Malaysian Journal Of Science 41(1): 54-75 (February 2022)

MEASUREMENT OF IRON, MANGANESE, COPPER, COBALT AND LEAD TO ASSESS THE EDIBLE FITNESS OF M. CEPHALUS COLLECTED FROM LASBELA (BALOCHISTAN), PAKISTAN, USING ATOMIC ABSORPTION SPECTROSCOPY

Fehmeeda Afzal[1b], Samiullah[2c], Ashraf Nadeem[3b], Muhammad Aslam[4b], Muhammad Shafi[5b], Khalil Hasni[6a], Abdul Hakeem[7b] and Sonia Tariq[8a*]

[a]Department of Coastal and Environmental Sciences, Lasbela University of Agriculture, Water and Marine Sciences Uthal, Lasbela, Balochistan, PAKISTAN. Email: soniatariq@luawms.edu.pk[8]; khalil_hasni1@yahoo.com[6]

[b]Department of Marine sciences, Lasbela University of Agriculture, Water and Marine Sciences Uthal, Lasbela, Balochistan, PAKISTAN. Email: fahmeedaafzal22@gmail.com[1]; ashrafbaluch@gmail.com[3]; aslambc@hotmail.com[4]; drmshafi3333@hotmail.com[5]; a.hakeem.faeem@gmail.com[7]

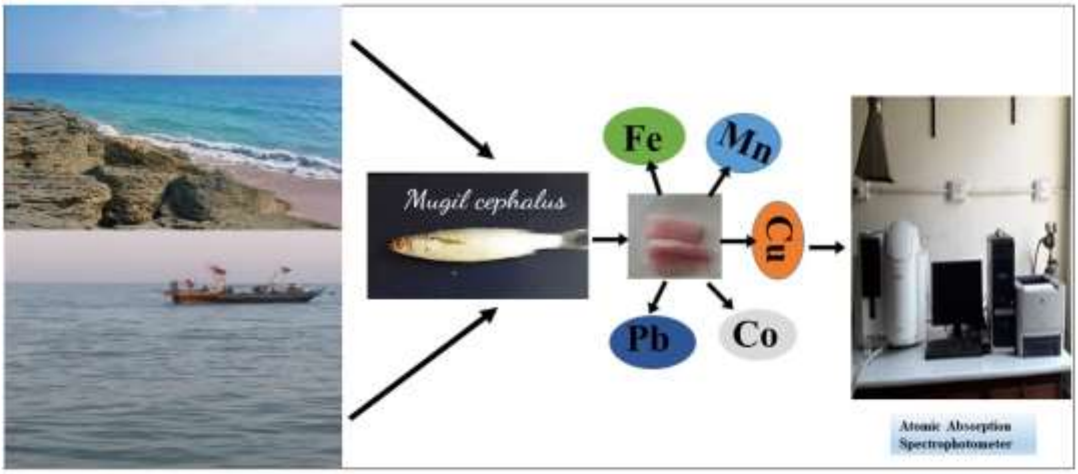
[c]Department of Chemistry, University of Balochistan, Quetta, PAKISTAN. Email: sami435889@gmail.com[2]

Corresponding authors: soniatariq@luawms.edu.pk

Received: 12th Sep 2020 Accepted: 7th Sep 2021 Published: 28th Feb 2022

DOI: https://doi.org/10.22452/mjs.vol41no1.4

ABSTRACT The sea serves as a good source of food. The direct discharge of pollutants into aquatic environments can result in exceeded levels of heavy metals. This can harm the organisms and cause serious health issues when consumed by humans. *Mugil cephalus* is consumed as food, and its continuous analysis for heavy metals is required for food safety. Some selected metals – namely iron, copper, manganese, cobalt and lead – were evaluated in muscle tissues of *Mugil cephalus* collected from two major fish landing sites (i.e. Gaddani and Damb) along the Lasbela coast of Balochistan). The concentration of metals was determined using an atomic absorption spectrometer. For this purpose, several digestion methods were used to digest the meat for comparison. Using H_2SO_4, HNO_3 and $HClO_4$ was the most effective method, which is an important analytical finding for future researchers to consider. The concentrations of metals assumed the order of Fe > Cu > Pb > Mn > Co in samples from Gaddani and Fe > Cu > Mn > Pb > Co in samples taken from Damb. Among the metals analyzed, iron levels at Gaddani and Damb were found within the permissible values suggested by the World Health Organization (WHO), Food and Agriculture Organization (FAO), and United States Environmental Protection Agency (USEPA). Copper and lead levels were higher than the safe limits in samples from both sites. The estimated daily and weekly metals intake in an average 70 kg person were lower than the limits proposed by the FAO and WHO for all metals. The overall results revealed that the selected fish species is safe for consumption and presents no hazardous effects on human health. However, Gaddani fish Harbor and Damb should be protected from the direct discharge of pollutants, and the future monitoring of *M. cephalus* fish is required to make sure the edible meat is safe for consumption.



Keywords: atomic absorption spectroscopy; heavy metals; *mugil cephalus*; muscular tissue; edible fitness; lasbela coast.

1. INTRODUCTION

Heavy metal accumulation in aquatic environments is among the most prominent global issues (Argamino and Janairo 2016; Ali and Khan, 2018; Ugonna *et al.,* 2020) due to their non-biodegradability, toxicity (Saher and Siddiqui, 2016; Islam *et al.,* 2018) and serious hazards to human health (Khemis *et al.,* 2017; Ali *et al.,* 2019). Natural and anthropogenic sources of heavy metals have gained significant global concern and attention from researchers in many developing countries (Ihedioha *et al.,* 2017; Ali and Khan, 2018; Odika *et al.,* 2020). Natural routes of heavy metal pollution include atmospheric pollutants, deposition on the surface water and soil erosion (Hosseini *et al.,* 2012; Odika *et al.,* 2020). Currently, seas and oceans receive significant amounts of heavy metal pollutants from various point (easily traced) and non-point (untraceable) sources. Examples of point sources are wastewater discharge by industries and metropolitan sewage released from domestic and urban regions; examples of non-point sources are waste products and chemicals that flow over land through coastal waters, lakes and rivers (Belabed *et al.,* 2017; Odika *et al.,* 2020). Metals' entrance into fish bodies occurs via three possible routes: through the body surface, gills or digestive tracts (Ben Ameur *et al.,* 2012). The concentrations of heavy metals (other than mercury) in the muscles of fish are usually lower than in other parts of the body. Nevertheless, it is important to detect metal levels in the muscles of fish due to its high consumption rate and association with health risks (Fazio *et al.,* 2020; Jaric *et al.,* 2011; Zhuang *et al.,* 2013; Stancheva *et al.,* 2013; Idriss and Ahmad; 2015). Some important elements, such as iron, copper, zinc, manganese and nickel, have fundamental roles in aquatic organisms' lives because of the environmental sustainability and human-ecological context (Ghaderpoori *et al.,* 2018; Harahap and Lubis, 2018). However, due to biomagnification and accumulation, their biological systems can get affected, causing many health problems (Gale *et al.,* 2004; Ali *et al.,* 2019). Mercury, cadmium, lead and arsenic are toxic, meaning they can harm aquatic organisms and result in potential carcinogenic effects (Table 1; Zhong *et al.,* 2018; Rajeshkumar and Li, 2018; Rahman *et al.,* 2019).

Fish are considered a great source of food with several health benefits. They are also good bioindicators because of their ability to uptake various trace metals from the aquatic environment and transfer them to humans (Diop *et al.,* 2016a; Fazio *et al.,* 2020). *Mugil cephalus* (commonly referred to as the flathead mullet, striped mullet or sea mullet) is a fish that inhabits marine, freshwater and intertidal estuarine environments worldwide (Nelson, 2006; Whitfield *et al.,* 2012; Diop *et al.,* 2016a). *Mugil cephalus* is a pelagic fish that usually lives in shallow water and enters estuaries and lagoons.

Mullets belong to the family *Muglidae*, consisting of 18 genera and 81 species. About three genera and 12 species of mullets have been found in Pakistan (Bianchi, 1985), and eight species of this family have been found along the Karachi coast alone (Fehmida, 2002). Mullets are often used in aquaculture due to their consumption rate. They are the dominant landing fish along the Pakistan coast (Zubia *et al.*, 2015). *M. cephalus* is generally considered to be highly susceptible to metal accumulation, mainly due to their long gut length, their uptake of metals via different routes in water columns and their ingestion of large amounts of organic matter, sand and mud from the sediments (Whitfield *et al.,* 2012; Stancheva *et al.,* 2013; Khemis *et al.,* 2017; Peycheva *et al.,* 2019; Ihunwo *et al.,* 2020). In addition, the uptake of metals in the organisms that inhabit the benthic area is usually high (Yilmaz, 2009; Stancheva *et al.,* 2013).

Table 1*: Metals with maximum safe health limits, sources and adverse effects.*

Metals	WHO limits (WHO, 1989)	Sources	Adverse effects on fish and Human
Zinc	40 µg/g	Weathering of rocks, industrial and domestic water wastes, soil erosion, die castings metals, mining and metal smelters activities, alloy, rubber and paints activities (Adeyeye, 1996; Wanna and Okieimen, 2011; Bowen *et al.,* 2006; Bhagure and Mirgane, 2010).	Hypoxia, immobile fins and restless swimming in fishes (Vosyliena and Jankaita, 2006; Olaifa *et al.,* 1998; Kori and Ubogu, 2008). Loss of appetite, anemia, vomiting, nausea, stomach cramps, immunological abnormalities and respiratory disorders in human
Copper	30 µg/g	Industrial discharges, municipal sewage, use of fertilizers and	Affects the sensory organs in fishes (Sandahl *et al.,* 2007)

		pesticides in agriculture (Eisler, 1998a).	Anemia, hypertension, pathological changes in brain tissues of human (Kudesia, 1990; Bhagure and Mirgane, 2010; Wuana and Okieimen, 2011).
Manganese	0.50 µg/kg	Wate water discharge, mining and emission of minerals from alloy	Neurological damage, cerebral dysfunction (Reilly, 2002).
Chromium	0.15 µg/kg	Discharge from electroplating, textile industries (Hardy *et al.*, 2008; Lenntech, 2010; Asio, 2009; Bhagure and Mirgane, 2010;	Anemia, eosinophilia and lymphocytosis, bronchial and renal lesion and gills damage in fishes. Kidney destruction, allergic disease, nose irritation and nose bleeds, respiratory tract cancer, gastrointestinal tract bleeding and lung cancer in human (Adelekan and Abegunde, 2011; Bhagure and Mirgane, 2010; Karadede *et al.*, 2004; Dayan and Paine, 2001).
Lead	2 µg/kg	Soil erosion, gasoline combustion (DWAF, 1996) and anthropogenic activities (WHO, 1985), such as combustion and emission of petroleum fuels from automobile, household plumbing, burning of fossil fuel and mining (Edwaed *et al.,* 2013; Muzyed, 2011; Juberg, 2000).	Damage the liver, kidney and spleen, causing spinal defects and death in fishes (Davies and Everhart, 1973; Haider, 1964). Lower IQ level and it is responsible for hyperactivity in children, loss of memory, anorexia, nausea, anemia, failure of reproduction, intestinal cramps, weakness of joints and miscarriages in pregnant women (Wuana and Okieimen, 2011; Asio, 2009; Hardy *et al.*, 2008; Umar *et al.,* 2001; Adelekan and Abegunde, 2011).

Recently, a lot of work has been published on the levels of heavy metals in fish samples from Pakistani coasts. Fish samples from Keti Bandar showed higher arsenic levels than samples from other locations due to industrial discharge (specifically, a significant amount of arsenic pollutant entered the area via the Indus River; Khattak *et al.*,2013). A study performed by Ahmed *et al.* (2014) in Gwadar, Balochistan, showed that the amounts of iron, manganese, lead and copper were higher in fish livers than in their muscle tissues; also, the concentrations of these metals remained the same throughout the year. The concentrations of zinc, lead, manganese, copper, chromium and mercury were high in the muscular tissues of *Mugil cephalus* from the Machilipatnam coast, causing concern about the health risks associated with its consumption (Krishna *et al.*,2014).

An assessment of metal concentrations in grey mullet fish from various hydro systems and sediments in the Gulf of Annaba (North Africa) was carried out. This study showed dangerous levels of zinc, lead and cadmium accumulation in the tissues of *Mugil cephalus* and revealed that regular monitoring of heavy metals is required (Ouali *et al.*, 2018). Another recent work on metal concentrations in different tissues of *Mugil cephalus* (Grey mullet) fish from Italy was carried out, as this species is a good indicator of environmental and coastal water quality (Fazio *et al.*, 2020).

Previous metal detection studies in *Mugil cephalus* samples collected from different parts of the world have been done, but no work has reported heavy metal measurements in *Mugil cephalus* collected from Balochistan, Pakistan, especially from the Lasbela coast. In this paper, we present the amounts of iron, manganese, copper, cobalt and lead in the muscles of *M. cephalus* collected from the Lasbela coast of Balochistan, Pakistan. We have also compared the recorded concentrations with the recommended safe edible standards of USEPA and FAO/WHO to assure that health risks are manageable, as *M. cephalus* is one of the most highly consumed fish along the Lasbela coast due to its high availability and moderate cost.

Figure 1.1: *District Lasbela map (the red arrows point to the study sites. (source: google maps).*

2.2. Sample collection and identification

About 50 samples of *Mugil cephalus* (Linnaeus, 1758) were collected from two different fish landing sites (i.e. Gaddani and Damb; twenty-five from each site) from March to December. The fish samples were immediately placed in an icebox in order to keep them fresh. Fish samples were identified using the FAO's species identification sheets (Bianchi, 1985) and stored at 20 ℃ for further processing.

2.3 Chemicals

Nitric acid (HNO_3), perchloric acid ($HClO_4$), hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) were supplied by Merck, Germany.

2.4. Drying and Digestion of samples

Fish samples were dissected to obtain fish muscles. Samples were dried in an oven (ESCO Isotherm, 5277) at 105 ℃ for three hours and then placed at room temperature for 24 hours for proper drying (Mwangi, 2013; Malik *et al.*, 2016; Adebayo, 2017). The dried samples were then weighed using an electric weighing balance (Sartorius, CP324S) and crushed into fine powders using a pestle and mortar. Three digestion methods were performed to find the best method for muscle tissue digestion of *M. cephalus* fish: HNO_3 and HCl (Method 1), HNO_3 and $HClO_4$ (Method 2) and H_2SO_4, HNO_3 and $HClO_4$ (Method 3). Details about these methods are given in the supplementary information.

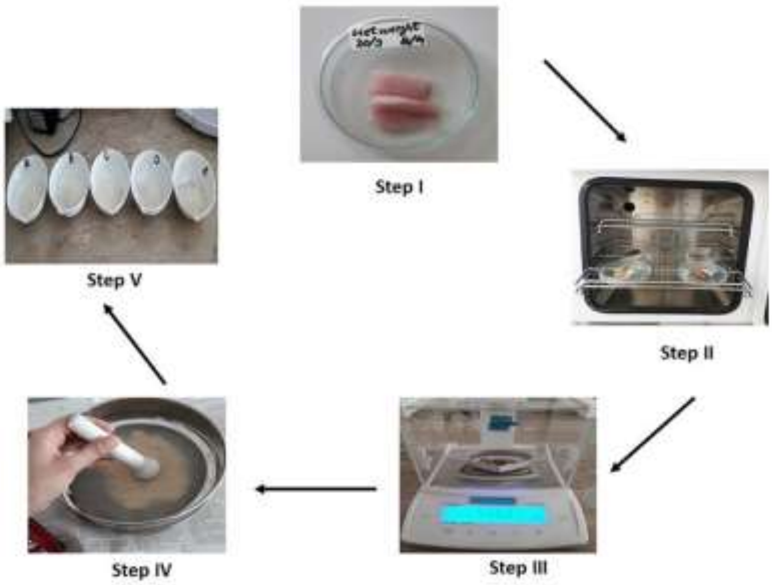


Figure 2.1: Process of fish muscle drying: Step I: dissected meat, step II: oven drying, step III: weighing the dried meat, step IV: crushing the dried meat into powder.

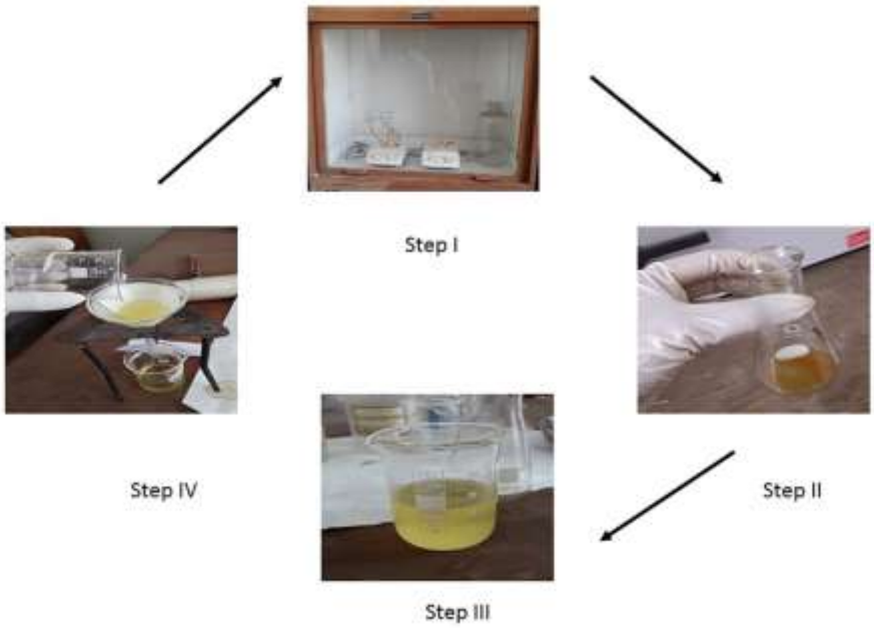


Figure 2.2: Process of sample digestion and filtration: Step I: digestion in acids, step II: digested sample, step III: dilution, step IV: filtration.

2.5 Analysis of metals in fish samples

Standard solutions (5 ppm, 10 ppm, 15ppm and 20ppm) of targeted metals to be detected were freshly prepared by diluting1000 mg/L of stock solutions (supplied by Merck, Germany) for calibration. The targeted metals (i.e. iron, manganese, copper, cobalt and lead) in muscle samples were detected using an atomic absorption spectrometer (Thermo-S4 AA).

2.6 Statistical analysis

In this study, Student's t-test was applied to test the equality of mean metal concentrations of the samples collected from the Gaddani and Damb areas using SPSS version 16.0.

3. RESULTS AND DISCUSSIONS

Digestion methods for fish. All digestion methods that we experimented on worked well, but digestion method 3, which uses H_2SO_4, HNO_3 and $HClO_4$ (as discussed in SI), was the best and fastest. Figures 3.1 and 3.2 summarize the mean concentrations of targeted metals in the samples taken from both sites. Detailed results are summarized in **Table 3.1**.

The concentrations of iron and copper were the highest among all metals, with significant differences ($p<0.05$), followed by lead and manganese. Cobalt had the lowest mean concentration at both sites, though its differences from the next-lowest concentrations were not significant ($p>0.05$). A detailed t-test table is given in the supplementary information. A comparison of the results with the relevant literature is also provided in **Table 3.2**.

Iron levels in the muscles of fish taken from Gaddani and Damb were 94.43 µg/g ± 1.68 and 83.29 µg/g ± 1.49 (dry weight), respectively. These concentrations are lower than reported by Prasath and Khan (i.e. 529 µg/g) in fish collected from Poompuhar, India, which neighbours Pakistan. They are also several times higher than the iron concentrations reported by Canli and Atli (38.71 µg/g dry weight), who collected samples from the Mediterranean Sea (**Table 3.2**; Canli and Atli, 2003; Prasath and Khan, 2008; WHO, 1989; FAO, 1983).

As far as the edible fitness of fish is concerned (specifically regarding the levels of iron), the mean values of Fe do not exceed the recommended safe edible limits set by the WHO and FAO (i.e. 100 µg/g), suggesting that these fish are safe for human consumption. However, the maximum values of Fe recorded in samples from Gaddani (110.8 µg/g) and Damb (92.30 µg/g) exceed and are close to the limit, respectively (**Table 3.1**).

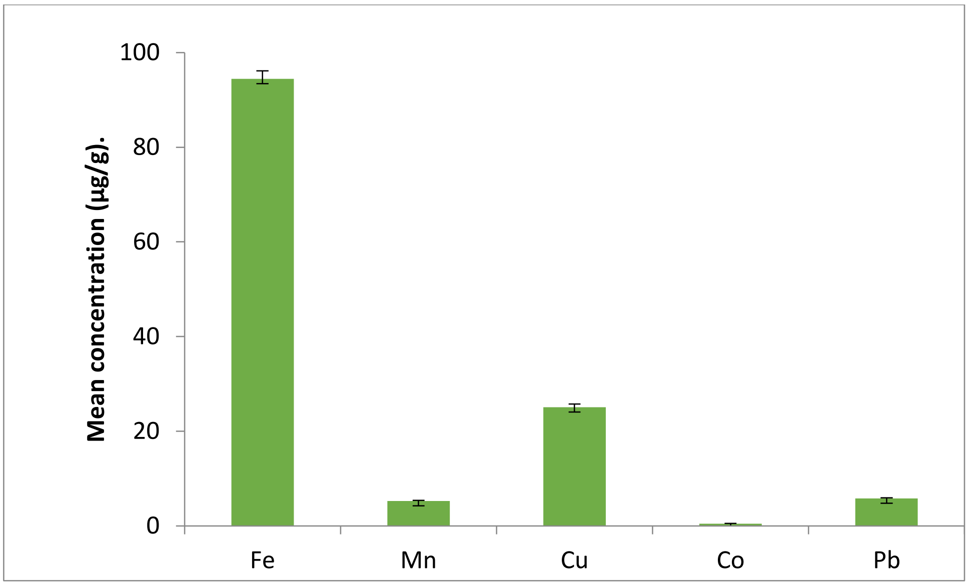


Figure 3.1: Mean concentrations of metals with standard error bars from Gaddani.

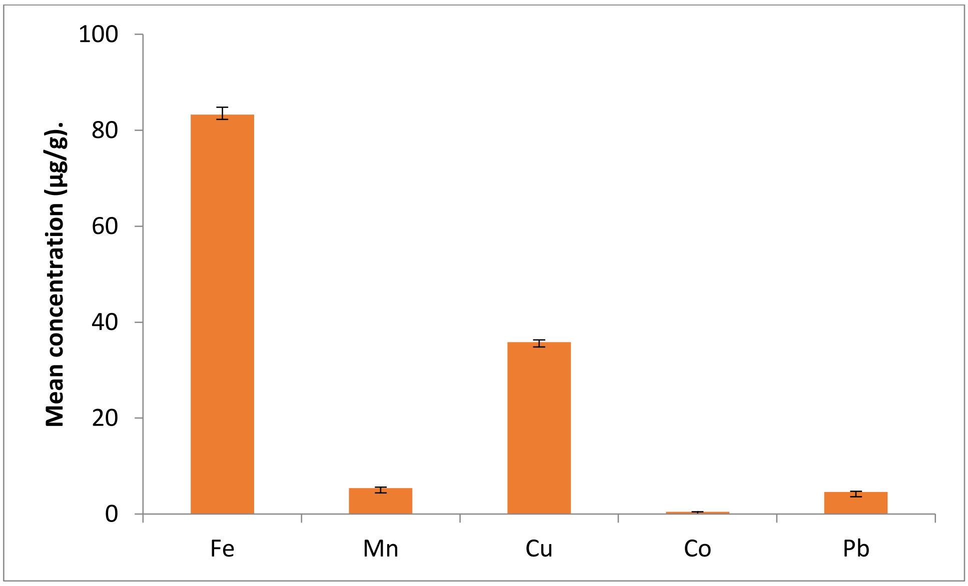


Figure 3.2: Mean concentrations of metals with standard error bars from Damb.

Manganese is an essential micro-nutrient and the 12th most common metal usually found in water (about 1000 ppm; i.e. 0.1%), which comes from the Earth's crust (Emsley, 2001). It is also an important metal for bone and skin cell formation. However, excessive amounts can have harmful effects. The manganese level measured in Gaddani samples was 5.22 µg/g ± 0.14, while in the Damb site, it was 5.43 µg/g ± 0.17. These results are very concerning, as the safe standard is 5.4 µg/g according to the FAO and WHO (FAO and WHO, 1984; Hisham, 2015). The Mg concentration in this study's samples is lower than the values reported by Prasath and Khan (samples from Poompuhar, India) and higher than the values reported by

Hisham and Tawfik *et al.* (Hisham, 2015; Tawfik *et al.*,2013).

Copper can last for 7000-8000 years because of its wide availability (Schroeder *et al.*, 1966). It is necessary for the production of hemoglobin in humans and the development of animals and plants (Wuana and Okieimen, 2011). However, it becomes toxic when its concentration exceeds the required limit. Copper concentrations from the samples taken from Gaddani and Damb were 25.06 ± 0.68 µg/g and 35.83 ± 0.45 µg/g, respectively. The Damb site showed higher copper levels, which exceeded the permissible limit set by the WHO (1989) and FAO (1983). These concentrations are higher than reported by Tawfik *et al.* and lower than obtained by Prasath and Khan, Hisham and Ahmed *et al*. (Tawfik *et al.*, ibid; Prasath and Khan, ibid; Hisham, ibid; Ahmed *et al.*,2015).

Table 3.1: Concentrations of selected metals (µg/g dry weight) in edible tissues of M. cephalus fish from Gaddani and Damb with mean±SD and SEM.

S/No.	Metals	Sample size	Minimum	Maximum	Mean	Std.Deviation	SEM
1	Fe	25	73.38	110.80	94.4300	8.41290	1.68258
2	Mn	25	4	6.8	5.2234	0.73870	0.14774
3	Cu	25	19.70	29.24	25.0642	3.42027	0.68405
4	Co	25	0.33	0.63	0.4788	0.07721	0.01544
5	Pb	25	4.19	6.86	5.7760	0.63753	0.12751

Table 3.2: Comparison of metals in muscle tissues of Mugil cephalus fish (µg/g dry weight) in the present study and other studies (NP = number of samples not presented in the paper, ND = metal not detected).

S. No	No. of *M. cephalus* samples	Location	Fe	Mn	Cu	Co	P b	References
1	20	Mediterranean Sea	38.71	ND	4.41	ND	5.32	Canli and Atli, (2003)
2	NP	Manzala Lake, Egypt	ND	ND	5.68	ND	2.98	Bahnasawy *et al.*, (2009)
3	36	Lake Macquarie, Australia	ND	ND	3.6	ND	ND	Kirby *et al.*, (2001)
4	NP	Poompuhar coast, India a) Pre tsunami	a) 479.0	18.43	14.4	0.001	ND	Prasath and Khan, (2008)
		b) Post tsunami	b) 529.13	25.48	20.48	0.003	ND	
5	NP	Gaza fishing harbor	ND	0.90	13.15	0.68	1.82	Hisham, (2015)
6	NP	Riyadh market, Saudi Arabia	ND	0.11	0.76	ND	0.002	Twafik *et al.*, (2013)

7	12	East Barbice-Corentyne, Guyana	0.152	0.278	1.206	ND	0.058	Sivakumar *et al.*, (2015)
8	56	Uppanar River, India a) dry season b) wet season	a) ND b) ND	26.269 10.12	0.181 0.127	ND ND	0.189 0.098	Usha and Reddy, (2013)
9	76	Tuzla lagoon, Turkey	11.2	ND	0.62	ND	1.19	Dural *et al.*, *(*2007)
10	70	Koycegiz lagoon, Turkey	ND	ND	29.901	ND	0.626	Genc and Yilmaz, (2018)
11	50	Lasbela coast a) Gaddani b) Damb	a) 94.4 b) 83.3	5.22 5.43	25.1 35.83	0.478 0.485	5.77 4.62	Present study
12	WHO, 1989		100	5.4	30	_	0.5-0.6	(WHO, 1989)
13	FAO, 1983		100	_	30	_	0.5	(FAO, 1983)
14								

	USEPA, 1998	0.5	0.02	2.25	_	0.11	(USEPA, 1998)
15	WHO, 1996	_	_	30	_	2	Cited in (Ikema and Egieborb, 2005)

Lead is an extremely toxic heavy metal and is greatly accumulated in sediments. Consequently, due to mullets' ingestion of sand and mud from sediments, lead uptake in these fish is relatively high (Eustace, 1974; Campbell, 1994). Lead values from the study sites were 5.77 ± 0.12µg/g and 4.61 ± 0.11 µg/g, both of which exceed the values recommended by the WHO (1989), FAO (1983) and USEPA (1998) (i.e. 2 µg/g, 0.5-0.6 µg/g and 0.11µg/g, respectively). The nearest lead concentrations in fish (5.32 µg/g) were found in the Mediterranean Sea by Canli and Atli. Zehra *et al.* (2003) reported a lead concentration of 0.5 µg/g in the muscles of Dandya fish from Gaddani, which is much lower than the value observed in the present study. The element's exposure levels in fish tissues are highly dependent on the selection of species because of their diets, behaviours, modes of life and time exposure to metals (Canli *et al.,* 1993; Zehra et al., 2003; Chen *et al.,* 1999; Yilmaz, 2005).

These values are lower than that detected by Hisham (2015) and higher than that found by Prasath and Khan (2008). The cobalt concentrations in the muscles of fish were substantially lower than any of the other metals analyzed in this study. The influx of these metals from both sites occurred because of rising pollution levels and shipbreaking activities in Gaddani (Khattak *et al.*, (2013).

Finally, the cobalt levels in samples from Gaddani were 0.47 ± 0.01 µg/g, while in samples from Damb, the concentration was 0.48 ± 0.01 µg/g.

3.1. Estimated health risks on a daily and weekly intake basis.

Daily and weekly metal intakes were calculated from Gaddani and Damb site samples to compare the fish consumption rates with the daily and weekly safe intakes set by FAO and WHO (1994, 2004, 2006). The daily provisional tolerable metal intake (in µg/day/70 kg person) and weekly intake (in µg/week/70 kg person) are shown in **Table 3.3** (70 kg is considered the weight of the average person).

Table 3.3: Estimated daily and weekly intake of metals (µg/g) from M. cephalus fish muscle samples for a person weighing 70 kg

S/No.	Metals	PTDI[a]	PTWI[b]	EDI[c] Gaddani, Damb	EWI[d] Gaddani, Damb
1	**Fe**	56000	392000	(472), (416.5)	(3304), (2915.5)
2	**Mn**	9800	68600	(26.1), (27.15)	(182.7), (190.1)
3	**Cu**	35000	245000	(125.5), (179.15)	(878.5),(1254.1)
4	**Co**	_	_	(2.5), (2.7)	(17.5), (18.9)
5	**Pb**	250	1750	(28.85), (23.1)	(201.9), (161.7)

[a]**PTWI** (Permissible tolerable weekly intake in µg/week/70 kg person) (FAO/WHO, 1994, 2004, 2006; Turkemen et al., 2009).

[b]**PTDI** (Provisional tolerable daily intake in µg/day/70 kg person) (FAO/WHO, 1994, 2004, 2006; Turkemen et al., 2009).

[c]**EDI** (Estimated daily intake of metals in µg/day/70 kg person) (Calculated from the present study).

[d]**EWI** (Estimated weekly intake of metals in µg/week/70 kg person) (Calculated from the present study).

According to the FAO (2010), the average fish consumption rate per 70 kg person in Pakistan is 5 g/person/day and 35 g/person/week (Waseem, 2007; Chughtai and Mehmood, 2012; Ahmed *et al.,* 2016). The estimated daily intake and weekly intake of metals were calculated using the following formula:

EDI = Mean concentration of metal in edible parts (µg/g) × average daily fish consumption (g) / body weight (70 kg)

EWI = Mean concentration of metal (µg/g) × average weekly fish consumption (g) / body weight (70 Kg) (as cited in Agusa *et al.,* 2007; Ikem and Egiebor, 2005).

Excluding lead and copper, the concentrations of all metals in the muscles of fishes collected from both sites along the Lasbela coast (Gaddani and Damb Harbor) were lower than the limits given by FAO and WHO (1994, 2004, 2006). This result suggests that the fish from both sites are safe for human consumption. When the daily and weekly estimated intakes of metals through the targeted fish were calculated and compared with the tolerable limits set by the FAO and WHO, the amounts were noticeably lower than the limits. This further suggests that these fish are safe for consumption. The estimated daily intakes of copper, manganese and lead were higher in the present study than the intakes estimated by Elnabris concerning *M. cephalus* from Gaza, Palestine, but lower than the daily and weekly values estimated by Zaqoot (Elnabris *et al.,* 2013; Zaqoot *et al.,* 2017).

4. CONCLUSION

We employed different digestion methods for metal detection in *Mugil cephalus* and found that the method using H_2SO_4, HNO_3 and $HClO_4$ was the best (i.e. most reliable and fastest) and gave the maximum recovery of the metals to be detected. The levels of metals obtained in this study (from both sites) are within the acceptable limits (with the exceptions of lead and copper). Lead levels were much higher than the limits suggested by the WHO (1989), FAO (1983) and USEPA (1998). In order to avoid increases in the metal concentrations in the edible tissues of *M. cephalus* (and other commercially important fish species) from both sites, future monitoring of these metals is recommended.

Gaddani Fish Harbor and Damb Bandar should be protected from the direct discharge of pollutants into the beach water. The health risk assessment based on daily and weekly fish consumption was lower than suggested by the FAO and WHO (1994, 2004, 2006). Thus, it is concluded that these fish are safe to eat without any hazardous health effects. Nevertheless, the continuous monitoring of the edible fitness of these fish by future researchers is recommended.

5. ACKNOWLEDGEMENTS

We are thankful to the team at the analytical chemistry lab for the AAS data analysis. We would also like to acknowledge the Lasbela University of Agriculture, Water and Marine Sciences, for providing financial support to collect fish samples. Finally, we wish to thank the University of Balochistan for research collaboration.

6. REFERENCES

Adebayo, I.A. (2017). "Determination of heavy metals in water, fish and sediment from Ureje water reservoir". *J. Envir. Analyt. Toxicolo,***7**(4): 2161-0525.

Agusa, T., Kunito, T., Sudaryanto, A., Monirith, I and kan-Atrieklap, S. (2007). "Exposure assessment for trace elements from consumption of marine fish in Southeast Aisa". *Enviro. Pollut,***145**: 766-777.

Ahmed, Q., Bat, L., and Yousuf, F., Aricri, E. (2016). "Heavy metals in *Achnthopagrusarabicus* Iwatsuki, 2013 from Karachi coasts, Pakistan and potential risk of human health". *Int. J. of. Fishe & Aquat. Stud,***4**(1): 203-208.

Ahmed, Q., Khan, D., and Elahi, N. (2014). "Concentration of heavy metals (Fe, Mn, Zn, Cd, Pb and Cu) in muscles, liver and gills of adult *Sardinella albella* (Valenciennes, 1847) from Gawadar water of Balochistan Pakistan". *FUUAST. J. Biolo,***4**(2):195-204.

Ali, H and Khan, E. (2018). "Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish.

Risk to human health". *Enviro, Chemist, Lett*, **16**: 903–917.

Ali, H., Khan, E and Ilahi, I. (2019). "Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation," *Journal of Chemistry*, Article ID 6730305, 14.

Alinnor,I.J., and Obiji, I.A. (2010). "Assessment of trace metal composition in fish samples from Nworie River". *Pak. j. Nutr,* **9**(1): 81-85.

Argamino, C.R. and Janairo, J.I.B. (2016). "Qualitative assessment and management of microplastics in Asian green mussels (Perna viridis) cultured in Bacoor Bay, Cavite, Phillipines". *Environ. Asia*, **9**, 48–54.

Belabed, B.E., Meddour, A., Samraoui, B., Chenchouni, H., (2017). "Modeling seasonal and spatial contamination of surface waters and upper sediments with trace metal elements across industrialized urban areas of the Seybouse watershed in North Africa". *Environ. Monit. Assess.* **189** (6): 265. https://doi.org/10.1007/s10661-017-5968-5.

Ben Ameur, W., de Lapuente, J., ElMegdiche, Y., Barhoumi, B., Trabelsi, S., Camps, L., Serret, J., Ramos-López, D., Gonzalez Linares, J., Driss, M.R., Borràs, M., (2012). "Oxidative stress, genotoxicity and histopathology biomarker responses in mullet (Mugil cephalus) and sea bass (Dicentrarchus labrax) liver from Bizerte Lagoon (Tunisia)". *Mar. Pollut. Bull*. **64** (2):241–251. https://doi.org/10.1016/j.marpolbul.2011.11.026.

Bianchi, G. (1985). "FAO species identification sheets for fishery purposes. Field guide prepared with the support of PAK/77/033 and FAO (FIRM) regular programe, *Rome, FAO,* 200.

Campbell, K.R. (1994). "Concentrations of heavy metals associated with urban runoff in fish living in stormwater treatment ponds". *Archi. Of. Enviro. Contam. And Toxicol,***27**: 352-356.

Canli, M., and Atli, G. (2003). "The relationship between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species". *Env. pollu,***121**: 129-136. Chughtai, M.I. and Mahmood, K. (2012). "Semi-intensive carp culture in saline water-logged area: A multi-location study in Shorkot (District Jhang), Pakistan*". Pak. J. Zool,***44**:1065-1072.

Diop, M., Howsam, M., Diop, C., Cazier, F., Goossens, J.F., Diouf, A., Amara, R., (2016a). "Spatial and seasonal variations of trace elements concentrations in liver and muscle of round Sardinelle (Sardinella aurita) and Senegalese sole (Solea senegalensis) along the Senegalese coast". *Chemosphere*, **144**:758–766. https://doi.org/10.1016/j.chemosphere.2015.08.085.

Elnabris, K.J., Muzyed, S.K., and El-Asghar, N.M. (2013). "Heavy metal concentration in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza strip (Palestine)". *J. Of. Assocu. Of. Ara. Uni. For. Basi. and. Appli. Sci*,**13**: 44-51.

Emsley, J. (2001). "Zinc. Nature's Building Blocks". An A-Z Guide to the Elements, Oxford, England, UK: Oxford University Press, 499-505.

EPA. (1999). "National guidance: Guideline for assessing chemical contaminant data for use in fish advisories: risk assessment and fish consumption limits". *US EPA Washington.***2**(3)

Eustace, I.J. (1974). "Zinc, cadmium, copper and manganese in species of finfish and shellfish caught in the Derwent Estuart, tasmania". *Aus. J. Mar, Freshwat. Res*,**25**: 209-220.

Fahmida, I. (2002). "Mullets of korangi creek karachi". *Rec. Surv. Pak*,**14**; 11-18.

FAO (Food and Agriculture Organization). (1999). "Standards, guideline and legislature regulation concerning metals and their compounds in environment". **1**: 687-711.

FAO, (2010a). "The state of world fisheries and aquaculture".*doi*:10.1111/jfb.12 187.

FAO. (1983). "Compilation of legal limits for hazardous substances in fish and fishery products. FAO*". Fish. Circ,* 464: 5-100.

FAO. (1992). "Committee for Inland Fisheries of Africa Report of the Third Session of the Working Party on Pollution and Fisheries". *Accra. Ghana*,

FAO. (2010). "The food consumption refers to the amount of food available for human consumption as estimated by the FAO Food Balance Sheets

FAO/WHO, (2004). "Summary of the evaluations performed by thejoint FAO/WHO expert committee on food additives (JECFA 1956-2003). International life sciences institute press Washington, DC.DOI: 10.13140/RG.2.1.2024.1044.

Food and Agriculture Organization/World Health Organization (FAO/WHO). (2006). "Joint FAO/WHO Food Standards Programme Codex Committee on Food Additives and Contaminants". Thirty-eighth Session, The Hague, the Netherlands, 24-28.

Fazio, F., Iglio, D., Capillo, G., Saoca, C., Peycheva, K., Piccione, G., and Makedonsk, L. (2020). "Environmental Investigations and Tissue Bioaccumulation of Heavy Metals in Grey Mullet from the Black Sea (Bulgaria) and the Ionian Sea (Italy)". Anim, **10**, 1739; doi:10.3390/ani10101739.

Gale, N.L., Adams, C.D., Wixson, B.G., Loftin, K.A., Huang, Y.W. (2004). "Lead, zinc, copper and cadmium in fish and sediments from the Big River and Flat River creek of Missouri" s old lead belt". *Envir. Geochem. Healt*,**26**: 37-49.

Genc, T.O and Yilmaz, F. (2018). "Heavy metals content in water, sediment and Fish (*Mugil Cephalus*) from Koycegiz Lagoon system in Turkey: Approaches for assessing environmental and health risk". *J. Agr. Sci. Tech*,**20**: 71-82.

Ghaderpoori, M., Kamarehie, B., Jafari, A., Ghaderpoury, A and Karami, M. (2018). "Heavy metals analysis and quality assessment in drinking water—Khorramabad city, Iran," *Data in Brief*, **16**: 685–692.

Damb, BA. (13 Jun. 2021). Google Maps. Google. Retrieved from https://www.google.com/maps/place/Damb,+Winder,+Balochistan,+Pakistan/@25.4489083,66.5620982,3279m/data=!3m2!1e3!4b1!4m9!1m2!2m1!1sdamb!3m5!1s0x3eb46254b86a2cd5:0xe6e73d55cf6db991!8m2!3d25.4477747!4d66.5729937!15sCgRkYW1ikgEMc3VibG9jYWxpdHkx

Gaddani, BA. (13 Jun. 2021). Google Maps. Google. Retrieved from https://www.google.com/maps/place/Gaddani,+Las+Bela,+Balochistan,+Pakistan/@25.0697169,66.6750259,26315m/data=!3m1!1e3!4m5!3m4!1s0x3eb37bcb04974843:0x2d56274478e56748!8m2!3d25.0861084!4d66.7307796

Harahap, F. S and Lubis, L. T. (2018). "Analysis of heavy metals distribution in the river town of hamasaki's rod padangsidimpuan," *EKSAKTA: Berkala Ilmiah Bidang MIPA*, **19**: 50–56.

Hisham, N.W., Adnan, M.A., Hossam. A.Z. (2015). "assessment of heavy metals contamination in the Mediterranena sea along Gaza coast: a case study of Gaza fishing harbor".

Hosseini Alhashemi, A., Sekhavatjou, M.S., hassanzadeh kiabi, B., and Karbassi, A.R. (2012). "Bioaccumulation of trace elements un water sediments and six fish species from a freshwater wetland, iran". *Michrochem. J*, 104: 1-6.

Ihedioha, J. N., Ukoha, P. O and Ekere, N. R. (2017). "Ecological and human health risk assessment of heavy metal contamination in soil of a municipal solid waste dump in Uyo, Nigeria," *Envir, Geochemis, and Health*. **39**: 497–515.

Ihunwo, O.C., Dibofori-Orji, A.N., Olowo, C., Ibezim-Ezeani, M.U. (2020). "Distribution and risk assessment of some heavy metals in surface water, sediment and grey mullet (Mugil cephalus) from contaminated creek in Woji, southern Nigeria". *Mar. Pollut. Bull.* **154**: 111042. [CrossRef] [PubMed].

Islam, M. S., Hossain, M. B., Matin, A., Sarker, M.S.I. (2018). "Assessment of heavy metal pollution, distribution and source apportionment in the sediment from Feni River estuary, Bangladesh". *Chemosphere.* **202**: 25–32. https://doi.org/10.1016/j.chemosphere.

Jaric, I., Visnjic-Jeftic, Z., Cvijanovic, G., Gacic, Z., Jovanovi, L., Skoric, S., & Lenhardt, M. (2011). "Determination of Differential Heavy Metal and Trace Element Accumulation in Liver, Gills, Intestine and Muscle of Sterlet (Acipenser Ruthenus) from the Danube River in Serbia by ICP-OES". *Microchem. J,* **98**: 77-81.

Khattak, M.I., and Khattak, M.I. (2013). "Evaluation of heavy metals (As and Cd) contamination in the edible fish along Karachi-Makran coast". *Pak. J. Zool,***45**(1): 219-226.

Khemis, I.B., Aridh, N.B., Hamza, N., M'Hetli, M., Sadok, S., (2017). "Heavy metals and minerals contents in pikeperch (Sander lucioperca), carp (Cyprinus carpio) and flathead grey mullet (Mugil cephalus) from Sidi Salem reservoir (Tunisia): health risk assessment related to fish consumption". *Environ. Sci. Pollut. Res.* **24** (24):19494–19507. https://doi.org/10.1007/s11356-017-9586-0.

Krishna, P.V., Rao, K.M., Swaruparani, V and Rao, D.S. (2014). "Heavy metals concentration in fish *Mugilcephalus* from Machilipatnam coast and possible health risks to fish consumption". *Briti. Biotech. Jour,***4**(2): 126-135.

Malik, Y., Achakzai, W., Muhammad, S., Mustafa, S., Saddozai, S., Qadir, A., Ahmed, N., Ahmed, I. (2016). "Determination of heavy metal concentration in six species of fresh water fish sold in Quetta city, Pakistan". *Pure Appl. Biol,*

Mwangi J.M. (2013). "Determination of concentration of selected heavy metals in Tilapia fish, sediments and water from Mbagathi and Ruiru Athi River tributaried, Kenya".

Nelson, J.S., (2006). "Fishes of the world. 4th ed, John wiley and sons, Inc, Hoboken, New jersy, USA". 601.

Odika, P.O., Anike, O.L., Onwuemesi, A.G., Odika,N.F and Ejeckam, R.B. (2020) "Assessment of environmental geochemistry of lead-zinc mining at Ishiagu area, lower Benue trough, southeastern Nigeria," *Earth Science Research,* **9**;1–31.

Ouali, N., Belabed, B.E., Chenchoun, H. (2018). "Modelling environment contamination with heavy metals in flathead grey mullet Mugil cephalus and upper sediments from north African coasts of the Mediterranean Sea". *Sci of the Total Environ,* **639:** 156–174.

Peycheva, K., Panayotova, V., Merdzhanova, A., Stancheva, R. (2019). "Estimation of THQ and potential health risk for metals by comsumption of some black sea marine fishes and mussels in Bulgaria". *Bulg. Chem. Commun*. **51**: 241–246.

Prasath, P.M and Khan, T.H. (2008). "Impact of tsunami on the heavy metal accumulation in water, sediments and fish at Poompuhar coast, Southeast coast of India". *E-J. of. Chem,*5(1): 16-22.

Rahman, M. S., Hossain, M. B., Babu, S. O. F., Rahman, M., Ahmed, A. S., Jolly, Y. (2019). "Source of metal contamination in sediment, their ecological risk, and phytoremediation ability of the studied mangrove plants in ship breaking area, Bangladesh". *Mar, Pol, Bul.* **141**:137–146. https://doi.org/10.1016/j.marpolbul.

Rajeshkumar, S and Li, X. (2018). "Biaccumulation of heavy metals in fish species from the Meillang bay, tahu lake China". *Toxicolo, Rep.*, **5**:288-295.

Saher, N.U., Siddiqui, A.S. (2016). "Comparison of heavymetal contamination during the last decade along the coastal sediment of Pakistan: multiple pollution indices approach". *Mar. Pollut. Bull.* **105** (1):403–410. https://doi.org/10.1016/j.marpolbul.2016.02.012.

Schroeder, H.A. (1965). "Cadmium as a factor in hypertension". *J. Of. Chro. dise,***18**(7): 647-656

Sivakumar, R., Dhivya, K., Grayson, H and Natesan, M. (2015). "An integrated use of histological and ultra-structural biomarkers in *Mugilcephalus* for assessing heavy metal pollution in east Berbic-Corentyne, Guyana". *Inter. J. Bioassys,***4**(11): 4541-4554.

Stancheva, M., Makedonski, L., Petrova, E. (2013). "Determination of heavymetals (Pb, cd, as and hg) in black sea grey mullet (Mugil cephalus)". *Bulg. J. Agric. Sci.* **19** (1), 30–34.

Tawfik, M.S. (2013). "Metals content in the muscle and head of common fish and shrimp from Riyadh market and assessment of the daily intake". *Pak. j. Agri. sci,***50**(3): 479-486.

Ugonna, C. Nkwunonwo., Precious O. Odika and Nneka I. Onyia. (2020). "A Review of the Health Implications of Heavy Metals in Food Chain in Nigeria". *The Scientific World Journal*, 2020, Article ID 6594109, 11. https://doi.org/10.1155/2020/6594109.

USEPA. (1989). "Risk assessment guideline for superfund human health evaluation manual Part A". *Washing. DC. Uni. Stat. Envir. Protec. Agen,* **1**: 498.

Virtanen, J.K., Tina, H.R., Sari, V., and Tomi-Pekka, T. (2007). “Mercury as a risk factor for cardiovascular diseases”. J. Of. Nutri. biochem, **18**(12): 75-85.

Waseem, M.P. (2007). “Issues, growth and instability of Inland fresh production in Sindh(Pakistan): Spatial temporal analysis”. *Pak. Econo. & Soc. Rev,* **45**(2): 203-230.

Whitfield, A.K., Panfili, J., and Durand, J.D. (2012). “A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 with emphasis on the biology genetics ecology and fisheries aspects of this apparent species complex”. *Rev. Fish. Biol Fishe,***22**: 641-681.

WHO (World Health Organization). (1985). “Guidelines for Drinking Water Quality Recommendations”. WHO, Geneva, Switzerland.

WHO. (1989). “Evaluation of certain food additives and contaminants”. Report of the Thirty-Third of the join FAO/WHO expert committee on food additives. *Techn. Rep. Ser No.* 776. Geneva.

WHO. (1996). “Trace elements in human nutrition and health”. ISBN: 92 4 156173 4. Geneva.

WHO. (2000). “Evaluation of certain food additivies and contaminants”. Report of the Fifty-Third of the join FAO/WHO expert committee on food additives *Techic. Repor. Serie No.* 896. Geneva. *DOI*: 10.13140/RG.2.1.2728.6165.

WHO. (2004). “Evaluation of certain food additivies and contaminants”. Report of the Sixty-First of the join FAO/WHO expert committee on food additives *Techic. Repor. Serie No.* 922. Geneva. DOI: 10.13140/RG.2.1.2024.1044.

Wuana, R.A., and Okiemen, F.E. (2011). “Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation*”. Isrn. Ecol, 2011.*

Yi, Y.J., Zhifeng, Y., and Shanghong, Z. (2011). “Ecological risk assessment of heavy metals in sediments and human health risk assessmeny of heavy metals in fishes in the middle and lower reaches of the Yangtze river basin”. *Enviro. Pollut,***159**: 2575-2585.

Yilmaz, A.B (2005). “Comparison of heavy metal levels of Grey Mullet (*Mugilcephalus*) and Sea Bream (Sparus *aurata*) caught in Iskenderun Bay, Turkey”. *Turk. J. Vet. Anim. Sci,***29**: 257-262.

Zaqoot, A.H., Aish, M.A., Wafi, N.H. (2017). “Baseline concentration of heavy metals in fish collected from Gaza fishing harbor in the Mediterranean Sea along Gaza Coast, Palestine”. *Turk. J. Fish. Aquat. Sci*, 17: 101-109.

Zehra, I., Kauser, T., Zahir, E., Naqvi, I.I. (2003). "Determination of Cu, cd, Pb and Zn concentration in edible marine fish *Acanthopagurusberda* (DANDYA) along Baluchistan Coast-Pakistan". *Int. J. Agri. Biol*, **5**(1): 80-82.

Zhong, W., Zhang, Y., Wu, Z., Yang, R., Chen, X., Yang, J. (2018). "Health risk assessment of heavy metals in freshwater fish in the central and eastern North China. Ecotoxicology and Environmental Safety". **157**: 343–349. https://doi.org/10.1016/j.ecoenv.2018.

Zhuang, P., Li, Z., Mcbride, M. B., and Zou, B. (2013). "Health Risk Assessment for Consumption of Fish Originating from Ponds Near Dabaoshan Mine, South China". *Enviro. Sci. Pollu.t Res*,

Zubia, M., Fariha, M., Shagufta, S., Nosheen, R., Wali, M.A., Nighat, D., Humera, Z., Wajeha, R and Farhat, I. (2015). "Study on copepod parasite, *Lernaeenicuslongiventris* in *Mugilcephalus* from Karachi Coast of Pakistan". *Glob. Veter*,**15**(3): 325-327.