



## Heavy metals in Fish Species from Mediterranean Coast, Tripoli Port (Libya): A comprehensive assessment of the potential adverse effects on human health

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### ARTICLE INFO

#### Article History:

Received: Sept.22,2018

Accepted: Oct.29,2018

Available online: Nov.2018

#### Keywords:

Fish species

Heavy metals

Assessing Potential Risk

Tripoli Port

Mediterranean Sea

### ABSTRACT

The Libyan coast plays an important role in terms of biodiversity and productivity of Mediterranean marine ecosystem. This study is designed to assess potential risks for human populations via fish intake. It enhances the information about anthropogenic impacts in Tripoli port (Libya) to understand the distribution of pollutants encourage appropriate common policies to predict potential risk zones for stakeholders. The levels of Iron, Zinc, Copper, and Cadmium in livers, gills, muscles, skin, and bones of five Mediterranean Sea fish species in Tripoli Port (Libya) namely; *Boops boops*, *Hemiramphus far*, *Sardinella aurita*, *Saurida undosquamis* and *Scomber japonicas* were evaluated. The results showed that considerable difference in metal concentrations among fish organs. The highest concentrations of Cd, Cu, and Fe were measured in livers, while gills and skins had higher concentrations of Zn. The ranges of heavy metal concentrations in different organs of studied fish species were reported (Zn; 7.18 - 21.94, Cu; 1.89-7.03, Fe; 0.93-4.05, Cd; 0.19 to 0.97  $\mu\text{g}\cdot\text{g}^{-1}$ wet wt.). Fortunately, the calculated weekly intakes of metals (EWIs) were much lower than provisional tolerable weekly intake (PTWI). So, human health risks resulting from consumption of studied fish species from Tripoli Port are inconsiderable, that fish muscles are not active tissues for metal accumulation, rendering them suitable for human consumption. In this study the hazard quotient (HQ) and the hazard index (HI) were  $<1$ , and so no potential health risk to the consumers. Principal component analyses specified that Cu and Cd were contributed from the same anthropogenic activity.

### INTRODUCTION

Evaluation and impact relationship of the effects of heavy metals in biological species is essential for the preservation of the aquatic ecosystem. Therefore, heavy metals pose serious threats due to their potential to enter aquatic organisms, and for their bio-accumulation and bio-magnification in the food chain (Dhanakumar *et al.*, 2015). Copper, Iron, and Zinc are classified as essential, but they may also cause toxic effects when the intake exceeds the safe consumption levels. Cu and Fe are essential parts of several enzymes and are necessary for the synthesis of hemoglobin (McCluggage, 1991). Zn contamination affects the hepatic distribution of other trace metals in fish. A high intake of Zn could cause neurotoxicity to humans; however, Cu

is associated with immune toxicity (ATSDR, 2005). Cd is associated with hepatic, skeletal, renal, and reproductive effects (Bosch *et al.*, 2016).

Iron is a cellular toxin, it damages and kills the cells that make up the tissues of organs like the liver. Iron toxicity can eventually lead to seizures, coma, multiple organ failure, and death (Osweiler *et al.*, 1985). So, the potential risks of human health associated with the consumption of aquatic products containing heavy metals must be continually subjected to research, regulation and debate.

Libya is almost self-sufficient in fish with a low estimated per capita consumption of fresh fish products of approximately 7 kg year<sup>-1</sup> (FAO, 2005). Nearly 95% of the total catches are for direct human consumption (European Commission, 2009). Contaminations of marine environment with heavy metals have been receiving worldwide attention, especially in developing countries like Libya, and have become a challenge for scientists. Metals triggered in the aquatic environment by atmospheric deposition and erosion of the geological matrix, or through anthropogenic sources (Alam *et al.*, 2002). Trace metals are foremost contaminating agents which deteriorated the aquatic ecosystems due to their toxicity, persistence, abundance, and subsequent bio-accumulation, and all have the potential to be toxic to living organisms (Storelli *et al.*, 2005; Zhang *et al.*, 2009; Ali *et al.*, 2013). Heavy metals can be strongly accumulated in sediments and biomagnified along the aquatic food chains. Because of the non-degradability of heavy metals, toxic effects are often observed at points far away from the sources (Guagliardi *et al.*, 2013, Yang *et al.*, 2011, Abdel Ghani, 2015). Fish lie in a higher level of the food chain, and they are widely used to biologically monitor the level of metal pollution in aquatic ecosystems (Al-Sayegh Petkovšek *et al.*, 2012; Abd-El-khalek *et al.*, 2012), as fish may consume large amounts of some metals from the water (Davignus *et al.*, 2002). Toxic bioactivity may pose serious threats to normal metabolic processes. In fish, gills are considered to be the dominant site for contaminant uptake because of their anatomical and/or physiological properties that magnify absorption efficiency from water (Hayton and Baron, 1990). Moreover, metal distribution between the different tissues depends on the mode of exposure, i.e., dietary and/or aqueous exposure, and can serve as a pollution indicator (Alam *et al.*, 2002). Heavy metals enter the aquatic food chain through the digestive tract and non-dietary routes across permeable membranes such as the muscle and gills directly consumption of water and food. Levels of heavy metals in fish frequently reflect levels found in sediment and water of the particular aquatic ecosystem (Rajeshkumar and Li, 2018). The main objectives of this work were: 1- Study the content of heavy metals in Mediterranean Sea fish in Tripoli Port (Libya), 2- Match their levels within liver, gill, muscle, skin, and bone tissues. 3- Comparing calculated weekly intakes of metals (EWIs) with the provisional tolerable weekly intake (PTWI). 4- Evaluate potential health risks associated with these metals via the consumption of the fish using the target hazard quotient (THQ

## MATERIALS AND METHODS

### *Study Area*

Libya lies in the Northern African coast in the eastern part of the Maghreb, and which lies between latitudes of 22° and 32° N and longitudes of 10° and 25° E, with a total area of approximately 1, 775, 500 km<sup>2</sup> (Myriam *et al.*, 2015). It is bordered by the Mediterranean Sea in the north, Tunisia and Algeria in the west, Egypt in the east, Sudan in the southeast, and Nigeria and Chad in the south (Otman and Karlberg,

2007). The distribution of population in Libya is very heterogeneous, and concentrated in the narrow coastal strip, which represents 10% of the country. The Port of Tripoli is the principal sea port in Tripoli (Figure 1), the capital of Libya, and one of the oldest ports in the Mediterranean. It lies on the shores of the Mediterranean Sea in northwest Libya. The port serves general cargo, bulk cargo & passengers.



Figure.1: Sampling area

### **Sample Collection and Preparation**

Samples of five fish species (a total of 100 fish specimens) were collected from Tripoli Port, Libya in Winter, 2017; *Boops boops*, *Hemiramphus distant*, *Sardinella aurita*, *Saurida undosquamis*, and *Scomber japonicus*. Twenty fish samples were arbitrarily collected from each species and transported in ice-packed coolers to the lab. The collected fish were selected according to the recommendation given by Haug *et al.* (1971).

The collected fish were washed with deionized water, protected in cleaned plastic sacks, and put away solidified until investigation. Fish weights wavered between 100 and 250 gm. The organs of each species were carefully evacuated with a plastic cut (gills, liver, muscles, skin and bones). The organs were homogenized in a blender, kept in plastic packs, and put away under  $-20\text{ }^{\circ}\text{C}$  earlier until investigation.

Composite samples for each tissue were digested according to FAO/SIDA (1983). 1 gm of a wet sample was placed in 50 ml Teflon containers with screw caps. A 10ml portion of freshly acids prepared ( $\text{HNO}_3$ :  $\text{HClO}_4$ ; 1:1) was added to each sample, covered with a watch glass, and the content was heated for 1 h on a hot plate in a fume cupboard at  $160\text{ }^{\circ}\text{C}$  until the solution was clear. After cooling, the digests were completed to 25 ml deionized water, filtered, and transferred to plastic bottles. A blank digest was carried out in the same way. All metals assurance was duplicated three times. The recovery values were about (extended; 91-95%) for the over assimilation strategy. The recovery tests were performed for the examined metals in tests by spiking with aliquots of the metal standards and at that point carried out absorption. The overall recovery rates (mean  $\pm$  SD) of Fe, Cu, Zn and Cd were  $92 \pm 7.6$ ,  $93 \pm 4.5$ ,  $95 \pm 5.3$  and  $91 \pm 3.9$ , respectively.

The coming solutions, analyzed utilizing the GBC atomic absorption reader, show Intellectual AA AAS with GF 5000. The results are communicated as  $\mu\text{g-g}^{-1}$  damp weight of fish tests. All mineral acids and oxidants  $\text{H}_2\text{O}_2$  used were of the highest quality grade (Suprapure, Merck, Darmstadt, Germany). All the plastic and

dish sets were cleaned by drenching overnight in a 10% (w/v) nitric acid solution and at that point washed with deionized water.

### Statistical Analysis

Multivariate statistical analyses in terms of principal component analysis (PCA) was used to obtain the distribution of heavy metals by detecting similarities or differences in samples using the statistical package, SPSS 19.0.

The total target hazard quotient (TTHQ or HI) was also calculated as the arithmetic sum of the THQ values (Chien *et al.*, 2002).

## RESULTS AND DISCUSSION

Fish is highly recommended as animal protein source instead of mutton in order to avoid high cholesterol level. So, the quality of fish is of a special concern.

Moreover, fish has been successfully employed in bio-monitoring programs of a wide range of pollutants including heavy metals in order to assess the quality of marine environment (Kuklina *et al.*, 2014). All examined metal concentrations were determined on a wet weight basis. In addition, metal levels in muscle tissue of these species were compared with the maximum permissible limits to ascertain whether this food could be considered suitable for human consumption. The levels of heavy metals concentration (Fe, Zn, Cu and Cd) in the different organs of the studied species are illustrated (Table 1).

### Copper

Copper impact is mainly from urban runoff and antifouling paint. It is utilized as herbicidal producing ingredient. Copper is a cofactor for enzymes involved in glucose metabolism and the synthesis of hemoglobin, connective tissue and phospholipids (Kennish, 1996). High concentration of Cu and Cd lead to growth reduction due to the inevitable energetic deficiency (Witeskaetal, 2014). The Cu concentration ( $\mu\text{g g}^{-1}$  wet wt.) in the different organs of the studied species varied from 0.45 to 4.06 in the muscles; 1.05 to 3.79 in the skin; 4.10 to 11.27 in the liver; 0.18 to 6.01 in the gill and from 0.44 to 3.31 in the bone. The levels of Cu content were below the maximum guideline of  $30 \mu\text{g g}^{-1}$  of Cu for safe human consumption (FAO/WHO, 1999). Copper concentration in all analyzed fish were much lower than that recorded in tissues collected from Benghazi markets in Libya ( $44.80 - 79.33 \mu\text{g g}^{-1}$ ) as achieved by Bader *et al.* (2018). Khalifa *et al.* (2010) reported much lower ranges for different organs of 0.007-0.116; 0.011- 0.026 and 0.007- 0.010  $\mu\text{g g}^{-1}$  for muscles, skin and bone, respectively.

### Zinc

Zinc is as an integral part of a number of metalloenzymes and as a catalyst for coordinating the activity of specific zinc dependent enzymes (Lall, 1995). Zn contents in the studied fish species ( $\mu\text{g g}^{-1}$  wet wt.) were in the range; 1.47-12.65 for the muscles. Zn concentration in skin ranged from 6.00 to 34.89  $\mu\text{g g}^{-1}$ . It extended from 4.76 to 33.35 for the liver, had a range of 8.22-37.09 in the gill, and from 8.94 to 12.98 in the bone. WHO 1989 showed concerns for human health risk from Zn in fish  $> 100.0 \mu\text{g g}^{-1}$ . High content of Zn in gills referred to substitution of Ca by Zn from intracellular Ca-phosphate granules in the fish's gill, since Zn has strong affinity for binding to calcium, binding proteins, and disruption of Ca homeostasis (Niyogi *et al.*, 2004; Goto and Wallace, 2010). Zn content in the five studied species were much less than that calculated in all fish species from Libyan coastal area ( $10.0-47.9 \mu\text{g g}^{-1}$ ) as reported by Bonsignore *et al.* (2018).

Table1: Trace metals concentrations ( $\mu\text{g g}^{-1}$  wet wt.) in organs of some fish species Tripoli, Libya 2017

| <b>Muscles</b>             |           |           |           |           |
|----------------------------|-----------|-----------|-----------|-----------|
| Species                    | <b>Cu</b> | <b>Zn</b> | <b>Cd</b> | <b>Fe</b> |
| <i>Boops boops</i>         | 1.34      | 2.76      | 0.75      | 0.76      |
| <i>Hemiramphus far</i>     | 0.84      | 10.51     | 0.19      | 0.41      |
| <i>sardinella aurita</i>   | 4.06      | 12.65     | 0.47      | 1.83      |
| <i>Saurida undosquamis</i> | 3.45      | 8.52      | 0.48      | 1.13      |
| <i>Scomber japonicus</i>   | 0.45      | 1.47      | 0.10      | 0.52      |
| Average                    | 2.03      | 7.18      | 0.40      | 0.93      |
| <b>Skin</b>                |           |           |           |           |
| Species                    | <b>Cu</b> | <b>Zn</b> | <b>Cd</b> | <b>Fe</b> |
| <i>Boops boops</i>         | 1.05      | 6.00      | 0.66      | 0.73      |
| <i>Hemiramphus far</i>     | 3.79      | 34.89     | 0.82      | 2.79      |
| <i>sardinella aurita</i>   | 2.66      | 8.45      | 0.26      | 1.79      |
| <i>Saurida undosquamis</i> | 1.96      | 10.95     | 0.33      | 1.59      |
| <i>Scomber japonicus</i>   | 1.48      | 47.07     | 0.18      | 1.85      |
| Average                    | 2.19      | 21.47     | 0.45      | 1.75      |
| <b>Liver</b>               |           |           |           |           |
| Species                    | <b>Cu</b> | <b>Zn</b> | <b>Cd</b> | <b>Fe</b> |
| <i>Boops boops</i>         | 7.70      | 4.76      | 1.62      | 2.74      |
| <i>Hemiramphus far</i>     | 5.77      | 33.35     | 1.82      | 7.49      |
| <i>sardinella aurita</i>   | 11.27     | 16.70     | 0.58      | 3.06      |
| <i>Saurida undosquamis</i> | 6.31      | 11.39     | 0.47      | 3.5       |
| <i>Scomber japonicus</i>   | 4.10      | 26.32     | 0.37      | 3.45      |
| Average                    | 7.03      | 18.50     | 0.97      | 4.05      |
| <b>Gill</b>                |           |           |           |           |
| Species                    | <b>Cu</b> | <b>Zn</b> | <b>Cd</b> | <b>Fe</b> |
| <i>Boops boops</i>         | 0.18      | 8.22      | 0.56      | 0.66      |
| <i>Hemiramphus far</i>     | 2.17      | 37.09     | 0.89      | 3.06      |
| <i>sardinella aurita</i>   | 2.56      | 13.43     | 0.13      | 1.42      |
| <i>Saurida undosquamis</i> | 6.01      | 30.44     | 0.62      | 2.31      |
| <i>Scomber japonicus</i>   | 0.65      | 20.51     | 0.18      | 0.87      |
| Average                    | 2.31      | 21.94     | 0.48      | 1.66      |
| <b>Bone</b>                |           |           |           |           |
| Species                    | <b>Cu</b> | <b>Zn</b> | <b>Cd</b> | <b>Fe</b> |
| <i>Boops boops</i>         | 1.46      | 12.84     | 0.80      | 1.16      |
| <i>Hemiramphus far</i>     | 2.07      | 12.98     | 1.15      | 1.91      |
| <i>sardinella aurita</i>   | 3.31      | 11.93     | 0.56      | 2.03      |
| <i>Saurida undosquamis</i> | 2.19      | 8.94      | 0.34      | 0.77      |
| <i>Scomber japonicus</i>   | 0.44      | 11.84     | 0.24      | 1.3       |
| Average                    | 1.89      | 11.71     | 0.62      | 1.43      |

### Iron

Iron is an essential mineral for life; it is found in every living cell and is necessary for the production of hemoglobin, myoglobin, and certain enzymes. Fe insufficiency can cause weakness, weak focus, and susceptibility to infection. According to the World Health Organization, iron scarcity anemia is one of the most common nutrient deficiencies in the world (Anderson and Fitzgerald, 2010). The Fe content ( $\mu\text{g g}^{-1}$  wet wt.) for (muscles, skin, liver, gill and bone) were between (0.41-1.83), (0.73-2.79), (2.74-7.49), (0.66-3.06), and (0.77-2.03), respectively. In the present study Fe has a range in muscles skin and bones much higher than that evaluated by Khalifa *et al.* (2010) in Libyan coastline (0.099-0.366; 0.088-0.756 and 0.078- 0.3  $\mu\text{g g}^{-1}$  for muscle, skin and bones, respectively.

### Cadmium

Muramoto (1981) reported that Cd effects on fish include gill and kidney damage, poor bone mineralization, and delayed growth. The main source of Cd exposure in humans is through food consumption. Cadmium is known to be an endocrine annoying substance and may cause development of prostate cancer and breast cancer in humans (Saha and Zaman, 2012). High levels of Cd ingestion can cause acute renal failure in humans (NAS-NRC, 1982). The main mechanism of toxicity of Cd is the antagonistic interaction between the uptake of  $\text{Ca}^{2+}$  and  $\text{Cd}^{2+}$ , which disrupts  $\text{Ca}^{2+}$  absorption leading to growth reduction (McGeer *et al.*, 2011).

Cd contents ( $\mu\text{g g}^{-1}$  wet wt.) in the analyzed fish for the different organs were fluctuated between (0.10-0.75), (0.18-0.82), (0.37-1.82), (0.13-0.89), and (0.24-1.15) for muscles, skin, liver, gill and bone respectively. From the results, it can be predicted that consumption of fish from the study area by humans would not cause any considerable health risk and under the permissible values ( $1 \mu\text{g/g}$  wet wt) indorsed by WHO (1999). Cd average concentration in muscles documented in this study ( $0.47 \mu\text{g g}^{-1}$ ) was lower than those reported in Gulf of Gabes (Tunisia) ( $1.0 \mu\text{g g}^{-1}$  in fish muscles of *Sardinella aurita* (Annabi *et al.*, 2017). However, Cd content in the present study (0.75) for muscles of boops boops exceeded that reported by TEKÝN-ÖZAN (2012) in Mediterranean sea, Turkey ( $0.0064 - 0.03 \mu\text{g g}^{-1}$ ).

The livers of the five fish species collected for this study had higher concentrations of Cu, Zn, Cd, and Fe than the levels in the muscles. Our observation is in agreement with findings by Ploetz *et al.* (2007) who observed that these metals concentration are higher in the liver than in the muscles of fish.

Fish and other vertebrates have metal binding proteins like metallothioneins in the liver. These proteins link to metals such as Cu, Cd, and Zn, allowing the liver to magnify higher levels of metals than other organs (Ploetz *et al.*, 2007; Atli and Canli 2003). Cadmium concentration increases 3,000-fold when it binds to cystein-rich proteins such as metallothionein. In the liver, the cystein-metallothionein complex causes hepatotoxicity and then it circulates to the kidney and gets accumulated in the renal tissue causing nephrotoxicity (Saibua *et al.*, 2018).

In all species, Zn is the highest average concentration in fish organs with ( $7.18-21.94 \mu\text{g g}^{-1}$  wet wt.), followed by Cu with range ( $1.89-7.03 \mu\text{g g}^{-1}$  wet wt.) and by Fe with range of  $0.93-4.05 \mu\text{g g}^{-1}$  wet wt., and the least concentrated is Cd, ranged from 0.19 to  $0.97 \mu\text{g g}^{-1}$  wet wt. The low concentration of Cd may result from its low tendency for bioaccumulation or its good ability to secrete from the body, similar to those obtained by Ibrahim *et al.* (2008) and Abdelrahman *et al.* (2016).

The metal concentration of Fe, Cu, Zn and Cd in these muscles of fish species declined in the subsequent sequence:  $\text{Zn} > \text{Cu} > \text{Fe} > \text{Cd}$ . These metals' bioaccumulations might be as a result of the different tendencies to multiply in the tissues of different species of fish. Generally, considerable differences were observed in the total metal concentrations in different species. This is related to the differences in feeding conditions, ecological needs, swimming behaviors, and metabolic activities among assorted species, the period during which the fish apprehended the differences in water quality (Abdel Ghani, 2015; Ibrahim *et al.*, 2008; Saeed, 2013; Younis *et al.*, 2014). The overall metal contents in the different muscle tissues of the studied fish species were outlined utilizing the metal pollution index (MPI) equation (Usero *et al.*, 1996):

$$\text{MPI} = (\text{M1} \times \text{M2} \times \text{M3} \times \dots \times \text{M}_n)^{1/n}$$

where  $\text{M}_n$  is the concentration of metal n expressed in  $\mu\text{g/g}$  of wet weight. MPI of the four studied heavy metals in the studied species decreases in the order *sardinella*

*aurita* (2.578) > *Saurida undosquamis* (1.999) > *Boops boops* (1.205) > *Hemiramphus far* (0.911).

### Maximum Admissible Fish Consumption Rate

The precise consumption limit safe dietary intake and maximum acceptable fish consumption set that it would be convenient to reduce the weekly meals of the analyzed fish species to minimize the risk for fish consumers and avoid the chronic systemic effects. The results of safe dietary intake ( $CR_{lim}$ ) and maximum admissible fish consumption rate ( $CR_{mm}$ ) are arrayed in Table 2.

**Table 2: Hazard Quotient (HQ) for individual metals, the total THQ (HI) and Risk Contribution % of the metals of fish muscles and skin in Tripoli port.**

| Species                    | HQ Muscles |        |        |        | THQ (HI) |       | Contribution % |       |      |    |
|----------------------------|------------|--------|--------|--------|----------|-------|----------------|-------|------|----|
|                            | Cu         | Zn     | Cd     | Fe     |          |       | Cu             | Zn    | Cd   | Fe |
| <i>Boops boops</i>         | 0.0039     | 0.0011 | 0.0870 | 0.0001 | 0.0921   | 4.23  | 1.19           | 94.46 | 0.11 |    |
| <i>Hemiramphus far</i>     | 0.0024     | 0.0041 | 0.0220 | 0.0001 | 0.0286   | 8.39  | 14.34          | 76.92 | 0.35 |    |
| <i>sardinella aurita</i>   | 0.0118     | 0.0049 | 0.0545 | 0.0003 | 0.0715   | 16.50 | 6.85           | 76.22 | 0.42 |    |
| <i>Saurida undosquamis</i> | 0.0100     | 0.0033 | 0.0557 | 0.0002 | 0.0692   | 14.45 | 4.77           | 80.49 | 0.29 |    |
| <i>Scomber japonicus</i>   | 0.0013     | 0.0006 | 0.0116 | 0.0001 | 0.0136   | 9.56  | 4.41           | 85.29 | 0.74 |    |
| Species                    | HQ Skin    |        |        |        |          |       | Contribution % |       |      |    |
|                            | Cu         | Zn     | Cd     | Fe     |          |       | Cu             | Zn    | Cd   | Fe |
| <i>Boops boops</i>         | 0.0030     | 0.0023 | 0.0766 | 0.0001 | 0.0820   | 3.66  | 2.80           | 93.41 | 0.12 |    |
| <i>Hemiramphus far</i>     | 0.0110     | 0.0135 | 0.0951 | 0.0005 | 0.1201   | 9.16  | 11.24          | 79.18 | 0.42 |    |
| <i>sardinella aurita</i>   | 0.0077     | 0.0033 | 0.0302 | 0.0003 | 0.0415   | 18.55 | 7.95           | 72.77 | 0.72 |    |
| <i>Saurida undosquamis</i> | 0.0057     | 0.0042 | 0.0383 | 0.0003 | 0.0485   | 11.75 | 8.66           | 78.97 | 0.62 |    |
| <i>Scomber japonicus</i>   | 0.0043     | 0.0182 | 0.0209 | 0.0003 | 0.0437   | 9.84  | 41.65          | 47.83 | 0.69 |    |

Safe dietary intake was declared in kilogram of fish per day if we confess that there are no other sources of Cu, Zn, Cd, and Fe found in the local people diet. The maximum safe daily consumption of fish ( $CR_{Lm}$ ), Safe dietary intake ( $CR_{Lim}$ , Kg/Week) and maximum allowable fish consumption rate (meals/month) were calculated using the consequent equations.

$$CR_{Lm} = RFD \cdot BW / C_m, CR_{Lim} = (RFD \cdot BW / C_m) \cdot 7 \text{ and } CR_{mm} = CR_{Lim} \cdot T_{ap} / MS.$$

The safe daily intake results designated admissible fish consumption rate of the studied fish. The maximum safe daily consumption of fish muscles ( $CR_{Lm}$ ) in the present study was in the range of 0.69-6.22 for Cu; 1.66-14.29 for Zn; 0.09-0.70 for Cd; and ranged between 26.78- 119.51 for Fe (Table 2 & Figures 2-5). These safe daily intake values were multiplied by seven to get a safe weekly intake and converted to maximum safe number of monthly fish meals according to Moreau *et al.* (2007).  $CR_{mm}$  is the maximum admissible fish consumption rate (meals/month);  $CR_{lim}$  is the maximum safe daily consumption rate of the fish samples (kg/week);  $T_{ap}$  is the average time period in a month (4.3 week/month) and MS is the meal size, 227 g for adults and 114 g for children (USEPA, 2000). The Fe concentrations in all studied fish samples gave rise to the highest maximum admissible fish consumption rates likened with others studied of heavy metals, it ranged from 0.01 to 0.09meals/month for adults and ranged (0.02-0.18 meals/ month) for children. On the other hand, Cd concentrations were the lowest in all the studied fish samples; ranged (3.55-15.85 meals/ month) for adults and ranged (7.07-31.56 meals/ month) for children (Table 2).

### Health risk assessment of heavy metals in the fish species

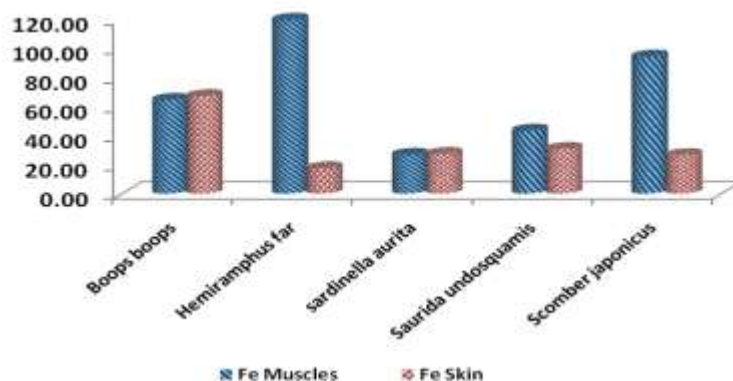
Metals concentration in eatable parts of fish tissue can summarize natural contamination and potential chance for consumers. It is assent that fish concentrate

distinctive contaminants depend on their trophic levels, life span, and living spaces in expansion chemical characteristics of particular toxin (Dizman *et al.* 2017).

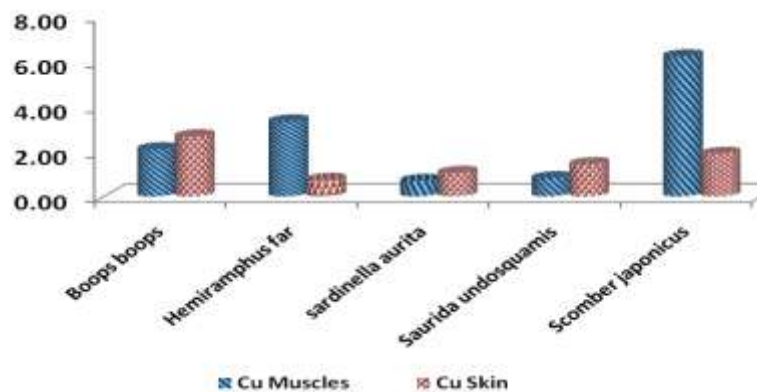
EWIs are assessed from the taking after equation:

$$EWIs = (C_{\text{metal}} \times W_{\text{food}} / WB) \times 7$$

Where  $C_{\text{metal}}$  ( $\mu\text{g/g}$ , wet weight) is the concentration of metal in fish muscles;  $W_{\text{food}}$  represents the daily average consumption of fish in this region; and  $Bw$  is the body weight. Stand on the dietary admissions overview by FAO 2005 the neighborhood occupants had normal utilization per individual (70 kg in body weight) of 20.83 g/day for fish. For Cu, Zn, Cd, and Fe the World Wellbeing Organization has set up a temporary middle of the road week by week admissions (PTWI) levels of 245,000, 490000, 490, and 392,000  $\mu\text{g}/\text{week}/70 \text{ Kg}$  (WHO, 1996, 2005).



**Figure2: Maximum Fe Safe Daily Consumption of Some Fishes**



**Figure3: Maximum Cu Safe Daily Consumption of Some Fishes**

The calculated week by week immaterial of metals through the utilization of fish were: Cd: 0.208–1.562  $\mu\text{g}/\text{kg b.w}$ , Cu: 0.937 - 8.457  $\mu\text{g}/\text{kg b.w}$ , Zn: 3.062-26.350  $\mu\text{g}/\text{kg b.w}$  and Fe: 0.854-3.812  $\mu\text{g}/\text{kg b.w}$ . It could be concluded that the EWIs values for all metals are below the WHO “safe” PTWI guideline levels for all species. Provisional tolerable weekly intake (PTWI) evaluation for certain metals was assessed by comparing the metal admissions from the utilization rate of fish with the Temporary Mediocre Week after week Admissions (PTWI) concurring to the calculation made by Turkmen *et al.* (2012)



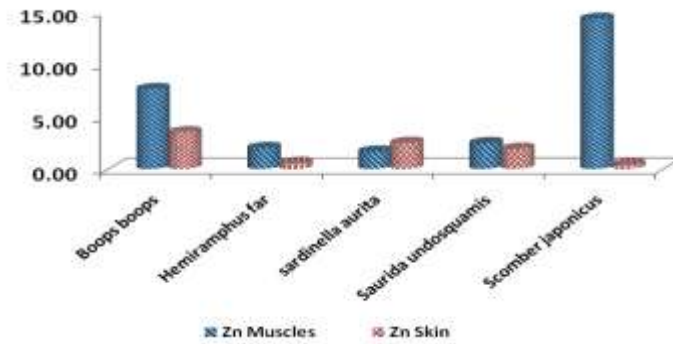


Figure 4: Maximum Zn Safe Daily Consumption of Some Fishes

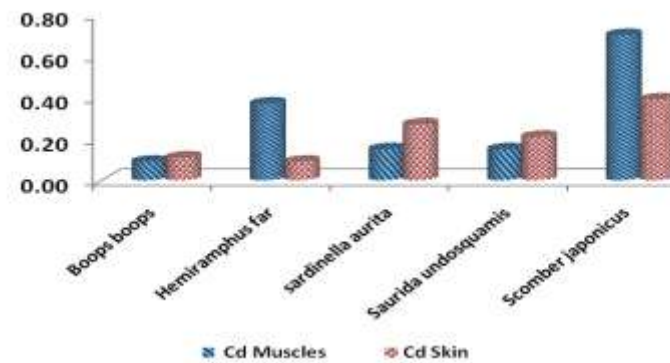


Figure5: Maximum Cd Safe Daily Consumption of Some Fishes

The total hazard quotient (HQ), which is the extent between the presentation and the reference estimations (a reference estimation or RfD), is utilized to come over the danger of non-carcinogenic impacts. Extent of less than 1 implies non-obvious danger. Conversely, any revealed people of concern will include prosperity risk in the occasion that the measurement is risen to or more than the RfD which is based on the upper level of admissions for an adult human with normal body weight of 70 kg, which does not cause deceptive impacts in the person's lifetime (USEPA, 2009). The way for the affirmation of HQ was given in the Joined Together States EPA Region III risk-based concentration table (USEPA, 1989). Hazard Index (HI) and Hazard Quotient (HQ) are applied to assess the health risk for humans from fish intake (USEPA, 2000; Goher *et al.*, 2015). The succeeding equations are used to compute the HQ and HI values as reported by the United States Environmental Protection Agency (USEPA, 2013). Where: HQ is the Hazard Quotient values for the individual heavy metal imitative by the method of Chien *et al.* (2002) and Amirah *et al.* (2013): Total HQ = HQ toxicant 1 + HQ toxicant 2+...+HQ toxicant. The human health risk assessment associated with the fish consumption was confirmed using Hazard Quotient (HQ) and Hazard Index (HI) developed by the subsequent equation:

$$HQ = [(EF \times ED \times FI \times Cm) / (RfD \times BW \times AT)] \times 10^{-3}$$

Where: Cm is the metal concentration in fish tissue (mg/kg, wet weight); EF: exposure frequency; FI is intake rate of fish per person per day represent about 19 g in Libya ( FAO, 2005); and ED is the life time exposure duration (70 years); BW is the body weight (70 kg is used as a default value for the adult (USEPA, 2013); AT is averaging time for non-carcinogens (365 days/year x number of exposure years); RfD is the oral reference dose, the RfD for Cu, Zn, Fe and Cd are  $4.0 \times 10^{-2}$ ,  $3.0 \times 10^{-1}$ ,  $7.0 \times 10^{-1}$  and  $1.0 \times 10^{-3}$  mg/kg/ day, respectively (USEPA,2000); and  $10^{-3}$  is the unit

conversion factor. The HQ is a ratio of estimated dosage of a pollutant to a reference dosage level (Goher *et al.*, 2015). HQ is known as the measurable magnitude of the possibility of health effects of non-carcinogenic for an average exposure period.

To estimate the risk to human health by more than one heavy metal, the hazard index (HI) has been developed (USEPA, 1989; Kumar *et al.*, 2013). The hazard index is the sum of the hazard quotients for all heavy metals, which was calculated by the subsequent equation (Guerra *et al.*, 2010):

$$HI = \sum HQ = HQ_{Fe} + HQ_{Cu} + HQ_{Zn} + HQ_{Cd}$$

When HQ is equal to or less than one, it signalizes no appreciable health risk, while if the  $HQ > 1$ , then it indicates a reason for health concern (USEPA, 2013). On the other hand, no health risk may happen as a result of ingestion of the fish at Hazard Quotient (HQ) or total Hazard Index (HI) below one. The greater the value of HQ and HI (if it is above 1), the greater the level of risk associated with fish consumption. Hence,  $HI < 1$  means no hazard;  $1 < HI < 10$  means moderate hazard while greater than 10 goes to high hazard or risk (Ukoha *et al.*, 2014).

The calculated HQ and HI values are acceded Table 3. The HQ due to ingestion of the studied metals through the five fish species from the consumption of fish muscles was in the range of Fe (0.0001-0.0003), Zn (0.0006-0.0049), Cu (0.0013-0.0118), Cd (0.0116-0.0870), while it was in the fish skin for the same metal in the range (0.0001-0.0005), (0.0023-0.0182), (0.0030-0.0110), and (0.0209-0.0951), for Fe, Zn, Cu and Cd, respectively. If HQ more than 1 is obtained, potential health risk is related to the studied heavy metal (Taweel *et al.* 2013). The mean values of HQ computed for the metals were below 1 for all fish species assessed, thus consumption of fish from study area poses no adverse effects of Cu, Zn, Cd, and Fe (Table 3). Similarly, local study conducted by Agusa *et al.* (2005), which addressed health risk assessment of heavy metal via fish consumption also documented that the daily intake for Cd and Cu did not exceed the guidelines set by EPA.

As illustrated in Table 3, the values of Hazard Index (HI) spread from the low value of 0.0136 for *Scomber japonicus* to the highest value of 0.0921 for *Boops boops* species for fish muscles; while in the fish skin the HI values fluctuated from 0.0415 and 0.1201 for *Sardinella aurita* and *Hemiramphus far*, respectively.

Stand on the average values of HQ, the Cd was the highest while the Fe was affirmed the lowest one. HI values due to expenditure of the five fish species from the Tripoli port were in the subsequent sequence: *Hemiramphus far* (0.1201) > *Boops boops* (0.0820) > *Saurida undosquamis* (0.0485) > *Scomber japonicus* (0.0437) > *Sardinella aurita* (0.0415).

The commitment percent of total Hazard Quotient for heavy metals was arrayed in Table 3. The most noteworthy THQ value has a place in relation to Cd and it was higher than comparable values for Cu, Zn. and Fe. This investigation settled that Cd was a major hazard supporter for common populace in Tripoli port, considered 94.46, 76.92, 76.22, 80.49, and 85.29 % of the add up to THQ for *Boops boops*, *Hemiramphus distant*, *sardinella aurita*, *Saurida undosquamis* and *Scomber japonicus*, individually. The next higher hazard donor metal was Cu, contributing extended (4.23-16.50 % of the total to THQ). The next higher hazard donor metal was Zn, taking part extend from 1.19 to 14.34% of the total THQ. Iron (Fe) was the most reduced hazard donor metal extended between 0.11 to 0.74 % for the five fish species.

Table 3: Safe dietary intake (CR<sub>lim</sub>; kg/week) and maximum allowable fish consumption rate (CR<sub>mm</sub>; meals/month) for studied heavy metal in fish samples

| Fish Species               |           |       |       |    | Muscles           |                   | CR <sub>mm</sub> |          |
|----------------------------|-----------|-------|-------|----|-------------------|-------------------|------------------|----------|
|                            | Cu        | Cm    | RfD   | Bw | CR <sub>lim</sub> | CR <sub>lim</sub> | adults           | children |
| <i>Boops boops</i>         |           | 1.34  | 0.040 | 70 | 2.09              | 14.63             | 0.28             | 0.55     |
| <i>Hemiramphus far</i>     |           | 0.84  | 0.040 | 70 | 3.33              | 23.33             | 0.44             | 0.88     |
| <i>sardinella aurita</i>   |           | 4.06  | 0.040 | 70 | 0.69              | 4.83              | 0.09             | 0.18     |
| <i>Saurida undosquamis</i> |           | 3.45  | 0.040 | 70 | 0.81              | 5.68              | 0.11             | 0.21     |
| <i>Scomber japonicus</i>   |           | 0.45  | 0.040 | 70 | 6.22              | 43.56             | 0.83             | 1.64     |
|                            | <b>Zn</b> |       |       |    |                   |                   |                  |          |
| <i>Boops boops</i>         |           | 2.76  | 0.300 | 70 | 7.61              | 53.26             | 1.01             | 2.01     |
| <i>Hemiramphus far</i>     |           | 10.51 | 0.300 | 70 | 2.00              | 13.99             | 0.26             | 0.53     |
| <i>sardinella aurita</i>   |           | 12.65 | 0.300 | 70 | 1.66              | 11.62             | 0.22             | 0.44     |
| <i>Saurida undosquamis</i> |           | 8.52  | 0.300 | 70 | 2.46              | 17.25             | 0.33             | 0.65     |
| <i>Scomber japonicus</i>   |           | 1.47  | 0.300 | 70 | 14.29             | 100.00            | 1.89             | 3.77     |
|                            | <b>Cd</b> |       |       |    |                   |                   |                  |          |
| <i>Boops boops</i>         |           | 0.75  | 0.001 | 70 | 0.09              | 0.65              | 0.01             | 0.02     |
| <i>Hemiramphus far</i>     |           | 0.19  | 0.001 | 70 | 0.37              | 2.58              | 0.05             | 0.10     |
| <i>sardinella aurita</i>   |           | 0.47  | 0.001 | 70 | 0.15              | 1.04              | 0.02             | 0.04     |
| <i>Saurida undosquamis</i> |           | 0.48  | 0.001 | 70 | 0.15              | 1.02              | 0.02             | 0.04     |
| <i>Scomber japonicus</i>   |           | 0.10  | 0.001 | 70 | 0.70              | 4.90              | 0.09             | 0.18     |
|                            | <b>Fe</b> |       |       |    |                   |                   |                  |          |
| <i>Boops boops</i>         |           | 0.76  | 0.700 | 70 | 64.47             | 451.32            | 8.55             | 17.02    |
| <i>Hemiramphus far</i>     |           | 0.41  | 0.700 | 70 | 119.51            | 836.59            | 15.85            | 31.56    |
| <i>sardinella aurita</i>   |           | 1.83  | 0.700 | 70 | 26.78             | 187.43            | 3.55             | 7.07     |
| <i>Saurida undosquamis</i> |           | 1.13  | 0.700 | 70 | 43.36             | 303.54            | 5.75             | 11.45    |
| <i>Scomber japonicus</i>   |           | 0.52  | 0.700 | 70 | 94.23             | 659.62            | 12.49            | 24.88    |

CR<sub>Lm</sub>: Maximum Safe Daily Consumption of Fish;  $CR_{Lm} = RFD \cdot BW / Cm$   
 CR<sub>Lim</sub>: Safe dietary intake (Kg/Week);  $CR_{Lim} = (RFD \cdot BW / Cm) \cdot 7$   
 CR<sub>mm</sub>: maximum allowable fish consumption Rate (meals/month);  $CR_{mm} = CR_{Lim} \cdot T_{ap} / MS$   
 RfD: Reference Doses (mg/kg/day)      BW: Average Weight (70Kg)  
 Tap: the average time period in a month (4.3week/month) Cm: metal concentration (mg/kg)  
 MS: The meal size 227 g and 114 g for adults and children, respectively.

The multivariate Principal Component Analysis (PCA) was conducted to create the relationship between the sources and the levels of metals in fish organs (muscles, skin, livers, gills, and bones). PCA analysis incorporates the four metal concentration data of five species and explores the possible similar distribution pattern of metals. Two principal components (PCs) were extracted approximately 82.18% (PC1: 51.48% and PC2: 30.70%). Similar loadings of Cu and Cd in fish indicated that these were mostly contributed by anthropogenic activities (Figure 6) (Shah and Shaheen, 2007).

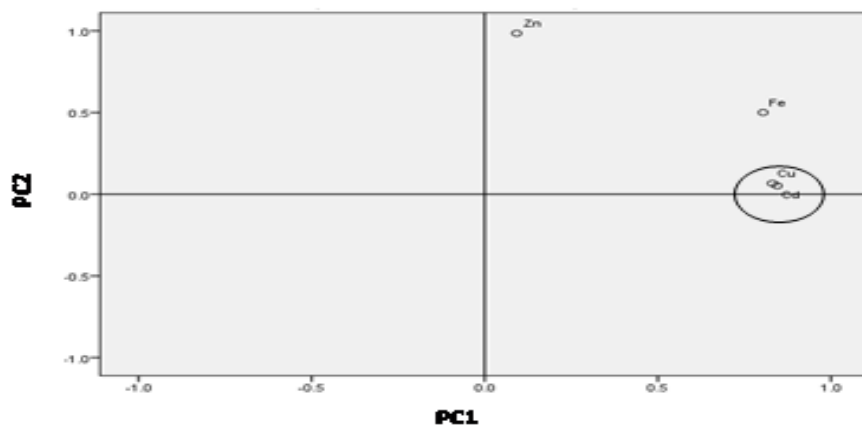


Figure 6: Component plot for heavy metals in fish species

## CONCLUSION

Two opposite opinions concerning the importance of fish consumption in the human diet are presented in the world literature. The main themes of the discussion are the benefits for consumer health from the nutritional properties of fish, especially component n-3 fatty acids, and the risks posed by the contamination of fish with pollutants like heavy metals. This study provides valuable information concerning comparison of heavy metal distributions, fish consumption and health risk assessment. Results showed that in spite of shipping, oil and gas activities on-shore and off-shore in Tripoli Port, no levels in the current study were beyond the allowable limits recommended by WHO (Zn; 100, Cu; 30, Fe; 100; and Cd; 1 µg/g wet wt ) for examined heavy metals. EWIs values for all metals are below the PTWI guideline levels for all species. Based on MPI of the four examined heavy metals in the studied species *sardinella aurita* recorded with the highest value; however, *Hemiramphus* had the lowest value. In common, schedule check and investigation of nourishment stuff is required to maintain a strategic distance from the hazard of surpassing the admissions past the tolerance limits measures. The concentration of the heavy metals were higher in skin samples than the muscles due to complex mixture of metal with the mucus that cannot be removed from the tissue prior chemical analysis.

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## المخلص العربي

المعادن الثقيلة في بعض فصائل الأسماك من ساحل البحر المتوسط ، ميناء طرابلس (ليبيا):

تقييم شامل للآثار الضارة المحتملة على صحة الإنسان

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يلعب الساحل الليبي دوراً هاماً من حيث التنوع البيولوجي وإنتاجية النظام البيئي البحري في البحر المتوسط. صممت هذه الدراسة لتقييم المخاطر المحتملة على السكان من خلال تناول الأسماك. وتعزيز المعلومات المتعلقة بالتأثيرات البشرية في ميناء طرابلس (ليبيا) لفهم توزيع الملوثات ووضع سياسات للتعويض بمناطق الخطر المحتملة للمستفيدين. تم تقييم مستويات الحديد والزنك والنحاس والكاديوم في الكبد والخياشيم والعضلات والجلد والعظام في خمسة أنواع من أسماك البحر المتوسط في ميناء طرابلس (ليبيا). أظهرت النتائج وجود فرق كبير في تركيزات المعادن بين أعضاء الأسماك. تم قياس أعلى التركيزات من Cu، Cd، و Fe في الكبد ، في حين أن الخياشيم والجلود احتوت على تركيزات أعلى من Zn. سجلت تركيزات المعادن الثقيلة في تلك الدراسة في مختلف فصائل السمك مدي (Zn) ؛ ٧.١٨ - ٢١.٩٤ و Cu ؛ ١.٨٩-٧.٠٣ و Fe ؛ ٠.٩٣-٤.٠٥ و Cd ؛ ٠.١٩ إلى ٠.٩٧) ميكروجم/جم. لحسن الحظ ، كانت (EWI) أقل بكثير من المسموح به (PTW). لذا لا يوجد مخاطر على صحة الإنسان ناتجة عن استهلاك أنواع الأسماك المدروسة من ميناء طرابلس ، وأن عضلات الأسماك ليست أنسجة نشطة لتراكم المعادن ، مما يجعلها مناسبة للاستهلاك البشري. في هذه الدراسة كان حاصل الخطر (HQ) ومؤشر المخاطر (HI) أقل من ١ ، وبالتالي لا يوجد خطر محتمل على الصحة بالنسبة للمستهلكين. حددت تحليلات المكونات الرئيسية أن النحاس والكاديوم لهم نفس الصدر الناتج عن النشاط البشري.