea for livers[36]; 0.65–2.56, 1.03–6.09 for muscles and livers, respectively, for fish from the Egyptian Mediterranean Sea[20].

4.1.6. The contents of Pb

Pb is a non-essential metal and a toxic element which can affect fish in high doses, lead to a decrease in survival, growth rates, development and metabolism and to the increased mucus formation. Pb may have many adverse health effects including neurotoxicity and nephrotoxicity[38]. This is due to the fact that Pb and Ca are similar in deposition and in mobilization from bones as previously reported by Moore and Ramamoorthy[39]. Fishbone is used in production of food additives, *i.e.,* gelatin. Therefore, its health quality is also very important. The maximum amount of Pb found in fishbone (0.16 µg/g) is higher than that determined in fish flesh (Figure 1), but stays within the limits of allowable amount of Pb (MTL is equal to 300, 1500, 1000 and 500 µg/kg for fish, bivalve molluscs, cephalopods and crustaceans, respectively[40]. Gills which are considered as hard tissues also recorded high value of Pb but still lower than its level in bone tissues. Livers of *M. barbatus* recorded the highest Pb content in the fish species under investigation. The relatively low binding lead with the SH group explained the lowest concentration of Pb in the muscles of all the tested fish species. Lead levels in the literature have been reported in the range of 0.22–0.85 µg/g for fish from the middle Black Sea[41]; 0.33–0.93 µg/g for fish from the Black and Aegean Seas for muscles[31]; 1.41–3.92 µg/g, 0.38–5.20 µg/g, 0.83–3.71 µg/g for fish livers[18,19,37]. The average concentration of Pb in the muscles measured in this study was considerable lower than the Turkish food codex (0.2 µg/g), the European commission (0.3 µg/g) and the Saudi Arabia Standards Organization (2 µg/g)[42].

4.1.7. The contents of Zn

Zn is one of the most important trace elements for normal growth and development of humans. Its deficiency results from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in Zn metabolism[43]. Moreover, Zn is also considered as highly bioavailable in the aquatic environment and thus may exhibit higher accumulation in various tissues[44,45]. Average concentrations of Zn in the muscles measured in the present study was within the permissible limits suggested by Turkish food codex (50 µg/g), FAO (30 µg/g)[15], Saudi Arabian allowable limits (50 µg/g). These amounts of Zn in the muscles cannot cause harm to the fish themselves as well as consumers.

Zn concentrations in the samples were compared well to the earlier reports on the fish species from the Black Sea coasts with 9.5–22.9 µg/g for muscles[17], 9.83–195 µg/g for livers of fish from Turkish seas[37], 21.7–99.8 µg/g for livers of fish from Tuzla Lagoon, Mediterranean sea region[18], 26.2–43.5 µg/g for livers of fish from Marmara, Aegean and Mediterranean seas[19], 16.1–31.4, 30.5–67.7 and 27.3–76.2 µg/g for muscles, gills and livers of fish from Mediterranean sea[46].

4.2. Calculation of the daily intake of metals

The potential health risks associated with fish consumptions may be linked to the carcinogenic and non-carcinogenic effects. Heavy elements may present in low levels in the water reservoirs. However, fish species can concentrate these contaminants by bioaccumulation and biomagnifications[46,47]. Fish consumption is a major source of human exposure to the above mentioned contaminants. Therefore, fish consumers can accumulate high levels of these contaminants in their bodies.

Based on the dietary intake survey by Dawoud, the local inhabitants had an average consumption per person (70 kg in body weight) of 36.03 g/day for seafood[48].

An important aspect in assessing associated risks to human health from potentially harmful chemicals in food is the knowledge of the dietary intake of such substances which must remain within determined safety margins. For Fe, Cu, Zn, Cd and Pb, the WHO has established "safe" intake levels *i.e.*, provisional tolerable weekly intake levels of 5600, 3500, 7000, 7, and 25 µg/kg of body weight, respectively[12], Mn and Cr of 980 and 21 µg/kg, respectively[11]. In this study, the weekly intakes of metals through the consumptions of muscles and skins were lower than the WHO's "safe" provisional tolerable weekly intake guideline levels for Cd, Cr, Cu, Fe, Mn, Pb and Zn.

4.3. Health risk assessment

The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed pollution is unlikely to experience obvious adverse effects.

The THQ has been recognized as a useful parameter for evaluation of risk associated with the consumptions of metal contaminated food[22]. The THQs values of Cd, Cr, Cu, Fe, Mn, Pb and Zn for the investigated fishes and mussels do not exceed one indicating so that there is no health risk from consuming the muscles of the investigated fish (Table 3). Furthermore, the TTHQ ranged from 0.343 to 0.421 (Table 3) and likely will not pose any potential risk to the local inhabitants through its consumption.

The targets of this study were to determine concentrations of Cd, Cr, Cu, Fe, Mn, Pb and Zn in the muscles, gills, livers, bones and skins of six common edible fish species in Alexandria, Egypt, and to assess their potential risks to human health. Results showed that metals are present in fish tissues at different concentrations. Fe and Zn were the most abundant among all fish tissues under investigation. Cr, Cu and Fe recorded their highest concentration levels in gills while Cd and Pb recorded their highest values in liver. The estimated values of all metals in muscles of fish in this study were below the permissible limits. Therefore, it can be concluded that these metals in edible parts of the examined species have no health problems for consumers. In general, routine check and frequent analysis of food stuff is required to avoid the risk of exceeding the intake beyond the tolerance limits standards. Moreover, the results of this work can also be used to understand the chemical quality of fishes and to evaluate the possible risk associated with their consumptions.

Conflict of interest statement

We declare that we have no conflict of interest.

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