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ORIGINAL ARTICLE **OPEN ACCESS**

Estimation of Trace Metal Concentrations in Muscle Tissues of Certain Fishes Available Commercially in United Arab Emirates

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ABSTRACT
Food contaminated with toxic metals could present a hazard for the consumers, depending on the metal concentration present and the amount of food consumed. To estimate the accumulation of metals in aquatic systems, it has been reported that fish may serve as a useful indicator. Hence, the present study was carried out to estimate and compare the levels of lead, cadmium and arsenic present in the muscle tissue in the three fish species. A total number of 72 samples from three fish species; Sardin (*Sardinella longiceps*), Mackerel (*Scomberomorus commerson*) and Greasy grouper (*Epinephelus tauvina*)*were collected twice weekly for 4 weeks from the fish markets of Ajman, Sharjah and Dubai, United Arab Emirates. The samples were analyzed in duplicates for lead, cadmium and arsenic using atomic absorption spectrophotometer. Lead and cadmium were determined using flame atomizer, while arsenic was determined using hydride generator. Provisional tolerable weekly intake (PTWI), hazard quotient (HQ) and total hazard quotient (THQ) were also calculated to estimate health risks associated with fish consumption. The presence of heavy metals was observed in all fish samples but within the permissible limits. Although the PTWI values for the estimated heavy metals were found to be lower than the permissible limits, the predicted HQ and THQ values were found higher than one. Since continuous exposure to heavy metals may have serious impact on human health, the potential public health risks from dietary exposure to hazardous contaminants from fish species must be continually subjected to research, regulation and debate.*
Keywords: *Atomic Absorption Spectrophotometer, Fish, Heavy Metals, United Arab Emirates, Provisional Tolerable Weekly*

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INTRODUCTION

Due to anthropogenic activities in the past few decades, the natural marine system has been reported to be highly contaminated with heavy metals [1, 2, 3, 4]. In the aquatic ecosystems, heavy metals can be mobilized from both sediment and water and are transferred through the aquatic food-web to macro-invertebrates, fish, piscivorous animals and eventually to humans. Thus contamination of food sources either natural or introduced is a concern for consumers health due to the adverse effects associated with the exposure [5, 6, 7, 8, 9].

Recent studies indicate that fish can be a useful bio-indicator tool to determine heavy metal pollution in aquatic system, since they occupy various trophic levels [10] As fishes are an important source for protein and a key component in human food chain [11, 12, 13, 14], consumption of fish contaminated with heavy metals can impose high risk on human health [15].Mercury (Hg), Cadmium (Cd), Lead (Pb), and inorganic arsenic have significant potential to affect human health at molecular, subcellular, cellular and organ level [16].

Lead (Pb) alters the hemopoietic system and can also damage heart, kidneys, liver, immune system and male reproductive system. There are reports that it causes lung fibrosis and emphysema by affecting the peripheral airway function [17]. Arsenic (As) can induce axonal degeneration leading to peripheral polyneuropathy, and adverse effects on gastro-intestinal tract and skin. Chronic exposure to As has been reported to be associated with visual disorder and blindness, liver cirrhosis, sensory disturbances and also carcinogenic effects[18]. Cadmium (Cd) enters the human body through food chain process and stays for a long time in the system. Throat dryness, kidney damage, vomiting, extreme restlessness, irritability, pneumonitis and bronchopneumonia are acute effects of Cd and it can also cause death due to severe lung damage [19,20].

Several studies have reported the presence of heavy metals in the different species of fishes available commercially [21, 22, 23, 24, 25, 26, 27]. Hence, it was considered worthwhile to estimate the levels of Pb, Cd and As in fishes available in the markets of Ajman, Sharjah and Dubai, UAE. Based on the common availability, geographical distribution and recent reports, three species selected were Sardin (*Sardinella longiceps*), Mackerel (*Scomberomorus commerson*) and Greasy grouper (*Epinephelu stauvina*)[28, 29] (Figure 1).

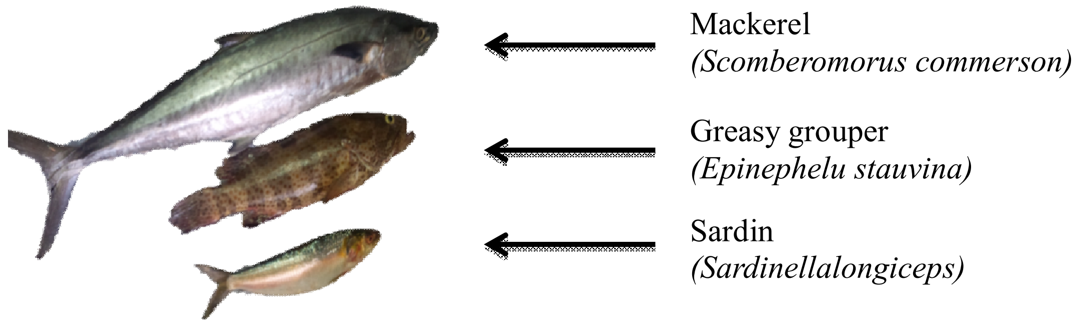

Figure 1: Fish Species used in the study

MATERIAL AND METHODS

Study design

The study took place in Centre for advanced biomedical research and innovation (CABRI), Gulf Medical University, Ajman, UAE. A total number of 72 samples from 3 fish species (Sardin (*Sardinella longiceps*), Mackerel (*Scomberomorus commerson*) and Greasy grouper (*Epinephelu stauvina*)) were collected. The samples were collected twice weekly for 4weeks from the Ajman, Sharjah and Dubai markets in UAE. The necessary approval has been obtained from the Institutional Ethics committee.

Samples and Chemicals

The samples were transported immediately to the laboratory in clean plastic containers packed with crushed ice. They were washed with ultra-pure water and the morphometric details were recorded. Dorsal muscle tissue (10–20 g) has been dissected (next to the dorsal fin) and stored at −20°C for heavy metal analysis.

Analytical standards of lead, cadmium, arsenic, and 30% hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitric acid and hydrochloric acid were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sodium borohydride was purchased from Fisher Scientific (Pittsburgh, USA).

Sample preparation

The muscle tissue samples were digested by wet digestion method [30]. In brief, fish was descaled and dried in an electric oven at 80°C for three hours. They were pulverized in a clean mortar, dried and stored in a polythene bag. Five milliliters of 65% nitric acid was added to 1g of sample powder in a 50 mL beaker and was heated on a hot plate at 120°C for 20 min; five mL of 65% nitric acid was added and heated for additional 20 min at 150°C. The digest was cooled, and 5 mL of concentrated hydrochloric acid was added and heated for 30 min at 120°C. The last step was repeated. The sample was cooled and 5mL of 1% nitric acid was added in order to wash the beaker walls from any precipitated heavy metals. The samples were filtered through a Whatman No. 42 filter paper and diluted to 100mL with 1% nitric acid in standard flask for further analysis.

Standards preparation

Stock standard (1000 ppm): Commercially available standard stock solutions for Pb, Cd, and As (1000 ppm, respectively) were used. Intermediate standard (10ppm):one mL of each stock solution was diluted to 100 mL by adding 99mL of 1% nitric acid.

Working standard: Intermediate standard was serially diluted to get desired working concentration.

Calibration curve

The calibration curve for each metal was achieved by measuring the absorbance for working standards. Heavy metal concentration in all fish species were calculated using their respective linear calibration curves.

Heavy metal analysis

Pb and Cd were analyzed using flame atomizer and As was analyzed using hydride generator in the atomic absorption spectrophotometer (PG 500 GF; PG Instruments Ltd, Leicestershire, UK). Standards and samples were analyzed using the analytical conditions described in the analytical cookbook and data entered using AAWin software (Version 1.0; PG Instruments, UK) (Table 1)

Table 1: Analytical conditions for heavy metal analysis

Analytical condition	**Lead (Pb)**	**Cadmium (Cd)**	**Arsenic (As)**
Wavelength (nm)	217.0	228.8	193.7
Bandwidth (nm)	0.4	0.4	0.4
Filter Factor	1.0	1.0	1.0
Lamp Current (mA)	5.0	5.0	10.0
Integration Time (sec)	3.0	3.0	15
Background	D2/SR	D2/SR	-
Gas type	Air/Acetylene	Air/Acetylene	-

Risk assessment

Different health risk estimation methods have been developed and used by some researchers for the evaluation of the risk hazards [31, 32, 33]. For certain metals, the human health risk assessment is estimated by comparing the metal intake from the consumption rate of seafood with the Provisional Tolerable Weekly Intake (PTWI). The amount of heavy metals taken weekly by a person can be calculated **(**Equation1).

$$\text{PTWI} = \frac{Metalconcentration\left(\frac{ug}{gm}\right) XAverageconsumption\ (gm)}{Bodyweight}$$

Equation1: Amount of heavy metals taken weekly by a person

The average consumption was calculated based on standard reference value "i.e." 7.8 Kg fish/year for 70 Kg body weight [34].

Estimated daily intake (Equation2) is useful to calculate the Hazard quotient (HQ) and the Total hazard quotient (THQ).

$$\text{EDI} = \frac{Conc\ (tissue)\ \ XIngR\ (tissue)\ XFXRAFXEFXEDXCF}{ATXBW}$$

Equation2: Estimated daily intake of heavy metals in the muscle tissue of fish species

*$Conc_{tissue}$ = Concentration of metals in tissue (mg/Kg), *Ing R_{tissue}= Ingestion rate for fish tissue (270 g/day), * F = Fraction of contaminant absorbed from site (1; 100% of contaminant assumed to be available at the site), * RAF = Relative absorption factor (1), * EF = Exposure frequency (365 days/year; conservative assumption), * ED = Exposure duration (60 year; conservative assumption), * CF = Conversion factor (1.0 × 10^{-3} Kg/g), * AT = Averaging time (21915 days; exposure frequency multiplied by exposure duration), * BW = Body weight (70 Kg) [35, 36].

Hazard quotient (Equation3) was calculated based on the ratio of EDI and Toxicological reference value (Toxicological reference values (TRV) for As, Pb and Cd are 0.0003, 0.0036 and 0.001 mg/kg/day, respectively) [36].

$$\text{HQ} = \frac{EstimatedDailyIntake\ (EDI)}{ToxicologicalReferenceValue\ (TRV)}$$

Equation3: Hazard quotient of heavy metals in the muscle tissue of fish species

While total HQ (THQ) of heavy metals in the muscle tissue of fish species(Equation4) is calculated as follows:

$$\text{THQ} = \frac{HQ(Lead) + HQ(Cadmium) + HQ\ (Arsenic\ )}{3}$$

Equation4: Total hazard quotient of heavy metals in the muscle tissue of fish species

Statistical analysis: One Way ANOVA followed by LSD Post Hoc was used to find the significance, where applicable. $P < 0.05$ considered as significant.

RESULTS

The mean concentration of Pb, Cd and As is shown in Table 2. The concentration of Pb in Mackerel, Sardine, and Greasy grouper ranges from 0-0.22 ppm (Mean: 0.041ppm), 0-0.24 ppm (Mean: 0.095 ppm) and 0-0.27 ppm (Mean: 0.116ppm) respectively. Cd concentrations in Mackerel, Sardine, and Greasy grouper ranges between 0-0.022 ppm (Mean: 0.014 ppm), 0.008-0.024ppm (Mean: 0.015ppm) and 0-0.026 ppm (Mean: 0.016 ppm) respectively. The concentration of As was found to be 0-14.32 ppb (Mean: 6.9 ppb), 0-13.20 ppb (Mean: 5.9 ppb), 0-14.88ppb (Mean: 5.8 ppb), respectively. Among the 3 species, maximum concentration of Pb and Cd was found in greasy grouper species, whereas the highest concentration of As was detected in mackerel species.

The concentration of Pb, Cd and As was also observed and compared between the species collected at different time interval of 4 weeks, but there were no significant differences observed. Table 3 depicts the variation in concentration between the samples collected from different sampling areas. It was found that As was significantly higher in samples from Sharjah, whereas no significant difference in Pb and Cd concentrations in the samples.

Table 4 shows the Provisional Tolerable Weekly Intake (PTWI)calculated on the basis of the average fish consumption of a 70Kg person [34]. The recommended PTWI values (μg/kg body weight) for Pb, Cd and As were 25, 2.5 and 15 respectively. We observed that the PTWI values were below the permissible limits.

Table 5 summarizes the values of estimated daily intake (EDI) that is calculated using equation 2. The EDI levels of heavy metals were then utilized to estimate hazard quotient (HQ) and total hazard quotient (THQ) using equation 3 and 4, as shown is Table 6 and 7 respectively. The calculated HQ and THQ for all the heavy metal in three species have been found higher than 1.

Table 2: Mean concentrations of lead, cadmium, and arsenic concentration in three fish species

Fish Species	**Lead (ppm)**	**Cadmium(ppm)**	**Arsenic (ppm)**
Mackerel	0.041±0.074	0.014±0.007	0.0069±-0.0043
Sardine	0.095±0.078	0.015±0.005	0.0059±0.0046
Greasy grouper	0.116±0.076	0.016±0.006	0.0058±0.0047

Data presented are Mean ± S.D.

Table 3: Heavy metal concentration in fish muscle tissue from three different sampling areas

Area	**Lead (ppm)**	**Cadmium (ppm)**	**Arsenic (ppm)**
Sharjah	0.09±0.09	0.01±0.02	0.008±0.005*
Ajman	0.08±0.08	0.02±0.01	0.005±0.004
Dubai	0.09±0.08	0.02±0.004	0.006±0.004

Data presented are Mean ± S.D. *P<0.05 One Way ANOVA followed by LSD Post Hoc

Table 4: Provisional tolerable weekly intake (PTWI)

	PTWI (μg/week/70 kg body weight)		
Species	**Pb**	**Cd**	**As**
Mackerel	0.09	0.030	0.015
Sardine	0.20	0.032	0.013
Greasy grouper	0.25	0.034	0.012

Table 5: Estimated daily intake (EDI)

	EDI (mg/Kg/day)		
Species	Pb	Cd	As
Mackerel	0.004	0.001	0.0007
Sardine	0.009	0.001	0.0006
Greasy grouper	0.011	0.002	0.0006

Table 6: Hazard quotient (HQ)

Species	Hazard quotient (HQ)		
	Pb	**Cd**	**As**
Mackerel	1.1	1	2.3
Sardine	2.5	1	2
Greasy grouper	3.1	2	2

Table 7: Total Hazard quotient (HQ)

Species	Total Hazard quotient (THQ)
Mackerel	1.5
Sardine	1.8
Greasy grouper	2.4

DISCUSSION

In the present study, the concentration of 3 heavy metals was estimated in the tissues of 3 species of fish available commercially in the markets of Ajman, Sharjah and Dubai in UAE. It is a well-known fact that consumption of heavy metal due to contaminated food can pose significant risk on human health, depending upon the concentration of heavy metals and quantity of food consumed over the period. Accumulations of metals were generally found to be species specific and may be related to their feeding habits and the bio-concentration capacity of each species [37, 38, 39, 40]. In the present study, the heavy metal concentrations detected were within the permissible limits for human consumption, as per the recommendations of permissible limits by Codex Alimentarius Commission (CAC), UN and WHO [41, 42, 43].

Majority of fishes are capable of accumulating metals from their environment such as food items, sediments, and water [44].They may accumulate metals in different tissues depending upon the intake and the elimination from their body [45].Needless to mention that they are recognized as an important part of the human diet, owing to their balanced ratio of proteins and polyunsaturated fatty acids [46].Hence, exceeding levels of accumulated metals in fishes can become a significant metal exposure pathway and a consequent health risk for fish consuming population [47, 48].

Although metals are present in the aquatic environment at low levels, but their concentrations in aquatic organisms could be higher due to bioconcentration and bioaccumulation processes. These processes may result in progressively higher concentrations of these pollutants and higher trophic levels in the food chain. Thus, we calculated the Provisional Tolerable Weekly Intake (PTWI) by comparing with the standard limits set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives [34]. Our results showed the PTWI levels were lower than the JECFA levels (lead – 25 μg/kg b.w; cadmium – 2.5 μg/kg b.w and arsenic – 15 μg/kg b.w) in the samples analyzed. Calculation of Hazard Quotient (HQ) and Total Hazard Quotient (THQ) clearly indicate the exact safety index of the fish for human consumption. The HQ for all the 3 species is higher than 1, hence the THQ was also higher indicating some degree of human health risk associated with the consumption of fish. The calculated PTWI and HQ suggest that the risk assessment can be conducted at a larger scale to calculate the real human health risk.

We could estimate the levels of 3 heavy metals in only 72 samples collected within a period of 4 weeks due to the scarcity of time, which is a limitation of our study. Further risk assessment studies with larger sample size in all 4 seasons are warranted to present a clear picture of human health risk associated with fish consumption.

In the present study, we observed that the PTWI values were below the permissible limits in the fish samples available in UAE markets. However, the calculated HQ and THQ for all the heavy metal in 3 species have been found higher than 1. Such studies are required to be done periodically to create awareness about the safety of marine food available and the potential health risks, if associated.

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