Evaluation of Lead and Cadmium Concentrations in The Muscles of Four Fish Species from Ain Al-Ghazala Lagoon, Libya

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Abstract

Evaluation of lead and cadmium concentrations in the muscles of four fish species: Trigla lucerna, Lithognathus mormyrus, Siganus rivulatus, Liza saliens, collected from Ain AL-Ghazala Lagoon, eastern Libya, during the summer of 2022, were analyzed by Atomic absorption spectrophotometer. The results indicated that the concentration of lead was ranged from 0.1485±0.0278 ppm in L. mormyrus to 0.2533±0.0044 ppm in L. saliens. The cadmium accumulation was ranged from 0.0004±0.000 to 0.0026±0.000 ppm in L. saliens, T. lucerna respectively. There was a significant positive correlation between the condition factor and the concentration of lead and cadmium in L. saliens and cadmium in T. lucerna. And there was a negative significant correlation between lead and cadmium in T. lucerna, while S. rivulatus and L.saliens recorded a positive significant correlation. We found a positive significant relationship between fish length and metals only in S. rivulatus (Cd; R^2:0.76, Pb; R^2: 0.56) and a negative significant relationship between length and cadmium in L. saliens (R^2:-0.69). The estimated daily intake results for lead and cadmium ranged between 0.1056 mg.day^{-1} to 0.1802 mg.day^{-1} and 0.0002 mg.day^{-1} to 0.00187 mg.day^{-1}. The mean of target hazard quotients and total target hazard quotient are below 1.00, so no adverse health effects are expected.

Keywords: *accumulation, Metals, Fish, Ain AL-Ghazala lagoon, Libya*

Introduction

Fish is an important part of the human diet due to its high nutritional quality (Sioen *et al.*, 2007). It provides an important source of proteins, vitamins, and, most importantly, fats, which typically contain a high amount of omega-3 fatty acids, especially alpha linolenic acid, Linoleic, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Bucher *et al.*, 2002). However, it can accumulate heavy metals from food, water, and sediment (Yilmaz *et al.*, 2007; Zhao *et al.*, 2012), which may have adverse effects on human health (Castro-Gonzalez and Mendez-Armenta, 2008). The bioaccumulation of metals can be affected by many factors, including sex, age, size, reproductive cycle, swimming patterns, feeding behavior, and living environment (Mustafa and Guluzar, 2003; Yilmaz, 2005; Zhao *et al.*, 2012; Afiyatillah *et al.*, 2022). Fish are one of the best indicators of heavy metal contamination in the coastal environment (Evans *et al.*, 1993; Rashed, 2001).

The Mediterranean Sea is a semi-enclosed sea with an area of about 2.5 million square kilometers. Oil-related human activities have a significant negative impact on the environment, in addition to industrial and agricultural waste, wastewater, and the release of environmental pollutants into the sea, which have often reached extreme values on a global scale (Meadows, 1992). Ain Al-Ghazala lagoon is located in the far eastern part of the Libyan coast and is about 60 km away from Tobruk. It is distinguished by its rocky coastline, coastal plain, interspersed sandy beaches, and deep, narrow valleys. With an estimated average depth of 2 meters and a maximum depth of 4.2 meters, the lagoon is a thumb-shaped depression of the Gulf of Bomba. It receives its water supply from freshwater springs located all along the southern shore. It was declared a protected area in 2011 (Badalamenti *et al.*, 2011). In this study, four species of fish were used: *Trigla lucerna* (Linnaeus, 1758); Tub gurnard from the Triglidae family, found at depths of 20 to 200 m, is characterized by the presence of dark blue spots with white on the inner side of the pectoral fin. It is a carnivorous fish; crustaceans are considered the most important prey in addition to Teleostei (Stagioni *et al.*, 2012). *Lithognathus mormyrus* (Linnaeus, 1758); striped sea bream from the Sparidae family, found in groups at depths of 50 m and is commercially important, is characterized by the presence of narrow bands of dark gray to black on the body. It is considered an omnivorous and herbivorous fish with a preference

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for animal materials, especially crustaceans (Hamida *et al*., 2016). *Siganus rivulatus* (Forsskal, 1775) is a marbled spine foot from the Siganidae family, found in rocky or sandy habitats covered with algae at depths of 30 m. It is a Red Sea fish that entered the Mediterranean Sea through the Suez Canal; it was first recorded in Libya in 1970. It is an herbivore, and Rhodophyta and Chlorophyta algae are major players in its diet (Shakman *et al*., 2009). *Liza saliens* (Risso, 1810); leaping gray mullet is from the Megelidae family, a coastal species that migrates along the coast, entering estuaries and highly saline lagoons. Juveniles (about 3 cm long) feed on zooplankton; when they reach 5 cm long, they feed on benthic organisms; and when they reach adults, they feed on algae and plant debris (Frimodt, 1995; Kottelat and Freyhof, 2007). These fish were selected to monitor the bioaccumulation of heavy metals (Cd and Pb) in the eastern coast of Libya, specifically in the Ain Al-Ghazala Reserve, due to their environmental and commercial importance, thus providing information that contributes to the evaluation of fisheries in this area. This study aims to determine the level of heavy metals in the muscles of four species of fish living in lagoon Ain Al-Ghazala, to determine the relationship between the total length and condition factor with the concentration of metal, and finally, to determine whether there are any human health hazards associated with these fish when consumed.

Materials and methods

Study site and sampling

Samples (*Trigla lucerne* 14, *Lithognathus momyrus* 20, *Siganus rivulatus* 15, and *Liza saliens* 20) were collected from Ain Al-Ghazala lagoon (32° 12'N – 23°20' E to 32°12' N – 23°21' E) (Figure.1), during July 2022 by local fishermen shown at Figure 1. They were placed in an ice box and transported to the laboratory. The specimens were identified in our laboratory using Golani *et al*. (2006). The total length (TL, cm) and total weight (TW, g) of the samples were measured. The condition factor was found by using the following equation: $K = TW/TL^3$ (Htun-han, 1978).

Analysis of heavy metal in fish muscle

Nitric acid 69% (analytical grade, BDH Ltd. Pool England) and perchloric acid 60% (analytical grade, Riedel- de Haen AG Germany) were used without additional purification. Deionized water from a Milli-Q water purification (Millipore, Bedford, MA, USA) was used for the preparation of samples and standards. The element standard solutions of lead used for calibration were prepared by diluting stock solutions of 1000 mg.L^{-1} of each element supplied from BDH. All containers and glassware were soaked in 20% nitric acid for at least 16 hours and rinsed with distilled and deionized water before use.

Sample (0.5±0.0005 g) was weighed into 150 ml pyrex beaker. 10 ml HNO_3 and 3 ml 60% $HClO_4$ were added and heated on hot plate, slowly at first, until frothing ceases. Samples were heated to white fumes of $HClO_4$. After cooling 10 ml HCl (1+1) were added and transfer quantitatively to 50 ml volumetric. Pb, and Cd concentration were determined as three replicates by ICE 3000 series M6 Graphite furnace atomic absorption spectrometer with Zeeman background, Thermal.

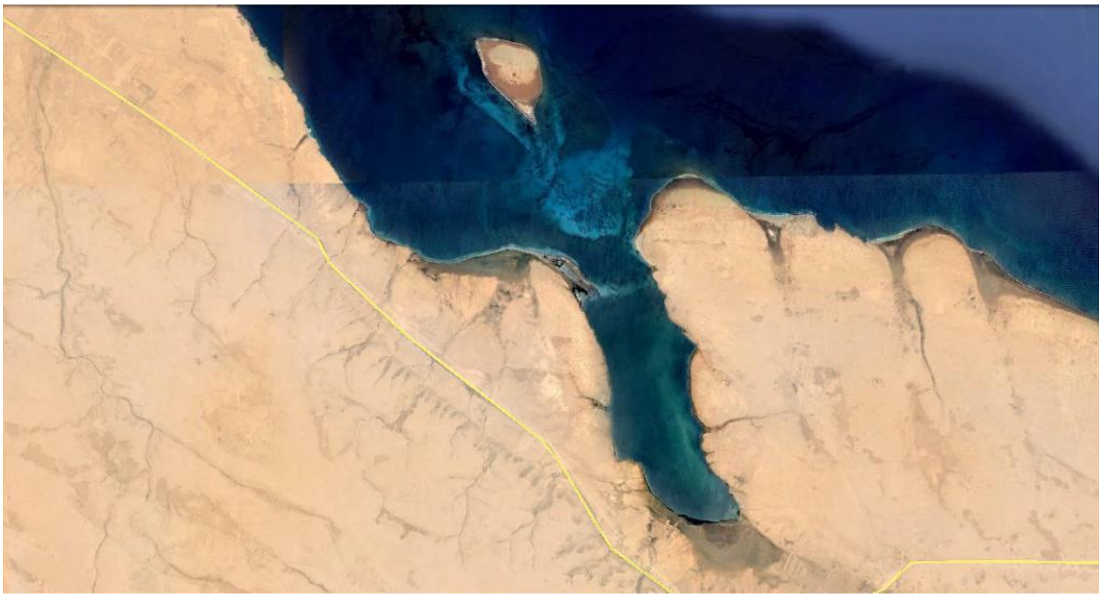

Figure 1. Studying area (Ain Al-Ghazala lagoon)

Human health risk assessment

Estimated daily intake (EDI) of metals (Cd and Pb) was analyzed by using the following equation (USEPA, 2000):

$$EDI = (C * FIR)/WAB$$

Where; C is the metal concentration in fish (μg.g^{-1}), FIR is the fish ingestion rate (47.67 g day for person in Libya; FAO/WHO, 2011), WAB is the average body weight of the adult consumer (67 kg).

No-carcinogenic risk for human health was assessed by calculating total target hazard quotient (THQ) values based on Cd and Pb measured in fish muscle, according to the following formula (USEPA, 1989):

$$THQ = \left(\frac{EF * ED * FIR * C}{RFD * WAB * TA}\right) 10^{-3}$$

Where; EF is the exposure frequency (365 days per year); ED represents the exposure duration (70 years), equivalent to the average lifetime, and RFD is the reference oral dose (USEPA, 2010); and TA is the average exposure time ($365\ \text{days/year} * \text{ED}$).

The hazard index or total target hazard quotient (TTHQ) it was calculated using the following equation:

$$TTHQ = THQCd + THQPb$$

Statistical analysis

The correlation coefficient was used to determine the relationship between lead and cadmium, in addition to its relationship with the condition factor, and linear regression analysis was used to find the relationship between total length and metal concentration. All statistical calculations were made with SPSS and Excel.

Results and discussion

A total of 69 fish were collected from Ain El-Ghazala lagoon, west of Tobruk. The mean lengths and weights of the study fish were as follow: *T. lucerna* 16.714±0.709cm (52.850±5.965g), *L. mormyrus* 15.701±0.299cm (57.690± 1.523g), *S. rivulatus* 17.873±0.474cm (74.306±5.084g), and *L. saliens* 22.445±0.402cm (114.525±7.193g). The value of the condition factor *was* highest in *L. mormyru* 1.563±.096 and the lowest value was in *L. saliens* and *T. lucerna* 1.040±.030 and 1.031±.029 respectively as shown at Table 1.

Metals concentration in fish species

The mean concentrations and associated standard error (SE) of lead and cadmium in fish (*T. lucerna, L. mormyrus, S. rivulatus* and *L. saliens*) are presented in Table 2. Low levels of cadmium were found in the fish species studied. In *L. saliens*, they were found to be 0.0004±0.00009 ppm of wet weight, while in *T. lucerna*, they were found to be 0.0026±0.00052 ppm of wet weight, while lead recorded higher levels from cadmium and ranged from 0.1485±0.02784 ppm of wet weight in the *L. mormyrus* to 0.2533±0.00443 ppm of wet weight in *L. saliens.*

In the research conducted by Yilmaz *et al.* (2010), the concentrations of lead and cadmium in *T.*

Table 1. Number of fish species, Total Length, Total Weight and Condition Factor.

Species	N	Total Length, TL (cm)		Total Weight, TW(g)		Condition Factor, K_F	
		Mean±SE	Min-Max	Mean±SE	Min-Max	Mean±SE	Min-Max
Trigla lucerna	14	16.714±0.709	11.60-19.50	52.850±5.965	16.50-86.90	1.031±0.029	0.93-1.17
Lithognathus mormyrus	20	15.701±0.299	14.33-20.33	57.690±1.523	46.80-74.00	1.563±0.096	0.88-2.02
Siganus rivulatus	15	17.873±0.474	15.10-22.10	74.306±5.084	48.20-130.50	1.272±0.029	1.15-1.40
Liza saliens	20	22.445±0.402	19.50-25.30	114.525±7.193	64.00-178.80	1.040±0.030	0.91-1.23

Table 2. Mean±SE and range (Min-Max) concentrations levels of metals in muscles of fish

Metal (ppm)	Species	Mean± SE	Min	Max
Lead	*Trigla lucerna*	0.2127±0.00617	0.1875	0.2394
	Lithognathus mormyrus	0.1485±0.02784	0.0750	0.2617
	Siganus rivulatus	0.2361±0.07718	0.1203	0.9260
	Liza salie	0.2533±0.00443	0.2377	0.2799
Cadmium	*Trigla lucerna*	0.0026±0.00052	0.0011	0.0052
	Lithognathus mormyrus	0.0011±0.00009	0.0006	0.0015
	Siganus rivulatus	0.0021±0.00051	0.0008	0.0052
	Liza saliens	0.0004±0.00009	0.00001	0.0008

lucerna were 0.14 μg.g^{-1} and 0.01 μg.g^{-1}, respectively, which were the lowest when compared *to L. budegassa* and *S. lascaris* in Iskenderun Bay. Turkmen *et al*. (2011) conducted a research in Paradeniz lagoon, revealing that the concentrations of lead and cadmium were elevated compared to those in *L. saliens*, measuring 0.52 μg.g^{-1} and 0.48 μg.g^{-1}, respectively.

In Turkey, the level of cadmium and lead for *L. saliens* collected from two sites in Adana coast (Tuzla lagoon and Camlik Lagoon) was higher than what we found in this study, as cadmium ranged from 0.06±0.01 mg.kg^{-1} wet wt. in Tuzla Lagoon to 0.36±0.05 mgkg^{-1} wet wt. in Camlik Lagoon, while lead was 0.78±0.14 mg.kg^{-1} wet wt. in Tuzla Lagoon to 0.98±0.21 mg.kg^{-1} wet wt. in the Camlik Lagoon (Tepe *et al*., 2017). In Mersin Bay, the average concentration of lead was 0.23± 0.35 μg.g^{-1} wet wt. in *S. rivulatus*, which was very close to what we found in our study, while the average lead level in the bays of Iskenderun, Mersin Bay, and Antalya was 0.06±0.01 μg.g^{-1}wet wt, 0.23±0.35 μg.g^{-1} wet wt., 0.06±0.03 μg. g^{-1} wet wt., which was lower than in our study while the level of cadmium was higher in the three bays (Kilic *et al*., 2021). In Egypt, Abdelsattar *et al*. (2022) recorded high levels of lead in *S. rivulatus* muscles, which were 1.054±0.106 mg.kg^{-1}, exceeding the permissible limits (0.5 ppm according to WHO, 2000). While in the city of Hurghada overlooking the Red Sea, the level of lead ranged from 0.39±0.15 μg.g^{-1} wet wt., in winter to 0.13±0.09 μg.g^{-1} wet wt., in the summer, and cadmium from 0.07±0.02 μg.g^{-1} wet wt. in the winter to 0.04± 0.02 μg.g^{-1} wet wt. in the summer (Zaghloul *et al*., 2022).

Several authors have stated that muscle is not an active tissue for heavy metal accumulation (Carpene and Vasak, 1989, Khan *et al*., 1989, Kargin and Erdem, 1991, Ünlü *et al*., 1994, Karadede and Ünlü, 1998). Therefore, it cannot represent the true impact of metal contamination on fish to assess potential pathological changes in fish species due to heavy metal contamination (Akoto *et al*., 2014), The liver is the most ideal organ for monitoring the level of heavy metals in fish and other aquatic species, while the gills are the most exposed (Agusa *et al*., 2007; Korkmaz Görür *et al*., 2012; Taweel *et al*., 2013) and the lower levels of heavy metals in fish may be due to a lower level of binding proteins (such as metallothionein) in fish muscles (Allen-Gil and Martynov, 1995). On the other hand, Voigt (2000) observed high levels of mercury in muscle tissue, higher than in the liver, for both *Osmerus eperlanus* and *Perca fluviatilis.*

Concerning lead and cadmium levels in the muscles of fish from the current research compared with the maximum allowable level (MPL) for public health risk assessment. The results showed that the concentrations of lead and cadmium in fish species are within the MPL for human consumption, according to the Food and Agriculture Organization (1983), the World Health Organization (1989), therefore, it is considered safe for human consumption.

Heavy metal concentration in relation to size and body condition

The mean Person's correlation coefficient was used to evaluate intermetal correlation, and the results are shown in Table 3. There was a positive significant correlation between the condition factor and the concentration of lead and cadmium in *L. saliens* and with cadmium in *T. lucerna*. And there was a negative significant correlation between lead and cadmium in *T. lucerna*, while *S. rivulatus* and *L. saliens* recorded a positive significant correlation. No significant correlation was obtained at the level of significance ($p < 0.05$) in *L. mormyrus* between lead and cadmium and between it and the condition factor, in addition, no correlation between the condition factor and lead in *T. lucerna* and *S. rivulatus.* In a study on the bioaccumulation of fourteen minerals and its relationship to the size and condition factor of the alien species in the Arno River, a significant association of magnesium and cobalt was recorded in one species, which is the *Silurus glanis* (Balzani *et al*., 2022).

In Gokova Bay, a positive and significant correlation was recorded between the condition factor and lead in *S. auratus*. Strong positive correlation between lead and cadmium in *P. erythrinus* and *E. costae* (GENC, 2021). One of the explanations given by Tenji *et al*. (2020) is that there may be physiological mechanisms that reduce the effect of minerals on the state of the body. Singh and Sharma (2024), in their review, mentioned that mechanisms that help alleviate oxidative stress caused by heavy metal exposure include the activation of antioxidant defense and the involvement of metallothionein in this response. In addition, the association of the Fulton factor with body fat content, which varies between individuals and species, and depends on the metal (Sassd, 2011; Schloesser and Fabrizio, 2017; Charette *et al*., 2021).

To determine the relationship between the lengths of the aggregated fish species and the heavy metal concentrations, linear regression was used. Table 4 showed the relationships between metal concentrations and fish size (Total length). A positive significant relationship was found between fish length and metals only in *S. rivulatus* (Cd; R^2:0.76, *P* value; 0.001, Pb; R^2: 0.56, *P* value; 0.013) and a negative significant relationship between length and cadmium in *L. saliens* (R^2:-0.69*P*value; 0.003). While there was no significant relationship between heavy metal and length in the rest of the species studied In the same line, Canli and Atli (2003), through their study on the relationship between size and the concentration of six heavy metals, including cadmium and lead, in the liver, gills, and muscles of six species of fish, showed that there is no significant relationship between muscle and the concentration of metals except for cadmium in the muscles of the *Mugil cephalus* (R value;-0.525, p<0.05) and lead in the muscles of the *Atherina hepsetus* (R value; -0.614, p<0.05), this agreed with our study where there was no relationship between lead and cadmium in *T. lucerna.*

While in the Yuangtze River, China, the study showed a positive relationship between size and heavy metal level in most species except for mercury and chromium in yellow catfish and yellow head catfish where the relationship was negative with size. Some researchers have interpreted the difference in the relationship between metal concentration and fish size as possibly related to differences in environmental needs, swimming behaviors, age and metabolic regulation (Douben, 1989; Yi and Zhang, 2012; Canli and Atli, 2003).

Table 3. Correlation coefficients of heavy metals and condition factor in fish

Species	Pb	Cd	K_F
Trigla lucerna			
Pb	1		
Cd	-.626	1	
K_F	-.172	.686	1
Lithognathus mormyrus			
Pb	1		
Cd	.153	1	
K_F	.038	.278	1
Siganus rivulatus			
Pb	1		
Cd	.604	1	
K_F	-.197	-.442	1
Liza saliens			
Pb	1		
Cd	.635	1	
K_F	.510	. 506	1

Table 4. Relationships between Total length (TL) and concentration of heavy metal (Pb and Cd) in muscle

Species	TL vs. con Pb			TL vs. con Cd		
	Liner equation	r^2	*P* value	Liner equation	r^2	*P* value
Trigla lucerna	y=-0.0046x+0.2910	-0.38	0.058	y=0.0003x+0.0029	0.27	0.120
Lithognathus mormyrus	y=0.01835x0.1355	0.13	0.306	y=-6.4225x+0.0011	-0.00	0.914
Siganus rivulatus	y=0.11872x-1.9744	0.56	0.013	y=0.00091x-0.0149	0.76	0.001
Liza saliens	y=-0.0053x+0.3801	-0.21	0.079	y=-0.0001x+0.0048	-0.69	0.003

Table 5. Comparison of the daily intake of heavy metals, Target hazard quotients, Total Target hazard from fish species

Species	Metal	EDI	THQ	TTHQ
Trigla lucerna	Pb	0.151356	0.0378±0.0011	0.0397±0.00091
	Cd	0.001878	0.0019±0.00037	
Lithognathus mormyrus	Pb	0.105635	0.0264±0.00495	0.0272±0.00496
	Cd	0.000754	0.0008±0.00006	
Siganus rivulatus	Pb	0.168012	0.0420±0.01373	0.0435±0.01395
	Cd	0.001522	0.0015±0.00036	
Liza saliens	Pb	0.180242	0.0451±0.00079	0.0453±0.00083
	Cd	0.000277	0.0003±0.00006	

Health risk for consuming fish

The estimated daily intake results of *T. lucerna, L. mormyrus, S. rivulatus,* and *L. saliens* for lead were as follows: 0.15135, 0.105635, 0.168012, 0.180242, and cadmium 0.001878, 0.000757, 0.001522, and 0.00277, respectively. Table 5 presents the estimated target hazard quotient (THQ) for each metal when ingested through fish. The accepted indicative value of THQ is 1.

According to USEPA (2011), the THQ for lead ranged from 0.0264±0.00495 in *L. mormyrus* to 0.0451±0.00079 in *L. saliens*, and cadmium ranged from 0.0003±0.00006 in *L. saliens* to 0.0019± 0.00037 in *T. lucerna*. The average value of the hazard quotient and the total quotient in the muscles of the studied fish were less than one, indicating no non-carcinogenic health risks from individual heavy metal intake. In the same direction, Okbab *et al*. (2018) reported during their study on three heavy metals (Cd, Cu, and Fe) for five species (*Boops boops*, *Hemiramphus far*, *Sardinella aurita*, *Saurida undosquamis,* and *Scomber japonicas*) collected from Tripoli port, western Libya, that the risks of weekly consumption of these fish are very small. The hazard ratio and the hazard index are less than one, and thus there are no potential health risks resulting from their consumption. However, on the western coast of the city of Zawiya in Libya, specifically in the city of Abu Kammash (where petrochemicals are located), the study indicated the presence of potential health risks associated with exposure to toxic metals due to the consumption of fish (*Mullus* spp., *Pagellus* spp., *Sardinella aurita*, and *Boops boops*) collected from that area (Bonsignore *et al*., 2018).

Conclusion

This study evaluated the levels of lead and cadmium in four fish species from Ain AL-Ghazala Lagoon. The overall results do not indicate a health risk from consuming fish from this site, as they were below the permissible limits according to international organizations. Finally, regular monitoring of water quality is essential to assess the long-term impact of pollution on aquatic ecosystems and human health, as well as taking proactive steps to address metal pollution and promote sustainable fishing practices, which helps maintain the ecological balance of aquatic ecosystems.

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